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Plant Posters

P-2000

Micropropagation and Transformation of Rhizomatous Plant Species. RUIFENG HE, Min-Jeong Kim, and David R. Gang. Laboratory for Cellular Metabolism and Engineering, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164. Email: rfhe@wsu.edu

The rhizome is the original stem of the vascular plant lineage, and the progenitor of modern stems. Rhizomes are still used as the stem type for many plants, from ferns to more advanced perennial crops and bioenergy grasses such as *Miscanthus* and switchgrass and important medicinal/herbal and spice plants such as ginger, turmeric and peppermint. On the other hand, many of the world's most significant weeds including bermudagrass, cogongrass and common reed, spread through and invade new territory, via rhizomes. Due to the importance of rhizomes and rhizomatous plants, it is essential to develop and establish *in vitro* micropropagation, regeneration and transformation systems to be able to better study such plants so that they can be better utilized or controlled. Most species in our collection of over 20 important rhizomatous species did not have available micropropagation and transformation systems. In recent years, we have optimized callus induction and shoot regeneration conditions and established micropropagation systems for several of these rhizomatous species including ginger, turmeric, aloe, peppermint, scouring rush, red rice, johnsongrass, bermudagrass and quackgrass. We have also developed *Agrobecterium*-mediated transformation in turmeric. Calli derived from turmeric nodes were used as target tissues. Various aspects of transformation and regeneration processes including callus induction and culture, *Agrobacterium* concentration and duration of co-cultivation, bacterial elimination and transformant selection were examined in order to improve the transformation efficiency. Transgenic plants and their vegetative progeny stably expressed the transgene as indicated by PCR, RT-PCR and GUS assays. Furthermore, we have cloned and identified four transcription factor genes including three MADS box genes and an ARF gene from ginger and turmeric. We have made genetic transformation to alter expression (overexpression and RNAi) of these genes in turmeric as well as in Arabidopsis. Results related gene function will be presented.

P-2001

Micropropagation and Large-scale Multiplication of *Stevia rabaudiana*-Bio-sweetener of the Future. A. SABITHA RANI [1], M. Satyakala[2], and V. Nagamani[1]. [1]Department of Botany, Osmania University College for Women, Koti Hyderabad, INDIA and [2] Biology Division Indian Institute of Chemical Technology, Hyderabad, INDIA. Email: sabitaammana@yahoo.com

The plant cell and tissue culture has been successfully exploited for *in vitro* conservation of several important medicinal plants. *Stevia rabaudiana* (Bertoni) is a small herb of Asteraceae family and emerged has as bio-sweetener of the future. The leaves of this plant possess intense sweet taste i.e. 200 to 300 times more than the sugar. The main constitutes are Steviosides (St) and Rebausioside A (R.A) which are non caloric and heat stable at 200°C, hence also used in bakery products. There is a high market demand for *Stevia* in India due to the increased incidence of diabetes and growing concern over the safety of chemical sweeteners. But, the major constraints being experience by farmers are high cost of planting material and low seed set and poor germination. Thus the *in vitro* production and multiplication of this species is of great importance. In the present study, nodal segments were collected from young branches of field grown plants; surface sterilized and inoculated onto MS media with various concentrations of BAP. Direct shoot regeneration was observed within one week of inoculation. Among all, high frequency of plant regeneration (83.4 %) was obtained on MS with 3 mg/l BAP concentration. The number of shoots varied from 6 to 8 per explants and also the length of the shoots is more at this concentration. Shoots were multiplied by sub culturing on lower concentration of BAP (1.0 mg/l BAP) for every week in first cycle and three weeks after second cycle. Through this procedure, a total of 60–80 were resulted from single nodal segment. Regenerated shoots were transferred to half strength MS medium supplemented with different concentrations of IAA and IBA. Among all, 5 mg/l IAA induced high frequency of rooting (60.2 %). The plantlets were transferred to portrays and later established in the field with 80 % survival rate.

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P-2002

In Vitro Screening Protocols for Assessing Drought and Salinity Tolerance in Poplar (*Populus* spp.) Germplasm. R. RAY[1], J. T. Bushoven[2]. [1]Department of Biology and [2]Department of Plant Science, 2145 East San Ramon Avenue, M/S AS72, California State University, Fresno, CA, 93740. Email: resh244@mail.fresnostate.edu, jbushoven@csufresno.edu

It has long been known that crop performance is dependent upon prevailing environmental conditions. This is especially evident in arid- and semi-arid regions where drought and high salinity often limit yields of many agriculturally important species, including woody-plant biofuel feedstock. For example, Poplar (*Populus* spp.) has great potential for use as a biofuel feedstock in marginal agricultural areas on which cultivation of food crops is not economically feasible. However, further genetic improvement of this species to better tolerate environmental stress may be possible due to its relatively small genome, rapid growth rate, and ease of which it can be vegetatively propagated. This study utilized *in vitro* callus induction and screening of existing Poplar germplasm for relative drought and salinity stress tolerance. Cultures were established from meristematic, nodal, petiole, leaf, and root tissue on Woody Plant Medium (WPM) supplemented with B5 organic additives, 2 % (w/v) sucrose.0.8 % (w/v) TC Agar and 5 mg/L 2,4 dichlorophenoxyacetic acid (2,4 D) and maintained for 10 weeks in the dark at 25±2°C. Individual media were supplemented with a range of increasing concentrations of Sorbitol or Polyethylene Glycol (PEG) to induce osmotic stress or with Sodium Chloride (NaCl) to induce salinity stress. Osmotic potentials (MPa) for each media were calculated to facilitate distinction between the osmotic and toxic effects of increasing salinity. The mean relative growth rate of established calli was determined. This data are essential for the success of efforts to rapidly screen woody-plant biofuel feedstock germplasm for tolerance to a range of sub-optimal environmental conditions found in many marginal agricultural areas.

P-2003

Prevention of Shoot Tip Necrosis Responses in *In Vitro*-proliferated Mature Pistachio Plantlets. H. AKDEMIR[1], H. Yıldırım[2], E. Tilkat[3], A. Onay[4], and Y. Özden Çiftçi[1]. [1]Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400, Kocaeli, TURKEY; [2]Dicle University, Faculty of Agriculture, Department of Horticulture, 21280 Diyarbakır, TURKEY; [3]Batman University, Department of Biology, 72100, Batman, TURKEY; and [4]Dicle University, Faculty of Science, Department of Biology, 21280 Diyarbakır, TURKEY. Email pinarakdemir@gmail.com, hakdemir@gyte.edu.tr

In vitro propagation of pistachio is still cannot achieve a maximum rate due to problems occurred in the micropropagation of this species such as low proliferation rate, basal callusing, browning of the explants and the media and shoot tip necrosis (STN). Among them, STN seems to be one of the major obstacles for large scale propagation of the species. Therefore, trials were carried out to overcome the STN problem in mature pistachio plantlets by assessing different calcium (Ca) (3, 6, 12 and 24 mM) and boron (B) (0.1, 0.2, 0.3 and 0.4 mM) concentrations used as medium components, while silver nitrate ($AgNO_3$, 12, 24 an 48 μM) was used as an ethylene inhibitor under different photon flux densities (30 or 50 μmol m^{-2} s^{-1}). Plantlets regenerated in a medium containing increased Ca or B concentrations showed fewer or no STN responses in comparison to normal MS medium (control). Although supplementation of $AgNO_3$ under both light intensities decreased STN, higher $AgNO_3$ concentrations (48 μM) had a toxic effect on the growth rate of the shoots. As a consequence, the STN problem in pistachio plantlets can be prevented with increased Ca or B concentrations under relatively low light intensities.

P-2004

Sequencing Coconut (*Cocos nucifera* L.) Expressed Sequenced Tags (EST) Using 454 Life Sciences Technology. H. D. D. BANDUPRIYA and J. M. Dunwell. Dept. of Biological Sciences, University of Reading, RG6 6AS, Reading, UNITED KINGDOM. Email: dbandupriya@yahoo.com

Coconut (*Cocos nucifera* L.) is a valuable plantation crop in the tropical region. As a complement to whole genome sequencing, EST analysis is a rapid and cost effective way to identify expressed genes. The focus of the work carried out in this study was to generate and compare a large set of coconut ESTs from different embryo development stages providing genomic resources for the discovery of novel genes associated with embryo development and to offer valuable genetic resources for coconut functional genomics. ESTs libraries were generated from four tissue samples including two zygotic embryo developmental stages; microspore derived somatic embryo and leaves. These four libraries were sequenced using 454-GS FLX platform which produced a total 223.7 Mb from 979428 high quality sequences. The assembly of quality filtered sequence reads using Newbler software provided with the Roche GS FLX sequencer led to construction of 32621 contigs with average length 460 bp. According to the BLASTX results, approximately 50 % of the sequences in each library showed significant sequence similarities to proteins in the NCBI databases. The unigenes were assigned into different gene ontology (GO) categories and summarized into broad

biologically functional groups according to similar functional characteristics or cellular roles by BLAST search against public databases. More than 45 % of the unigenes in each library were assigned in to GO categories. The Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway assignments were used for the identification of sequences encoding enzymes in biochemical pathways. This attempt predicted a total of 139 pathways for coconut ESTs. This collection EST data generated from different tissue types of describes the first large scale EST collection of coconut thus provides significant resource for genome wide studies and gene discovery of coconut; a non-model species which represented poorly in databases.

P-2005

Migration of Organellar DNA into the Nucleus of Maize. R. A. KUMAR, D. J. Oldenburg, and A. J. Bendich. Department of Biology, University of Washington, Box 351800, Seattle, WA. Email: rachana@uw.edu, bendich@uw.edu

The transfer of plastid DNA and mitochondrial DNA to the nucleus is an ongoing process. These transferred DNAs, called nuclear integrated plastid DNA (NUPTs) and nuclear integrated mitochondrial DNA (NUMTs), can confound the analysis of authentic organellar DNA during PCR when total tissue DNA is used. We have used bioinformatics to develop a method to design organellar DNA-specific primers and tested them for their ability to amplify NUPTs/NUMTs using methylation-sensitive PCR (NUPTs/NUMTs are highly methylated whereas organellar DNAs are unmethylated). The method is useful for analyzing phylogenetic and clinical data, as well as copy number changes during development for both organellar DNAs and NUPTs/NUMTs. We find that NUPT/NUMT copy number is higher in differentiated (leaf) than meristematic (stalk) tissue, whereas DNA polymerase-blocking lesions (reflecting DNA damage) are more frequent and DNA fragmentation is greater in leaf than stalk. The increase in NUPTs/NUMTs during development may result from increased release from the organelles of fragmented DNA that accompanies differentiation from meristematic cells. Although this method only quantifies methylated NUPTs/NUMTs, it can be used to identify events that affect the formation of NUPTs/NUMTs during plant development.

P-2006

Genetic Diversity of NBS-LRR Class Disease Resistance Gene Analogs in Cultivated and Wild Eggplants. Y. ZHUANG, X. H. Zhou, J. Liu, J. Jiang, and S. B. Wang. Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, 50 Zhongling St, 210014, CHINA. Email: yongzh1973@yahoo.com.cn

The genes encoding the nucleotide-binding site (NBS) and leucine-rich repeat (LRR) motifs constitute a large gene family in plants and have received great interest since most of the plant disease resistance genes that have been cloned are from this gene family. In the present study, degenerate oligonucleotide primers, designed based on conserved regions of Nucleotide Binding Site (NBS) domains from known plant resistance genes, were used to isolate Resistance Gene Analogs (RGAs) from cultivated and wild eggplants, including *Solanum melongena*, *S. aethiopicum* gr. *Gilo*, *S. linnaeanum*, *S. integrifolium*, *S. sisymbriifolium* and *S. khasianum*. Sequences analysis indicated that the cloned eggplant RGAs belong to non-TIR-NBS-LRR type, which showed strong similarity with *R* genes or the RGAs identified in other plant species, especially Solanaceae plants, suggesting the existence of common ancestors. Wide genetic diversity of eggplant RGAs was observed both in interspecific sequences and intraspecific sequences, and eight distinct families of eggplant RGAs were identified. Further studies showed the high average ratio of synonymous to non-synonymous substitution and low level of recombination. Above results suggested that NBS-encoding sequences of RGAs in cultivated and wild eggplants are subject to a gradual accumulation of mutations leading to purifying selection. These results represent the first report of NBS-LRR class RGAs in eggplants.

P-2007

Factors that Affect the Recovery of *Taxus* Transgenic Cell Lines. J. VAN ECK[1], P. Keen[1], S. Wilson[2], and S. Roberts[2]. [1] Boyce Thompson Institute, 533 Tower Road, Ithaca, NY 14853 and [2]Department of Chemical Engineering, University of Massachusetts at Amherst, Amherst, MA 01003. Email: jv27@cornell.edu

Paclitaxel or Taxol®, an effective chemotherapeutic drug, is produced by several species in the *Taxus* genus, which are slow growing yew trees. Two to four mature trees are required to provide sufficient paclitaxel for treatment of one cancer patient. Alternative sources of paclitaxel are *Taxus* cell cultures derived from yew embryos or other organs. Metabolic engineering of cell cultures can be used to elucidate the mechanism of paclitaxel biosynthesis and regulation and this knowledge could lead to strategies for increased production. However, a reliable and robust transformation system is needed for these studies, therefore, we investigated factors that promote efficient *Agrobacterium tumefaciens*-mediated transformation. Suspension and callus cultures of *Taxus cuspidata* cell lines were infected with *Agrobacterium* strains (LBA4404, GV3101, EHA105, C58C1) containing the binary vector pCAMBIA1301, which contains a GUS reporter gene and selectable marker gene for hygromycin resistance. Factors identified as being critical for recovery of stable transgenic

lines included the *Agrobacterium* strain, inclusion of an antioxidant cocktail during infection and washing, which was dependent on the cell line, optimization of the hygromycin concentration, and timing of transfer to hygromycin-containing selective medium post cocultivation. Application of the key factors resulted in recovery of numerous stable transgenic calli based on histochemical analysis for GUS expression in approximately 100 % of the transformation experiments where these parameters were tested.

P-2008

Expression of Anthocyanin MYB Autoregulatory Transcription Factors in Petunia. M. R. BOASE[1], C. Brendolise[2], K. E. Schwinn[1], K. M. Davies[1], R. V. Espley[2], and R. P. Hellens[2]. [1]The New Zealand Institute for Plant & Food Research Limited, Palmerston North 4442, NEW ZEALAND and [2]The New Zealand Institute for Plant & Food Research Limited, Mt Albert Research Centre, Auckland 1025, NEW ZEALAND. Email: murray.boase@plantandfood.co.nz

Previously we showed that an allelic rearrangement in the promoter of an anthocyanin-regulating transcription factor, MdMYB10, generated an autoregulatory gene, which occurred in all red foliage apple varieties. We also investigated how the complex floral and vegetative pigmentation patterns in petunia are derived from anthocyanin regulation by MYB, bHLH and WDR co-regulators. In the current work, by using a dual luciferase transient assay in tobacco, a 35SCaMV construct for one of the petunia MYBs, DEEP PURPLE (*35 S:DPL*), was shown to strongly activate the promoter of a petunia anthocyanin biosynthetic gene (DFR). Using the same transient assays we also found that *35 S:DPL* can activate the promoter of the apple self-regulatory allele (R6MYB10) to a similar extent as the apple protein MdMYB10. Based on these results, we constructed two new autoregulatory *DPL* alleles, the first an intragenic one with the petunia DFR promoter and the second a heterologous construct with the apple MYB10 promoter. A third autoregulatory construct of the R6MYB10 promoter driving MdMYB10 was already available. Using a particle inflow gun, we compared transient expression of the three autoregulatory constructs in petunia leaf discs. Subsequently we produced numerous independent transgenic lines containing each of the three autoregulatory constructs, via *Agrobacterium* mediated transformation. The phenotypes obtained from transient and stable transgenic experiments will be shown and discussed.

P-2009

Bioreactor Design for *In Vitro* Plant Propagation and Somatic Embryo Induction Using Transient Expression of Transcription Factors. S. FLOREZ, S. Shaw, and W. R. Curtis. Dept. of Chemical Engineering, Penn State University, University Park, PA 16802. Email: Wayne.Curtis@curtislab.org

Our research seeks to examine the feasibility of inducing somatic embryos from cultured plant tissues using agrobacterium-mediated transient expression of transcription factors in temporary immersion bioreactors. The commercial target plant is cacao (chocolate tree) and using the recent genome sequence, homologs of the "baby boom" transcription factor have been cloned (as well as several other embryogenesis-related genes). We are currently transforming Arabidopsis with this gene to confirm the "baby-boom" phenotype as a step toward the goal of introducing this gene into recalcitrant cacao genotypes using our *Agrobacterium* auxotroph to facilitate transient gene expression – and hopefully enhanced embryo formation. This work is being facilitated using a newly designed temporary immersion bioreactor (TIB) that utilizes gravity feed instead of typical pneumatic displacement of media. This design avoids use of pressurized gas and uses a simple plastic bag and headplate design with the intention of being implemented easily in a large-scale format. Another important aspect of this design is that it can be implemented with a small amount of gas flow which facilitates inexpensive control of the gas phase. Supplementation with CO_2 and removal of sugar is being tested in this system as an approach to further reduce plant propagation cost and minimize contamination.

P-2010

Metabolite Production in Plant Cell Cultures of Burdock (*Arctium sp.*). BIZHEN HU, Joseph C. Scheerens, John Cardina, and John J. Finer. The Ohio State University, 1680 Madison Ave., Wooster, OH, 44691. Email: hu.327@osu.edu

Use of plant secondary products for pharmaceutical applications has received recent attention as holistic approaches have become more widespread. Consistency in biochemical accumulation of secondary products in source plants can vary due to inconsistencies in environment and genetics. As the genotype is predominantly preserved from a donor plant to its tissue cultured propagules, plant tissue culture can be used to efficiently increase homogeneous plant material with superior metabolite profiles for use in clinical studies. Therefore, plant tissue culture has some utility for production of plant-based metabolites, especially for herbal pharmacy. Burdock *(Arctium lappa* L. and *Arctium minus*) is a potential source of herbal medicine with its plentiful secondary metabolites. Although Burdock has been used as a folk medicine for treatments of burns , no research is available on production of medicinal metabolites in this species. This research is aimed at

determining and developing an efficient procedure of plant tissue culture for burdock medicinal metabolite production. Callus culture was initiated from burdock cotyledon sections on MS media containing 1.5 mg/L BA and 1 mg/L NAA. Callus was then transferred and maintained on MS media containing 1.5 mg/L NAA and 1 mg/L 2,4-D. The effects of abiotic stimulus on medicinal metabolite accumulation in callus were studied by adding different concentrations of methyl jasmonate, salt, suguar, and MS inorganic nutrients. Total phenolics were extracted from callus and analyzed by HPLC over various time intervals. The biochemical production in callus culture was compared to that in plants grown in the field. Our preliminary data showed similar biochemical composition of burdock callus cultures compared to burdock leaves from field-grown plants, indicating that tissue culture is a potential way to produce medicinal compounds from burdock.

P-2011

Characterisation of the *Pistacia* Genus via Retrotransposon Based Marker Systems. E. KIRDOK and Y. Özden Çiftçi. Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400 Kocaeli, TURKEY. Email: emrahkirdok@gmail.com

The genus *Pistacia* contains at least 11 species including the commercially important pistachio. Up to now, genetic relatedness among the species were tried to be classified by using different genetic markers such as RAPD, AFLP, ISSR and SSR. However, retrotransposon based markers could also aid the classification of the genus. Marker systems established for detection of the polymorphism within the genome, based on retrotransposon structural properties: LTR' s (long terminal repeats) and PBS (primer binding site, universal and conserved tRNA primer binding site for reverse transcription which is found all retrotransposons) regions. Because of the universal structure of retrotransposons, a novel marker system called iPBS, based on PBS regions, could be useful for detecting not only polymorphism between species but also cloning new retrotransposons to develop IRAP and REMAP markers. In this context, the aims of this study concern to isolate retrotransposon fragments via iPBS PCR and then characterization the genus by using IRAP and REMAP markers. Thus, genomic DNA was isolated with using a modified CTAB protocol from germinated seedlings or cotyledons of *P. khinjuk* and used as template for iPBS PCR reaction. 9 bands were obtained with the usage of 4 iPBS primers. Then successful isolation and sequencing of these fragments resulted with identification of LTR regions thus provided information for designing primers for IRAP and REMAP markers. The genetic similarities and polymorphisms will be detected among individuals of *P. vera*, *P. khinjuk*, *P. lentiscus*, *P. mutica, P. palestina, P. atlantica* by using IRAP and REMAP markers.

P-2012

Progress Toward Castor (*Ricinus communis* L.) Transformation and Silencing the Ricin Gene. D. J. BARNES[1], N. A. Reichert[2], and B. S. Baldwin[3]. [1]Dept. of Mol. Bio., Biochem., Ent., and Plant Path., Mississippi State University, Starkville, MS 39762; [2]Dept. of Biological Sciences, Mississippi State University, Starkville, MS 39762; and [3]Dept. of Plant and Soil Sciences, Mississippi State University, Starkville, MS 39762. Email: djb70@msstate.edu

Castor (*Ricinus communis* L.) is a high-yielding oilseed crop native to tropical Africa. The seed contains ~60 % oil by weight, yielding approximately 1,200 kg of oil per hectare. The oil is composed of ~90 % ricinoleic acid, a unique hydroxyl-fatty acid. This unique composition provides castor oil with unique characteristics important for industrial use. Unfortunately this valuable oilseed has not been domestically cultivated since 1972, due in part to the presence of ricin in the seed. Ricin is a highly toxic lectin found in the endosperm of mature castor seed. This project seeks to remove ricin content via RNA interference. An *A. tumefaciens*-mediated transformation protocol is under development, and has shown putative success in transforming the *gusA* gene into castor. Current research is seeking to optimize this protocol in order to insert the experimental RNAi element into castor explants. The existing procedure utilizes direct transformation of intact embryo axes; however in order to increase the efficiency of the process, somatic embryogenesis and organogenesis protocols are being developed. After transformation the explants are screened for *hptII* activity on hygromycin b selection media. Putative transformants are then assayed for the presence of the *gusA* gene via PCR and X-Gluc assay. Explants that are confirmed via PCR and X-Gluc staining will then be rooted, acclimatized, and planted in the greenhouse. Transformants will then be allowed to flower, be self-pollinated, and the seed will be collected. Seed from each transformant will then be analyzed for ricin expression via Western blot and RT-PCR.

P-2013

RNAi Mediated Silencing of Hydroxycinnamoyl-CoA Quinate-Hydroxycinnamoyl Transferase Alters Phenylpropanoid Metabolism in Potato. R. S. PAYYAVULA[1], D. A. Navarre[1, 2]. [1]Irrigated Agricultural Research and Extension Center, Washington State University, 24106 N. Bunn Rd, Prosser, WA 99350 and [2]USDA-Agricultural Research Service. Email: raja.payyavula@ars.usda.gov; navarrer@wsu.edu

Plants produce many phenylpropanoid compounds that differ in abundance and function. In white potato (*Solanum tuberosum*), chlorogenic acid (CGA) is the major soluble phenolic sink. Several pathways are proposed for CGA biosynthesis, but hydroxycinnamoyl-CoA:quinate hydroxycinnamoyl transferase (HQT) mediated synthesis is considered the primary route. Genes involved in regulation and biosynthesis of the CGA pathway in potato were characterized by silencing of HQT and the effect of this redirecting of carbon flow within the phenylpropanoid pathway was characterized using LCMS. In HQT silenced transgenics, a sizeable reduction was observed in levels of CGA. Interestingly, total phenolics and antioxidant capacity were only modestly reduced. Perturbations elsewhere in the phenylpropanoid pathway were observed, including a decrease in caffeic and feruloyl quinic acids, rutin and kaempferol in leaves and tubers. Consistent with reduced phenolics, phenylalanine ammonia lyase activity decreased. HQT suppression increased carbon flux to polyamines such as caffeoyl putrescine, dihydrocaffeoyl spermines and additional unidentified compounds. Expression of several genes in the phenylpropanoid pathway, including transcription factors MYB1 and a basic helix loop protein (BHLH1), were reduced in HQT silenced plants. Sucrose feeding induced accumulation of CGA, and correlated with the increased expression of PAL but not HQT. Over expression of both MYB1 and BHLH1 in transient assays increased CGA. Our results suggest that in potato, CGA is synthesized mostly through HQT, but levels are regulated by additional factors including PAL, MYB1 and BHLH1.

P-2014

Fungal Mitochondrial DNases: an Unlimited Source of Genes for Engineering Disease Resistance in Plants. LEE A. HADWIGER. Department of Plant Pathology, Washington State University, Pullman, WA 99164–6430. Email: chitosan@wsu.edu

The ORF of a DNase gene from *Fusarium solani* f. sp. *phaseoli* (FsphDNase) was constructed in line with a promoter from a pea PR (pathogenesis-related) gene and inserted into the tobacco genome to develop resistance against *Pseudomonas syringae* pv tabacci. A number of pea PR genes, individually, have been shown to augment disease resistance transgenically in other plant species, indicating their general effect in developing resistance. Pea PR gene promoters are rapidly activated in pea when challenged with fungal mitochondrial DNase (mitoDNase) or by fungi not normally pathogens of pea (nonhost resistance response). This report identifies the ORF sequences, characteristics of gene product action and release mechanisms of mitoDNases from a wide array of fungal pathogens of both plant and animal. The DNase cellular exit and plant entry, the mode of defense gene activation and gene functions resulting in induced resistance by DNases have been characterized.

P-2015

Effect of Various Sterilization Procedures on the In Vitro Germination of Cotton Seeds Collected from the Open Field. S. KRASNYANSKI[1], S. Barampuram[1], C. Haigler[2], and G. Allen[1]. [1]North Carolina State University, Dept. of Horticulture, Campus Box 7550, Partners II Bldg., Room 1208, Raleigh, NC 27606 and [2]North Carolina State University, Dept. of Crop Science, 4405 Williams Hall, Campus Box 7620, Raleigh, NC 27695. Email: sfkrasny@ncsu.edu

In vitro manipulation of cotton often requires the production of high quality sterile seedlings as a source of hypocotyl and cotyledon explants to initiate embryogenic cultures. However, if seeds are collected from the open field of various locations and exposed to unknown weather and storage conditions, a very high level of seed contamination, associated with endogenous and soil borne pathogens, may result. Often the seed supply available for sterilization is limited. The major challenge in this situation is to produce contamination-free seedlings at a high germination rate. The aim of this study is to identify the most efficient surface sterilization protocol by determining an optimal sterilizing agent, time exposure, and other factors that affect *in vitro* seedling germination and quality. Three the most commonly used sterilizing agents (sodium hypochlorite or commercial bleach, chlorine gas, and hydrogen peroxide) were examined. Additional modifications to improve the sterilization procedure included hot soapy water wash, concentrated sulfuric acid treatment, and Plant Preservative Mixture (PPM) rinse. The sterilized seeds were then germinated on Linsmaier & Skoog medium gelled with 0.6 % agar and incubated under the light in the growth chamber for 8 days. Results were recorded as the percentage of contamination-free seeds, seed germination, and the final number of sterile well-developed seedlings. We found that washing the seeds with hot soapy water, followed by sterilization with 3 % hydrogen peroxide for 7–8 h, and an additional PPM (1 %v/v) rinse showed significantly higher sterilization and germination rates when compared to other treatments. In addition, this sterilization protocol was the most efficient for all 17 genotypes tested so far for producing high quality seedlings. This protocol may also be useful for sterilizing other seeds, besides the cotton, that were collected from the open field and likely to have high levels of contamination.

P-2016

Overexpression of PvMYB4 in Switchgrass Leads to a Three-fold Increase in Ethanol Production without Pretreatment.

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Recalcitrance of the cell wall is a major hurdle in the conversion of lignocellulosic biomass to ethanol from switchgrass. Genetically engineered plants optimized for bioethanol production will enable us to even skip the pretreatment step thus reducing the cost for ethanol production. Overexpression of PvMYB4, an R2R3 type MYB repressor of the lignin pathway, in switchgrass down-regulated most of the monolignol biosynthetic genes. As a result, total lignin content and ester linked p-CA/FA ratio was reduced. The plants had reduced stature and increased number of tillers and there was no change in biomass yield compared to controls. Sugar release assays demonstrated an approximately 3-fold increase in sugar release efficiency. Simultaneous saccharification and fermentation (SSF) of untreated biomass resulted in an about threefold increase in ethanol production compared to controls. Consolidated bioprocessing (CBP) of hot water pretreated biomass using *C. thermocellum* is being done to analyze potential inhibitors of fermentation. These results indicate that overexpression of a key transcription factor that represses lignin pathway will significantly impact ethanol production by reducing the cost for biomass pretreatment.

P-2017

EMS Mutagenesis from Embryogenic Cell Suspension and PCR-HRM Screening for SNPs in Grapevine. Y. ACANDA, O. Martinez, M. J. Prado, and M. Rey. Department of Plant Biology and Soil Science, University of Vigo, 36310, SPAIN. Email: yosvani.acanda@gmail.com

Grapevine (*Vitis vinifera* L.), the most important fruit crop grown worldwide, is also considered a model species in woody plants biotechnology. Somatic embryogenesis is an excellent system for mutation induction and clonal propagation in woody plants. Our work was focused on establishing one procedure for inducing EMS mutagenesis in grapevine. Somatic embryos were induced from staminal filaments using TDZ and 2,4-D and embryogenic cell suspension was established in the same liquid media. Embryogenic cell aggregates (ECA) were treated with increased concentration of EMS (0, 1, 3, 10, 20 and 30 mM). In order to determine mutagen dosage, percentages of survival and cell aggregates generating embryo (CAGE) were recorded. Embryo masses generated from treated ECA were analyzed by PCR - HRM in order to detect SNPs on VvNCED1 1200 bp encoding fragment as target gen. Mutation on regenerated plants was verified by PCR-Sequencing. We have found that EMS affects viability drastically at concentration above 20 mM (25.51± 2.98 ECA survival percentage) while concentration above 10 mM seriously compromises embryogenic potential (22.07±1.71 CAGE percentage). Analysis of the difference plots for the fluorescence signals detected a mutation at one sample from 10 mM EMS treatment. Embryos from this clump were germinated and leaves collected for PCR-Sequencing analysis. The alignment of the sequences from regenerated plants and the wild type sequence revealed a transitional mutation G/C to A/T in the VvNCED1 encoding region. In conclusion, ECA are suitable for EMS mutagenesis and PCR-HRM analysis allows SNPs detection before plant regeneration in grapevine.

P-2018

RNAi-suppressed Lignin Biosynthesis in Sugarcane. F. ALTPETER, J. H. Jung, Y. Xiong, J. Y. Kim, W. Fouad, W. Vermerris, and M. Gallo. Agronomy Department, Plant Molecular and Cellular Biology Program and Genetics Institute, University of Florida - IFAS, Gainesville, FL. Email: altpeter@ufl.edu

A large amount of lignocellulosic biomass such as leaf litter residues and bagasse are generated during the sugarcane harvest or after the sugar refining process, respectively. Therefore, lignocellulosic biomass from leaf and processing residues will likely become a valuable feedstock for future biofuel production. However, lignin is recognized as the major limitation to efficient conversion of lignocellulosic biomass to biofuel. Therefore, altering lignin composition or reducing lignin content via RNAi suppression of lignin biosynthetic genes is a promising strategy to increase the efficiency of biofuel production from lignocellulosic sugarcane residues. In the lignin pathway, 4-coumarate-CoA ligase (4CL) and caffeic acid 3-*O*-methyltransferase (COMT) are key enzymes that catalyze the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates or the methylation of 5-hydroxyconiferaldehyde to sinapaldehyde, respectively. In this study, *COMT* and *4CL* genes were isolated from the commercially important sugarcane cultivar CP 88–1762 by a combination of cDNA library screening and PCR based approaches. More than 100 transgenic lines harboring *COMTi* or *4CLi* or both

COMTi and *4CLi* constructs were generated via biolistic gene transfer. Quantitative real-time PCR identified transgenic lines ranging from no suppression to almost complete suppression of the target genes. Accumulation of siRNAs was confirmed in 23 transgenic lines by Northern blot analysis of low molecular weight RNA. These results demonstrate that RNAi is effective in suppression of individual or co-suppression of multiple endogenous genes of the complex sugarcane genome. The transgenic lines were vegetatively propagated and grown to maturity in a replicated and randomized design. Data describing the analysis of lignin content and composition as well as performance of the transgenic plants will also be presented.

P-2019

Agrobacterium- and Biolistics-mediated Stable Gene Expression in Apomictic *Brachiaria brizantha*. G. B. CABRAL[1,2], A. P. Martinelli[1], V. T. C. Carneiro[2], and D. M. A. Dusi[2]. [1]University of São Paulo, CENA, Av. Centenário, 303, Piracicaba, SP, 13416–903 BRAZIL and [2] EMBRAPA Genetic Resources and Biotechnology, PO Box 02372, Brasilia, DF, 70770–900, BRAZIL. Email: gbcabral@cena.usp.br

Brachiaria brizantha cv. Marandu is an aposporic apomictic forage grass of great economic importance, as the basic feed for beef cattle production, in Brazil and other tropical countries. The apomictic reproduction impairs the introduction of variability in the species to produce new cultivars. Moreover, crosses between the sexual and apomictic plants are impracticable due to the difference in ploidy. A methodology to introduce genes of interest is highly desirable and useful to conventional and molecular breeding of *Brachiaria*. Furthermore, it will allow the study of gene function during apomictic and sexual reproduction in *Brachiaria*. *Brachiaria* is still recalcitrant to genetic transformation and there are very few reports in the literature. To establish a system that allow the introduction of genes of agronomic interest and to study gene function in apomictic *Brachiaria*, we have been developing transient and stable gene expression protocols for *B. brizantha*. Embryogenic cell suspensions, induced in two different media, were co-cultivated with *A. tumefaciens* strain LBA4404 or EHA105 harboring a binary vector containing *gus/gfp* under the control of *Ubi1* promoter and *hptII* gene controlled by the *Act1* promoter (pGPro1). Liquid and solid co-culture was carried out for 3 days. The same binary vector was bombarded into cell suspensions that were further selected in hygromycin containing medium. GFP and GUS expression were confirmed in cultures and hygromycin resistant cells were selected to obtain transgenic plants. Acknowledgements: CAPES, CNPq

P-2020

Promoting Conservation and Sustainable Use of Rare Species of Himalayan Orchid *Habenaria edgeworthii* Through *In Vitro* Propagation. L. GIRI[1], A. Jugran[1], I. D. Bhatt[1], R. S. Rawal[1], S. K. Nandi[1] and V. Pande[2]. [1]G. B. Pant Institute of Himalayan Environment and Development, Kosi- Katarmal, Almora, Uttarakhand 263 643, INDIA and [2]Biotechnology Department, Kumaun University Nainital, Uttarakhand 263 001, INDIA. Email: lalitorchid@gmail.com

Habenaria edgeworthii (family Orchidaceae), native to Himalaya, is used in 'Chyavanprash', a popular Ayurvedic rejuvenating tonic in India. Large scale harvesting is done from wild, affecting availability in nature. Species is considered rare and needs appropriate strategies of conservation and sustainable utilization. Present study attempts to: (i) investigate genetic and phytochemical variation amongst populations, (ii) standardize *in vitro* propagation protocol for secondary metabolite production and (iii) suggest appropriate strategy of its conservation and sustainable utilization. Plant samples were collected from nine populations in Uttarakhand, west Himalaya (India) for genetic, phytochemical and *in vitro* propagation studies, and analyzed using standard methods. Genetic variability, using ISSR marker, among populations ranged from 0.1048 to 0.3016, with maximum diversity in Mussoorie population. Total phenolic content varied between 3.78 to 5.78 mg Gallic acid equivalent (GAE) / g dry weight (DW), maximum being for Pithoragarh population. The antioxidant activity using different *in vitro* assay showed significant variation across populations. *In vitro* propagation protocol using seed derived callus was established, to increase biomass and phytochemical. Maximum callus biomass (2.61 g fresh weight) was obtained in 1.0 μM BA. Total phenolic content varied significantly and ranged from 10.33 to 14.30 mg GAE/ g DW. This study identifies as Mussoorie (genetic) and Pithoragarh (phytochemical) populations as elite. *In vitro* propagation through callus emerges good for increasing callus biomass; secondary metabolite production. These approaches have commercial applications as increased biomass production of calli may be used for obtaining desired yield of active compounds. If this happens, pressure will be reduced from natural populations thereby contributing for *in situ* conservation of this rare species in the Himalaya.

P-2021

Improving Mineral Nutrition of Micropropagated Hazelnuts. CHARLES HAND[1] and Barbara M. Reed[2]. [1]Department of Horticulture, 4017 ALS, Oregon State University, Corvallis, OR 97331 and [2]USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333–2521. Email: handc@onid.orst.edu, Barbara.Reed@ars.usda.gov

New hazelnut (*Corylus avellana* L.) cultivars resistant to Eastern Filbert Blight are in demand. Micropropagation is used to rapidly increase plant availability, however micropropagtion rates and plant quality vary greatly and improved media are needed. This study tested five *C. avellana* selections on modified Driver and Kuniyuki [DKW (1984)] medium containing 30 g glucose/liter and 200 mg/liter Sequestrene 138 Fe (Yu and Reed 1995). The mineral nutrients were separated into five factors: NH_4NO_3, Ca (NO_3), mesos ($MgSO_4$ and KH_2PO_4), K_2SO_4, and micro-elements (B, Cu, Mn, Mo, and Zn). The factors ranged from 0.5x-2.0x standard DKW and 33 treatments were designed using a surface response program. Shoots were grown on treatment medium for a total of thirteen weeks, medium was refreshed every 3 weeks. BA standardized at 8 μM and IBA 0.05 μM based on an earlier experiment. Multifactor response surface analysis showed optimum shoot proliferation in response to mineral nutrients was genotype specific. Nitrogen ratios and increased mesos were important to overall quality and shoot length for all genotypes. Four of the five genotypes showed increased minors nutrients improved general quality and shoot length. Nitrogen ratios were important for shoot multiplication, as were increased minors for most genotypes. Data indicated there is no one "general" medium was optimum for all genotypes, was clear that the nitrogen ratios were important and minor elements should be increased.

P-2022

Next Generation Gene Silencing Vectors in Soybean. THOMAS B. JACOBS[1], Peter R. LaFayette[1], Lila O. Vodkin[2], and Wayne A. Parrott[1]. [1]University of Georgia, Center for Applied Genetic Technologies, Athens, GA and [2]University of Illinois, Department of Crop Science, Urbana, IL. Email: tbj03001@uga.edu

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

P-2023

Long-term Preservation of *Pycnanthemum* Genetic Resources. M. M. JENDEREK[1], G. E. Holman[1], D. Ellis[1], and B. M. Reed[2]. [1] National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, CO 80521 and [2] National Germplasm Repository, USDA-ARS, Corvallis, OR 97333. Email: maria.jenderek@ars.usda.gov

Plants of *Pycnanthemum* Michx. (Mountain mint; *Lamiaceae*) are native to North America. They naturally grow in prairies, forest edges, pastures and along roadsides. Their flowers provide abundant nectar for honey bees, wasps, butterflies and other insects. Mountain mint leaves are very scented and pungent making them usable as spices. The plants also contain fragrant oils that are used in pharmaceutical and cosmetic industries. Some species have ornamental value and are sold in nurseries. The genus has over 20 different species. The National Plant Germplasm System (USDA, ARS) has 120 different accessions of Mountain mint and several of them are maintained vegetatively as tissue culture. Our study investigated the possibility of placing 27 different in vitro accessions (12 species) in long-term storage to avoid periodic subculturing. Shoot tips were isolated from three-week old cultures, cold hardened for two additional weeks (22°C/8 hr light and −1°C/16 hr dark). The shoot tips consisted of basal stem tissue, 1–2 young leaf bases and 1–2 leaf primordia. The cryopreservation followed an encapsulation-dehydration protocol. After 24 hours of storage in liquid nitrogen, the encapsulated shoot tips were warmed to 20-25°C and plated on a recovery medium (MS+BA 0.5 mg/L and IBA and 0.1 mg/L). After two weeks, the shoot tips were transferred to a growth medium (MS without growth regulators) for another three weeks. For 23 accessions, the post cryo viability (culture with developed leaves and roots) was 60 to 100 %. The viability for the remaining four accessions was 40 to 50 %. The encapsulation-dehydration cryopreservation method proved adequate for long-term preservation of the 27 *Pycnanthemum* germplasm accessions.

P-2024

Impact of Habitat Type and Altitudinal Range on Morphological and Genetic Diversity of *Valeriana jatamansi* in Western

Himalaya, India. A. JUGRAN[1], I. D. Bhatt[1], R. S. Rawal[1], S. K. Nandi[1], and V. Pande[2]. [1]G. B. Pant Institute of Himalayan Environment and Development, Kosi- Katarmal, Almora, Uttarakhand 263 643, INDIA and [2]Biotechnology Department, Kumaun University Nainital, Uttarakhand 263 001, INDIA. Email: arunjugran@gmail.com

Reduction in habitat and population size, disturbances in pollination activity and seed dispersal are amongst most common consequences of habitat fragmentation causing loss in genetic variation, increased differentiation and genetic drifts which lead to reduced ability of populations to adapt to changing environments. Besides habitat fragmentation, altitude impacts genetic diversity. Present study focuses on populations of *Valeriana jatamansi* (Indian Valerian; family Valerianaceae) a high value medicinally important herb, at diverse habitat and altitudes and attempts to: (i) investigate morphological and genetic diversity, and (ii) assess the impact of habitat type and altitude range on its morphological and genetic diversity. Plants were collected from twenty five populations of west Himalaya, India. Morphological parameters, including above ground fresh and dry weight (AGFW & AGDW), and below ground fresh and dry weight (BGFW & BGDW) were recorded. Significant variation in AGFW, AGDW and BGDW across populations was revealing. High genetic variability amongst populations was observed using ISSR markers. 159 clear and reproducible fragments, ranging from 200 – 4000 bp were detected. Of these, 125 ISSR loci were polymorphic, with a range from 65.41 % to 91.19 % and mean as 78.62 %. Across habitat types, maximum AGFW (21.88 g), BGFW (2.72 g), AGDW (1.20 g) and BGDW (0.90 g) were recorded in mixed forests and minimum in pine forests. Populations growing in grassy slopes showed highest genetic diversity (He=0.334; Pp=84.38). Across altitude, maximum AGFW (5.05 g), BGFW (3.15 g), AGDW (1.91 g), BGDW (1.2 g) were recorded for populations located>2400 m asl. Analysis of genetic diversity, however, revealed a decreasing trend (Pearson $r=-0.386$; $p<0.05$) with increasing altitude. Study recommends: (i) populations growing on grassy habitats of low altitudinal areas, with higher genetic diversity, should be considered as elite stock (ii) population in mixed forest habitats is good for production of biomass.

P-2025

Improvement of Switchgrass C4 Photosynthetic Efficiency for Increased Biomass Production. M. C. HALTER[1], J. Mitchell[2], D. G. J. Mann[1], M. Balasubramaniam[1], E. T. Nilsen[2], and N. Stewart[1]. [1]Department of Plant Sciences, University of Tennessee, Knoxville, TN and [2]Department of Biological Sciences, Virginia Polytechnic University, Blacksburg, VA. Email: mhalter@utk.edu

As the demand for biofuels in the United States and worldwide increases, so too does the need for a reliable and high yielding feedstock. Switchgrass (*Panicum virgatum* L.), a cellulosic biomass crop native to North America, has been proposed as an alternative to starch-based crops in the U.S. Ongoing research is currently exploring multiple modes of improving this feedstock, both through traditional breeding practices and biotechnology. Among the major targets of research are improving upon the recalcitrance of the switchgrass cell wall to enzymatic pretreatment, as well as improvement of biomass yields. Improvement of cold temperature photosynthesis has been proposed as a means to the latter. As a C4 plant, switchgrass is prone to poor cold temperature photosynthesis. We have shown in this study that switchgrass experiences a 46 % reduction in growth under cold temperatures (14°C) relative to ideal temperatures (28°C). *Miscanthus* x *giganteus*, another C4 monocot, has been shown to photosynthesize efficiently at 14°C, only experiencing a 31 % reduction in growth. This has been shown to be at least partially due to the up-regulation of the expression of the *pyruvate orthophosphate dikinase* (PPDK) gene in cold conditions. This gene catalyzes the regeneration of phospho*enol*-pyruvate (PEP) from pyruvate in the C4 photosynthetic pathway, and has been shown to be the rate limiting enzyme in this pathway. It has been proposed that overexpression of *M. x giganteus* PPDK in switchgrass will increase the cold temperature photosynthetic capacity of switchgrass by increasing the concentration of PEP available for carboxylation with CO_2. Using the pANIC vector set developed for switchgrass transformation, T0 switchgrass plants constitutively expressing *M. x giganteus* or *Zea mays* PPDK have been produced. These plants are currently being analyzed at 14°C and 25°C for photosynthetic capacity relative to non-transgenic control plants of the same genotype. The effect of increased photosynthetic flux on cell wall structure is also currently being characterized.

P-2026

Cryopreservation of *Eucalyptus uro-grandis* (*E. grandis x urophylla*) via Droplet Vitrification. E. KAYA[1], A. Ozudogru[2], M. Jenderek[1], and D. Ellis[1]. [1]National Center for Genetic Resources Preservation, Fort Collins, CO and [2]Gebze Institue of Technology. Kocaeli, TURKEY. Email: david.ellis@ars.usda.gov

Eucalyptus sp. is a widely as a forest tree, covering more than four million hectares in 58 countries. *E. uro-grandis*, a hybrid of *E. grandis* and *E. urophylla*, is an important hybrid for paper products, building materials, and essential oils. Droplet vitrification was used for cryopreservation of 1.5 mm shoot tips from in vitro shoot cultrues of *E. uro-grandis*. Shoot cultures were maintained on MS medium,

supplemented with 1 % sucrose, charcoal ($10gl^{-1}$), benzyladenine ($0.04\ mgl^{-1}$), gelrite ($1{,}5gl^{-1}$) and agar ($4{,}5gl^{-1}$). For cryopreservation, excised shoot tips were precultured for 24 hr on solid MS medium containing 0.25 M sucrose followed by 24 hr on MS medium contianing 0.625 M sucrose. Following sucrose preculture, indivdual shoot tips were placed in 4–5 µl PVS2 drops on sterile aluminium foil strips (~ 5 x 15 mm strips, five PVS2 drops per strip) on ice for 15, 30, 45, 60, 75 or 90 min. Following PVS2 exposure, the aluminium foil strips containing the shoot tips in PVS2 droplets were directly plunged into liquid nitrogen (LN) and transferred under LN into cryovials. Thawing was done after a minimum of 24 hr LN exposure by rapidly removing the frozen aluminium foil strips from the cryovials and immediately immersing the frozen strips into room temperature washing solution (liquid MS medium, containing 1 M sucrose). When the shoot tips floated off the aluminum foil strips, they were transferred onto the MS maintenance medium. Control shoot tips consisted of shoot tips exposed to PVS2 for the same time intervals as LN-treated material but immediately placed in washing solution following PVS2 exposure. Using this approach, 60 % of cryopreserved *E. uro-grandis* shoot tips exposed to PVS2 for 60-min developed into plants.

P-2027

Cryopreservation of *Musa velutina* Seed via Dehydration. D. Ellis[1], E. KAYA[1], E. A. Ozudogru[2], and B. Irish[3]. [1]National Center for Genetic Resources Preservation, Fort Collins, CO; [2]Gebze Institute of Technology. Kocaeli, TURKEY; and [3]Tropical Agriculture Research Station, Mayaguez, PR. Email: david.ellis@ars.usda.gov

A fungal disease threatening global banana production has intensified interest in breeding bananas resistent to the current threat. However, bananas are predominately vegetatively propagated, seed is difficult to germinate and further, seed does not store well. *Musa velutina* H. Wendl. & Drude, a species originally from India, is fast growing, smaller in stature and a valued ornamental. *M. velutina* also produces large amounts of seed making it an ideal model for the development of methods for long-term storage of *Musa* seed. As a first step we determined how much dehydration (based on seed moisture content [MC]) the *M. velutinea* seed could survive. Surface-sterilized *M. velutina* seeds were placed in a laminar flow hood for varying periods of time for controlled dehydration, and seed MC was determined every hourly for up to 9 hours when seed MC was below 20 %. To assess seed sruvival, embryos were aseptically excised from the dehydrated seed and placed on germination medium (MS medium supplemented with 0.1uM GA, 20 gl^{-1} sucrose, 1.5 gl^{-1} phytagel and 4 gl^{-1} agar). Embryo survival was good even when seeds were dried to below 20 % MC. For cryopreservation, five dehydrated seeds (MC of 15 %-30 %) were placed in each 1.5 ml cryovial and directly plunged into liquid nitrogen. After at least 24 hr of LN exposure, the seeds were rewarmed by in cryovials in a laminar flow hood at room temparature for 15 min. Embriyos were then aseptically excised from the seeds and transferred to germination mediumThe highest survival was obtained 8 hr dehydration (84.3 % germination rate; MC: 16,7 %) of the seed.

P-2028

In Vitro Propagation of Peanut (*Arachis hypogea* L.) via Temporary Immersion Bioreactor Systems. E. KAYA and E. A. Ozudogru. Gebze Institute of Technology, Department of Molecular Biology and Genetics, Istanbul cad. no: 101, 41400 Gebze, Kocaeli, TURKEY. Email: kayaer19@gmail.com

Peanut (*Arachis hypogaea* L.) is very rich in herbal oil, protein, aminoacids and vitamins, and thus is widely used for human nutrition and animal feed. However, descent planting of an American cultivar 'Virginia' with relatively bigger seeds gives rise to negligence of many other cultivars, among which also the valuable Turkish local cultivars exist, and thus they face a serious risk of loss in nature. Mass propagation of threaten species via *in vitro* regeneration systems makes available the maintenance and storage of their germplasm. Nevertheless, peanut possesses a recalcitrant nature to *in vitro* regeneration procedures. Indeed, although several protocols of somatic embryogenesis, organogenesis and micropropagation of peanut have been developed up to date, plant regeneration obtained in those studies remained relatively low. Thus, development of advanced protocols and approaches for *in vitro* propagation of peanut is required. Recently developed technique, TIS, Temporary Immersion Bioreactor System provides a new approach in mass propagation of plants. In fact, TIS bioreactor system not only combines the advantages of liquid and semi-solid cultures, but also decreases to minimum the difficulties of such classic propagation techniques. The aim of the present study was the development of protocols for the use of TIS Bioreactor System for the mass propagation of peanut, and application of optimized procedures to the valuable Turkish local cultivars (COM, NC7, 7X77). In conclusion, use of liquid MS medium supplemented with 110 µM BA (6-Benzyladenin) or 10 µM TDZ (Thidiazuron), in TIS bioreactor system with 16 hour / 16 minute immersion, resulted with the highest multiple shoot formation on cotyledones devoid of embryos. Although the system was tested for its utility in mass propagation of several different species, it has not been utilized yet for the propagation of peanut plant. Thus, the present study was of great importance in this aspect.

P-2029

FLP/FRT Recombination for Plant Genetic Engineering. L. D. NGUYEN, S. Nandy, and V. Srivastava. Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR. Email: ldnguyen@uark.edu

Site-specific recombination systems are powerful tools for genetic engineering, particularly for excising selection marker gene from transgene locus. Cre/*lox* system from bacteriophage P1 and FLP/*FRT* system from the yeast *Saccharomyces cerevisiae* are the two most widely used recombination systems in eukaryotes. However, efficiency of FLP/*FRT* system in plant genome is often reported to be below practical level. To solve this problem, improved versions of FLP protein called FLPe, the thermostable derivative of the wild-type FLP (FLPwt), and FLPo, the mouse codon-optimized version of FLPe, were developed by other groups. In the present research, the relative recombination efficiencies of FLPwt, FLPe and FLPo for marker gene excision from the transgene locus in rice will be evaluated. Rice lines expressing FLP proteins were tested for their relative recombination efficiency using a transient expression assay. This analysis showed that FLPe and FLPo expressing rice lines display higher recombination efficiency compared to FLPwt. In future, each FLP line will be crossed with a recombination-target line to evaluate the recombination efficiency in rice plants, and inheritance of the recombinant locus by the progeny. This information will be critical for developing protocols for marker gene excision using FLP/*FRT* system.

P-2030

An Approach to Limit the Cry Endotoxin Protein Production to Insect Bite Sites in Cotton Using a Wound Induced Promoter. S. ÖZCAN, A. Bakhsh, E. Anayol, S. Onarıcı, M. Aasım, C. Sancak, K. M. Khawar, S. F. Özcan, and L. Ünlü. Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110 Ankara, TURKEY. Email: ozcans@ankara.edu.tr

Insect resistance in plants is created by insertion of *cry* genes regulated by strong constitutive promoters such as CaMV 35 S. As a result, cry toxin proteins are produced in all organs throughout the entire life cycle of the plant. There are anxieties towards the accumulation of endotoxins in soil and in different living beings due to prolonged exposure of these proteins. Previous studies have shown that when AoPR1 promoter isolated from *Asparagus officinalis* mesophyll cells suspension and cloned in front of *gus* gene; when transferred to tobacco and potato plants, results in showing high activity of AoPR1 promoter in the wounded region only and in the plant leaves, stems, roots, tubers, seeds and pollens mostly showed no or very low rates of expression. As a result chewing leaves of tobacco transformed by cloned *cry1Ac* gene after AoPR1 promoter, the toxic cry proteins results in rapid and 100 % death of insects. However, no or very low-level expression of *cry1Ac* gene was observed in non-injured or non-chewed portions of the plant. In this study, regeneration capacity of different cotton cultivars was first checked. Then, *cry1Ac* gene expression cassette under the control of AoPR1 promoter was cloned into the binary vector carrying *bar* gene. Regenerative explants were inoculated with *A. tumefaciens* containing AoPR1-cry1Ac plasmid to produce transgenic cotton, in which cry endotoxins were eliminated.

P-2031

RNAi Mediated Viral Resistance in Transgenic Wheat: Stability Over Five Generations. JESSICA L. RUPP[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

Wheat streak mosaic virus (WSMV) and Triticum mosaic virus (TriMV), are two viruses of the wheat mosaic complex affecting wheat in the Great Plains of the United States. The current disease management strategy incorporates the deployment of resistant varieties, 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. RNAi expression vectors were independently created from the sequences of the coat proteins (CP), the HC-Pro, 6 K2-Nia and a portion of the VPG of both WSMV and TriMV. Immature embryos of the wheat cultivar 'Bobwhite' were independently co-transformed by biolistic particle delivery system with RNAi expression vectors and pAHC20, which contains the *bar* gene for glufosinate selection. After tissue culture, putative transformed plants were analyzed through PCR for the presence of the appropriate RNAi 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 the corresponding virus. Viral presence was established by ELISA. In the T_1 generation, resistance was seen in up to 60 % of the plants evaluated for both CP constructs, although some events that showed transgene presence did not exhibited resistant phenotype. Analyses of transgene presence and expression in T_2 generation evidenced events of transgene silencing and deletion. Regardless of these phenomena, a consistent resistance response in two lines of WSMV CP construct and one TriMV CP transgenic line was found. T_5 generations have shown continuing high

levels of resistance among the CP constructs. While these findings are scientifically significant; ultimately a stable resistant line is required for commercial use. Thirty two crosses have been made to the commercially desirable cultivar 'Overly' followed by 3 backcrosses. These plants have shown continued resistance to *Wheat Streak Mosaic* Virus thereby offering a possible stable transgenic method of disease management.

P-2032

Studies on Sexual Dimorphism in *Simmondsia chinensis* (Link) Schneider: Differential Morphogenic Behaviour and DNA Fingerprinting. KULDEEP SHARMA, Monika Haijkuram, and Veena Agrawal. Department of Botany, University of Delhi, New Delhi, 110007, INDIA. Email: kdsharmadu@gmail.com, monica_honev11@yahoo.co.in, drveena_du@yahoo.co.in

Simmondsia chinensis (Jojoba) is a monogeneric and multipurpose dioecious shrub, of simmondsiaceae, has emerged as a cash crop all over the globe as its seeds store liquid wax (40- 60 % dry wt. basis) of commerce. Determination of sex prior to flowering, which takes almost 3–4 years, has been a serious problem in this taxon where male and female ratio remains 5: 1. Realizing the aforesaid problems, influence of different stressors *viz*. different growth regulators [cytokinins (BA, Kn and 2iP), and auxins (IAA and NAA)], on *in vitro* morphogenesis of node and shoot- tip explants of the male and female individuals of two cultivars *i.e.*, Q-104 and C- 64, has been evaluated separately. An attempt has also been made to ascertain the sex in this species with the help of PCR- based molecular markers. Differential morphogenic behaviour was recorded in both the cultivars under same regime of growth regulators and male and female individuals showed differential hormonal requirement for their growth and development. BA was found to be the cytokinin of choice, with 10 μM concentration being optimum for male individuals while, 20 μM for the female individuals. Overall auxins played an inhibitory role but NAA proved better than IAA in differentiating relatively high number of shoots among auxins. However, in combination of cytokinin (10 μM or 20 μM BA) and auxins morphogenic responses of female individuals (C- 64) were better. Employing the PCR based markers, i.e. RAPD and ISSR with bulk segregate analysis (BSA), two putative sex linked markers (OPG-5_{1400} and UBC-807_{1200}) have been obtained in the selected clones of jojoba. The unique fragments of ~1400 bp and ~1200 bp were present in all the male individuals of all the clones and were completely absent in respective female individuals tested. Further work is in progress to generate a SCAR marker and characterization of the particular fragment which would be of immense use for better understanding of the developmental as well as evolutionary pathways of sexual dimorphism. Nevertheless, this will be quite helpful in uprooting the male plants, thereby, saving resources like labor, water, fertilizers and the space for highly desirable female plants.

P-2033

Development of Transgenic Rice Lines Expressing Coleoptera-resistant Genes and Insect Bioassay on Rice Water Weevil. K. S. SHIN[1], J. H. Lee[1], S. L. Rhim[2], S. A. Lee[1], M. H. Lim[1], H. J. Woo[1], H. I. Ahn[1], Y. H. Lee[1], S. J. Kweon[1], and S. C. Suh[1]. [1]National Academy of Agricultural Science, Rural Development Administration, 225 Seodun-dong, Gwonseon-gu, Suwon, Gyeonggi-do 441–707, KOREA and [2] Department of Biomedical Science, Hallym University, Chuncheon, Gangwon-do 200–702, KOREA. Email. kongsiks@korea.kr

The rice water weevil (RWW), *Lissorhoptrus oryzophilus* are major pests of aquatic rice plant in Korea as well as throughout the country. Larvae of RWW sucking the nourishment on roots, causes a stunted root system and reduces grain yields. To prevent these damages, we constructed various plant expression vectors, which were harbored by insecticidal genes, *cryBP1* and *cryIIIa*, and fused with the actin promoter and/or the modified RCg2 root-preferential promoters for expressing the insect-toxic genes in leaves and roots. A *cryBP1* was cloned from *Bacillus popilliae*, producing crystal toxin against Japanese beetle, and *CryIIIa* was modified from the δ-endotoxin gene of *Bacillus thuringiensis* ssp. tenebrionis, encoding the coleoptera-specific toxin. The vectors containing the insecticidal genes were transferred into *Oryza sativa* japonica cultivar, Nakdong, by *Agrobacterium*-mediated transformation method. Several independent transgenic lines were selected by Southern blotting and Western blotting, confirming that *cryBP1* and *cryIIIa* genes were stably integrated into the plant genomes and were expressed in transgenic plants. Upon insect bioassay using RWW, the mortality of insect larvae on *cryBP1* and *cryIIIa* transgenic rice lines recorded up to 41 % and34%, respectively. These results suggested that the transgenic lines can be used to develop Coleoptera-resistant cultivars and could be valuable for later application in crop breeding for insect resistance.

P-2034

Cryopreservation and In Vitro Germination of *Aechmea bicolor* L. B. Sm. (Bromeliaceae) Pollen Grains. E. H.

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Pollen grain conservation is important for fundamental and applied aspects of breeding and conservation of plant genetic resources. The possibility of pollen grains conservation allows for crossings between genotypes, which may be difficult to obtain due to asynchrony of flowering, facilitating the production of hybrids and genetic compatibility studies. In this study we evaluated the efficiency of different methods of pollen grains conservation in *Aechmea bicolor* through in vitro germination in different culture media and pollen grains ultrastructure following preservation treatments. Fresh pollen grains obtained at anthesis were initially cultured in different culture media (BM, BKM, MBKM, SM, MSM) to define the best medium for pollen grains germination and tube growth. Viability was also verified in Carmine, Alexander and Sudan IV stains. Preservation methods were tested using pollen grains collected at anthesis, consisting of four freezing temperatures (freezer at −5°C, ultra-freezer at −80°C and liquid nitrogen at −196°C), with or without prior desiccation in silica gel for 3 hours. Pollen grains in vitro germination and tube growth was measured at 1 h, 24 h, 8d, 30d, 6 and 12 months. Pollen grains morphology and ultrastructure was evaluated after 24 h, 30d and 12 months. A completely randomized design was used, in a 2 x 3 +1 factorial (2 dehydration conditions, 3 conservation temperatures and 1 control) with a subdivided parcel in time, being the parcels composed of the factorial and the subparcels composed of time and its interactions with the parcels treatments. After conservation treatments the pollen grains were cultivated in MSM medium (0.01 % H_3BO_3, 0.03 $Ca(NO_3)_2$, 0.02 % $MgSO_4$. 0.01 % KNO_3, 12 % sucrose; 0.8 % agar; pH 6,5). The results showed that dehydration followed by liquid nitrogen conservation (−196°C) gave the best results for in vitro germination and pollen tube growth. Morphological and ultrastructural integrity was also maintained in this treatment, with considerable morphological and structural damage observed in other treatments. (Acknowledgements: FAPESP - Process 2009/18255-0, CNPq, CAPES).

P-2035

Restriction Enzyme Mediated Pollen Ablation Promises to be an Effective Species-independent Male Sterility and Bioconfinement Tool. C. NEAL STEWART, JR., Hong S. Moon, Jason M. Abercrombie, Laura L. Abercrombie, Reginald J. Millwood, Charleson R. Poovaiah, and Shigetoshi Eda. Department of Plant Sciences, University of Tennessee, Knoxville, TN 37919. Email: nealstewart@utk.edu

Type II restriction endonucleases, such as EcoRI are very well characterized and have long-served as workhorses for molecular biology. We placed an *EcoRI-GFP* fusion gene under the control of a pollen-specific promoter, which was stably integrated in transgenic tobacco. The resultant plants were self-fertilized and a flow cytometry method detected very few GFP-positive pollen grains, yet some events had decreased pollen viability. A test-cross to a male-sterile tobacco mutant (used as the female parent) was made, seeds were collected from the male-sterile parent, germinated on selective media and PCR-confirmed. Several of the transgenic lines displayed 100 % efficacy for the male-sterile trait (of ~40,000 seeds plated). Pollen-specific *EcoRI* expression appears to be an effective and potentially universal pollen ablation tool that could be used in any plant species for transgene bioconfinement. In addition to its efficacy, there is no indication of any off-target effects to the plant, since plants appeared morphologically normal. Currently the system is being further validated in test-crosses being performed in the field and the construct and variants are being engineered into other plant species.

P-2036

Cotton Plants Expressing a Hemipteran-active *Bacillus thuringiensis* Crystal Protein Impact the Development and Survival of *Lygus hesperus* Nymphs. SHUBHA SUBBARAO, James A. Baum, Jeanne G. Layton, Uma R. Sukuru, Stephen R. Penn, Steven E. Meyer, Xiaohong Shi, Nina V. Sidorova, Elizabeth H. Jakse, Pamela A. DeLaquil, Jeannie M. Washam, Stanislaw Flasinski, Gregory R. Heck, Robert S. Brown, and Thomas L. Clark. Monsanto Company, St. Louis, MO. Email: shubha.subbarao@monsanto.com; Corresponding author: james.a.baum@monsanto.com

The plant bugs *Lygus hesperus* Knight (Hemiptera: Miridae) and *L. lineolaris* (Palisot de Beauvois) have emerged as economic pests of cotton in the United States. These hemipteran species are refractory to the insect control traits found in genetically-modified commercial varieties of cotton. The isolation and characterization of a 35 kDa crystal protein from *Bacillus thuringiensis*, designated TIC807, causes reduced mass gain and mortality of *L. hesperus* and *L. lineolaris* nymphs when presented in an artificial diet feeding assay. Cotton plants (Coker130) expressing the TIC807 protein were observed to impact the survival and development of *Lygus hesperus* nymphs in a concentration-dependent manner. These results, demonstrating *in planta* activity of a Lygus insecticidal protein, represent an important milestone in the development of cotton varieties protected from Lygus feeding damage.

P-2037

Deletion Analysis of Anther-specific Region in *gALCHS7* Promoter from Lily (*Lilium hybrid* cv. 'Acapulco') by Petunia Transformation. E. J. SUH [1], B. H. Han[2], B. W. Yae[3], D. H. Kim[1], M. J. Jeong[1], S. K. Lee[1], An-Cheol Chang[1], and S. C. Bae[1]. [1] Bio-crop Development Division, National Academy of Agricultural Science, RDA, 441–857, KOREA; [2] Dept. of Seed Service, Foundation of Agri. Tech. Commercialization & Transfer, 441–100, KOREA; and [3]Floriculture Research Division, National Institute of Horticultural & Herbal Science, RDA, 441–440, KOREA. Email: seji00@empal.com, seji00@korea.kr

Tissue-specific promoter (*gALCHS7* , 1584 bp) which was cloned from lily was confirmed high GUS (*β-glucuronidase*) expression in anther. But some transgenic plants (five of ten lines) also showed weak GUS expression in ovule. To identify the functional elements of the *gALCHS7* promoter involved in anther–specific expression, *cis*-regulatory elements were predited using the PLACE (http://www.dna.affrc.go.jp/cDNA/Place) database. Many cis-acting elements associated with anther specific expression were found in the promoter region. Especially POLLEN1LELAT52 motif which were dispersed full promoter sequence was estimated as major contribution to anther expression. We constructed several deleted construct based on these elements and tried to find expression regioin in anther . Among them, *gALCHS7-4* (1017 bp) and *gALCHS7-7* (270 bp) was expressed only anther and not detected ovule as contrast to *gALCHS7* promoter by GUS staining. But GUS activity by the minimal promoter was down as compared with *gALCHS7-4* promoter by naked eyes. Through dark-field microscopic analysis, GUS signal of two promoter was found in pollen, endothesium and epidermis. Therefore *gALCHS7-4* (1017 bp) promoter was enough to identify the function of genes from anther with no side effects.

P-2038

Mesos Components Improve Pear Germplasm Micropropagation. SUGAE WADA[1], Randall P. Niedz[2], Terrence J. Evens[2], and Barbara M. Reed[3]. [1]Dept. of Horticulture, Oregon State University, 4017 Agriculture & Life Science Bldg. , Corvallis, OR97331; [2]Horticulture and Breeding Unit, 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 97333–2521. Email: wadas@hort.oregonstate.edu, Barbara.Reed@ars.usda.gov

The USDA-ARS National Clonal Germplasm Repository *in vitro* collection contains over 200 pear accessions in 18 species. Due to the wide genetic diversity of this collection there is also a diverse response to growth on standard tissue culture media. An initial study of mineral nutrition using a systematic response-surface approach with five mineral stock solutions (ammonium nitrate, potassium nitrate, mesos, minors and iron) found that the mesos factor affected the most responses and the most genotypes. This study was designed to optimize the mesos (Ca, Mg, P) components on 10 pears (5 *Pyrus communis* cvs, *P. calleryana*, *P. dimorphophylla, P. koehnei*, *P. ussuriensis* and *P. pyrifolia*). Sixteen factors were evaluated including overall quality, shoot multiplication and shoot height. Short stature, leaf spots, edge necrosis and red or yellow coloration were some of the main symptoms of poor nutrition in these diverse pears. Increased concentrations of all three constituents of the mesos solution decreased leaf symptoms, and significantly increased overall plant quality ($P=0.0001$). Treatments with >1.5X the MS concentrations of all three chemicals produced the best quality ratings for all 10 genotypes. Concentrations producing the best growth varied by genotype, but all required higher than MS concentrations for the best growth and multiplication. A further test of 16 genotypes found that increasing all the components equally to 1.5 or 2.0X the MS levels produced high quality plants for all genotypes. Resulting shoot growth was healthier and taller, leaves were greener plus leaf spotting, discoloration and edge burn were mostly eliminated.

P-2039

Efficient Transformation of Cytokinin Pathway Genes Controlled by Root-specific Promoters in Barley (H. vulgare L.). H. Gnad[2], J. WEYEN[1], E. Ramireddy[3], and T. Schmuelling[3]. [1]SAATEN-UNION BIOTEC GmbH, Hovedisser Str. 92, D-33818 Leopoldshoehe, GERMANY; [2] SAATEN-UNION BIOTEC GmbH, Am Schwabeplan 6, D-06466 Stadt Seeland, OT Gatersleben, GERMANY; and [3]Dahlem Centre of Plant Sciences (DCPS), Institute of Biology/Applied Genetics, Albrecht-Thaer-Weg 6, D-14195 Berlin, GERMANY. Email: weyen@saaten-union-biotec.com

In the Plant-KBBE II project ROOT (funded by the German Ministry of Education and Research) we focus on the genetic transformation of cytokinin pathway genes under the control of root-specific promoters to modify root morphology, nutrient acquisition and drought resistance in barley. Cytokinins regulate cell division and development and are negative regulators of root growth and lateral root formation. Transgenic Arabidopsis plants with reduced function of cytokinin oxidase/dehydrogenase 1 and 2 (CKX1 and CKX2) show increased cytokinin breakdown and increased primary root growth. Finally the produced transgenic lines will be analyzed by automated phenotyping tools. To identify the impact of CKX genes in barley, four root-specific promoters were identified in rice and combined with CKX1 and CKX2 genes of

Arabidopsis. Transformations of immature barley embryos were made using Agrobacterium-mediated gene transfer. The transformation efficiencies ranged between 3,0 and 9,3 %. This range was even broader when reporter genes GUS and GFP were used and rose up to 14,8 %. Primary transformed plants are transferred into soil and first results will be presented. Finally the produced transgenic lines will be analyzed by automated phenotyping tools.

P-2041

In Vitro Screening of Salt-tolerant Somatic Mutants in *Pennisetum purpureum.* XIAOXIAN ZHONG, Zhiwei Liu, and Jianli Zhang. Institute of Animal Science, Jiangsu Academy of Agricultural Sciences, Jiangsu Province, Nanjing 210014, CHINA. Email: xiaoxian@jaas.ac.cn

Pennisetum purpureum (Napier grass or elephant grass) is an important forage and energy crop. Inducing salt tolerant mutants for breeding new cultivars is of great significance when utilizing plentiful saline land to satisfy the needs of livestock and production of biomass energy in the world. To achieve this, a system for screening salt tolerant elephant grass mutants was established. Immature inflorescences were used as explants, and calli were induced on MS medium supplemented with 3.0 mg l^{-1} 2, 4-dichlorophenoxy acetic acid (2, 4-D) and 0.2 mg l^{-1} 6-benzylaminopurine (6-BA). White and compact embryogenic calli were subcultured on the same callus induction medium supplemented with 0, 1.2, 1.4, 1.6, 1.8, and 2.0 gl^{-1} NaCl. After 42 days, embryogenic callus was differentiated on MS medium with 0.01 mg l^{-1} naphthyl acetic acid (NAA) and 2.0 mg l^{-1} N-(2-Chloro-4-pyridyl)-N′-phenylurea (CPPU) supplemented with the same NaCl concentration as the subculture medium. All cultures were incubated at 26°C in a growth chamber under 16 h of diffuse light. At 0, 1.2, 1.4, 1.6, 1.8, and 2.0 gl^{-1} NaCl concentration, the ration of compact callus was 55.3 %, 49.3 %, 36.2 %, 32.8 %, 31.1 %, and 15.4 %, respectively during the subculture, and the percentage of regenerated plantlets was 8.4 %, 4.0 %, 1.8 %, 1.6 %, 0.3 %, and 0.0 %, respectively. The lethal NaCl concentration was 20 gl^{-1} in differential culture. The shape of almost all plantlets was abnormal from 1.4 gl^{-1} to 1.8 gl^{-1} NaCl in subculture and differentiation medium. The suitable NaCl concentration for salt tolerant mutant screening was determined to be below 1.4 gl^{-1}.

P-2042

Somatic Embryogenesis and Plant Regeneration from Calluses Derived from Shoot-tips of Forced Softwood Shoots of Teak (*Tectona grandis* L. f.). M. AKRAM and F. Aftab. Department of Botany, University of the Punjab, Lahore-54590, PAKISTAN. Email: faheem.botany@pu.edu.pk

Softwood shoot forcing is relatively a newer approach for *in vitro* establishment and multiplication of several recalcitrant tree species. In the present study, softwood shoots were used as a starting material for somatic embryogenesis (SE) and plant regeneration of teak (*Tectona grandis* L. f.). Completely randomized design was used and the data were analyzed by ANOVA and Regression analysis ($p<0.05$). Shoot tips, nodal and internodal explants were cultured on Murashige and Skoog (MS) medium supplemented with α-Naphthaleneacetic acid (NAA; 1, 3, 6, 10, 15 μM) or Thidiazuron (TDZ; 0.001, 0.01, 1, 4, 8, 10, 12 μM) for callus induction and further growth of callus on the same levels of TDZ or N^6-benzyadenine (BA; 0.4, 1, 4, 8, 10 μM)+1 μM Indole-3-butyric acid (IBA) or Gibberellic Acid (GA_3) in glass jars (55 x 125 mm). Highest callus induction (100 %; $p< 0.0442$) with nodulated callus masses (45 mm^3), and dry weight (5.7 g) were obtained at 0.1 μM TDZ from shoot tip explants after 35 days. Calluses were then grown at 6, 8 or 12 μM TDZ+2.2 μM BA or IBA along with 5 mM ascorbic acid (AA) for SE. High frequency (100 %; $p=0.0221$) with 36.4 globular ($p< 0.0321$) and 5.5 ($p=0.0022$) heart shaped somatic embryos were obtained at 8 μM TDZ+2.2 μM BA after 63 days. Such cultures when further maintained on the same medium for up to 150 days resulted in 100 % shoot regeneration ($p=0.0321$) with 16.4 mean number of shoots. Shoots were elongated (50 mm) on MS agar medium+8 μM BA+1 μM IBA for 40 days and then rooted on half MS supplemented with 6, 8, 10 μM IBA and NAA alone or in combinations with each other+charcoal (0.1 %). Rooting was 70 % with 4.5 mean number and 49.1 mm length at 8 μM IBA+8 μM NAA after 36 days with 56.6 % acclimatization.

P-2043

Somatic Embryogenesis and Plantlet Regeneration in Amla-A Medicinal Plant. L. AL-SABAH, C. Sudhersan, and S. Jibi Manuel. Biotechnology Department, Food Resources Division, Kuwait Institute for Scientific Research, P. O. Box 24885, Safat 13109, KUWAIT. Email: lsabah@kisr.edu/kw

Amla, botanically known as *Emblica officinalis* is a medicinally important perennial tree species cultivated for its fruits. A high efficient *in vitro* plant regeneration technique is necessary for the crop improvement, micropropagation and germplasm conservation in this species. The aim of the current study is to develop a protocol for high efficient simple plant regeneration protocol via somatic embryogenesis for amla. Immature cotyledons were isolated from the sterilized immature seeds of amla and used as explants for the experiments. Cotyledon explants were inoculated onto MS basal culture medium supplemented with auxins (2,4-D or NAA at 0–10 mg/l) and cytokinin (1 mg/l Kinetin). After 3–4 weeks in culture under 16 h light, direct somatic embryo regeneration

was noticed on the fully expanded cotyledon explants inoculated onto MS medium with 1–5 mg/l 2,4-D and 1 mg/l Kinetin. Somatic embryo proliferation, maturation and germination occurred when the cultures were transferred on to growth hormone free MS basal medium. Explants maintained further for long time on the 2,4-D media produced somatic embryogenic callus. Somatic embryo desiccation improved the plant regeneration. Quarter strength MS media containing 1–3 mg/l rooting hormone IBA enhanced the root induction and plantlet growth *in vitro*. Photoautotrophic culture system supported 100 % plant survival during greenhouse *ex vitro* hardening. A simple high efficient protocol for *in vitro* plant regeneration via somatic embryogenesis has been developed for the genetic improvement, germplasm conservation and micropropagation of amla through this study.

P-2044

A Somoclonal Variant of Potato cv. Spunta Developed via In Vitro Tissue Culture. C. Sudhersan, Y. AL-SHAYJI, S. Jibi Manuel, and J. Ashkanani. Biotechnology Department, Food Resources and Marine Sciences Division, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, KUWAIT. Email: schellan@kisr.edu.kw

Genetic variation is the source for crop improvement. Somoclonal variation is the genetic variation induced during in vitro cell and tissue culture. The aim of the present study was to get somoclonal variants with salt tolerant characters. In this study we used potato cultivar Spunta for the *in vitro* experiments. Somatic embryogenic callus cultures were developed from stem nodal explants of potato cultivar Spunta using higher concentrations of 2,4-D under longer duration. The regenerated plantlets via somatic embryogenesis were screened for salinity tolerance and variation in tuber characteristic features *in vitro*. Few plants tolerant to high salinity was selected among the regenerated potato plantlets and multiplied for microtuber induction at high salinity *in vitro*. The highest salinity tolerance of the selected variant was 9770 ppm TDS in the culture media. This variant produced microtubers under the same salinity *in vitro* with variation in microtuber shape. The *in vitro* produced microtubers and the rooted cuttings when transferred to the field condition showed plant growth and survival under brackish water irrigation. However, the tuber size was smaller than the control plants irrigated with fresh water. The shape of the tubers was similar in both type of irrigation and varied from the mother cultivar Spunta on salinity tolerance, tuber shape and yield. This study demonstrated and conformed that somoclonal variants can be developed through *in vitro* cell and tissue culture techniques in potato.

P-2045

Screening and Quantification of an Antiseptic Alkylamide, Spilanthol from In Vitro Cell and Tissue Cultures of *Spilanthes Acmella* Murr. RAKHI CHATURVEDI and Mithilesh Singh. Department of Biotechnology, Indian Institute of Technology - Guwahati, Guwahati - 781039, Assam, INDIA. Email: rakhi_chaturvedi@yahoo.co.uk, rakhi_chaturvedi@iitg.ernet.in

The study revealed, for the first time, accumulation of spilanthol, an antiseptic alkylamide, in *in vitro* cultures of *Spilanthes acmella* Murr., a medicinal plant of immense commercial value. To achieve this, in vitro shoots were regenerated via direct organogenesis from leaf-disc explants of *Spilanthes*. Shoots were induced in the presence of N6-benzylaminopurine (BAP) alone or in combination with either alpha-naphthalene acetic acid (NAA) or Indole-3-acetic acid (IAA) in Murashige and Skoog medium. The best treatment for shoot regeneration was MS+BAP (5.0 μM)+IAA (5.0 μM), which promoted adventitious shoot proliferation in >82 % cultures with an average of 5.3 shoots per explant. Regenerated shoots rooted spontaneously with a frequency of 100 % on half strength MS medium (major salts reduced to half strength) containing 50 gl^{-1} sucrose. The plantlets were acclimatized successfully with 90 % survival rate. Additionally, ploidy stability of the regenerated plants was assessed by flowcytometry which showed that all investigated plants had the similar ploidy as that of the mother plant. For spilanthol identification, peaks eluted from HPLC were analyzed by mass spectrometry with its characteristic fragmentation pattern. For quantification studies, calibration curve was generated, which revealed a higher amount of spilanthol content (3294.36±12.4 μg/g DW) in the leaves of *in vitro* plants compare to those of in vivo plants (2703.66± 9.6 μg/g DW of spilanthol). An efficient multiplication frequency, ploidy stability and enhanced spilanthol accumulation ensure the efficacy of the protocol developed for this industrially important medicinal plant.

P-2046

Tissue Culture Responsiveness of Immature Embryos of Grain and Sweet Sorghum (*Sorghum bicolor* L. Moench) Towards Different Callus-inducing Media. I. S. CURTIS, R. Kaur, and O. Folkerts. Chromatin Inc., 2109 S. Oak St., Suite 101, Champaign, IL 61820. Email: icurtis@chromatininc.com

Sorghum ranks fifth worldwide in terms of production among cereals. Grain sorghum is important as a source of food for human and animals especially on lands marginal for the production of many other crops due to its ability to tolerate drought, disease, poor soils and water-logging. Forage

sorghum is an important animal feed crop, and its significant biomass yields per acre make it suitable as a biomass/bioenergy crop for co-firing or cellulosic feedstock applications. Sweet sorghum has major potential as a biofuel feedstock due to its ability to accumulate high levels of fermentable sugars in the stalk. Genetic engineering offers the opportunity to increase crop yield and increase fuel and fibre production, but this will rely on an efficient gene transfer system, which has remained a major bottleneck in sorghum. A critical factor in the success of producing transgenic events is to establish an efficient and reproducible system for the production of embryogenic callus from immature embryos which can be used as target material for gene transfer. Here, we have evaluated three media: mM11, PHI-T and DC on the responsiveness of immature embryos of four grain (BTX623, TX430, BNB381, N246) and two sweet sorghums (Rio, PI563917) in terms of growth of explant, type of callus produced, phenolic production and frequency of explants producing embryogenic callus. In terms of growth, mM11 medium promoted strong coleoptile growth over callus production for all genotypes. The initiation of callus from immature embryos was much delayed by all genotypes when cultured on PHI-T and showed more phenolics compared to other media. Callus formed on PHI-T medium was white and compact, whilst those from other media were friable and cream/yellow in color. Maximum number of explants producing embryogenic callus ranged from 77 % for BTX623 and 39 % for Rio (both on DC), 76 % for TX430 and 83 % for N246 (both on PHI-T), 20 % for BNB381 on mM11 and 17 % for PI563917 on PHI-T with 0.64 g/L MES. Such embryogenic callus will be used in particle bombardment transformation experiments with *npt*II and *pmi* selection to further optimize transgenic plant production.

P-2047

Development of a Rapid Regeneration Method for Runner-type peanut (*Arachis hypogaea* L.). P. M. DANG, C. Y. Chen, and M. C. Lamb. USDA, ARS, National Peanut Research Laboratory, 1011 Forrester Drive, Dawson, GA 39842. Email: phat.dang@ars.usda.gov

Application of biotechnology through plant transformation can facilitate the development of new varieties with "hard to breed for" traits such as disease or stress resistance. A rapid peanut regeneration process, especially following a gene transfer event such as biolistic gene gun or *Agrobacterium* mediated, can minimize stress to tissue in culture and produce fertile plants. Current regeneration methods have been successful with Spanish, Valencia, and Virginia peanut plant types but limited success in the regeneration of runner-type which represents 75-80 % of all peanuts grown in the U.S. A rapid organogenesis-regeneration method for runner-type peanut was developed utilizing cotyledonary nodes as starting explants to produce normal healthy plants within 4 to 6 months. Regenerated plants produced seeds (F1) which germinated and grew similarly to control plants. This new method will be incorporated into *Agrobacterium* mediated peanut transformation. If successful, this approach can significantly increase the number of genes that can be introduced into peanuts for new variety development as well as to provide a strategy to define gene function.

P-2048

Effective Anti-browning Treatments for *Musa* Meristem Bud Clumps. J. Y. FANG and M. Cherry. Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, No.1 Shueh Fu Road, Neipu, Pingtung 91201, TAIWAN. Email: jyfang@mail.npust.edu.tw

The study aimed at investigating effective methods for reducing browning in meristem cultures of *Musa* 'Pei-Chiao'. The extent of browning of the meristem bud clumps was evaluated following four weeks culture on media supplemented with different anti-browning agents [i.e. ascorbic acid (AA), citric acid (CA), activated charcoal (CH) and polyvinylpyrrolidone (PVP)] and different sugars (i.e. glucose, fructose, sucrose, maltose, mannitol and sorbitol) based on their fresh weight gain and total phenolic content. It was observed that the total phenolic content of the meristem bud clumps was the highest with 2000 mg l^{-1} of PVP and 1000–2000 mg l^{-1} of CH, followed by 50–200 mg l^{-1} of AA and the control. The lowest phenolic production was observed with 50–200 mg l^{-1} of CA, 500–1000 mg l^{-1} of PVP, and 500 mg l^{-1} of CH. These treatments which produced the lowest amount of total phenolics coincided with those which promoted the highest fresh weight gain, in exception of the 500–1000 mg l^{-1} PVP treatments. It was also found that the incorporation of 4 % maltose, mannitol and sorbitol was effective in reducing the total phenolic content in the clumps compared to sucrose. However, the latter two treatments were also the ones which inhibited the growth of the clumps. Only maltose allowed an increase in fresh weight of the clumps, although the increase was inferior to sucrose. In conclusion, it is suggested that 50–200 mg l^{-1} of CA, 500 mg l^{-1} of CH and maltose can be used in the future for the browning control of *Musa* meristem clumps.

P-2049

In Vitro Germination and Acclimatization Studies in *Vriesea* species with Ornamental Potential. A. P. MARTINELLI, T. J. Kievitsbosch, and M. L. Rossi. University of São Paulo, CENA, Av. Centenario, 303, Piracicaba, SP, 13416–903 BRAZIL. Email: adriana@cena.usp.br

Many bromeliad species are valued for their ornamental characteristics. The improvement of *in vitro* propagation is highly necessary in order to meet market needs, preventing illegal extraction of these from their natural habitat. This study aimed to improve the protocol for *in vitro* propagation of *V. carinata, V. friburgensis, V. paraiba* and *V. simplex* and characterize the morpho-anatomy of the seedling development and leaf during acclimatization. Seeds were disinfested and introduced *in vitro* at: 22°C, 27°C and 32°C in MS medium modified with ¼ of the major nutrients, minor nutrients, vitamins, coconut water (10 mL L-1), banana extract (60 gL-1), sucrose (20 gL-1), GA3 (10 uM), solidified with agar (8 gL-1). Additionally, seeds of these species were sown in trays and maintained in a greenhouse at ambient conditions. The post-seminal development was described by light and scanning electron microscopy and the anatomical and morphological characteristics of leaves of the species cultivated *in vitro* and during acclimatization were compared. Seed germination *in vitro* varied from 35-78 % at 14 d, varying according to the species at 22°C, 27° C. At 32°C we observed the highest mortality rates with time. Germination in the greenhouse showed higher mortality and lower germination rates than *in vitro* germination. The morpho-anatomical description of the post-seminal development allowed for the characterization of five developmental stages. The morpho-anatomical analysis of leaves of plants grown in vitro and during acclimatization showed the presence of typical structures of Bromeliaceae such as stomata and scales, the mesophyll with uniseriate epidermis, water storage tissue, collateral vascular bundles and air channels. Structural changes during acclimatization is presented. Detailed studies of the propagation and reproduction characeristics of bromeliads are important for the conservation of these species, many of them vulnerable, or under risk of extinction in the Atlantic Forest.. (Acknowledgements: FAPESP and CNPq for research support, AN Aranda-Peres for seeds).

P-2050

Factors Affecting Rooting of In Vitro Shoots of Three Endangered Florida Pawpaws. VALERIE C. PENCE. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

Asimina tetramera, Deeringothamnus rugelii, and *D. pulchellus* (Annonaceae), three federally endangered species endemic to Florida, produce few seeds, and the seeds appear to be recalcitrant, making seed banking a limited option for ex situ conservation. Shoot cultures of multiple genotypes have been established for cryopreservation, as well as for producing plants for restoration, but shoots show little or no rooting on standard rooting media. Preliminary evidence suggested that the ethylene inhibitor, silver thiosulfate (STS), could stimulate rooting in *A. tetramera*, and this response as well as other media components have been investigated further with all three species. Shoot cultures were maintained on MS medium with 1 mg/L BAP and transferred to WP medium with 1 mg/L IBA for rooting. Only very rare root initiation occurred without the addition of 25, 50, 100, or 200 μM STS, which gave rooting of 5 to over 70 %, depending on the line and the age of the culture. Younger, shorter shoots had higher rooting rates than older, taller explants. The addition of 1 μM GA_3 increased rooting, whereas the addition of the uniconazole or higher basal salt formulations inhibited rooting. These results suggest that ethylene is inhibiting rooting in these species and provide guidance in formulating improved protocols for producing plants for restoration projects.

P-2051

Micropropagation of Heirloom and Commercial Tomato Varieties by Nodal Buds Sprouting. MARTÍNEZ O. VÁZQUEZ[1], Balch E. M. Pérez-Molphe[2], Castorena O. E. Medina[1], Fuentes Y. M. Ochoa[1], Gourcy F. Ramos[1], and Gavilán M. Urrestarazu[3]. [1] Universidad Autónoma de Aguascalientes, Dep. of Plant Breeding, Av. Universidad #940 col. Ciudad Universitaria, MÉXICO; [2] Universidad Autónoma de Aguascalientes, Dep. of Chemistry, Av. Universidad #940 col. Ciudad Universitaria, MÉXICO; and [3] Universidad de Almería, Dep. of Plant Production, Carretera a Sacramento s/n. la Cañada, SPAIN. Email: omartnez@yahoo.com

Tomato is one of the major crops in the world and most of the tomato imports in the United States come from México. This study contributes to obtain a protocol for *in vitro* multiplication of tomato varieties of distinct origin and high value (different fruit shapes and sizes, flavors and colors, etc). Plant growth regulators (PGR) have been used to induce direct or non-direct regeneration on leaf discs, hypocotyl or cotyledonary explants but limited reports are available on axillary shoot multiplication trough nodal segments in tomatoes. Our objectives were: (1) to evaluate four different PGR in order to determine their effect on sprouted nodal segment number and length in 10 Heirloom and commercial tomato varieties; and (2) to determine percentage plantlet establishment in response to different rooting and acclimatization methods. We evaluated a general method to micro propagate different Heirloom tomato varieties and also other commercial genotypes. At least ten varieties were established *in vitro* using one-single node when placed in MS medium without adding any plant growth regulator. Kinetin, TDZ, Meta-topoline, Benzyladenine, 2iP and different TIBA/2iP concentrations were tested to enhance breaking of auxiliary bud growth. Two sporadic shoots per node were grown in the highest cytokinin or antiauxin levels (0.0 -

10.0 mg L $^{-1}$) but in many varieties, the rate of axillary bud breaking per-node was observed to be enhanced when cytokinins were added to the medium. Apical shoots 3.0 cm long were easily rooted in MS medium, when transfered to pots containing a peat-moss mix, and were acclimatized after 22 days. The same rate of plantlets established on substrate was achieved when shoot apices of the same length were transferred and directly rooted on substrate from multiplication phase, thus avoiding the rooting phase.

P-2052

A New Wheat Anther Culture Technology Preventing Albinism in the EU Winter Wheat Gene Pool. J. WEYEN[1], J. Orsini[1], and H. Gnad[2]. [1]SAATEN-UNION BIOTEC GmbH, Hovedisser Str. 92, D-33818 Leopoldshoehe, GERMANY[1]; and [2]Am Schwabeplan 6, D-06466 Gatersleben, GERMANY. Email: weyen@saaten-union-biotec.com

Wheat doubled haploids are a major tool for breeders worldwide to speed up the development of improved varieties. Total homozygosity of those plants and their progenies offers extremely useful advantages such as easier selection of quantitative traits, easier and cost reduced handling in breeding logistics and maintenance breeding etc. DH technologies using the totipotency of androgenic cells ensure best efficiencies. Unfortunately anther and microspore culture in EU winter wheat pool was reported to be sensitive in respect of albinism, genotype dependency and poor induction and regeneration. We have now identified major keys to prevent those disadvantages in a significant range of germplasm out of EU winter wheat breeding programs and are able to reach regeneration rates which are suitable to produce anther culture derived lines economically in comparison to the established maize wheat hybridization protocol. In average more than 3,5 green plantlets per ear can be produced. We are still working on the optimization of the logistics and problems as rooting, optimal regeneration and handling of the plants in the field.

P-2053

Isolation, Cloning and Molecular Characterization of Circadian Clock Associated Genes of Rice. ASHOK CHAUDHURY[1,2], Minesh Patel[1], Sang Yoon Lee[1], and Rongda Qu[1]. [1]Department of Crop Science, 1200 Partners II, 840 Main Campus Drive, NC State University, Raleigh, NC 27606 and [2]Department of Bio & Nano Technology, Guru Jambheshwar University of Science & Technology, Hisar-125001, Haryana, INDIA. Email: ashokchaudhury@hotmail.com

In higher plants, circadian rhythms regulate various biological processes including metabolism, photosynthesis, onset of flowering, seed setting, water uptake, hormones, light and dark response to environmental cues. The central oscillator is comprised of several positive and negative regulators which modulate expression of several cascades of genes through transcriptional and post translational feedback loops. Molecular mechanisms regulating circadian clock genes have been reported in *Arabidopsis thaliana,* wherein the two morning element transcription factors CCA1 (Circadian Clock Associated 1) and its redundant homolog LHY (Late Elongated Hypocotyl) of a single MYB domain repress the transcription of TOC1 (Timing of Cab Expression 1) gene by recognizing the evening element in TOC1 promoter by forming an auto regulatory negative/positive feedback loop. The PRRs (Pseudo Response Regulators) are the key to the plant central oscillators and TOC1 is one of its representatives. Studies in Arabidopsis have found that suppression of CCA1 and altered circadian clock rhythms maybe a source of the hybrid vigor. To investigate whether a similar approach can promote plant growth in cereal crops, rice TOC1 gene promoter and CCA1 gene has been cloned and various constructs were made and will be introduced into rice. To develop a transformation system, embryogenic calli were obtained from mature seeds of Taipei 309 and *Agrobacterium*-mediated transformation of rice calli were performed using a GFP construct. High level of GFP expression in hygromycin B resistant calli was observed. Attempts are underway to transform the rice calli with circadian clock gene constructs to study the regulation of gene expression and the effects on plant growth.

P-2054

Isolation and Characterization of *TERMINAL FLOWER 1* Homolog from Black Cherry (*Prunus serotina* Ehrh.). Y. WANG[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: wang4@purdue.edu, ppijut@purdue.edu

Flowering control is one of several strategies for gene-containment of transgenic plants. *TERMINAL FLOWER 1* (*TFL1*) is known to be involved in the transcriptional repression of genes for inflorescence development. A *TFL1* homolog was cloned from black cherry (*Prunus serotina* Ehrh.) using a reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) methods. Two *PsTFL1* sequences, 1248-bp and 1579-bp in length, were obtained and both contained the same 519-bp open reading frame (ORF) which encodes a putative protein of 172 amino acid residues. The phylogenetic analysis of amino acid sequence showed high identity of *PsTFL1* to *TFL1* orthologs of other *Prunus* species, including Yoshino

cherry (*Prunus x yedoensis*), peach (*Prunus persica*), apricot (*Prunus armeniaca*), and Japanese apricot (*Prunus mume*). The gene expression of *PsTFL1* was examined in several tissues including the stem, leaves, and shoot apical meristem (SAM) from in vitro black cherry shoot cultures using quantitative RT-PCR (qRT-PCR). The highest mRNA level was detected in SAM and the lowest level in the leaves. DNA-blot analysis and functional analysis by transformation of *Arabidopsis thaliana* over-expressing *PsTFL1* are currently under way.

P-2055

Variations in Duration of Dormancy, Morphological and Molecular Markers Among White Yam Accessions from South-western Nigeria. ADEOLA O. BAMIKOLE and M. O. Balogun. University of Ibadan, Ibadan, Oyo State 234, NIGERIA. Email: abamikole@yahoo.com

DNA fingerprint was generated using Ten (10) R.A.P.D (Random Amplification of polymorphic DNA) Markers, on 7 white yam varieties. The 10 random primers were used to test the level of polymorphism in the yam varieties. The morphological and dormancy duration variations were estimated. Three categories were identified into short, medium and long dormancy period. Morphological characteristics measured were spiny vines, flowering period, duration of flowering period. Dormancy duration was measured by recording the date of tuber initiation to date of tuber sprouting. The study is based on the premise that duration of the dormancy period is under endogenous (genic) control, as very little exogenous control was achieved in earlier studies. Studying the diversity in duration of dormancy among collections will serve as baseline information in breeding for specific duration-varieties, marker-assisted selection and identification of quantitative trait loci linked to the trait.

P-2056

Development and Comparative Assessment of *In Vivo* and *In Vitro* Techniques for Determination of Photoperiod Sensitivity in Kenaf (*Hibiscus cannabinus*). M. O. BALOGUN[1], S. R. Akande[2], J. A. Raji[2], A. O. Olabisi[1], and B. A. Ogunbodede[2]. [1]Department of Crop Protection and Environmental Biology, University of Ibadan, NIGERIA and [2]Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Ibadan, NIGERIA. Email: kemtoy2003@yahoo.com

In equatorial climates like Nigeria, photoinsensitive kenaf cultivars yield more fibre than photosensitive ones. Delineating photoinsensitive genotypes is therefore critical for the development of cultivars adapted to Nigerian agroecologies. This study developed and compared *in vivo* and *in vitro* techniques in screening for photoperiod sensitivity in Kenaf. Seven genotypes were grown in a plywood-insulated metal semi-controlled growth room provided with white fluorescent tubes at 14, 12 and 10 hours and control in the open at natural photoperiod regimes. Planting was done in pots in a completely randomized design with four replicates. Data were collected on number of days to flowering, growth rates before and after flowering as indicator of photosensitivity and fibre yield per plant. For *in vitro* studies, stem and leaf explants of genotypes Tainung and $V_1$400, which expressed contrasting photoperiodic responses *in vivo*, were tested for callus induction at 0 and 12 hours photoperiod in completely randomized design with three replicates. Four weeks later, the degrees of callus induction were recorded. Calli were transferred to modified MS differentiation medium, incubated at 12 hours photoperiod for two weeks and numbers of green spots per callus were recorded. Thereafter, half of the calli were each incubated at 12 and 9 hours photoperiod for 1 week and numbers of green spots and somatic embryos per callus were recorded. All genotypes flowered earliest when grown under natural photoperiod (53 days) and latest at 14hours photoperiod (157 days). There was no significant difference in number of days to flowering between 12 hours (118 days) and 10 hours (114 days) photoperiod regimes in all genotypes except $V_1$400 which flowered 22 days earlier at 10 than at 12 hours. Growth rates were higher before than after flowering in all varieties except $V_1$400 in which growth rate increased after flowering. $V_1$400 had highest fibre yield of 83.4 g and 76.7 g/plant recorded at 10 hours and natural photoperiod regimes respectively, significantly higher than 32.9 g at 12 hours. Other genotypes had highest yield at 12hours photoperiod. $V_1$400 was classified as photoinsensitive and others photosensitive. In Tainung cultured in darkness, the degree of callus formation by leaf explants (100 %) was higher than stem explants (55 %) while in light, stem explants had a higher degree of callus formation (75 %) than leaf explants (42 %). In $V_1$400 however, stem explants had a higher degree of callus formation (75 %, 92 %) than leaf explants (53 %, 49 %) in light or darkness respectively. Tainung had at least 5 times more green spots and somatic embryos per callus than $V_1$400. Incubating callus in differentiation medium in 12 hours light and evaluating for greenness by counting number of green spots will indicate photosensitivity among genotypes. Both *in vivo* and *in vitro* results showed that Tainung is photosensitive while $V_1$400 is relatively photoinsenstive. This *in vitro* screening method is faster, cost effective in space and labour requirements and therefore more efficient than *in vivo* methods. $V_1$400 will be a good parent in kenaf breeding for high fibre yield and adaptation to Nigerian agroecologies.

P-2057

Detection of Resistant to Glyphosate Maize Transformation Events Ga21, Mon88017 and Nk603 Among Ukrainian Market Samples. T. FEDORENKO [1,2], O. Markovskiy [1,2], O. Vlasova [1,2], M. Bannikova[2], M. Kuchuk[2], and B. Morgun[2]. [1]National Technical University of Ukraine, 37 Peremogy Avenue, Kiev, 03056, UKRAINE and [2]Institute of Cell Biology and Genetic Engineering, 148 Akademika Zabolotnoho Str., Kiev, 03680, UKRAINE. Email: maidanyuk_tanya@ukr.net

The number of genetically modified organisms (GMOs) has grown steadily over the past few years. Uncontrolled dissemination of transgenic organisms is a problem nowadays. According to this it is necessary to create reliable and specific systems for detection and monitoring of GMOs in Ukraine. The objects of our research were 210 samples of domestic maize from small markets. The aim was to detect accidentally brought in Ukraine transgenic forms of *Zea mays* such as GA21 (Syngenta Seeds), NK603 and MON88017 (Monsanto Company). These registered in the European Union transformation events impart to plants resistance to glyphosate, a widespread herbicidal agent. During the study total DNA was extracted from plantlets and purified from RNA by enzymatic hydrolysis. The DNA concentration was measured by spectrophotometry and normalized with TE buffer pH 8.0 up to 10 ng/μl. To control the quality of extracted DNA and its usefulness for further research the polymerase chain reactions (PCRs) for the presence of reference genes *zein* and *adh-1* were carried out. All samples were positive on before-mentioned genes. Event-specific as well as gene-specific PCR was employed to detect transgenes. Amplified products were separated by electrophoresis in agarose gel. The GA21 presence was identified with primer pair GA21F and GA21R where the length of amplicon was 112 bp. Primer pair NK603F and NK603R (108-bp amplicon) was used for detection of NK603. The MON88017 presence was tested with primer pair M7R8 та M7F8 (amplicon is 313 bp). Well-defined DNA from transgenic lines GA21, NK603 and MON88017 served as positive controls. Reaction mix without template DNA was used as a negative control. Presence of NK603 and MON88017 positive signals has not been found in any samples. In contrast there have been observed three GA21 positive samples. Thus, we observe weak unauthorized transgene flow from abroad and maintenance of live genetically modified maize in Ukraine.

P-2058

Towards Automation of Micropropagation Using a Mist Bioreactor. L. FEI and P. J. Weathers. Worcester Polytechnic Institute, Dept. of Biology & Biotechnology, 100 Institute Road, MA 01609. Email: lwfei@wpi.edu; weathers@wpi.edu

Micropropagation is challenging to automate mainly because of the diverse morphology of plant shoots. Previously we showed that the mist reactor can be used for 1-step propagation of carrot cells into embryos and then fully rooted plantlets using poly-L-lysine (PLL) coated polypropylene strips to which cells were attached prior to growth while hanging in the nutrient mist. Cell development into rooted plantlets occurred in 20 days. It is also possible to propagate attached small attached explants of leaves; *Artemisia annua* leaf explants, for example, attached by their filamentous trichomes to PLL polypropylene or nylon. Although use of an increased misting cycle led to an increased ratio in post-heart embryo formation after 14 days, continuous misting did not further increase embryogenesis. Carbon dioxide enrichment on carrot embryogenesis was also investigated. Compared with embryos developed in unvented culture chambers, after 14 days more embryos formed with 3 % carbon dioxide enrichment and after 20 days the average length of rooted embryos was increased by almost 50 % compared to ambient air controls. When only a sucrose solution was used during the adhesion step, cell attachment to PLL coated 50 micron nylon, 70 micron nylon and 74 micron polypropylene mesh was 4.4, 3.3 and 2.3 fold, respectively, compared to half strength B5 salts. Almost 90 % of the originally attached cells remained on the 50 micron nylon mesh 24 hrs later after spraying with B5 medium in the mist reactor. Together these results demonstrate that both the mist reactor and our attachment technology may offer opportunities for at least partial automation of micropropagation.

P-2059

Influences of Different Medium Compositions and Cytokinins on Micropropagation of Fraser Photinia. D. GÜMÜŞEL, H. Akdemir, V. Süzerer, and Y. Özden Çiftçi. Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400, Kocaeli, TURKEY. Email: gumuseld@hotmail.com

Fraser photinia, is a woody ornamental plant that is widely used in the parks, gardens and roadsides with its remarkable red foliage and white flowers. Although photinia is able to grow rapidly, problems exist on rooting of its cuttings that reduce its vegetative production. Thus, development of efficient micropropagation methods is important not only to overcome rooting difficulty problems but also to fasten its clonal production, and gene transferring studies. Therefore, four different medium compositions (MS, WPM, QL and DKW) were assessed by using binodal segments excised from *in vitro*-grown shoots of fraser photinia together with different cytokinin types [BA(6-benzyladenine), TDZ (thidiazuron), KIN (kinetin) and 2-iP (2-isopentyl adenine)] supplemented with varying concentrations (1, 2, 4 mg l^{-1}) to each media. Our

results showed that relatively higher shoot proliferation (100 % and 98 %, respectively) was achieved on WPM and QL media with supplementation of different amounts of cytokinins. Among tested cytokinins, the highest multiple shoot formation (2.22 shoots per explant) and longest shoots (5.04 mm) were obtained by supplementing relatively lower concentrations of BA, irrespective of the tested media. Shoots that were subcultured at least three times on proliferation medium were transferred to indole-3-butyric acid (IBA) containing medium and rooted successfully. The obtained results are expected to be used for rapid and effective propagation and conservation of fraser photinia.

P-2060

Banana Micropropagation Using an Exclusive Temporary Immersion Bioreactor. S. HESSAMI[1] and A. Babaei[2]. [1]Hessami Plant Tissue Culture Laboratory (HPTCL), Giah CT., Next to Golchin tea St, Shahrake Naaz, Fardis, Karaj 31796, IRAN and [2] Faculty of Agriculture, Tarbiat Modares University, PO Box: 14115–365, Tehran, IRAN. Email: sh.hessami@hptcl.com

Typical methods in plant micropropagation in comparison with new techniques are more expensive and time consuming. Therefore development of new methods for production of less expensive *in vitro* plants has steadily increased in recent years. In this study micropropagation of different cultivars of banana using an efficient and exclusive Temporary Immersion Bioreactor (TIB) has been carried out. In order to decrease the primary investment, application of disposable polyethylene terephthalate (PET)-based tank instead of glass tank considered. As this kind of container is not autoclavable, an exclusive chemical sterilization has been applied. It does not require much time for preparing medium and transplanting of multiplied plantlets under aseptic condition. Therefore it does not need any special equipment such as autoclave. In addition the mentioned TIB system is light and easy transferable. Using this bioreactor is a great step forward in lab automation and increase lab efficiency. A pilot of this TIB system has been setup for the commercial production of Banana in multiplication and rooting phases in HPTC laboratory.

P-2061

Preliminary *In Vitro* Studies of *Victoria* and *Nymphaea* hybrids. N. H. HOANG and M. E. Kane. Environmental Horticulture Department, University of Florida, Bldg. 68, PO Box 110675, Gainesville, FL 32611–0675. Email: nhhoang@ufl.edu

Victoria and *Nymphaea* hybrids (Nymphaceae) are commercially important horticultural plants. We are seeking to develop propagation procedures to overcome difficulties encountered in clonal propagation. Sterile seedlings could serve as an explant source to optimize media components and culture conditions. The effectiveness of different procedures for *in vitro* culture establishment of *Victoria* cv. Longwood Hybrid and the tropical water lily hybrid *Nymphaea* Madame Ganna Walska were examined. For *Victoria*, a surface sterilization protocol and culture conditions have been established. *Victoria* seed were surfaced sterilized using a two-step process consisting of agitation in 3 % sodium hypochlorite for 30 minutes after which zygotic embryos were excised and further surface sterilized in 0.6 % sodium hypochlorite for 1 minute before being cultured *in vitro*. Cultures were indexed for contamination on Leifert and Waites sterility test media. *Victoria* plantlets only grew in MS medium supplemented with 2 mg/L BA. Seed sterilization of the tropical water lily hybrid *Nymphaea* Madame Ganna Walska resulted in very low germination and very high contamination rates. For culture establishment of this hybrid excised unfertilized and fertilized ovules may provide a more reliable explant source for *in vitro* regeneration. To determine the optimal stage for ovule explantation, the time course of ovule and zygotic embryo development was examined using histological sectioning. Initiation of zygotic embryo development was observed within 6 days post-pollination and cotyledonary embryos present by day 10 post-pollination. Our preliminary results provide a step by step procedure which will allow further investigations on the *in vitro* culture of water lilies.

P-2062

Assessment of Genetic Stability of *In Vitro* Micrografted and Propagated Almond cv. Texas. V. SÜZERER[1], H. Akdemir[1], H. Yildirim[2], A. Onay[3] and Y. Özden Çiftçi[1]. [1]Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400, Kocaeli, TURKEY; [2]Dicle University, Faculty of Agriculture, Department of Horticulture, 21280 Diyarbakır, TURKEY; and [3]Dicle University, Faculty of Science, Department of Biology, 21280 Diyarbakır, TURKEY. Email: beyso1985@gmail.com, vsuzerer@gyte.edu.tr

Almonds are members of the *Rosaceae* (rose) family, along with many other tree fruits such as peaches, apples, pears, plums, cherries, and apricots. Within the genus *Prunus*, almond is most closely related to the peach, and the two crops share the subgenus *Amygdalus*. "Texas" is one of the mostly used cultivar as pollinator for different almond cultivars (especially cv. Non-pareil). As the species faces difficulty in rooting of its cuttings, development of *in vitro* micrografting protocols that do not cause extreme genetic instability in clones is essential to overcome this problem. Thus, successful micrografting technique was developed for "Texas" cultivar by using *in vitro* germinated seedlings,

which was developed 14 days after culturing in the modified Murashige and Skoog (MS) medium, as rootstock and axenic shoot cultures established from mature tree sources as microscions. Shoot culture initiation from paper-shell almond cultivar "Texas" was successfully achieved by culturing mature shoot tips from forced nodal buds, about 4–6 mm, on 0.7 mg/L BA and 0.01 mg/L NAA containing a modified MS medium. The regenerated adventitious shoots from *in vitro* cultures were maintained and proliferated by subculturing on a fresh medium every three to 4 weeks up to 9 months. When almond scions, about 1.5 cm long, were micrografted on germinated seedling and cultured on proliferation medium (PM), the mean shoot length was 19.84 mm. Graft success was changed between 83.3 % and 100 %. Moreover, genetic fidelity of the micrografted plantlets was also assessed by RAPD (randomly amplified polymorphic DNA) markers. A total of 81 bands were obtained in which only 3 of them were polymorphic (3.70 %) with all the tested primers. The similarity values were ranged from 0.963 to 1.000 with a mean of 0.982. As the genetic instability was only 3.70 % after 9 month of subculture, developed micrografting technique could be used safely for rejuvenation of shoot explants of mature elite almond cultivars.

P-2063

Genetic Reduction of Inositol Triphosphate ($InsP_3$) in Tomato Plants Affects Signal-transduction Pathways Controlled by Light. M. ALIMOHAMMADI, K. de Silva, and M. Khodakovskaya. Department of Applied Science, University of Arkansas at Little Rock, AR, 72204. Email: mxalimohamma@ualr.edu, mvkhodakovsk@ualr.edu

Many plant stress signal transduction pathways are regulated by light. Light is also a key factor in biosynthesis of several secondary metabolites in plants, including carotenoids and phenylpropanoids. It has been demonstrated that the phosphoinositol pathway interacts with light signaling in plant cells. Recently, it was shown that the genetic reduction of inositol triphosphate ($InsP_3$), a major second messenger of the phosphoinositol pathway, through over-expression of the human InsP 5-ptase gene, leads to a significant increase of lycopene in transgenic tomato fruits (Khodakovskaya *et al.*, 2010). We found that tomato plants expressing InsP 5-ptase were able to withstand prolonged high-light conditions. We hypothesized that increase of lycopene in InsP 5-ptase expressing tomato fruits can be associated with modifications in light signaling through genetic reduction of $InsP_3$ in transgenic lines. In order to test this hypothesis, the expression level of key regulators of light-signaling (*LeHY5, SlMYB12 and LeELIP*) was determined in tomato wild type and InsP 5-ptase expressing tomato lines by real-time PCR. It was found that all tested genes are up-regulated in InsP 5-ptase tomato fruits compared to control fruits. Taking in account that many genes of phenylpropanoid pathway are light-regulated, we monitored expression of genes of key enzymes of phenylpropanoid metabolism (*CHS1, HCT*) and production of major flavonoids (chlorogenic acid, rutin) in fruits of control and transgenic tomato lines. Up-regulation of *CHS1* and *HCT* genes and enhanced accumulation of chlorogenic acid and rutin was detected in transgenic fruits compared to control tissues. These results demonstrate how modification of phosphoinositol pathway can lead to massive changes in production of secondary metabolites in plants and prove an existence of link between phosphoinositol metabolism, light signaling and secondary metabolites production in plants.

P-2064

Production of Flavonoids from *Vaccinium bracteatum* Thunb. in Bioreactors. L. LANGHANSOVA, P. Landa, P. Marsik, and T. Vanek. Rozvojova 263, Laboratory of Plant Biotechnologies, Prague 6, Institute of Experimental Botany ASCR v.v.i., 165 02, CZECH REPUBLIC. Email: langhansova@ueb.cas.cz

Vaccinium species are popular for a high content of biologically active compounds, possessing anti-inflammatory and anti-carcinogenic activity. Recently a biological activity was proven in *Vaccinium bracteatum* Thunb., however no paper on micropropagation of this species was reported. In our study we succeed to initiate *in vitro* cultures of *V. bracteatum* and scale-up to bioreactor cultivation systems. Callus was induced from leaf discs on WPM medium supplemented with plant growth regulators (5.4 μM NAA, 0.45 μM 2,4-D and 2.3 μM Kin), 600 μM FeEDTA, 3 % sacharose and solidified with 0.25 % Phytagel. Suspension culture from friable callus was initiated on liquid WPM medium supplemented with 5.4 μM NAA, 2.3 μM Kin, 200 μM FeEDTA and 0.85 mM L-Ascorbic acid. We found dichloromethane/methanol *V. bracteatum* leaves extract to be active in cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibition. COX-2 is primarily involved in inflammation and cancer. Similar results on COX-1 and COX-2 inhibition were observed on dichloromethane/methanol extract of our callus cultures. Applying GC-MS chromatography on callus extract, we identified some phenolic, steroid and terpenoid compounds including ursolic acid known to have anti-proliferative activity. Phenolic compounds are considered to be responsible for anti-inflammatory activity. Bioreactor cultivation is a promising technology to produce these desired valuable compounds from cells of *V. bracteatum*. We tested few types of large-scale systems with different mixing and aeration conditions to produce the biomass. Acknowledgement: This study was supported by project ME08070.

P-2065

In-Vitro Antioxidant Activity and Genetic Diversity in *Hedychium spicatum* from West Himalaya, India. S. RAWAT[1], A. Jugran[1], I. D. Bhatt[1], R. S. Rawal[1], S. K. Nandi[1], and V. Pande[2]. [1]G. B. Pant Institute of Himalayan Environment and Development, Kosi- Katarmal, Almora, Uttarakhand (INDIA) 263 643 and [2]Biotechnology Department, Kumaun University Nainital, Uttarakhand 263 001, INDIA. Email: sandeep_rawat15@rediffmail.com

Hedychium spicatum is a plant of Himalayan region used in traditional and modern medicinal system and cosmetic and perfumery industries. Species is used in Tibetan medicines, ayurvedic and many folk preparations such as Chyawanprash, which is known for its antioxidant properties. Total phenolic content and *in-vitro* antioxidant activities among 16 populations were analysed from Indian west Himalaya. Total phenolic content, using Folin-Ciocalteu's reagent method, ranged between 2.84 mg to 4.68 mg gallic acid equivalent (GAE)/g and varied significantly among populations. All the three *in-vitro* antioxidant assays [i.e., azinobisethylbenzothiazoline-6-sulphonic acid radical scavenging (ABTS) assay, diphenyl-2-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP)] showed significant ($p<0.05$) difference across populations. The diversity and genetic relationship among and within 16 populations were also analyzed using ISSR markers. High percentage of polymorphism indicated a high level of genetic diversity across populations. Analysis of molecular variance (AMOVA) revealed higher within population variation (94 %). The UPGMA clustering exhibited grouping of most populations from the same or adjacent regions. Study revealed that variation in phenolic content and antioxidant activity may be related to genetic factors along with environmental conditions. The results exhibited that the high genetic diversity of this species can be attributed to its wider adaptation. An appropriate strategy for conserving has been proposed with a recommendation for *in-situ* conservation of populations like Shitla, Thakurh and Kalika.

P-2066

Harnessing Rhizospheric Bacterial Diversity of Important Medicinal Plants of Central India for Bioprospecting. VASUDHA SINGH, Shivesh Sharma, and Keshav Prasad Shukla. Department of Applied Mechanics (Biotechnology), Motilal Nehru National Institute of Technology, Allahabad (UP), INDIA. Email: vasudhasingh1@gmail.com

Tribal regions of India has valuable heritage of indigenous medicinal plant diversity that are of great importance for mankind. Overexploitation of natural resources due to increase in population has lead many medicinally important plants on the verge of extinction, hence the study was undertaken to document the ethnobotanical knowledge of Baiga tribes of Amarkantak as well as to increase the population of some of the medicinal plants that are known to grow only in their indigenous regions. Three plants namely, *Litsea glutinosa, Rubia manjith* and *Pueraria tuberosa* with their endangered, vulnerable and threatened status respectively were taken for studies of growth enhancement and harvest yields. Antimicrobial activity of extracts of these plants were tested against different pathogenic organisms viz., *Salmonella typhimurium, Vibrio cholera, Klebsiella pneumoniae, Proteus mirabilis, Bacillus cereus, Micrococcus luteus, Shigella flexneri* and *Fusarium udum.* Identification and characterization of rhizospheric microorganisms associated with medicinal plants was carried out to screen out strains exihibiting PGP activity. Plant growth promoting traits and antagonistic activity was best exhibited by two isolates of plant growth rhizobacteria designated as VS-5 and VS-21 identified as *Pseudomonas* spp.

P-2067

Isolation and Identification of Pathogenic Fungi on Some Indian Currencies. CHANDAN KUMAR, Gazala Tabassum, and Rashmi Komal. Plant Pathology and Microbiology Laboratory, Department of Botany, Patna University, Patna, Bihar, INDIA. Email: ckscience@gmail.com

Paper currency is used in exchange for goods and services and so the circulation of paper currency from one individual to another potentially spreads microorganisms. Paper currency can be contaminated by droplets during coughing, sneezing, touching with previously contaminated hands or other materials and placement on dirty surface. Paper currency is commonly handled by various categories of people during transaction. Contamination of objects by pathogenic microorganisms is of much public health concern as contaminated materials can be sources of transmitting pathogens. If pathogenic fungi contaminate these currencies, the rate of infection from these infectious agents will continue to rise. This study was conducted to survey the pathogenic fungal contamination of paper currency in India. Sample of paper currency were collected during February 2011 to October 2011. Total 45 paper currency were collected from different source such as railway ticket counter, vegetable market, chicken market and bus passenger were selected. The values of currencies were 5, 10, 50 and 100 Indian rupees which were collected randomly and put in sterile polythene bags. They were immediately transferred to the plant pathology and microbiology laboratory, department of Botany, Patna University to apply all microbiological examinations for the different values of collected currencies. The pathogenic fungi were isolated in different culture media using standard techniques.

Among the nearly 16 pathogenic fungi were identified. Identification and characterization of the fungi were made with help of authentic manual of fungi. A species of *Trichophyton sp., Mycrosporum sp., Candida sp., Epidermophyton sp., Saccharomyces sp., Aspergillus niger, Apergillus flavus* were commonly observed in the Indian paper currency.

P-2068

Seasonal Influence on Fungal Diversity of Some Common Food in Rural Areas of Bihar, India. GAZALA TABASSUM, Chandan Kumar, and Rashmi Komal. Plant Pathology and Microbiology Laboratory, Department of Botany, Patna University, Patna, Bihar, INDIA. Email – gazalaarshad@gmail.com

With the improved living conditions in all over the world in general and in India, we can preserve our foods through different means but still a substantial percentage of population living in rural areas of Bihar do not have such facility. In such a circumstances, the deterioration of food stuffs due to microbial infections are observed accordingly.The basis framed for fungal diversity and its frequency determinations were importantly dependent on the environmental conditions especially in Bihar where diverse climatic conditions are found. In the present investigation, during December 2007 to October 2008 fungal diversity was estimated on some common food consumed in Bihar. For that, food samples were considered viz; chapati,boiled potato, mutton, dal, boiled rice.The samples from different rural areas were prepared from home and collected in sterilised plastic containers. The fungi isolated from these infected foods were identified and characterized with the help of authentic manuals of fungi. A mean of eight months data were calculated and placed in the table according to month wise frequency. Factors regulating the microbial growth were p^H, moisture content, nutrient content, and fluctuating temperature and relative humidity of environment etc. Thus a large range of fungal diversity was found during entire observation causing spoilage of food.The mycofloras which were isolated from different types of foods in different seasons, most of them belonged to the species of *Aspergillus* and *Penicillium* viz; *Aspergillus flavus, Aspergillus niger, Aspergillus versicolor* and *Penicillium citrinum ,Penicillium funiculosum.* Although some of them also belonged to class Deuteromycetes and Zygomycetes like *Cladosporium herbarum, Curvularia lunata* and species of *Geotrichum* and *Fusarium.*

P-2069

Obtaining of Transgenic Carrot (*Daucus carota* L.) and Celery (*Apium graveolens* L.) Plants Expressing the Recombinant Gene of Thaumatin II Protein. YU. LUCHAKIVSKA, I. Komarnitskii, and M. Kuchuk. Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine and Akad. Zabolotnogo str. No 148, Kiev, UKRAINE, 03680. Email: yu.luchakivskaya@gmail.com

Thaumatin is sweet tasting protein isolated from *Thaumatococcus daniellii* (Benth) fruits and known for its strong homology to thaumatin-like proteins that are assumed to induce in plants in response to stress or plant pathogen infection and thus to play a role in the plant defense system. Recent studies have shown the enhanced plant abiotic stress tolerance in addition to the plant resistance to fungal pathogens for the transgenic plants carrying the recombinant thaumatin gene. Four varieties of carrot (Nantskaya, Perfection, Red giant and Korotel) and two varieties of celery (Zakhar, Paskal) were used for *Agrobacterium tumefaciens*–mediated transformation (nopaline strain GV3101). The plasmid vector construct pCB171 contained the sequence coding for recombinant thaumatin II gene fused with plastid targeting transit peptide sequence driven by *35 S* CaMV promoter and the selective gene of neomycin phosphotransferase II (*nptII*). *Agrobacterium*-mediated transformation of 2-week-old seedlings led to formation of primary callus clones with the further regeneration of kanamycinsulphate-resistant plants *via* somatic embriogenesis in 3–4 month period in case carrot and in 4–5 month time after genetic transformation in case of celery. The presence of trangenes in selected carrot and celery plants was confirmed by PCR-analysis. RT-PCR analysis proved the transcription for selective and target genes. The transformed plants were transferred to the greenhouse in 1–2 weeks after their being rooted and are being analyzed for their resistance to fungal pathogens.

P-2070

Development of a Genetic Transformation Protocol for Interspecific Hybrids of Elephantgrass (*Pennisetum purpureum*) x Pearl Millet (*Pennisetum glaucum*). E. B. MAYERS [1], M. Sobanski[2], F. Faleiro[3], B. Kannan[1], H. Wu[1], J. Y. Kim[1], and F. Altpeter[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida – IFAS, Gainesville, FL; [2]Current address: Universidad Nacional del Sur, Bahía Blanca, ARGENTINA; [3]Current address: Embrapa Cerrados, Planaltina, DF, BRAZIL. Email: ebmayers@ufl.edu

Interspecific hybrids of elephantgrass (*Pennisetum purpureum*) x pearl millet (*Pennisetum glaucum*) are among the most productive perennial grasses for biofuel and forage production in subtropical and tropical regions. Elephantgrass produces wind dispersed seeds which may contribute to invasiveness. In contrast, triploid, interspecific hybrids of elephantgrass x pearl millet are completely sterile and seedless which will enhance biocontainment. We recently generated and selected triploid, sterile, interspecific hybrids with superior biomass yields and persistence. A genetic transformation protocol for

further improvement of interspecific hybrids of elephantgrass x pearl millet was lacking and its development was our primary objective. This involves the optimization of tissue culture, gene transfer and selection parameters. Five breeding lines with superior biomass yield and persistence were evaluated for tissue culture response. All of the lines were able to induce regenerable callus with line PMN 51 regenerating the most shoots per explant and callus. This line was therefore selected for biolistic gene transfer with selectable marker (*nptII*) constructs differing in the constitutive promoter (enhanced 35 S or ubiquitin). The timing of selection as well as concentration and type of the selective agent (paromomycin or geneticin) were also compared. Transgenic nature of the regenerated plants was confirmed by PCR and NPTII-immuno- chromatography. Data from Southern blot analysis and NPTII-ELISA will also be presented. This is the first report of transgenic interspecific hybrids of elephantgrass x pearl millet. This genetic transformation protocol will allow the introduction of transgenes to enhance biomass production and stress tolerance while providing elevated biocontainment through the sterile, seedless nature of this promising biofuel feedstock.

P-2071

RNAi Induced Root Lesion Nematode Resistance in Transgenic Wheat. KERRI NEUGEBAUER, Jiarui Li, Timothy C. Todd, Tom R. Oakley, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506. Email: hnt@ksu.edu

Root lesion nematodes *Pratylenchus neglectus and Pratylenchus thornei* are serious pests affecting wheat production in the world. Current management practices for root lesion nematodes are either ineffective or nonexistent. Our lab is exploring the use of RNA interference (RNAi), targeting specific root lesion nematode genes, as a strategy to improve nematode resistance in wheat. We cloned two gene fragments *PnY1* and *PnR1* from *Pratylenchus neglectus* by PCR, separately. Sequencing results showed that *PnY1* gene fragment shares 99 % similarity between *P. neglectus* and *P. thornei*. RNAi constructs of *PnY1* and *PnR1* were made by cloning inverted repeats of these two gene fragments in pANDA-mini, separately. Using biolistic-mediated gene transformation, the two RNAi constructs were transferred into the wheat cultivar 'Bobwhite'. Five and ten different transgenic lines were confirmed to have transgenes by PCR for *PnY1* and *PnR1*, respectively. Reverse transcription (RT)-PCR and semi-quantitative RT-PCR were conducted to confirm the transcriptional expression of RNAi constructs. Southern blot analysis and root lesion nematode bioassay of transgenic plants will be discussed.

P-2072

Agrobacterium-mediated Transformation of *Fraxinus americana* L. Hypocotyls. KAITLIN J. PALLA[1] and Paula 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: kpalla@purdue.edu, ppijut@purdue.edu

Fraxinus americana (white ash) trees provide both economic and ecological benefits. White ash is a valuable hardwood tree species that provides both food and shelter for wildlife, and the wood is highly valued in the hardwood industry. The emerald ash borer (EAB) is an invasive non-native beetle that threatens all *Fraxinus* species growing in North America, and there are no known means of complete eradication of this beetle or of any innate resistance in the native tree populations. Therefore, the development of white ash with resistance to the EAB is an urgent goal. The objective of this research was to develop a successful protocol for genetic transformation and regeneration of white ash. Aseptic mature embryos were cultured on Murashige and Skoog (MS) medium with Gamborg B5 vitamins supplemented with 10 μM 6-benzyladenine (BA) and 10 μM thidiazuron (TDZ) for 5 days before hypocotyls were excised and used for transformation. White ash hypocotyls were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring the binary vector pq35GR containing the neomycin phosphotransferase (*nptII*) and β-glucuronidase (GUS) genes. Hypocotyls were transformed in a bacterial suspension with 100 μM acetosyringone using 90 s sonication and 10 min vacuum-infiltration. Hypocotyls were then co-cultured for 2 days in the dark on MS medium containing 22.2 μM BA, 0.5 μM TDZ, 50 mg L^{-1} adenine sulfate (AS), and 10 % coconut water (CW). Kanamycin-resistant shoots were selected on MS medium with 22.2 μM BA, 0.5 μM TDZ, 50 mg L^{-1} AS, 10 % CW, 500 mg L^{-1} timentin, and 30 mg L^{-1} kanamycin. Explants with adventitious shoots were then transferred to MS medium containing 10 μM BA and 10 μM TDZ, 500 mg L^{-1} timentin, and 30 mg L^{-1} kanamycin to elongate shoots. The presence of GUS and *nptII* were confirmed by polymerase chain reaction. This research provides the framework for genetic transformation of white ash with a gene specific for EAB resistance.

P-2073

Monitoring *Ds* Transposition in the Soybean Genome. MANMEET SINGH[1,2], Hanh Nguyen[2], Shirley Sato[2], Saadia Bihmidine[2], Fareha Razvi[2], Tryuen Quach[2], and Thomas Clemente[1,2]. [1]Department of Agronomy and Horticulture; and University of Nebraska-Lincoln and [2]Center for Plant

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The maize two-component transposon system *Ac*/*Ds* has been used in many plant species as a means to generate insertional and activation tagged mutants. The long-term goal of this program is to develop a repository of germinal transposition events harboring mapped *Ds* elements that will have utility in soybean functional genomics. We generated ~500 events carrying activation tag construct delineated by *Ds* termini to act as initial launch sites. To investigate *Ds* transposition in soybean genome we stacked events harboring activation tag or enhancer trap elements delineated by Ds termini with an *Ac* cassette under control of constitutive 35 S CaMV promoter. We have generated 551 crosses carrying the activation tag and 144 crosses carrying the enhancer trap. Among 17 F2 activation tagged stacks genotyped to date we observed 26 unique germinal transpositions with a frequency of 3.15 %, whereas we found only six unique germinal transpositions from 22 F2 populations derived from enhancer trap stacks with a frequency of 0.5 %. In soybean it appears that *Ds* transposes often to unlinked positions in the soybean genome. Two germinal transpositions we are currently characterizing are a *Ds* delineated enhancer trap element that inserted into the third intron of Glyma06g08110, a cyclic nucleotide binding domain gene, resulting in a phenotype with reduced pollen germination and shorter pollen tubes, along with a germinal transposition of the *Ds* delineated activation tag element that landed upstream of Glyma15g21240, IMP/GMP specific nucleotidase, causing miss-expression of the gene activity.

P-2074

Overexpression of Tocopherol Cyclase Resulted in an Increase Tocopherol Content Drought Stress Tolerance of Rice. HEE-JONG WOO, Soo-In Sohn, Jae-Kwang Kim, Kong-Sik Shin, Myung-Ho Lim, Soon-Jong Kweon, and Seok-Cheol Suh. National Academy of Agricultural Science, Rural Development Administration (RDA), Suwon, REPUBLIC OF KOREA. Email: woo001@korea.kr

Tocopherols(α-, β-, γ- and δ-tocopherols) represent a group of lipophilic antioxidants which are synthesized only by photosynthetic organisms. It is widely believed that protection of pigments and proteins of photosynthetic system and polyunsaturated fatty acids from oxidative damage caused by reactive oxygen species (ROS) is the main function of tocopherols. In the present study, *NtTC*, which encodes a tobacco tocopherol cyclase ortholog, was cloned and characterized. Compared with control plants, *NtTC* transgenic rice showed higher tolerance to drought stress, and total tocopherol content increased by 52 % in leaf. Additionally, total antioxitant activity of *NtTC* transgenic lines was increased significantly by 19 %. These results demonstrate that over-expressing *NtTC* could improve the tolerance to abiotic stress in rice, and tocopherols play a crucial role in the protection of oxidative stress.

P-2075

In Vitro Selection to Develop Salt Tolerant Plants of Sweet Potato, Giant Swamp Taro and Soft Taro. 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 salt tolerant plants. Sweet potato, giant swamp and soft taro 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. These crops are placed on high agricultural priority but limitations in availability of salt tolerant germplasm, disease-free and elite seedlings, is a major bottleneck in production. Therefore, the study was undertaken to develop efficient *in vitro* protocols for salt tolerance selection and mass multiplication of sweet potato *(Ipomoea batatas* (L.) Lam.), giant swamp taro (*Cyrtosperma merkusii* (Hassk.) Schott) and soft taro (*Colocasia esculenta* (L.) Schott). A series of experiments were performed to establish aseptic cultures and to develop efficient and reproducible *in vitro* multiplication protocols by manipulation of various plant growth regulators, media composition and culture conditions. Different concentrations of sodium chloride (50.0 mM-200.0 mM) were used for *in vitro* selection of salt tolerant germplasm. *In vitro* selected germplasm will be further evaluated for salt tolerance in the greenhouse and field.

P-2076

RNAi Based Resistance to Three Different Cucurbit-infecting Viruses Through Expression of Chimeric Hairpins. VINAY PANWAR. Pacific Agri-Food Research Center, Summerland, BC V0H 1Z0, CANADA. Email: gene_silencing@yahoo.com

Viral diseases are a major concern in agriculture and globalization of economic activities will boost existing problems

demanding improved strategies to control viral pathogens. Recently RNA interference (RNAi), whereby double-stranded RNA (dsRNA) induces sequence specific degradation of complimentary mRNA, has emerged as a method of choice to develop resistance against plant viruses. Since under field conditions plants can be attacked by several viruses, developing transgenic varieties resistant to a single virus has practical limitations. Therefore, pyramiding gene segments from different viruses in a single chimeric form can be used as a potential alternative to generate broad-spectrum multiple virus resistance. To test this hypothesis in a commercial cucurbit crop, untranslatable coat protein (CP) gene segments of *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* type watermelon (PRSV-W) and a *Cucumber mosaic virus* (CMV) were fused together in self-complimentary inverted repeat form under the control of a single promoter. The constructed binary vectors were used to genetically modify melon (*Cucumis melo* L.) by *Agrobacterium*-mediated T-DNA transformation. Several transgenic lines were generated and tested for resistance to the three target viruses. Upon challenge inoculation by single and mixed virus inoculum transgenic lines exhibited various degrees of resistance to the corresponding viruses. Transgene induced resistance was associated with production of small interfering RNAs (siRNAs), indicating that post-transcriptional gene silencing was the mechanism involved. Together, this work demonstrates that the chimeric transgene strategy offers great potential for developing multiple virus resistance in an agriculturally important crop.

P-2077

Development of Efficient *In Vitro* Propagation and Conservation Techniques for Lentisk. İ.KOÇ and Y. Özden Çiftçi. Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400, Kocaeli, TURKEY. Email: koc_ibrahim@yahoo.com, ikoc@gyte.edu.tr

Lentisk, which produces valuable mastic used in toothpaste, gum, perfume and medicine industry, faces genetic erosion due to problems faced with its generative and vegetative propagation and rapid urbanization. *In vitro* propagation and conservation methods could aid to safeguard its germplasm and complement conventional conservation methods. Thus, different explants types (shoot tips, nodal buds, cotyledonary nodes) were evaluated together with various cytokinins types (BA, KIN, TDZ, 2-iP) and carbon sources (sucrose and glucose) supplemented to various medium compositions (MS, WPM, QL, DKW). Efficient proliferation rate (%71.4) with the highest multiple shoot formation (1.87) was obtained from nodal explants cultured on MS medium supplemented with 1 mg l^{-1} BA and 30 gl^{-1} sucrose. In the case of *in vitro* conservation of lentisk, results revealed that encapsulated shoot tips of lentisk could be conserved for up to 6 months at 4°C in dark with a 87.5 % plant retrival. *In vitro* propagated and conserved microshoots were rooted on 2 mg l^{-1} IBA and successfully acclimatized to *in vivo* conditions. The developed micropropagation and conservation protocols could help to minimize its genetic erosion and to protect its germplasm.

P-2078

In Vitro Propagation of *Barringtonia acutangula* – a Medicinal Tree through Shoot Tip and Axillary Bud Culture. SANDHYA RANI KONDABATHINI. Department of Botany, Kakatiya University, Warangal - 506009, Andhra Pradesh, INDIA. Email: sandhyaraj29@yahoo.com

Micro propagation of *Barringtonia acutangula* is described for the first time. *B. acutangula* is a non-exclusive type mangrove, belongs to the family *Barringtoniaceae*. It is distributed in the tropical regions of Asia, Malaysia and Pacific. *B. acutangula* is also known as fresh water mangrove, Indian oak, itchy tree, mango- pine and stream *barringtonia.* It is a highly medicinal plant. Every part (bark, roots, leaves and fruits) of the plant is useful. Nine triterpene saponins, acutangulosides A-F, acutangulosides D-F methyl esters and a single triterpene aglycone were isolated from the bark. Ethanolic study of fruit extracts showed saponins, on hydrolysis yielded triterpenoid sapogenins, barringtogenol B, C & D and two triterpenoid acid sapogenins. The bark is used for fish intoxication. An efficient micro propagation protocol was established for *B. acutangula*. Proliferation was obtained from shoot tips and axillary buds, which served as initial explants. The regeneration capacity of explants was influenced by factors such as concentrations of growth regulators and the type of medium (MS, B5 or WPM). Among the three media tested MS medium was found to be effective. In both explants the highest proliferation rate was obtained on the MS medium supplemented with BAP 2 mg/L. The regenerated shoots were rooted (60-65 %). In both explants, best rooting response was observed with ½ MS media+IBA (0.5 & 1.00 mg/L). The rooted plantlets were successfully acclimatized and 90 % survival rate was observed.

P-2079

The Reason Why Low Fruit Set Was Harvested When Hybridization of Black Locust Was Carried Out. PENG SUN, Li Dai, Yan Sun, Ruiyang Hu, Cunquan Yuan, Yunhan Sun, and Yun Li. National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, P. R. CHINA. Email: sunpeng1017@gmail.com, yunli63@gmail.com (Corresponding Author)

The hybridization of black locust which has been carried out for many years reflected that the fruit set of hybridization (flowers of which were emasculated and bagged) was 3.56 %, whereas the fruit set of natural flowers (untreated) and self-pollinated flowers (flowers of which were bagged) were 40.66 % and 18 %, respectively. Experiments of other experts have also demonstrated the similar result. Emasculation was inferred to cause low fruit set of hybridyzation. However, the exact reason has not been clarified and it strongly limits the hybridization of black locust as well as other Papilionaceae plants. In our previous study, we found that the content of ethylene and ABA (abscisic acid) increased and the content of JA (jasmonic acid) and reducing sugars decreased in the emasculated flowers at 5, 24, 72 and 120 h after emasculation. These facts may cause premature flower drop of the emasculated flowers and result in low fruit set. Furthermore, the emasculation was speculated to change the floral transcriptome during the floral development, pollination, fertilization and ovarian development. These changes might lead to the alterations of related physiological substances and their signaling systems. Subsequently, senescence and programmed cell death of the emasculated flowers might be accelereated, which would negatively affect the floral development, pollination, fertilization and ovarian development. Finally, few fruit of the hybridization was got because of these reasons. To explore the mechanism, GC-MS, cDNA-AFLP method, TUNEL and DNA gradient electrophoresis methods is being used to determine the changes of hormones, transcriptome and cell apoptosis during the floral and ovarian developments. In addition, transmission electron microscopy is also being used to observe the position and condition of signal substances such as the calcium, free IAA, and methyl ester pectin in the styles during the growth of pollen tubes. These results may help us to reveal the reason why the fruit set of hybridization was low and make a foundation for the improvement of the hybridization of black locust as well as other monoclinous plants.