Plant Posters

P-2000

Improving Soybean Resistance Against Soybean Cyst Nematode (*Heterodera glycines*) by Stacking RNAi Constructs. CHAD R. BRADY, Jiarui Li, Timothy C. Todd, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Corresponding author. Email: hnt@ksu.edu, crbrady@ksu.edu

The soybean cyst nematode (SCN), *Heterodera glycines*, is the primary biotic stress limiting soybean production, accounting for $4 billion loss per year worldwide and $460-818 million annual loss in the USA. Current SCN management choices such as nematicides, crop rotation and resistant varieties all have serious limitations. To explore alternative methods of SCN control, we are deploying RNAi strategy for controlling SCN. As shown previously, we have improved soybean resistance against SCN significantly by transgenic composite plants expressing dsRNA targeting several SCN genes, such as Y-1 and P-6. To achieve increased and possibly durable resistance in soybean, we have created an RNAi construction targeting two traits through the ligation of the Y-1 and P-6 target fragments together by digestion, ligation, and finally the Gateway cloning process. Using the rapid soybean transgenic system established in our lab, we have transformed composite plants with RNAi constructs targeting the Y-1 and P-6 genes individually as well as stacking both targets in one vector. Preliminary bioassay results showed that composite plants with stacking construct displayed better resistance against SCN compared to plants transformed with only one RNAi construct. Stable soybean transformation with stacking RNAi constructs is in the process in hopes to provide a more durable resistance trait against SCN.

P-2001

Improve Resistance to Brown Patch and Gray Leaf Spot in Tall Fescue. BINBIN ZHOU and Rongda Qu. Crop Science Department, North Carolina State University, Campus Box 7287, Raleigh, NC 27695. Email: bzhou2@ncsu.edu

Widely used in home lawns, parks, golf courses and athletic fields, tall fescue now is a turf and forage grass species of great economic importance in Europe, Asia, North Africa and North America. The main problems in growing tall fescue are two fungal diseases: brown patch caused by *Rhizoctonia solani*, and gray leaf spot caused by *Maganporthe grisea*. Previous researchers have introduced several genes into tall fescue and other turfgrasses, like T4 Lysozyme gene, alfalfa β-1,3-glucanase AGLU1 gene, dermaseptin SI gene, and rice *Pi9* gene, which confer turfgrass improved resistance to the diseases. In this project, we are trying to introduce a novel synthetic gene into tall fescue in an attempt to provide higher resistance to both diseases, particularly to brown patch. The transgene has been introduced into tall fescue through Agrobacterium-mediated transformation.

P-2002

Role of Tobacco Methyl Esterase in Plant Disease Resistance Signaling and Its Subcellular Localization. DHIREN德拉 KUMAR, L. Y. Fai, T. Hotz, Y. Patel, A. Ingram, J. P. Yuh, M. A. Hossain and P. C. Chigurupati. Department of Biological Sciences, East Tennessee State University, Johnson City, TN 37614. Email: kumard@etsu.edu

Plants are regularly attacked by microbial pathogens as easy source of nutrients. Over time plants have developed elaborate mechanisms to detect and respond to invading pathogens. One such signaling mechanism is mediated by plant hormone, salicylic acid (SA). Level of salicylic acid quickly increases several folds in plants resisting infections. SA is synthesized in the plastids and is converted to methyl salicylic acid (MeSA) by salicylic acid methyl transferase (SAMT), which then diffuses out of the chloroplasts. SA-binding protein 2 (SABP2), a 29 kDa tobacco protein has been shown to catalyze the conversion of MeSA to SA which is critical for activating plant defenses. Increased levels of SA in infected and in cells immediately surrounding infected cells leads to hypersensitive responses resulting in death these cells. Some MeSA also travels through phloem to yet uninfected parts of the plant where it is converted into SA by SABP2 to induce Systemic Acquired Resistance (SAR). SAR helps prepare uninfected parts of the plant for future attack. Tobacco plants silenced in SABP2 expression, show suppressed local resistance to...
Tobacco Mosaic Virus, are unable to induce defense genes/proteins and are unable to develop robust SAR. Precise location of SABP2 in plant cells is not clear. Analysis of SABP2 protein does not reveal any signal peptide signal sequence. We have used combination of cellular fractionation, biochemical and immunological techniques to determine subcellular localization of SABP2 in tobacco cells. A better understanding of the subcellular localization of SABP2 will help understand metabolic pathway involved in SA-mediated defense response, and also the possibility of predicting SABP2 interactions which may be vital for its function. Study and better understanding of SA-mediated defense pathway may help to improve plants own natural defenses and could be used to reduce large scale use of pesticides in agriculture, which are environmentally unfriendly and are health hazard for humans and animals alike.

P-2003

Specific Knock-down of Multiple Genes by Artificial MicroRNA in *Populus trichocarpa*. RUI SHI1, Chenmin Yang2, Ronald Sederoff2, and Vincent L. Chiang1,2

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Artificial microRNAs (amiRNAs) are similar to microRNAs (miRNAs) in that they are able to reduce the abundance of specific transcripts by RNA-Induced Silencing Complex (RISC)-mediated cleavage and degradation, but differ in that they are designed for knock-down of specific target genes. The long generation times of forest trees have limited the discovery of mutations by conventional genetics. AmiRNAs can create gene-specific transcript reduction in transgenic trees in a single generation and may have broad application for functional genomics of trees. Here we describe the specific down-regulation of multiple genes in the phenylalanine ammonia-lyase (*PAL*) gene family of *Populus trichocarpa* using amiRNA sequences incorporated in a *P. trichocarpa* miRNA-producing precursor, *ptc-MIR408*. Two different amiRNAs were designed to down-regulate two different subsets of *PAL* genes. By using modified polyA tailing based RT-PCR and 5’ RACE, we have validated the efficiency and specificity of amiRNA expression and down-regulation of target transcripts in transgenic trees. Our analyses also revealed the differential regulation and potential feedback regulation within the *PAL* gene family: down-regulation of subset A (*PAL2, PAL4* and *PAL5*) transcripts by amiRNA led to an increase in transcript abundance of subset B (*PAL1* and *PAL3*). The reciprocal effect was not observed.

P-2004


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Ethylene responsive factors (ERFs) are a major group of plant transcription factors with either activation or repression capabilities on gene transcription. Repressor-type ERFs are characterized by an intrinsic motif, namely the ERF-associated amphiphilic repression motif (EAR). Here we report the identification of a gene from peach (*Prunus persica*), *Pp-EFR3.b*, encoding for an ERF repressor. The transcription kinetics of this gene was investigated by qRT-PCR after inoculation of peach leaves with *Xanthomonas campestris* pv. *pruni*. The more accumulation of *Pp-EFR3.b* transcripts in susceptible, i.e. BabyGold 5, than in resistant, i.e. Venture, peach varieties indicates a negative role for this gene in disease resistance. In response to defense elicitors, *Pp-EFR3.b* was upregulated by jasmonic acid (JA) and ethylene (ET) and to less extent by salicylic acid (SA). The functional potency of *Pp-EFR3.b* in vivo has been confirmed by its ability to repress the expression of GUS-reporter gene. To better understand the functional role of *Pp-EFR3.b*, the full-length gene and the gene without EAR motif (*Pp-EFR3.bΔEAR*) were overexpressed in tobacco (*Nicotiana tabaccum*). The site-branching was the most notable phenotype in both types of transgenic plants which suggests interference with auxin-mediated dormancy of lateral shoots. Consistent with that, the expression of auxin-responsive factors (*Nt-ARF1, Nt-ARF6* and *Nt-ARF8*) was significantly downregulated in transgenic plants compared to wild-type. Although site-branching was independent on EAR motif, the response of transgenic plants to inoculation by *Pseudomonas syringae* pv. *tabaci* was EAR-dependent. Transgenic plants overexpressing *Pp-EFR3.bΔEAR* were more resistant to bacteria than those overexpressing the full-length gene. This resistance was associated at the molecular level with more induction of Pathogenesis–Related (PR) genes (*Nt-PR1a, Nt-PRb1, Nt-PR2* and *Nt-PR4*). All together, these results indicate that repressor-type ERFs might act through pathways that are dependent or independent of the EAR motif.

P-2005

Adding Value to Sugarcane Leaves by in Planta Expression of the Hyperthermostable GH10 Xylanase *Xyl10B*. JAE
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Biofuel production from lignocellulosic biomass depends on technology that efficiently and economically releases fermentable sugars from multi-polymeric cell wall components. Xylan is after cellulose, the most abundant polysaccharide in grass and wood biomass and must be hydrolyzed to its component sugars (xylose or xylobiose) before fermentation to ethanol. Endoxylanases are the main enzymes involved in xylan hydrolysis. In planta production of cell wall degrading enzymes will reduce costs of enzyme production. Sugarcane (Saccharum sp. hybrids) is the main source for production of table sugar and is the most efficient photosynthesizer in the plant kingdom. Stalks from which the juice for sugar crystallization is extracted represent about 75% of the above ground biomass at harvest. The remaining 25% of the biomass are leaves (10 to 25 tons per hectare) which are typically reduced by open air burning to suppress pests and diseases in the following ratoon and facilitate harvest. Adding value through in planta production of cell wall degrading enzymes offers an environmentally friendly alternative use for this abundant leaf biomass resource. Constitutive, apoplast or chloroplast targeted expression cassettes of the codon optimized, hypothermostable GH10 xylanase from Thermotoga maritima (xyl10B) were generated for in planta expression. Transgene integration, expression and enzymatic activity were evaluated following biolistic co-transfer of the xyl10B and the selectable nptII expression cassettes by Southern blot analysis, PCR, RT-PCR, ELISA, Western blot analysis, flourometric xylanase activity analysis, Congo red assay and sugar release assay. 17 transgenic sugarcane lines showed clearly detectable xylanase activity. Highest expression was detected in mature leaves. The in planta produced enzyme was purified and sugarcane xylan was used as a substrate. TLC analysis confirmed the superior catalytic activity and stability of the in planta produced enzyme with directly fermentable xylobiose as the main degradation product.

P-2006

Characterization of Activation Tagged Potato (Solanum tuberosum L.) Mutants. SUKWINDER S. AULAKH1,2, Richard E. Veilleux1, and Barry S. Flinn1,2. 1Dept. of Horticulture, Virginia Polytechnic and State University, Blacksburg, VA, 24060, and 2Institute for Sustainable Resources and Research (ISRR), Institute for Advanced Learning and Research (IALR), Danville, VA, 24540. Email: ssaulakh@vt.edu, sukhwinder.aulakh@ialr.org, barry.flinn@ialr.org

Improvement in any crop plant is based on genetic and phenotypic resources, understanding of gene functions, and the availability of tools and techniques to use and/or modify these resources. Due to the complex, tetraploid (2n=4x= 48) nature of potato, activation tagged mutants are invaluable resources for functional genomics studies. Two activation tagged mutant lines generated by the Canadian Potato Genome Project were characterized in the present study. These lines (AT601 and AT615) were generated from cv. Bintje using the activation tagging vector pSKI074. In vitro and greenhouse phenotypic studies revealed that the AT601 mutant exhibited short stature, little rooting or tuberization whereas AT615 exhibited abundant axillary shoot growth, reduced tuber dormancy and size but greater tuber yield compared to wild type Bintje. Southern blot analyses of both mutants indicated the presence of single T-DNA insertions. The genomic DNA sequences flanking each T-DNA insertion were identified using Genome Walker technology, and flanking gene predictions were made using the FGENESH program. Initial gene expression studies for several genes at the flanking ends between mutants and WT were done by reverse transcriptase PCR, using primers directed against the predicted genes. We are in process of identifying and verifying the function of several candidate genes in the observed mutant phenotypes.

P-2007

Identification, Characterization and Expression Analysis of MicroRNAs in Cotton. GUILING SUN and Baohong Zhang. Department of Biology, East Carolina University, Greenville, NC 27858. Email: sung@ecu.edu

Cotton is one of the most important economic and fiber crops. In this study, using a well-established in silico approach, we identified 30 cotton miRNAs from genomic survey sequence database. These miRNAs belong to 22 miRNA families. miRNAs were differentially expressed in cotton organs, with certain classes expressed preferentially in both a spatiotemporal and tissue-specific manner. miR-156 was highly expressed in cotyledon whereas miR-172 was highly expressed in young leaves at fruit branch, young flower buds, 0 d post-anthesis (DPA) ovules and 0 DPA petals. However, miR-172 was not highly expressed in all parts of flowers; in contrast, miR-172 was only highly expressed in petal but not in stamen and carpel. Interestingly, miR-162 was highly expressed in immature fiber, 2 DPA ovules and 0 DPA mixtures of stamen and carpel, suggesting miR-162 may play a role in cotton fiber
Iron chlorosis in plants is one of the severe problems in alkaline/calcareous soil, which results in yield loss and nutrition limitation. Researches suggested that iron chlorosis could be caused either by iron deficiency in the environment or dysfunction of iron absorption and transportation of plants. As a key transporter of iron, the IRT (Iron Regulated Transporter) genes play an important role in transportation of iron from roots to other parts of a plant. In this research, putative IRT genes were identified from the genome sequence of *Populus trichocarpa*. The phylogenetic analysis showed that these genes are closely related to the *IRT* genes from other plant species. Using specific primers designed based on the gene information, four *IRT* genes (*PtIRT1-4*) were isolated from both the iron-deficiency tolerant (*PtG*) and the iron-deficiency susceptible (*PtY*) trees of *Populus tremula*. The sequence analysis showed that *PtIRT1-4* had the similar gene structure to the *IRT* genes of other species. Sequence comparison of the *PtIRT1-4* genes between *PtG* and *PtY* indicated no significant difference in nucleotide sequences. They all expressed in young leaves. Tissue-specific expression of *PtIRT1-4* in *PtG* and *PtY* under iron-deficient and sufficient conditions is being determined. Functions of *PtIRT1-4* will be also investigated in other plant species including aspen hybrids and tobacco. The research will help understand the role of *IRT* genes in iron utilization and address iron chlorosis in plants.

**P-2008**

Characterization of the Iron Regulated Transporter (*IRT*) Genes in *Populus tremula*. D. HUANG and W. Dai. Department of Plant Sciences, North Dakota State University, Fargo, ND 58108. Email: wenhao.dai@ndsu.edu

Iron chlorosis in plants is one of the severe problems in alkaline/calcareous soil, which results in yield loss and nutrition limitation. Researches suggested that iron chlorosis could be caused either by iron deficiency in the environment or dysfunction of iron absorption and transportation of plants. As a key transporter of iron, the IRT (Iron Regulated Transporter) genes play an important role in transportation of iron from roots to other parts of a plant. In this research, putative IRT genes were identified from the genome sequence of *Populus trichocarpa*. The phylogenetic analysis showed that these genes are closely related to the *IRT* genes from other plant species. Using specific primers designed based on the gene information, four *IRT* genes (*PtIRT1-4*) were isolated from both the iron-deficiency tolerant (*PtG*) and the iron-deficiency susceptible (*PtY*) trees of *Populus tremula*. The sequence analysis showed that *PtIRT1-4* had the similar gene structure to the *IRT* genes of other species. Sequence comparison of the *PtIRT1-4* genes between *PtG* and *PtY* indicated no significant difference in nucleotide sequences. They all expressed in young leaves. Tissue-specific expression of *PtIRT1-4* in *PtG* and *PtY* under iron-deficient and sufficient conditions is being determined. Functions of *PtIRT1-4* will be also investigated in other plant species including aspen hybrids and tobacco. The research will help understand the role of *IRT* genes in iron utilization and address iron chlorosis in plants.

**P-2009**

Cloning and Characterization of PR5 Gene from *Curcuma amada* and *Zingiber officinale* in Response to *Ralstonia solanacearum* Infection. D. PRASATH1,2, I. El-Sharkawy1,3, S. Sherif4,5, K. S. Tiwary4 and S. JAYASANKAR1,3. 1University of Guelph, Department of Plant Agriculture, 4890 Victoria Ave. N., PO Box 7000 Vineland Station, ON, L0R 2E0 CANADA; and 2Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1, CANADA. Email: prasath_d@yahoo.com; jsubrama@uoguelph.ca

**P-2010**

MSSS: Bioinformatics Method Facilitates Searching Large Set of Peptide Spectra Against Large Nucleotide Databases for Structural- and Onco-proteogenomics. M. HELMY1,2, N. Sugiyama1, N. Tomita1, and Y. Ishihama1,3. 1Institute for Advanced Biosciences, Keio University, JAPAN; 2Systems Biology Program, Graduate School of Media and Governance, Keio University, JAPAN; and 3Graduate School of Pharmaceutical Science, Kyoto University, JAPAN. Email: mohamed@sfc.keio.ac.jp, y-ishii@ttck.keio.ac.jp

Liquid chromatography-mass spectrometry (LC-MS/MS)-based proteomics is an incomparable approach to study
uncharacterized genome-wide proteome. Since LC-MS/MS is high-throughput approach, it produces large set of peptide spectra. To identify the sequence corresponds to each peptide spectra, the spectra set should be searched against database of putative sequences. Searching such large dataset against protein database or small-sized nucleotide sequence database is effortless task. However, searching such large dataset against big-sized nucleotide sequence database, such as six-frame translation of genome database, remains major challenge due the linear relationship between the search time and the database size. Here, we present MSSS (Mass Spectrum Sequential Subtraction), a novel bio-informatics method to compare the large sets of peptide spectra resulted from LC-MS/MS against series of protein and nucleotide sequence databases to find novel peptides. The main principle in MSSS is to search the peptide spectra set against the protein database then remove the spectra corresponding to the indentified peptides and search the remaining peptide spectra against the nucleotide sequence database. Therefore, we reduce the spectra number to be processed instead of the size of the database to be searched. We used MSSS in analyzing our MS/MS data of rice (eukaryotic genome), Archea (prokaryotic genome) and Hela S3 cell-line (cancer genome). MSSS was able to reduce the search time and the required computational demands without affecting the accuracy. Further, the novel peptide sequences identified through MSSS pointed several novel structural genomic features e.g. new ORFs, genes or alternative-splicing isoforms (in rice and Archea genomes) and candidate onco-peptides and cancer-related mutations (in the Hela S3 genome).

**P-2011**

Bioinformatics Tools and Analysis to Identify Lethal Genes in Root Knot Nematode (*Meloidogyne* spp.), A. ISMAIL and N. Alkharouf. Towson University, York Rd., No 7800, Dept. of Computer and Information Sciences (COSC), Towson MD 21252. Email: aig20779@gmail.com, nalkharouf@towson.edu

Root Knot nematode (RKN; *Meloidogyne* spp.) is one of the most devastating obligate parasites that infect the roots of thousands of plant species. RKN cannot live independently from their hosts and are the biggest contributors to the loss of the world's primary foods by an average of 12.3%. In the United States, the cost of the losses that these parasites cause annually is estimated to be about 12% or $8 billion. The RKN genome sequence is not well characterized or fully sequenced. We are seeking to create bioinformatics tools to identify genes that are lethal at any stage of RKN development. These genes could act as targets for RNAi constructs or gene knockout experiments. To that end we compared RKN sequences to the well-characterized and fully sequenced genome of the free-living nematode *C. elegans* (*Caenorhabditis elegans*). The primary database for *C. elegans* (www.wormbase.org) contains a huge repository of RNAi and mutation experiments on *C. elegans* genes. We conducted a comparative genomics analysis, comparing RKN genes of six species: *Meloidogyne arenaria*, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne Paranaensis* to *C. elegans* via wormbase. The RKN sequences were obtained from nematode.net. Our analysis yielded a total number of homologous lethal genes between *Meloidogyne spp* and *C. elegans* as follow; 5 from *M. Arenaria*, 1491 from *M. chitwoodi*, 3556 from *M. hapla*, 3949 from *M. incognita*, 2406 from *M. javanica*, and 959 from *M. paranaensis*. We then create a public database that links RKN ESTs and proteins to their *C. elegans* homologous and provided search capabilities for scientists in the field. The database was created using SQL-Server 2008 and ASP.NET for the web user interface.

**P-2012**

In Vitro Chromosome Doubling for Crop Improvement. D. H. TOUCHELL, J. Smith, and T. G. Ranney. Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, North Carolina State University, 455 Research Drive, Mills River, NC 28759-3423. Email: darren_touchell@ncsu.edu

In vitro regeneration systems provide a powerful tool for manipulating ploidy levels to facilitate the breeding and development of improved crops. The development of polyploids may enhance ornamental characteristics and environmental tolerances, expand breeding opportunities, assist with the development of non-invasive triploid cultivars, and restore fertility in sterile hybrids. In vitro chromosome doubling is commonly induced using antimitotic agents such as cochicine, oryzalin and triflurilin. Successful induction is dependent on the length of exposure and concentrations of antimitotic agents, explant types and interactions between basal media and plant growth regulators. Current research is focused on optimizing and exploring the effects of in vitro polyploid induction on taxa with ornamental and bioenergy applications such as Miscanthus, Acer, Hydrangea, Rudbeckia, Azalea, Baptisia, Liriope, Hypericum and Magnolia. In vitro conditions vary among taxa and individual genera, species and cultivars often require unique treatments to optimize polyploidy induction. In some taxa, the induction of polyploidy influences in vitro growth, development and root formation, leading to further studies on regeneration and rooting. Here we provide an overview of the application
of in vitro chromosome doubling for crop breeding and improvement.

P-2013

From Cells to Field-ready Plants: One-step Micropropagation in a Mist Bioreactor. L. FEI and P. J. Weathers. Worcester Polytechnic Institute, 100 Institute Road, Dept. of Biology & Biotechnology, MA 01609. Email: lwfei@wpi.edu, weathers@wpi.edu

Micropropagation is a widely applied technique for rapid regeneration of whole plants in large numbers using plant tissues ranging from single cells to shoot segments. The scaling of this technique, on the other hand, is hampered by its high labor costs for subculture and acclimatization. A mist bioreactor may provide cost effective scale-up of the entire process of micropropagation beginning with cells to field-ready plants in one step. This bioreactor is mainly composed of a growth chamber made of disposable plastic, an ultrasonic nozzle with a conical tip, gas inlet, medium reservoir, timer and peristaltic pump. Using carrot as a model plant, undifferentiated carrot cells were sprayed through the ultrasonic nozzle onto nylon screens placed in the growth chamber, fed with B5 medium mist in different feeding cycles and harvested after 2 wk to measure both cell viability, after this mode of inoculation, and development of embryos. After inoculation using 4.5 watts of ultrasonic power, 51.2% cells remained viable. Using a feeding cycle of 1 min mist on every 15 min at 38 ml/min, the total embryos produced per gram fresh weight were 11,322 and 10,138 for nylon screens with openings of 50 micron (CMN50) and 90 micron (CMN90), respectively. Of these embryos, there was no difference in which nylon screen was used; about 3.5% were developed to the torpedo or cotyledon stage. When a more frequent misting cycle was used that fed the same volume of medium per hour, there was no significant change in embryo formation. On the other hand, the yield of embryos beyond the heart stage tripled. This ongoing study includes additional experiments to maximize embryo formation with full development to field-ready plants and scale-up.

P-2015

Optimization of Conditions for Enhanced Biomass Accumulation and Phyto-chemicals Production in Callus Cultures of Ficus religiosa Linn. PRIYANKA SIWACH, Anita Rani Gill, Shilpa Thareja, Dipti Rani, Anita, and Kavita Kumara. Department of Biotechnology, Ch Devi Lal University, Sirsa, Haryana, INDIA. Email: psiwach29@gmail.com

In the past century, the chemical investigation and purification of extracts of plants have yielded numerous purified compounds with novel medicinal properties like aspirin, quinine, taxol, vincristine etc. Ficus religiosa L. is a multipurpose long-lived forest tree having great religious and medicinal importance in the culture of India and Nepal since times immemorial. Various extracts of this tree have been documented for applications like - for the treatment of diabetes, asthma, inflammations and glandular swelling, wound healing, stomatitis, skin diseases and female genital problems. Recently stem bark is also reported to have remarkable acetylcholinesterase inhibitory activity, latter being very effective for the treatment of Alzheimer disease. Despite its impressive medicinal applications, the tree has not been exploited commercially. The religious holdings and social values associated with this tree restrict its use for isolation, identification, characterization and commercial supply of secondary metabolites. Hence, there is an urgent need for alternative source. Plant cell cultures were
introduced as an important tool for studying and producing plant secondary metabolites in the mid 1960s. Some highly effective secondary metabolites that are used in pharmaceuticals, food industry and cosmetics have been produced through plant cell cultures, callus cultures, shoot cultures and root cultures. The application of tissue culture techniques to woody perennials has always been a challenge. Till now, there is no report regarding the use of any in vitro raised tissue of Ficus religiosa L. for secondary metabolite extraction. So we were interested in developing a suitable in vitro tissue source of Ficus religiosa which can be used for commercial level secondary metabolite extraction. As a preliminary step, we optimized various in vitro conditions for efficient induction and enhanced biomass production of callus cultures for more than one year followed by comparative analysis of various phytochemicals among methanolic extracts of stem segments of mother plant, 6-mo-old callus and 12-mo-old callus tissues, raised on different growth conditions. We will discuss about the effect of different growth regulators as well as modification in the MS basal medium on callus induction and proliferation from nodal segments of 45–50 yr old tree. Optimization of various conditions like inoculum size and agar concentration on biomass accumulation of callus cultures will be discussed. Monthly callus biomass increase on different in vitro conditions will be presented. Various qualitative tests for different phytochemicals showed the presence of some novel metabolites in callus cultures as these were absent in mother plant extracts. Total phenolic content and total flavonoids were many folds more in callus cultures as compared to in vivo source, as indicated by quantitative tests. A discussion will also be made about the effect of different medium composition and age of callus tissue on secondary metabolite production.

P-2016
Transformation with Anthocyanin Transcription Factors Produced Novel Purple Leaved Petunias and Red Leaved Cymbidiums. M. R. Boase1, H. B. Zhang1, D. H Lewis1, N. W. Albert2, L. X. Wang1, H. S. Arathoon1, H. Ngo1, S. C. Deroles1, K. E. Schwinn1, and K. M. Davies1. 1The New Zealand Institute for Plant & Food Research Limited, Private Bag 11-600, Palmerston North, NEW ZEALAND and 2Formerly, The New Zealand Institute for Plant & Food Research Limited, Private Bag 11-600, Palmerston North, NEW ZEALAND. Email: murray.boase@plantandfood.co.nz

Anthocyanin pigments are a key driver of consumer preferences in ornamental and food crops. In plants, MYB and bHLH transcription factors (TFs) control where, when and how much anthocyanin pigment is produced. Therefore, these TFs are important targets to incorporate in molecular breeding of novel cultivars with high market value. We have used both bHLH and MYB-type TFs from petunia and maize to transform petunia and cymbidiums. Over time, as scientific advances permitted, our approach has progressed from using transgenic plasmids and Agrobacterium based transformation to particle bombardment and DNA expression fragments of non-pathogenic origin. This shift was to avoid integration of DNA from plasmid backbones and to reduce IP constraints and the potential regulatory burden involved in commercialization. We experimented with petunia first as a model crop and then moved to apply our technology and experience to an important New Zealand export crop, cymbidium orchids. Novel transgenic petunia phenotypes displaying deep purple foliage due to anthocyanin production have been produced and tested in glasshouses and the field in New Zealand. Phenotypes varied from a purple blush to a dark purple coloration, depending on the TF used and the environmental conditions. Recently, petunia plants with novel phenotypes due to expression of petunia MYB TFs under control of petunia promoters and terminators have been produced and are currently undergoing glasshouse assessment overseas. Towards the production of red-leaved cymbidiums, protocorm-like bodies that were bombarded with plasmids containing maize bHLH and MYB TFs have been selected and some shoots with a red phenotype have emerged. Progress has also been made towards the goal of producing red-leaved intragenic cymbidiums by cloning an anthocyanin MYB cDNA from cymbidium petals.

P-2017
An Assessment of Fungal Diversity in Food Commodities. Gazala Tabassum, Chandan Kumar, and Rashmi Komal. Plant Pathology and Microbiology Laboratory, Department of Botany, Patna University, Patna, Bihar, INDIA. Email: gazalaarshad@gmail.com

Most of us know that food itself cannot be considered poisonous. Very few of us know that pleomorphic bacteria, yeast, and fungus and their toxins, which are characteristically present in stored and fermented food, are using our food chain as Trojan horse. The mycoflora of any food increasingly depends upon the contaminating flora which is influenced by many factors both external and internal to the food. In the present investigation, during August 2007 to March 2008 an assessment of fungal diversity was done on some common food consumed in Bihar. For that, food samples were considered viz; chapati, dal, boiled rice, chicken, mixed vegetables in Bihar, INDIA. The samples were prepared from home collected in sterilised plastic containers. The fungal analysis of food yields many diverse
Aspergillus viz; be saprophytic in nature and belonged to the genus foods in different seasons, most of them were found to mycofloras which were isolated from different types of isolated diverse fungi were placed in the table. The incubated on different media for 5 to 7 d at a temperature of 27±2°C. The percentage frequencies of the identified isolated diverse fungi were found to be saprophytic in nature and belonged to the genus Aspergillus viz; Aspergillus flavus, Aspergillus niger, Aspergillus glaucus, Aspergillus japonicus, Aspergillus fumigatus. Penicillium citrinum showed their presence and were also found in most of the seasons. The fungal metabolites transform reactions including alakalization, oxidation, reduction, hydrolysis, hydration and conjugation in foods. Mycotoxins can cause a variety of short term as well as long term health effects, ranging from immediate toxic response to potential long term carcinogenic and teratogenic effects. Hence, extraction of toxin penicillic acid was detected from Penicillium cyclopium, Penicillium citrinum because of their growth and aggressiveness on different food commodities.

P-2018

Plastid Transformation: Improved Efficiency with Linear Vectors and Mechanism of Transgene Integration. DELENE J. OLDENBURG, Anna Kovac, and Arnold J. Bendich. Dept. of Biology, Box 355325, University of Washington, Seattle, WA 98195-0001. Email: delene@uw.edu

For many plants, plastid DNA (ptDNA) is inherited maternally. Consequently, plastid transformation provides natural biological containment of the transgene against dispersal by pollen to non-target plants. Plastid transformation employing a circular transgene vector is routine in some plants, but the transformation efficiency is low. Most ptDNA, however, is comprised of linear molecules (monomer, dimer, etc.) and branched concatemers. Thus, we may anticipate improved transformation by using linear vectors. We have used two model systems – liverwort cell cultures and tobacco leaves – to compare plastid transformation efficiency with circular and linear vectors. With liverwort, we find a higher efficiency (usually 3-10-fold, but 200-fold in one case) with a linearized vector than with the circular vector. For tobacco leaves, efficiencies were similar with the linear and circular vectors, although better transformation was found with young leaves than older leaves. For a circular transgene vector with two plastid border sequences, the likely mechanism of integration is double-reciprocal crossover with the homologous ptDNA. For a linear vector, however, integration may proceed by double-reciprocal crossover, end-joining, or strand invasion. Verification of transgene integration is typically performed using PCR and plastid primers flanking the crossover site. If insertion proceeds by end-joining, however, assessing insertion is problematic because no amplification (except wild type) would be expected using the typical PCR primers. We have tested alternative primer sets and dot blot hybridization to confirm transformation with the linear vector in liverwort. We found that integration occurred by strand invasion in 21% of the transformants and by end-joining in 78%.

P-2019

Efficient Agrobacterium-mediated Transformation of a Commercial Wheat Cultivar. Terry Hu1, JIANPING XU1, Fengming Lu1, Anthony Paisley1, Ashok Shrawat1, Jack Berg1, Eric Godsy1, Kwan Thai1, Andrew Mrozek2, Wei Zheng2, Beth Savidge2, Dale Clark3, and Charles Armstrong1. 1Monsanto Company, 700 Chesterfield Parkway West, St. Louis, MO 63017; 2Monsanto Co., Davis, CA; and 3WestBred, Bozeman, MT. Email: jianping.xu@monsanto.com

An efficient Agrobacterium-mediated transformation system has been established in wheat. The Monsanto ABI strain carrying a triple-gene construct (pMON129444) with gfp, uidA, and bar genes driven by different promoters was used for transformation. In initial experiments, pre-cultured immature embryos (PCIEs) of the model genotype Bobwhite were used to develop the transformation system. A total of 149 independent transgenic events were produced in two experiments using 3 mg/L bialaphos selection, with transformation frequencies of 10.0 and 14.5%. Molecular analysis demonstrated that 13% of the events contained a single copy of all three genes and were backbone-free. 45% of the events contained 1–2 copies of each of the three genes. This system was extended to a commercial wheat cultivar using either 3 mg/L bialaphos selection or 25 mg/L LG418 selection. pMNON95881 carrying gfp and nptII genes driven by different promoters was used for transformation. Over 110 independent transgenic events were produced using this system. Transformation frequencies varied from 2.4% to 10.4%, with an average of 4.7%. Molecular analysis confirmed that 19% of the transgenic plants carried a single copy of each of the introduced genes and were backbone-free, while 40% of the transgenic plants contained 1–2 copies of each gene. The system has been extended for the use of cp4 as a selectable marker using glyphosate selection, and the use of a 2T-DNA vector system for production of marker-free plants.

P-2020

Metabolic engineering of plants usually requires transformation of multiple genes - over expression of the key enzyme gene(s) in the target pathway and reduction of gene(s) expression in non-target pathway(s). Sometimes, it is desirable to over express multiple enzyme genes in the target pathway. Although, a polycistron approach can be used in plant chloroplast transformation, it lacks efficacy for plant nuclei transformation. In Agrobacterium-mediated transformation, each introduced gene needs one promoter and one terminator. The more genes stacked in a construct, the longer the T-DNA and the lower the transformation efficiency. To reduce the T-DNA size, we have successfully used a 2A peptide strategy to link two genes, shared with one promoter and one terminator. However, the 2A peptide originates from foot-mouth virus and therefore may delay field trial approvals. Here we report a different strategy, using ribosome binding sequences to link the genes for multiple-gene transformation. An NPTII gene, a GUSplus gene, and a BAR gene, were linked by two different plant ribosome binding sequences (one from rice and the other from Arabidopsis), shared with one promoter and one terminator. Most of the transgenic tobacco plants were not only resistant to kanamycin, but also resistant to glufosinate. They showed GUS activity as well. The plant ribosome binding sequences showed similar GUS activity and glufosinate-resistance as the virus ribosome binding sequences or as separate genes. Each has one promoter and one terminator for GUSplus gene and BAR gene. This strategy can also be combined with RNAi approach. We conclude that this strategy is suitable for multiple-gene transformation.

P-2021

GmERF Promoters Give High Wound-inducible Expression in Transgenic Soybean. CARLOS M. HERNANDEZ-GARCIA1, Cheri Nemes1, Carola De la Torre1, Nuananong Semsang1,2, Michelle L. Jones1, and John J. Finer1. 1Department of Horticulture and Crop Science, OARDC/ The Ohio State University, 1680 Madison Ave., Wooster, OH 44691 and 2Biology Department, Faculty of Science, Chiang Mai University, 239 Huay Kaew Road Muang District, Chiang Mai, THAILAND, 50200. Email: hernandez-garcia.1@buckeyemail.osu.edu

Analysis of promoter responses to specific stimuli is not only important to gain a better understanding of the regulation of gene expression but also for the development of crops with enhanced disease/pest resistance. As plants show similar responses to mechanical wounding, pathogen invasion and damage from chewing insects, studies of wound-inducible promoters could provide insights to the mechanisms of regulation of stress-responsive genes. Here we studied induction of Glycine max Ethylene Response Factor (GmERF) genes and their promoters. Transcript analysis of 10 GmERF genes revealed that GmERF genes are highly responsive to wounding, methyl jasmonate and ethylene in hypocotyls and cotyledons of soybean seedlings. Four GmERF promoters were subsequently isolated, fused to the Green Fluorescent Protein (gfp) gene and introduced into soybean. In transgenic plants, the GmERF3 and GmERF10 promoters directed low background GFP expression in roots, pod tissues, epidermis, and vascular tissues. However, these promoters were highly inducible in wounded cotyledons, hypocotyls and young leaves. GFP was not induced in wounded roots, indicating that wound induction of these promoters is tissue specific. We also found no induction of expression in wounded roots for most of the 10 GmERF genes studied. Further analysis of induction under different stimuli, along with fine analysis of promoter sequences, will allow us to expand our knowledge of the regulation of gene expression under stress conditions.

P-2022

Tools for Fern Functional Genomics. MUTHUKUMAR BALASUBRAMANIAM1, Blake Joyce1, C. Neal Stewart Jr 1, David Lee2 and Mark Elless2. 1Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996 and 2Edenspace Systems Corporation 13800 Coppermine Road, Herndon, VA 20171. Email: mbalasub@utk.edu

Ferns occupy an important role in the evolution of angiosperms. Ferns are composed of about 250 genera and are the most ancient extant vascular land plants. Despite their prevalence, they are among the least-studied group of plants with regards to genetic research for stress tolerance, pest resistance, and allelopathy. The lack of any stable transformation methods inhibits progress for either practical improvement for fern usage or their functional genomics. Efforts are underway to stably transform ferns, including the arsenic hyperaccumulator Pteris vittata, using strong native- and canonical constitutive promoters as well as enhanced promoters that regulate various marker genes, including fluorescent proteins. Developing these tools will enable overexpression and knockdown studies for metabolic and physiological pathways, RNAi studies in all life stages, and in ecological genetics. The isolation of putative arsenic-responsive promoters will not only be utilized to study inducible promoters in ferns but also may be used in developing arsenic phytosensors in the future.
Nanoparticles Affect the Growth and MicroRNA Expression in Plants. C. E. BURKLEW, T. P. Fraizer, G. Sun, and B. H. Zhang. Department of Biology, East Carolina University, Greenville, NC 27858. Email: burklew06@ecu.edu

Titanium Dioxide (TiO$_2$) is one of the most widely used pigments in the world. Due to its heavy use in industry and daily life, such as food additives, cosmetics, pharmaceuticals, and paints, many residues are released into the environment and currently TiO$_2$ nanoparticles are considered an emerging environmental contaminant. Although several studies have shown the effect of TiO$_2$ nanoparticles on a wide range of organisms including bacteria, algae, plankton, fish, mice, and rats, little research has been performed on land plants. In this study, we investigated the effect of TiO$_2$ nanoparticles on the growth, development, and gene expression of tobacco, an important economic and agricultural crop in the southeastern United States as well as around the world. We found that TiO$_2$ nanoparticles significantly inhibited the germination rates, root lengths, and biomasses of tobacco seedlings after 3 wk of exposure to 0.1%, 1%, 2.5%, and 5% TiO$_2$ nanoparticles and that overall growth and development of the tobacco seedlings significantly decreased as TiO$_2$ nanoparticle concentrations increased. Overall, tobacco roots were the most sensitive to TiO$_2$ nanoparticle exposure. Nano-TiO$_2$ also significantly influenced the expression profiles of microRNAs (miRNAs), a recently discovered class of small endogenous non-coding RNAs (~20-22 nt). miRNAs are considered important gene regulators and have been shown to play an important role in plant development as well as plant tolerance to abiotic stresses such as drought, salinity, cold, and heavy metal. Low concentrations (0.1% and 1%) of TiO$_2$ nanoparticles dramatically induced miRNA expression in tobacco seedlings with miR395 and miR399 exhibiting the greatest fold changes of 285 fold and 143 fold, respectively. The results of this study show that TiO$_2$ nanoparticles have a negative impact on tobacco growth and development and that miRNAs may play an important role in tobacco resistance to heavy metals/nanoparticles by regulating gene expression.

P-2024

In Vitro Propagation and Cryopreservation for the Conservation of Endangered Species of Saintpaulia. V. C. PENCE. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine St., Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

Species of Saintpaulia (Gesneriaceae) have been used to develop thousands of hybrids for commerce, but the species are endangered in their native habitats in Kenya and Tanzania. Although Saintpaulia produces orthodox seeds and propagates well from cuttings, in vitro methods can play a supplemental role in supporting conservation, especially when there are few or no seeds available for germplasm storage. In vitro collecting can be used to collect tissues in the field, but leaves can also remain viable during several days of shipment, and such can be used to initiate cultures in the laboratory. Aseptic shoot cultures can be maintained to avoid infection with nematodes or disease that could negate the possibility of reintroducing propagated plants into a wild habitat. Tiny shoot buds can be initiated on leaf pieces in vitro, and these buds can be cryopreserved with very little dissection, using encapsulation dehydration. Survival after exposure to liquid nitrogen has ranged from 33% to 100% with $S.$ grandifolia, $S.$ confusa, $S.$ brevipilosa, $S.$ rupicola, and $S.$ tongwensis. These in vitro methods provide workable tools for collecting, propagating, and cryopreserving germplasm from threatened genotypes, even when seeds are not available, and can play a role in developing conservation strategies for this genus. Saintpaulia also provides a model for methods that could be utilized in the conservation of other endangered Gesneriaceae.

P-2025

Somatic Embryogenesis and Plant Regeneration of Superior Interspecific Hybrids of Elephant Grass and Pearl Millet. FABIO G. FALEIRO$^{1,2}$; Baskaran Kannan$^1$, and Fredy Altpeter$^1$. $^1$Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611 and $^2$Future address: Embrapa Cerrados, P.O.Box 08223, 73310-970 Planaltina, DF, BRAZIL. Email: altpeter@ufl.edu

Elephant grass is one of the most productive biomass producers in tropical and subtropical regions and considered an important forage and future bioenergy crop. Sexual compatibility between elephant grass (Pennisetum purpuraceum $2n = 4x = 28$) and pearl millet (Pennisetum glaucum $2n = 2x = 14$) allows the development of interspecific triploid hybrids for the introgression of favorable alleles by traditional plant breeding. In contrast to elephant grass, which produces large amounts of wind dispersed seeds, interspecific hybrids between elephant grass and pearl millet are male and female sterile due to triploidy ($2n = 3x = 21$) and do not produce seeds. Male and female sterility of interspecific hybrids will enhance containment of genetically improved bioenergy feedstocks. Alternatively,
doubling of chromosomes of the triploid hybrids may restore seed production and enhance biomass production. The requirement for both genetic engineering of interspecific hybrids and in vitro chromosome doubling is an efficient plant regeneration protocol by tissue culture. We selected the most persistent and productive interspecific hybrids in replicated field studies and evaluated the callus induction and plant regeneration response of seven superior interspecific hybrids of elephant grass and pearl millet. Significant genotypic differences were observed for callus induction, necrosis and quantity or quality of embryogenic callus. The results of this study supported identification of genotypes with induction of highly embryogenic calli with superior plant regeneration frequency. These tissues are currently providing the basis for development of in vitro chromosome doubling and genetic transformation protocols.

P-2026

Dissection of the Strong Soybean Ubiquitin Promoter (Gmubi) Reveals Essential Regions Responsible for Regulating High Levels of Gene Expression. CAROLA M. DE LA TORRE, Carlos M. Hernandez-Garcia, Robert A. Bouchard, and John J. Finer. Department of Horticulture and Crop Science, OARDC/ The Ohio State University, 1680 Madison Ave. Wooster, OH 44691. Email: de-la-torre.cuba.1@buckeyemail.osu.edu

The novel ubiquitin promoter (Gmubi) expresses constitutively in soybean at higher levels than the widely known cauliflower mosaic virus (CaMV35S) promoter. Gmubi is 912 bp in length containing a 5'UTR intronic region of 592 bp. To further analyze Gmubi regulatory regions, we built a set of Gmubi derivatives fused to the gfp reporter gene. Constructs were introduced via particle bombardment in lima bean cotyledons and GFP expression was monitored over 100 h using a robotic image collection system. Gmubi constructs were also transferred to Agrobacterium rhizogenes and used to produce stably-transformed soybean hairy roots. Gmubi derivatives include a minimal promoter sequence of 93 nt (GmuCore) enough to give very low levels of expression. Addition of the intron sequence to GmuCore and translocation of the intron upstream the promoter region increased GFP expression levels suggesting the presence of regulatory elements within the intron. Furthermore, duplication of internal intron sequences within the intron resulted in high expression levels further confirming an intron positive role in gene expression. Finally, a 1500 bp 5’ Gmubi extension showed expression levels 2X higher than Gmubi in transiently-transformed lima bean cotyledons and stably-transformed soybean hairy roots suggesting the presence of additional regulatory elements within this 5’ upstream region.

P-2027

Development of a Transformation and Regeneration System for Sunflower (Helianthus annuus). ZHIFEN ZHANG and John J. Finer. Department of Horticulture and Crop Science, OARDC/The Ohio State University, 1680, Madison Ave. Wooster, OH 44691. Email: zhang.653@buckeyemail.osu.edu, finer.1@osu.edu

Sunflower (Helianthus annuus) is the third most important oil-seed crop in the world. Although sunflower tissues are very responsive to Agrobacterium infection, production of transgenic sunflower remains terribly inefficient. This problem may result from the inability to target regeneration-competent cells for transformation. In order to take full advantage of the available biotechnological tools for both basic research and sunflower improvement, more routine transformation methodologies must be first developed. RHA280 was identified as a sunflower line with high response to shoot induction. Cotyledons of dry, mature seeds were excised and plated cut side down on a shoot induction media containing MS salts, B5 vitamins, 0.1 mg/L NAA, 1.5 mg/L BA, 3% sucrose (w/v) and 0.2% Gelrite (w/v). In the absence of Agrobacterium, over sixty shoots could be obtained, on average, from each (1×3 mm) cotyledon explant. Agrobacterium strain LBA4404 was initially selected from twelve stains as it yielded high transformation efficiency but low hypersensitive response from sunflower cotyledon explants. Transformation efficiency was evaluated by using the gfp reporter gene driven by a native sunflower polyubiquitin promoter (HaUbi) which directed high transgene expression in sunflower tissue. Consistently, over 80% of explants yielded high transient GFP expression, and several different types of cells within the cotyledonal tissues were targeted. Using Agrobacterium-mediated transformation of cotyledonary tissues, transformed shoots expressing GFP were occasionally obtained. Efforts are underway to increase the efficiency of plant recovery and improve Agrobacterium targeting of shoot-producing cells and selection for transgenic shoots.

P-2028

Dual Function of Thiosulfate in Regulation of Plant Tissue Culture Growth. B. STEINITZ and Y. Tabib. Institute of Plant Sciences, ARO, The Volcani Center, Bet Dagan 50250, ISRAEL. Email: steinitz@volcani.agri.gov.il
Silver ions (Ag⁺, provided as dissolved AgNO₃) are as a rule toxic to unicellular and multicellular organisms. In plant cells, non-toxic low Ag⁺ concentrations interfere with ethylene and auxin signaling, and thereby modulate cell development. Accordingly, AgNO₃ has often been used as a medium supplement to successfully regulate plant tissue culture development. Surprisingly, no references on Ag⁺ toxicity to plant tissue cultures were found in the literature. Ag⁺ reacts with thiosulfate ion (S₂O₃²⁻) to form silver thiosulfate complex (STS). Recently, we demonstrated the superiority of STS over AgNO₃ in controlling in vitro rooting and plant development in Corymbia maculata. In the present work, the impact of S₂O₃²⁻ on growth of a tomato root organ culture was explored, and the dependence of Ag⁺ toxicity on chemical formulation (AgNO₃ versus STS) was examined. We found that (a) Sodium thiosulfate (Na₂S₂O₃) medium supplement (without Ag⁺) augmented root growth and development. (b) Non-toxic concentrations of AgNO₃ stimulated root elongation. (c) Supra-optimal AgNO₃ concentrations completely inhibited root growth, and silver precipitate particles in the medium appeared to be toxic as well. (d) Toxic levels of Ag⁺ (when provided as AgNO₃) were non-toxic when supplied to roots as STS. The results suggest that STS superiority over AgNO₃ in control of root organ culture growth was due to two distinguishable functions of thiosulfate, namely, stimulation of root development by S₂O₃²⁻ and prevention of Ag⁺ toxicity by S₂O₃²⁻. Possible broader implications of the results will be discussed.

P-2029

Screening Rice Cultivars for Elevated Vitamin C Content. KATHERINE A. LISKO¹, John F. Hubstenberger¹, Helen Belefant-Miller², Gregory C. Phillips¹-³, Wen Gui Yan², Anna McClung², and Argelia Lorence¹-⁴. ¹Arkansas Biosciences Institute, Arkansas State University; ²USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR; ³College of Agriculture and Technology, Arkansas State University; and ⁴Department of Chemistry and Physics, Arkansas State University. Email: Katherine.lisko@mail.astate.edu, alorence@astate.edu

Vitamin C (ascorbic acid, AsA) is essential for human health, however, despite our dependency on plants as dietary sources of this key nutrient, little is known about its metabolism in crops of agricultural importance. As the most abundant antioxidant in plant tissues, AsA protects cells and organelles from oxidative damage by scavenging reactive oxygen species (ROS) that are produced in response to abiotic and biotic insults. Ascorbate is also a cofactor of multiple enzymes, controls cell division, and affects cell expansion. In addition, AsA is a substrate for the production of tartaric and oxalic acids, and is a modulator of plant senescence. Biosynthesis of AsA in plants is carried out by a complex metabolic network which has yet to be fully characterized. Our laboratory has shown that Arabidopsis lines over-expressing enzymes that participate in the myo-inositol pathway accumulate 2-3 times more AsA, and are tolerant to multiple abiotic stresses such as salt, cold, and heat compared to wild type controls. Our studies have also shown a positive influence of elevated AsA on the growth of both aerial and underground tissues. In Arabidopsis AsA is known to peak in young tissues and decrease throughout development. Multiple studies also indicate that the size of the AsA pool depends on light intensity. With the goal of gaining a deeper understanding of the basal steady state of AsA levels and the metabolism of this key molecule, in this work we studied ontogenetic changes in AsA in selected rice varieties. These cultivars were selected based on agronomical and biotechnological characteristics as well as differences in regeneration potential. Rice seeds were planted in soil and grown under controlled conditions. Leaf samples were collected at specific developmental stages and quickly frozen and stored at −80°C for subsequent analysis. Ascorbate was extracted using meta-phosphoric acid and total, reduced, and oxidized AsA pools were measured using a spectrophotometric-based method. Our results indicate that metabolism of vitamin C in rice follows a very different pattern from that seen in Arabidopsis, tomato, and members of the Nicotiana and Ipomoea genera. In rice, AsA peaks at two developmental stages: early during vegetative growth (V2) and at the beginning of the reproductive phase (R4). Our findings also indicate significant variation on AsA foliar levels among accessions. These studies are paving the way to identifying varieties with naturally high-AsA levels that may be used in the future as breeding materials to generate rice germplasm that is able to prosper under stressful conditions.

P-2030

In Vitro Inhibition of Growth of Mycelium of Aspergillus flavus. LIZA CHEN, Ying-Tung Lin, and Yuan Lin. Chinese Association of Human Evolution. No. 7-1, Shunsing Village, Shuulin Township, Yunlin County, TAIWAN. Email: lhchen2001@hotmail.com

Aspergillus flavus is a fungus. It grows by producing thread like branching filaments known as hyphae. A network of hyphae known as the mycelium secretes enzymes that break down complex food sources. The resulted small molecules are absorbed by the mycelium to fuel additional fungal growth. Aspergillus flavus can be pathogenic on several plant and animal species, including humans and domestic animals. The fungus can infect seeds of corn, peanuts,
cotton, and nut trees. Growth of fungus on food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound, which can cause acute hepatitis, immunosuppression, and hepatocellular carcinoma. Aspergillus flavus is favored by hot dry conditions. The optimum temperature for growth is 37°C, but fungus readily grows between the temperature of 25–42°C. Such a high temperature is optimum contributions to its pathogenic on humans. Fungus was obtained from spoiled peanuts and cultured in Petri dished filled with PDA medium. The purpose of this study is to compare the growth of mycelium under different treatments. The treatments included (1) energy in homeostasis, (2) hot, and (3) cold effects for three minutes and no treatment as control. All samples were incubated at 27°C and the cross section diameter of growth of mycelium was first measured twelve hours after treatment and subsequent diameter measurement was done at three to four hours interval. The test results showed that the diameter of mycelium treated by energy in homeostasis was lower than the other two treatments and the control. The hot treatment stimulated the growth of mycelium because Aspergillus flavus favored hot dry conditions and the cold treatment inhibited the growth to some degree as compared to the control.

P-2031

A High Efficient System for Micropropagation of Large-scale and High-quality Miscanthus × giganteus Plants. SEONHWAS KIM, Kedong Da, and Chuansheng Mei. Institute for Sustainable and Renewable Resources, Institute for Advanced Learning and Research, Danville, VA 24540. Email: seonhwa.kim@ialr.org

Miscanthus × giganteus is considered one of the most promising biofuel and bioenergy crops and has been grown in Europe as a biofuel and bioenergy crop for more than a decade. It is a tall, warm-season perennial grass, and is characterized as a low input and low maintenance plant with a high yield, little or no susceptibility to pests and diseases, and low moisture and low ash contents at harvest. In comparison with switchgrass, it was reported that M. × giganteus produced 2 times more biomass than switchgrass (Heaton et al. 2004, Biomass Bioenergy 27: 21–30). However, it is a sterile triploid (3N=57) generated from the hybridization of the diploid M. sinensis (2N=38) with the tetraploid M. sacchariflorus (4N=76) and cannot produce viable seeds. Plants can only be propagated from rhizomes or through tissue culture-based micropropagation. The cost for producing plants from rhizomes is expensive and low throughput, yielding low numbers of plants. Hence, more practical and high throughput protocols are needed to provide the large number of plants for the large-scale plantings required to make this a viable energy crop in the US. We initially tried to use young leaves and apical meristem tissues as explants for callus induction, but these failed due to excessive tissue release of phenolic compounds into medium, which caused browning. We then tried to obtain aseptic plants from tiller shoots in pots, but this did not succeed due to endogenous microorganisms, resulting in contamination in vitro. According to previous reports, the immature inflorescence is the best explant for callus induction (Holme and Petersen 1996, Plant Cell Tissue and Organ Culture 45: 43–52; Lewandowski 1997 In: Biotechnology in Agriculture and Forest, ed. by Bajaj YPS, Springer-Verlag Berlin Heidelberg. Vol. 39: pp240-255). We obtained immature inflorescences of M. × giganteus from field-grown material and induced calli and propagated and selected embryogenic calli in either solid medium or liquid medium. Then we obtained high plantlet regeneration from the embryogenic calli. More important is we achieved shoot multiplication rate average to 12 from one single shoot in one month, which is twice of that reported before. We have modified various media from callus induction, callus multiplication, plantlet regeneration, shoot multiplication, shoot quality improvement and rooting, and greatly improved plant survival in the greenhouse and in the field. We have developed a system for high-quality and large-scale micropropagation of M. × giganteus and could theoretically produce more than 700 billion plants from one single shoot in one year.

P-2032

Interactions Between Genotype, Explants and Media Composition for Tissue Culture Response of Elephant Grass (Pennisetum purpureum Schum.). FABIO G. FALEIRO1,2 and Fredy Altpeter1. 1Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611 and 2future address: Embrapa Cerrados, P.O.Box 08223, 73310-970 Planaltina, DF, BRAZIL. Email: altpeter@ufl.edu

Elephant grass (Pennisetum purpureum Schum.) has been introduced to all tropical and subtropical areas of the world because of its ability to produce large amounts of forage biomass, with very good forage quality. The feasibility of producing ethanol from cellulosic crops such as elephant grass has greatly improved in recent times. Elephant grass is considered the best adapted perennial feedstock for biofuel production in the southern US. Transgenic modifications of cell wall composition of bioenergy or forage grasses or expression of cell wall degrading enzymes is a promising approach to reduce pretreatment costs for ethanol production or enhance digestibility of the forage. However, a genetic transformation protocol is currently lacking for elephant grass. The first step to enable biotechnological
approaches is the development of an efficient plant regeneration protocol from tissue culture. Among the most important factors influencing tissue culture response are genotype, explant and media composition. In this study, fifteen elephant grass genotypes, two explants (immature leaves and young inflorescences) and two auxin types (CPA and 2,4D) were compared in a completely randomized factorial design with 5 replications. Each replication was represented by two petridishes with six explants each. The callus induction, necrosis, quantity and quality of embryogenic callus were evaluated during the first 60 d after culture initiation. The means were compared using the Tukey test at 1% level of probability. Significant effects of genotype, explants, auxin type and particularly interactions among these factors were observed for all characteristics evaluated. The results of this study supported induction of elephant grass calli with superior plant regeneration frequency and is currently providing the basis for development of a genetic transformation protocol for elephant grass.

P-2033

Cryopreservation of Walnut (Juglans regia L.) Embryogenic Cultures. Dave Ellis1, Margarita Hernandez-Ellis1, Chuck Leslie2, and MARIA JENDEREK1. 1USDA-ARS National Center for Genetic Resources Preservation, Fort Collins, CO and 2University of California – Davis, Department of Pomology, Davis, CA. Email: maria.jenderek@ars.usda.gov

California walnuts (Juglans regia L.) make up 99% of the U.S. and 2/3 of the global walnut production. While only four cultivars make up 75% of the California production, new cultivars are continually being released, indicating a strong interest in breeding programs. To support the breeding programs, the availability of diverse material is key, as is the secure back-up of the genetic resources to ensure long-term germplasm availability. Our research focuses on developing reliable methods for the long-term back-up of Juglans spp. We report a successful cryopreservation of three walnut embryogenic lines as a method for long-term preservation of walnut cultivars. Briefly 2–3 wk-old embryogenic cultures grown on solid medium (DKW hormone free) were cold acclimated (20°C for 8 h/−1°C for 16 h) for 7–12 d prior to placement on preculture medium (DKW+DMSO+proline) for 48 h. The embryogenic material was then chopped finely by hand and encapsulated in alginate beads followed by a passage through a sucrose gradient (0.25M, 0.625M and 1M), 8–10 h each. The beads containing the embryogenic cell masses were then air dried in a laminar flow hood to ~30% moisture content. The viability (% beads with cell proliferation after 2 mo) was 80% regardless of whether the alginate beads were exposed to the sucrose gradient in either solid or liquid medium (DKW, sucrose with and without agar). Growth of the embryogenic material following cryopreservation was slower than of the pre-cryopreserved material (all steps to desiccation to 30% moisture content). The initial success in survival of the cryopreserved embryogenic material suggests that this method may be applicable to preserving other walnut embryogenic lines.

P-2034

In Vitro Propagation and Cryopreservation of an Endemic Species of Turkey, “T. cариensis Hub.-Mor. & Jalas”. ERGUN KAYA, Emrah Kirdok, and Elif Aylin Ozudogru. Gebze Institute of Technology, Faculty of Science, Department of Molecular Biology and Genetics, Istanbul cad., no: 101, 41400 Gebze, (Kocaeli), TURKEY. Email: kayaer19@gmail.com, e.kaya@gyte.edu.tr

Among the aromatic plants belonging to Lamiaceae family, the genus Thymus is remarkable for the numerous species and varieties of wild-growing plants, many of which are typical for the Mediterranean area. In Turkey, Thymus is represented by 38 species, with a 53% of endemism ratio. Present study investigated in vitro propagation and cryopreservation of Thymus cариensis Hub.-Mor. & Jalas, an endemic species from western Anatolia. 1–1.5 cm long shoot tips, excised from mature plants of T. cариensis, in Mugla, were decontaminated and used to initiate in vitro shoot cultures Semi-solid MS medium, supplemented with 1 mg l−1 kinetin and 0.3 mg l−1 GA3, provided 95.7% of regeneration, with 1.27 shoots produced per explant. Shoot rooting was obtained on semi-solid MS medium, supplemented with 0.05 mg l−1 2,4-D. Indeed, this medium formulation enabled 100% rooting, each microshoot having 11.95 adventitious roots, on average. Rooted plantlets were transferred to 250-ml plastic pots and acclimatized successfully by gradually reducing the relative humidity. As for cryopreservation, following 2-wk cold hardening (at 4°C, in darkness) of in vitro shoot cultures and 48-h sucrose preculture (on semi-solid MS medium, containing 0.25M sucrose) of excised shoot tips, vitrification-based one-step freezing approach, ‘droplet-freezing’, was applied. Accordingly, shoot tips were placed into 4–5 μl PVS2 drops on sterile aluminium foil strips (~5×15 mm) resting in a Petri dish on ice block (3 drops per aluminium foil), and treated with the solution for 15, 30, 45, 60, 75, 90, 105 or 120 min. Aluminium foils were then transferred into cryovials and directly plunged into liquid nitrogen (LN). Thawing was done at room temperature by removing the aluminium foils from LN and immersing them in washing solution (i.e., liquid MS medium, containing 1.2M sucrose). When the explants were...
totally melted, they were transferred on above-mentioned regeneration medium and incubated in standard culture conditions for recovery. Using this approach, 20% viability and 18.3% recovery of T. cariensis shoot tips was obtained following 90-min PVS2 treatment. To our knowledge, this is the first report where in vitro propagation and cryopreservation of T. cariensis is succeeded.

P-2035

In Vitro Propagation of Turkish Olive Cultivar “Edremit yaglık” via Temporary Immersion Bioreactor Systems. ERGUN KAYA, Hulya Akdemir, Elif Aylin Ozudogru, and Yelda Ozden. Gebze Institute of Technology, Department of Molecular Biology and Genetics, Istanbul cad., no: 101, 41400 Gebze, (Kocaeli), TURKEY. Email: kayaer19@gmail.com, e.kaya@gyte.edu.tr

Olive is an economic and social resource for many Mediterranean countries, such as Turkey. Micropropagation in olive has not replaced the traditional propagation systems (cutting and grafting) yet, due to many difficulties, which inturn increase cost of the technique, as well. In fact, micropropagation can be applied for mass scale production and may represent an effective alternative to the traditional techniques for the olive cultivars that show easy- and medium-adaptation to in vitro conditions. However, further investigations are needed to optimize the technique and adapt it according to specific requirements of different cultivars. In the present study, effects of different carbon sources (sucrose, mannitol and glucose) and plant growth regulators (zeatin and dikegulac) on in vitro proliferation of nodal buds, obtained from seedlings of Turkish olive cultivar “Edremit yaglık”, were investigated by using both semi-solid medium and temporary immersion bioreactor system (TIS). The results showed that inclusion of mannitol to both semi-solid and liquid OM medium as carbon source was essential for micropropagation of nodal explants. When semi-solid OM medium was used, only elongation of single shoots was observed due to the presence of strong apical dominance. However, our results showed that it was possible to break this dominance in TIS system. Moreover, inclusion of dikegulak to zeatin-containing liquid medium was also showed to induce multiple shoot formation in TIS system. In conclusion, use of liquid OM medium supplemented with 10 μM zeatin and 66 μM dikegulac, in TIS bioreactor system resulted with the highest multiple shoot formation (3.2 shoots/explant) in nodal explants of olive.

P-2036

Addressing Trade-offs in Engineering Disease Resistance Using NPR1. RAJINI KANTH MOHAN1, Steven Spoel2, Yasuomi Tada3, and Xinnian Dong1. 1Dept of Biology, Duke University, Durham, NC 27708; 2University of Edinburgh, Institute of Molecular Plant Sciences, Mayfield Rd, Edinburgh, EH9 3JR, UNITED KINGDOM; and 3Institute of Research Promotion, Kagawa University, 2393 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0795, JAPAN. Email: mr90@duke.edu

The transcription cofactor, NPR1, is a master-regulator of plant defense responses and has been tapped as a candidate to engineer broad-spectrum disease resistance in plants. NPR1 overexpression confers heightened resistance to a wide range of diseases in a variety of crops. However, the increased resistance is accompanied by trade-offs, such as conditional lesioning in low light-grown rice plants. We investigated the basis of these trade-offs using Arabidopsis as a model. Plants overexpressing NPR1 in short day photoperiod displayed severe stunting and lesioning, which was rescued upon transfer to longer daylength. This phenotype was a result of constitutive salicylic acid accumulation and biologically depleting salicylic acid eliminated the phenotype. The phenotype was only observed when NPR1 was over-expressed in the npr1-1 mutant background, and not in wild type or other npr1 mutant backgrounds, suggesting a dominant negative effect of npr1-1 mutant protein. Indeed, overexpressing npr1-1 mutation in wild type background rendered plants hypersensitive to growth on media containing salicylic acid, confirming the dominant negative nature of the mutant protein. Plants overexpressing cysteine mutations of NPR1, C82A and C156A do not show accumulation of npr1-1 mutant protein in short day, do not have elevated levels of salicylic acid, and show normal growth in short day. Based on these results, we discuss how the interactions between the engineered protein and endogenous native protein can potentially influence the outcome of a genetically engineered crop.

P-2037

DNA Fingerprinting of Terminalia arjuna and Its Conservation Through Tissue Culture. AZMEERA SEETARAM NAIRK. Department of Botany, Kakatiya University, Warangal −506 009, Andhra Pradesh, INDIA. Email: asmaik9@gmail.com

Terminalia species belonging to Combretaceae family, are ever green forest trees growing in sub-tropical parts of India. Terminalia arjuna is one of the important tree with multiple uses. Besides yielding timber, the tree is primary food plant of tropical Tasar silk worms (Antheraea mylitta). Most of tribals derive their livelihood by collecting Tasar silk worm cocoons, since time immemorial. They are unable to apply scientific approach for selection of elite
trees. There is an urgent need for identification of such trees with higher nutritional value for silk worm growth to produce quality silk by using molecular markers. In this study we have selected *Terminalia arjuna* biotypes from different forest areas of Andhra Pradesh and Jarkhand and isolated DNA from leaves, generated Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) polymorphism. We have correlated the polymorphism with quality traits and identified elite trees. We have used tissue culture technique to micropropagate the selected tree of *T.arjuna* and transplanted them in vivo conditions. The results of the experiments will be presented.

**P-2038**

Genotype, Media and Temperature Influence Regeneration of Tomato Cultivars from Anthers. Bal K. Joshi, Darren H. Touchell, DILIP R. PANTHEE, and Thomas G. Ranney. Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, North Carolina State University, Mills River, NC 28759. Email: Dilip_panthee@ncsu.edu, Darren_touchell@ncsu.edu

Double haploid production reduces the development time of seed propagated crops as complete homozygosity can be achieved in a single generation. Anther culture, one of the methods for double haploid production, was initiated with the objectives of assessing responses of tomato genotypes for callogenesis and organogenesis, determining callusing media and effect of cold treatment (4°C for 4 d) of anthers on callusing. Two experiments, differing in media composition were conducted with a factorial combination of six genotypes and two temperature pretreatment of anthers with six replications arranged in a completely randomized design. All anthers of two flower buds with 4.5 to 5.9 mm length of each genotype were cultured and incubated at 25°C in dark for a month and then under a 16/8 hrs photoperiod. Genotypes were significantly different in number of callus or embryos per anther; however, this was found to be dependent on the media composition. Both pathways i.e. direct embryogenesis and callogenesis were observed in anther culture of some cultivars. Three genotypes NC 1 CS, NC 2 rin EC and NC 30P were found more responsive for callus induction and green shoot development. Genotype with male sterile gene e.g. NC 30P produced callus more effectively. Genotypes of cultivated tomato responded better than wild species. All regenerated plants were diploid. Plantlets developed from the callus frequently undergo spontaneous chromosome doubling, therefore it is unclear if callus-derived plantlets originated from microspore or sporophytic tissue. Molecular markers could be of greatly helpful to determine the origin of these plants.

**P-2039**

Functional Characterization of a Novel *Arabidopsis* Jacalin-lectin Gene and the Potential Use of Its Promoter in Driving Root-specific Transgene Expression. S. REIGHARD, Z. Li, Q. Hu, L. Wang, and H. Luo. Clemson University, Department of Genetics and Biochemistry, 110 Biosystems Research Complex, Clemson, SC. Email: shaner@clemson.edu

Carbohydrate-binding proteins, called lectins, are involved in a diverse array of biochemical pathways including biotic stress response, cell recognition, and hormonal control. A novel jacalin-lectin gene from *Arabidopsis thaliana* was studied to elucidate its tissue-specific expression and regulation under abiotic stress (salt and drought). RT-PCR analysis of the gene expression pattern indicates that this gene exhibits strong root-specific expression with undetectable expression in other plant tissues. A promoter-reporter plasmid construct was synthesized by inserting the jacalin-lectin promoter (~2.9 kb) region between the GFP and GUS reporter sequences. GUS expression would be indicative of jacalin-lectin expression while GFP expression would provide evidence for the expression of an uncharacterized hypothetical protein that is adjacent to the jacalin-lectin gene. Transgenic *Arabidopsis* lines were generated via *Agrobacterium*-mediated transformation, and the transgenic tissues (roots, leaves, and flowers) were assayed for GUS expression. Transgenic plants overexpressing the gene will be analyzed for their performance under drought and salt stress in comparison to wild-type controls and knock-out mutants. Biochemical and physiological characterization of transgenic plants will shed light on the possible role of this novel root-specific jacalin-lectin gene in plant response to environmental stress. Our research will provide insight on this gene’s function, tissue-specific expression, and the promoter region’s suitability for future biotech applications.

**P-2040**

Hypocotyl Derived In Vitro Regeneration of Pumpkin Ash (*Fraxinus profunda*). M. STEVENS¹ and Paula M. Pijut².
Pumpkin ash (Fraxinus profunda), along with other members of its genus, is at risk for extirpation by the exotic insect, emerald ash borer (EAB). The range of pumpkin ash is limited to wetland areas of the Eastern United States and it is listed as endangered in two states and threatened in another. Pumpkin ash provides many benefits to the ecosystem, such as providing food for a variety of wildlife, and its wood is commonly used for tool handles and the manufacturing industry. In vitro regeneration provides an integral tool for the mass propagation and genetic transformation of desired plant material to combat this threat. Though much work has been done developing regeneration systems for green (F. pennsylvanica), black (F. nigra), and white ash (F. americana), an in vitro protocol for pumpkin ash has yet to be determined. The objective of this research was the development of a successful in vitro regeneration protocol for pumpkin ash. This protocol would provide a means for genetic transformation for EAB resistance and mass propagation for conservation. Aseptically extracted hypocotyls successfully formed adventitious shoots following four weeks on Murashige and Skoog (MS) medium supplemented with 13.3 μM 6-benzylaminopurine (BA) and 4.5 μM thidiazuron (TDZ) then transferred for another four weeks on MS medium with Gamborg B5 vitamins (MSB5) containing 6.7 μM BA, 1 μM indole-3-butyric acid (IBA), and 0.29 μM gibberellic acid (GA3). As adventitious shoots developed, they were transferred to a MSB5 medium with 13.3 μM BA, 1 μM IBA, and 0.29 μM GA3 for shoot elongation. Elongated shoots were successfully micropropagated using MSB5 medium with 13.3 μM BA, 1 μM IBA, and 0.29 μM GA3. Formal rooting trials are ongoing, but preliminary evidence would suggest that pumpkin ash is very amenable to adventitious root formation.

P-2041

Optimizing Agrobacterium-mediated Gene Transfer to Sugarcane. QIANCHUN ZENG, Jae Yoon Kim, Maria Gallo, and Fredy Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611. Email: altpeter@ufl.edu

Sugarcane (Saccharum sp. hybrids) is a highly productive C4 grass used as the main source of sugar and more recently to produce biofuel. Transgenic sugarcane with improved agronomic and value added traits has been reported. Ongoing biotechnology research is expected to lead to commercial release of transgenic sugarcane and may include its development into a biofactory for high value products. Agrobacterium mediated transformation results in simple transgene integration which likely will enhance the performance of the transgenic plants. A reproducible Agrobacterium-mediated transformation protocol for generating transgenic sugarcane with simple transgene integration patterns has been established in our laboratory as demonstrated by Southern blot analysis. This protocol employs immature leaf derived embryogenic callus as target for the Agrobacterium mediated gene transfer. Current research evaluating alternative regeneration pathways and inoculation procedures to accelerate Agrobacterium-mediated sugarcane transformation will also be presented.

P-2042

Ascorbate Regulation in Arabidopsis Jasmonate, Abscisic Acid and Ethylene Mutants. J. A. RADIN1,2, W. Suza1, F. Goggin1, and A. Lorence1,2. 1Arkansas Biosciences Institute at Arkansas State University, Jonesboro, AR; 2Department of Chemistry and Physics, Arkansas State University, P.O. Box 639, State University, AR; and 3Department of Entomology, University of Arkansas, Fayetteville, AR. Email: jonnysemail@hotmail.com

Vitamin C (ascorbate, AsA) is the most abundant water-soluble antioxidant in plants. Ascorbate is critical for plant health, as evidenced by the fact that no plant completely devoid of AsA has ever been described. Despite its importance, factors regulating AsA metabolism are not fully understood. Plant defenses are dependent on hormones such as abscisic acid (ABA), which has been shown to improve adaption to environmental stresses such as cold, drought, and wounding, and also jasmonates (JA), which promote accumulation of reactive oxygen species (ROS). We have previously shown that wounding and jasmonates (JAs) influence AsA accumulation in Arabidopsis and tomato (Suza et al., 2010, Plant Physiology and Biochemistry 48: 337–350). In both species, certain mutations that impair JA metabolism and signaling influence foliar AsA levels, suggesting JAs involvement in regulating steady-state AsA levels. However, the impact of wounding on AsA accumulation was similar in JA mutants and wild type controls indicating that other signals are involved in regulating AsA. In order to gain a deeper understanding of other possible AsA regulators, we have compared baseline AsA levels in ABA and ethylene mutants and have performed experiments to test the impact of mechanical
wounding on AsA content in ABA mutants. Our preliminary results indicate that AsA content is higher in the Arabidopsis
abi3-1 mutant compared to wild type following wounding, suggesting that ABA regulates AsA metabolism in response
to wounding. Additional data from wounding experiments
with other hormone mutants including ethylene will be
discussed in this presentation.

P-2043

Somatic Embryogenesis in Icelandic Poppy. K. DA and B. S. Flinn. Institute for Sustainable and Renewable Resources
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Somatic embryogenesis (SE) is a powerful tool for clonal
propagation that has opened avenues for the deployment of
superior planting stock for ornamentals. Although the SE
process can be used with some plant species with high
efficiency, there remain many, including Icelandic poppy
(Papaver nudicaule), which require more research for
protocol establishment. Icelandic poppy “Temptress” is
regarded as the best source of cut and pot flowers from
the ornamental poppy family. Problems exist in “Temptress” production including mixed flower color and low seed
germination rates, phenomena associated with a traditional
seed production system. In an attempt to establish “Temptress” stock plant production through cell suspension
cultures as well as a system allowing an evaluation of the
secondary metabolites, we established a highly efficient
Icelandic poppy “Temptress” somatic embryogenesis sys-
tem for three color types, which include pink, white, and
orange. The embryos germinate with high efficiency and
develop into normal plantlets in germination medium. More
than 800 embryos can be induced from 1 ml of a 20%
suspension in a 3-inch Petri plate. Potted plants showed
flower colors and overall phenotype conservation with
original donor plants.

P-2044

The Effect of Explant Node Position on the Amount and
Type of Bacterial Contamination in Hazelnut Shoot Cultures. CHARLES P. HAND1 and Barbara M. Reed2.
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New hazelnut (Corylus avellana L.) cultivars resistant to
eastern filbert blight are in demand and micropropagation is
used to rapidly increase plant availability. Hazelnut trees
contain many endogenous microorganisms, making it
difficult to initiate axenic cultures. This study was designed
to determine the effect of explant location on bacterial
contamination of cultures and what types of bacteria are
present as contaminants. Plants of three genotypes were
grown in the greenhouse to reduce external contaminants
and explants were taken from new growth in late spring.
Single-node explants were collected from each of five
branches, from the first node below the apical meristem to
the sixth node. Standard surface sterilization techniques
were used. The explants were placed in pH 6.9 half-
strength liquid Murashige and Skoog (1962) medium for
one week, followed streaking on nutrient agar (NA) at each
transfer to detect bacteria. Bacterial contamination in-
creased with distance from the apex. After 6 mo the node
one had 85% bacteria-free explants and node two 68%, but
<33% of explants from the fourth to sixth nodes were
axenic. About half of all the explants were free of bacteria
and most were from the first two nodes. Pure bacterial
cultures were isolated from the explants and identified
through 16 S ribosomal DNA sequences. Many of the same
bacteria were found in each of the three genotypes. The best
procedure for collecting axenic hazelnut explants is to
collect from the first three nodes of fast-growing protected
source plants and use indexing techniques to identify
bacteria contaminated plant cultures.

P-2045

Initiation of Somatic Embryogenesis from Immature
Zygotic Embryos of Pinus oocarpa. ALEJANDRA
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Our aim was to establish somatic embryogenesis protocols
for the tropical pine species P. oocarpa using immature
zygotic embryo explants. A novel culture medium (PO),
based on megagametophyte mineral content was tested in
combination with different plant growth regulator concen-
trations and compared with standard P. taeda media for the
initiation of somatic embryogenesis from immature zygotic
embryos of P. oocarpa. Immature megagametophytes
containing immature zygotic embryos of two mother trees
were used with 11% and 7% extrusion rates for mother tree
genotypes 3 and 5, respectively. In both mother trees the
percentage of capture was 2%. Multiplication of two captured cell lines was improved by lowering the concentrations of plant growth regulators. Mature somatic embryos formed on 40 μM ABA, 6% (w/v) maltose, 12% (w/v) PEG 8000 and 0.6% (w/v) Phytagel. Germination was preceded by partial desiccation for a period of two to three weeks before embryo transfer to germination medium. Germination was observed after seven days under low light, and apical primordia slowly expanded after transfer to ex vitro conditions. To our knowledge, this is the first report on the production of somatic seedlings in *P. oocarpa*.

**P-2046**

Micropropagation and In Vitro Chromosome Doubling of Norway Maple (*Acer platanoides* L.). J. D. LATTIER, D. H. Touchell, and T. G. Ranney. Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, North Carolina State University, 455 Research Drive, Mills River, NC 28759-3423. Email: jdlattier@gmail.com

Norway maple is a valuable landscape tree known for its attractive foliage and architecture. Its pest and disease resistance, and tolerance of poor soils also make it a popular choice as a municipal street tree. However, Norway Maple has become invasive the North East and Mid West United States. Inducing polyploidy may improve ornamental features of Norway Maple while providing a platform for breeding new triploid, seedless cultivars. In this study, protocols were developed for in vitro multiplication and rooting, as well as induction of autopolyploids in the fastigate cultivar ‘Crimson Sentry’. Multiplication was optimized by culturing explants (10–15 mm in length) on combinations of MS, WPM, and QL basal salts supplemented with 2 μM BAP, mT, 2iP, Kin, or TDZ arranged in a factorial design. The combination MS and 2 μM BAP produced the highest shoot regeneration (3.2 shoots per explants), longest shoots (30 mm), and highest multiplication rate (4-fold over 6 wk). A further study was conducted to optimize cytokinin concentrations. Explants were cultured on MS basal salts with varying amounts of BAP (0, 2, 4, 8, 16 μM). Regression analysis showed MS and 2 μM BAP again produced the highest multiplication rate with a 4-fold increase. For the rooting trial, explants were cultured on half-strength WPM supplemented with 0, 5, 10, 20, 40, or 80 μM IBA. Regression analysis showed half strength WPM combined with 40 μM IBA produced the highest rooting percentage (70%), highest number of roots (2.5) and longest roots (15 mm) per explant. Futher, explants containing two nodes were subcultured in liquid MS media containing varying concentrations of BAP (0 or 2 μM BAP) and the dinitroaniline herbicide oryzalin (0, 15, 30, or 45 μM Oz) for a duration of 3, 5, or 7 d to induce polyploidy. Both oryzalin concentration and length of exposure influenced survival and induction of polyploidy. The greatest percentage (33%) of polyploids was induced after 3 d on MS supplemented with 0 μM BAP and 15 μM Oz. The combination of BAP with oryzalin significantly reduced explant survival and inhibited the induction of polyploidy.

**P-2047**

In Vitro Propagation of Persimmon (*Diospyros virginiana* L.). KAITLIN J. PALLA¹, Rochelle R. Beasley¹, and Paula M. Pijut². ¹Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907 and ²USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907. Email: kpalla@purdue.edu, ppijut@purdue.edu

The hard, strong, very close-grained wood of common persimmon (*Diospyros virginiana* L.; Ebenaceae) is desirable for specialty products such as golf-club heads, percussion sticks, billiard cues, and for wood turnery. The edible fruit of cultivated varieties is sold as pulp for use in puddings, cookies, cakes, and custards. Persimmon is usually propagated by grafting. Own-rooted clonal persimmon could offer several advantages to specialty fruit growers, such as elimination of grafting, graft-incompatibility issues, and improved rootstocks for variety testing. Four, mature grafted (male and female) persimmon genotypes and one hybrid were used for nodal explant culture. Single-bud nodal stem explants were cultured on Murashige and Skoog medium containing 10 μM zeatin, 3% sucrose, and 0.7% Difco-Bacto agar. Explants were routinely transferred to fresh medium every 3 wk until shoot cultures were established. One-hundred percent of nodal explants excised from the grafted greenhouse plants produced at least one shoot. For in vitro rooting, half-strength Murashige and Skoog medium with 0, 5, 10, or 15 μM indole-3-butric acid, 0.1 g L⁻¹ phloroglucinol, 3% sucrose, and 0.7% Difco-Bacto agar were tested with a 10-d dark culture treatment followed by culture in the light. Best rooting was achieved on medium containing 5 μM indole-3-butric acid for the common persimmon genotypes with an average of 3.5±1.1 roots per shoot. Ninety-one percent rooting with 5.3±2.6 roots per shoot was achieved for the hybrid persimmon. Rooted plants were successfully acclimatized to the greenhouse.

**P-2048**

Optimization of *Miscanthus Xgiganteus* In Vitro Regeneration. DINUM PERERA¹, Brian S. Baldwin¹, and Nancy A.
Miscanthus x giganteus (giant Miscanthus; Mxg) is a superior bioenergy crop with the potential to produce liquid fuel from cellulosic biomass. Since Mxg is seed sterile, it can only be propagated vegetatively; often through rhizomes, nodal sections, or by tissue culture. The purpose of this research was to study explant type, callus type and callus induction medium in order to develop an efficient in vitro propagation technique for Mxg. Immature inflorescence explants had the highest callus induction percentage (i.e. 96%) compared to all the other explants (i.e. shoot apex and leaf explants of in vitro and greenhouse grown plants) tested. Shoot-forming callus had the highest regeneration rate. A plant growth regulator (PGR) combination of 13.6 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.44 μM 6-benzylaminopurine (BAP) resulted in highest percentage of shoot-forming and embryogenic calli whereas a medium containing only 2,4-D (4.5-31.7 μM) primarily produced root-forming and non-morphogenic calli. The micro-shoots of Mxg introduced to the rooting medium produced in vitro roots. Rooted plantlets were acclimatized and transferred to the greenhouse. Optimization of culture conditions, including PGR combination and concentration and explant and callus type, resulted in efficient in vitro proliferation of Mxg.

P-2049

Improving Mineral Nutrition of Micropropagated Red Raspberry. SUKALYA POOTHONG1 and Barbara M. Reed2. 1Department of Horticulture, 4017 ALS, Oregon State University, Corvallis, OR 97331 and 2USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521. Email: sukalya_n@hotmail.com; Barbara_Reed@ars.usda.gov

In vitro propagation is important for fast multiplication of a wide range of nursery crops, including red raspberry. The variation in genetic background of the many red raspberry cultivars makes it difficult to successfully use one growth medium for all. Although most cultivars will grow on Murashige and Skoog (1962) medium (MS), some are stunted, or display hyperhydricity, chlorosis, callus, leaf spots, reddening or necrosis. These symptoms indicate suboptimum growth medium or growing conditions resulting in low quality shoot cultures. Mineral nutrition is a crucial component of growth media and the poor growth symptoms are likely caused by suboptimum concentrations of important mineral salts. This study investigated the effect of mineral nutrition using five MS salt solutions in a 5-dimensional experimental design. We tested five red raspberry cultivars with 46 treatments selected from the design sphere of five concentrations each of NH4NO3, KNO3, meso elements (CaCl2, KH2PO4 and MgSO4), minor elements (Zn-Mn-Cu-Co-Mo-B-I), and iron. Shoot cultures were grown for three 3-wk transfers before data was taken. Plant quality, multiplication rate, shoot height, and 10 other parameters were evaluated. Results varied by cultivar for some characteristics, but all cultivars had improved growth or appearance with some treatments compared to MS controls. Specific nutrients were associated with changes in growth characteristics and some resulted in improved plant quality. Future experiments will optimize concentrations of the most influential salt formulations and improved media will be developed for use in red raspberry micropropagation.

P-2050

In Vitro Hormonal Effect of Adenium Latex on Potato, Lycium and Date Palm. C. SUDHERSAN. Biotechnology Department, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, KUWAIT. Email: schellan@kisr.edu.kw

Influence of Adenium obesum latex on in vitro plant growth of potato, Lycium and somatic embryogenesis in date palm was investigated. While working on Adenium obesum (Forssk.) Roem. & Schult. multiplication in vitro, the author noticed continuous callusing at the cut end of 100% explants in the absence of any plant growth regulators in the culture media. This made the author to conclude that Adenium latex has some hormonal effect. This latex was experimented on in vitro plant growth of a native plant of Kuwait Lycium shawii and Solanum tuberosum (potato) belong to the botanical family Solanaceae and somatic embryogenesis on date palm (Phoenix dactylifera) belongs to Palmae. The aqueous solution of Adenium latex was prepared and 0.01 to 10 ml/l was included in the MS culture media. At lower concentrations (0.01-1.0 ml/l) the Adenium latex enhanced rooting and plant growth both in Lycium and Potato. In date palm callus culture, at 1.0 to 5.0 ml/l, it induced somatic embryogenesis, embryo multiplication and enhanced plantlet production. At higher concentrations above 5 ml/l it showed inhibitory effect on all the three plant species. Perhaps this is the first report on the hormonal effect of Adenium latex on these three plant species. Further study related to hormonal effect of Adenium latex on other plant species is ongoing in our laboratory.

P-2051

MicroRNAs and Their Diverse Functions in Plants. GUILING SUN and Baohong Zhang. Department of
Micro RNAs (miRNAs) are a class of newly identified non-protein-coding small RNAs. miRNAs posttranscriptionally regulate the expression of protein-coding genes. To date, a total of 3070 miRNAs were obtained from 43 plant species, which include 10 monocots, 28 dicots, 2 species from Embryophyta, 2 species from Coniferophyta and one species from Chlorophyta. Of them, 1769 miRNAs were obtained from dicots, 887 from monocots and 414 from lower plant species. A majority of plant miRNAs are highly evolutionary conserved from moss to higher flowering plants. miRNAs regulate the expression of many important genes; a majority of these genes are transcriptional factors. miRNAs control many biological and metabolic progress, including plant growth and development, response to environmental stress, signal transduction, protein degradation, and regulate their own biogenesis.

P-2052

Isolation and Characterization of the GmScream Promoter Family from Soybean. N. SEMSANG1,2, C. Nemes1, C. D. Torre1, C. M. Hernandez-Garcia2, and J. J. Finer1. 1Department of Horticulture and Crop Science, OARDC/The Ohio State University, 1680 Madison Ave., Wooster, OH 44691 and 2Biology Department, Faculty of Science, Chiang Mai University, 239 Huay Kaew Rd. Muang District, Chiang Mai 50200, THAILAND. Email: nsemsang@gmail.com

Promoters are one of the key regulatory elements required for precise control of gene expression. The isolation and functional analysis of promoters can provide valuable insight into factors which modulate gene expression and supply useful components for genetic engineering. Recovery of novel promoters which give high expression in plant tissues may have application in situations where high expression levels are required. Here, we present isolation and early characterization of the Glycine max high expression level (GmScream) family of promoters. Using soybean transcriptomic database generated by RNA-Seq technology, the promoters of genes which have high expression were first identified. GmScream promoters fragments (about 1.5 kb) were amplified from soybean genomic DNA and cloned upstream the green fluorescent protein (gfp) gene. The expression level of GFP driven by each GmScream promoter was determined using both transient and stable expression. For transient expression studies, promoter constructs were introduced into lima bean cotyledons via particle bombardment and subsequently transient expression intensity profiles were collected over 100 h using an automated image collection and image analysis system. GmScream promoter constructs were also cloned into a binary vector and introduced into Agrobacterium rhizogenes K599 for production of soybean hairy roots, where GFP expression levels were also evaluated for rapid analysis of gene expression in stably-transformed tissues. These rapid validation methods can be used for quantification of promoter strength. The GmScream family of promoters was also analyzed for the presence of common cis regulatory elements. We hope that this new family of soybean promoters will be useful for both basic research and production of transgenics for crop improvement.

P-2053

Identification of Molecular Markers Linked to X-disease Resistance in Chokecherry (Prunus virginiana). H. WANG1, J. A. Walla2, and W. Dai1. 1Department of Plant Sciences, North Dakota State University, Fargo, ND 58108 and 2Department of Plant Pathology, North Dakota State University, Fargo, ND 58018. Email: wenhao.dai@ndsu.edu

X-disease, caused by phytoplasmas, is one of the destructive diseases in stone fruit trees, causing yield loss and poor fruit quality. So far no effective methods are available to control X-disease. X-disease resistance has been first discovered in Chokecherry (Prunus virginiana, 2n=4x=32), which is a native woody species of North America. To identify molecular markers linked to X-disease resistance, simple sequence repeat (SSR) markers were used to construct genetic linkage maps for chokecherry and to identify markers associated with X-disease resistance in chokecherry. In this research, three segregating populations of chokecherry were developed by crossing one X-disease resistant (CL) with three susceptible chokecherry lines (a, c, and d), of which the progenies were 101, 179, and 82, respectively. A total of 366 pairs of SSR primers, including 258 pairs developed in chokecherry and 108 pairs developed in other Prunus species, were used to screen individuals of all three populations. The software TetraploidMap and JoinMap were both used to construct the genetic maps. Using TetraploidMap, 159, 165, and 98 loci were mapped in 16 linkage groups for each population, with which the average distance between loci of each map was 8.94, 9.31, 11.0 cM, respectively. The total length of the genetic map was 1421, 1537, and 1075 cM, respectively. The maps constructed by JoinMap were composed of 112, 114, and 62 loci in 15, 18, and 11 linkage groups with the total length of 1116, 1228, and 649 cM for each population, respectively. The average distance between loci was 9.96, 10.77, and 10.47 cM, respectively. The linkage maps from TetraploidMap resulted in a different marker order compared to the ones created by JoinMap. One SSR marker segregating with the
X-disease resistance trait, identified by bulked segregant analysis (BSA), was mapped in linkage group 3 and 15 based on population CL x a and CL x d, respectively. The genetic map and the molecular marker identified in this research will benefit the molecular research and marker-assisted selection for both chokecherry and other Prunus species.

P-2054

Functional Characterization of AtPK1, AtPK2 and AtPK3, the Three Root-specific Protein Kinases That Confer Salt Tolerance in Arabidopsis. NING YUAN, Zhigang Li, Shane Reighard, Liangjiang Wang, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: hluo@clemson.edu; ning@clemson.edu

Many abiotic stresses such as drought, salt can limit plant growth and development. When subjected to environmental adversities, root as the first affected tissue plays an important role in plant response to stress. Root-specific genes responsive to salt or drought stress may have crucial functions for plant resistance to these adverse circumstances. Using bioinformatics approaches analyzing Arabidopsis cDNA microarray data, we have identified three novel genes, AtPK1, AtPK2 and AtPK3 encoding putative leucine-rich repeat (LRR) family protein kinases. RT-PCR analysis indicated that AtPK1, AtPK2 and AtPK3 were all specifically expressed in roots, and their expression in plants was either up- or down-regulated by salt stress. Knock-out mutant analysis indicated that suppression of these genes led to decreased or enhanced salt tolerance in the mutant plants, suggesting their importance in plant defense mechanism. Further studies using reporter gene analysis and transgenic approaches to elucidate the expression profile and the functions of these genes as well as the molecular mechanisms of the AtRSPK1, 2, 3-mediated plant abiotic stress resistance will lead to development of novel molecular strategies to genetically engineer crop species for enhanced performance under unfavorable environmental conditions, contributing to agriculture production.

P-2055

Two Novel Root-specific Protein Kinases AtRSPK1 and AtRSPK2 Function in Salt Stress Response in Arabidopsis thaliana. SHUANGRONG YUAN, Zhigang Li, Shane Reighard, Liangjiang Wang, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: hluo@clemson.edu

Abiotic stress is a crucial limiting factor in plant growth and development, of which soil salination resulting from global warming, decreasing rainfall, rising temperature and increasing evapotranspiration rate significantly impacts agriculture production. Increasing evidences suggest that protein kinases play an important role in plant abiotic stress response. Protein kinase modifies other proteins by transferring a phosphate group from ATP and attaches it to hydroxyl group of amino acid. Previous evidences indicate that this kind of reversible protein phosphorylation is in response to environmental stress. For example, ten members of the SnRK2 (Sucrose non-fermenting-1-related protein kinase 2) protein kinase gene family were identified in Arabidopsis and some of them have been found to be activated by abscisic acid, drought, hyperosmotic and salinity stresses. To better understand the role protein kinases play in plant response to environmental stress, we have identified two putative protein kinases, AtRSPK1 (root-specific protein kinase 1) and AtRSPK2 through bioinformatic analysis of cDNA microarray assay data. RT-PCR analysis indicated that both AtRSPK1 and AtRSPK2 were specifically expressed in roots, and their expression in plants was up-regulated by salt stress. The knockout mutants of these two genes are more susceptible to salinity treatment. The involvement of these two genes in plant response to other environmental stresses and the molecular mechanisms of the AtRSPK1- and AtRSPK2-mediated plant abiotic stress resistance are currently being investigated. The results obtained so far point to the great potential of using these two novel kinase genes in crop species for enhanced performance under environmental stress, contributing to agriculture production.

P-2056

Target-align: a Tool for Plant MicroRNA Target Identification. FULIANG XIE1, Peng Xiao2, and Baohong Zhang1.1Department of Biology, East Carolina University, Greenville, NC 27858 and 2Department of Mathematics, East Carolina University, NC 27858. Email: xief@ecu.edu

MicroRNA (miRNA) is a new class of small RNA molecules, which result in translational repression and gene silencing by complementarily or near complementarily binding to their target mRNAs. It has been widely proved that miRNAs are involved in a series of biological regulation during plant development, phase change, stress response and metabolism. Although many miRNAs have been identified, the function of most of miRNAs is still unknown. It is crucial to elucidating miRNA function in identifying potential miRNA targets. However, few reliable tools have been developed for identifying miRNA targets in
plants. We developed a Smith-Waterman-like alignment tool named Target-align to accurately predict miRNA targets. Dynamic programming was used to build a score matrix based on the complementarity of nucleotides in order to trace the optimal local alignments. Important parameters, such as maximum mismatches and maximum consecutive mismatches between miRNAs and their targets, were also used for filtering the optimal local alignments. Almost all of the parameters in this alignment tool can be adjusted by users. Compared to other target prediction tools, Target-align exhibits strong sensitivity and accuracy for identifying miRNA targets. Windows, web, and command-line versions were developed to better serve different users. Target-align is freely available at http://www.leonxie.com/targetAlign.php.

P-2057

Antagonistic Activity of Some Indian Medicinal Plants Against Pathogenic Fungi. CHANDAN KUMAR, Gazala Tabassum and Rashmi Komal. Plant Pathology and Microbiology Laboratory, Department of Botany, Patna University, Patna, Bihar, INDIA. Email: rashmi0911@gmail.com

The indiscriminate use of chemicals for the past many years to control the menace of microbial infection on plants of economic importance, have lead to serious environmental threats for plants and human life around the world. The problems of chemical control have been reviewed amply, leading to a ban of many chemicals. There is always a need for safe and selective chemicals in agriculture. India has a rich history of using plants to combat various problems. The leaf, stem and root leachates of some medicinal plants viz: Adhatoda vesica, Achyranthus aspera, Tagetes erecta and Oxalis Corniculata were prepared. These leaves, stems and roots were dried and weighed. Extracts were obtained by maceration of leaves, stems and roots separately with ethanol solution. The leachates were screened for their antifungal activity, using disc diffusion method. Leaf, stem and root leachates of Tagetes erecta exhibited maximum in vitro antifungal activity against all the three test pathogenic fungi. The results revealed that these fungi were almost insensitive to the leachates of Oxalis corniculata. The leaf leachates were more effective, compared to stem leachates against the test fungi. This indicates that the plants have the potential to generate herbal metabolite. The crude extracts demonstrating antifungal activity could result in the discovery of new chemical classes of antifungal drugs that could act as selective agents for the maintenance of plant and human health and provide biochemical tools for the study of infectious diseases.

P-2058

Cell Line Selection and Media Optimization for Enhanced Azadirachtin Production from In Vitro Cultures of Neem (Azadirachta indica A. Juss.). RAKHI CHATURVEDI and Mithilesh Singh. Department of Biotechnology, Indian Institute of Technology- Guwahati, Guwahati – 781039, Assam, INDIA. Email: rakhichaturvedi@iitg.ernet.in

Azadirachtin (C_{35}H_{44}O_{16}), a tetranortriterpenoid, is the principal secondary metabolite of neem tree (Azadirachta indica A. Juss., family Meliaceae). It has been established as an efficient biopesticide against a wide range of insects and pests. The biotechnological production of azadirachtin in plant cell or organ cultures is an attractive alternative to extraction of whole plant material. However, this approach has been hampered by low yield. To improve azadirachtin production, selection of elite cell lines and optimization of medium components is a necessary prerequisite. For selection of an elite in vitro cell line, calli originated from various explants of neem like, zygotic embryos, leaves, anthers and ovaries, were established and screened on the best culture medium. Of these in vitro cell lines, the highest azadirachtin yield (2.33 mg/g DW) was observed in redifferentiated calli raised from zygotic embryo cultures. Further, Plackett–Burman design with five medium components- MS major salts, sucrose, CH, IAA and BAP- was performed to screen the variables that were significantly affecting the azadirachtin production. The three variables, MS major salts, sucrose and BAP, significantly affected the azadirachtin production and were considered to be significant factors for optimization using response surface methodology. The experimental results were fitted to a second-order polynomial model with correlation coefficient (R^2) of 0.9582. The optimal concentrations of variables for maximum azadirachtin production were full MS major salts; 5.68% sucrose and 10.42 μM BAP. The maximum azadirachtin production by the predicted model was 5.13 mg/g dry weight that was in agreement with the actual experimental value of 4.97 mg/g dry weight.

P-2059

In Vitro Toxicity of Jatropha Curcas Oil Phorbol Esters. R. K. DEVAPPA, J. Roach, H. P. S. Makkar, and Klaus Becker. Institute for Animal Production in the Tropics and
Currently Jatropha curcas is receiving considerable attention as an energy plant. Its seeds contain 25–35% oil which could be converted to high quality biodiesel. The oil is rich in phorbol esters (PEs). In this study, PEs from the oil were purified using column chromatography/LCMS/NMR and their concentrations were expressed equivalent phorbol 12-myristate 13-acetate. The PEs christened as factor C1, C2 and C3 were purified to homogeneity, whereas factor C4 and C5 were obtained as a mixture. These PEs exhibited acute toxicity in snail bioassay (Physa fontinalis) with an LC50 of 0.17, 0.27, 0.28 and 0.09 ppm for factor C1, C2, C3 and (C4+C5) mixture respectively. They also showed moderate toxicity in Artemia salina bioassay with an LC50 of 17.9, 624.9, 44.7 and 1.8 ppm respectively. The (C4+C5) mixture had highest activity in both these bioassays. Further, dermal effects of PEs upon topical application (time of exposure 1 h) were evaluated using reconstituted human epidermis (RHE). The effects were compared with that of 70% ethanol (a commonly used hand sanitizer). At 100 ng equivalent of PEs, both toxic Jatropha oil and PEs-mixture (all 5 factors as present in the oil) exhibited marked oedema, less viable cell layers and/or cellular alterations, partial tissue necrosis and partial tissue disintegration in RHE. Similar effects were observed for purified individual PEs [C1 (52.3 ng), C2 (20.61 ng), C3 (16.5 ng) and C4+C5 (10.4 ng); these are the amounts of individual PEs in 100 ng of a mixture of PEs as present in the oil]. Other toxic effects included increase in cutaneous inflammatory substances such as interleukins (IL-1α) and prostaglandin (PGE2). Application of 70% ethanol (10 μl) on RHE produced 42 pg/ml IL-1α and 4.67 ng/ml PGE2. While IL-1α production by toxic oil; purified PEs-mixture; C1, C2, C3 and (C4+C5) was higher by 2.8; 3.4; 1.8. 2.3, 2.6 and 3.7 folds; and PGE2 production increased by 1.27; 1.66; 1.42, 1.05, 1.41 and 1.55 folds respectively. The histological alterations and production of IL-1α and PGE2 were less evident by the nontoxic oil and these effects were comparable to those by 70% ethanol. Our data suggest that PEs from J. curcas are highly bioactive and their in vitro topical application exhibit inflammatory response and cellular alterations. PEs could be exploited for various agricultural and pharmaceutical applications; and while using preparations containing PEs, use of protective gloves and glasses is advised.

**P-2060**

Doubled Haploid System for Wheat through Wide Crosses with Maize in Korea. YOUNG JIN KIM, Hag SinKim, Chon Sik Kang, Kyeong Hoon Kim, Induck Choi, Young Keun Cheong, Kwang Won Lee, and Kee Jong Kim. Winter Cereal and Forage Crop Research Div., National Institute of Crop Science, RDA, Iksan 570-080, REPUBLIC OF KOREA. Email: yjikim@korea.kr

Doubled haploid system is a biotechnological tool which has been widely applied in wheat breeding programmes. Wide-hybridization, wheat x maize cross, is used for the production of wheat doubled haploids (DH) because of its efficiency. We carried out the experiment for development on effective method of producing haploid in wheat. Emasculated spikelets of wheat are pollinated with maize pollen and sprayed with 100 mg/t 2,4-D, and then incubated for 14 days in 100 mg/t 2,4-D solution containing 40 g/t sucrose and 15 MU/t sulfuric acid. The haploid embryos were rescued and cultured for plant regeneration. Significant genotypic differences in seed set (36–98.4%) were obtained. There was also varied efficiency in embryo formation (4.3-25.3%). Maize genotypes showed a significant influence on plant regeneration of haploid embryos. Regeneration of haploid embryos into plants varied from 3.1%-13.2%. Wheat genotypes also did affect haploid plant production. The correlation between embryo formation frequency and haploid regeneration frequency across maize genotypes was highly significant. Analysis of variance for seed set and embryo formation showed highly significant effects of wheat parents and maize pollinators, whereas their interaction effect was only significant for seed set. The effect of maize genotypes was greater than that of wheat genotypes. The roots of regenerated plants were immersed in colchicine solution at 0.05 and 0.1% supplemented with 2% dimethyl sulphoxide and were incubated for 7 h. The chromosome doubling efficiency varied from 25.2 to 97.6%.

**P-2061**

In Vitro Plant Regeneration, Flowering and Fruiting in Cucumber. C. Sudhersan, Y. AL-SHAYJI, S. Jibi Manuel, and M. Saleem. Biotechnology Department, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, KUWAIT. Email: schellan@kisr.edu.kw

Mature zygotic embryos of cucumber (Cucumis sativus L) cultivar Rawa Sluis isolated from the imbibed sterilized seeds were planted on low strength MS basal medium containing 10 g/l sucrose for germination. Mature zygotic embryos germinated and grew into whole plants within 20 d time. Cotyledon, root tip, epicotyl and hypocotyl segment explants isolated from the in vitro grown seedling planted on MS medium containing 0.1-1.0 mg/l 2,4-D produced somatic embryogenic callus and 0.1–1.0 mg/l BA produced organogenic callus within 30 d after the culture
initiation. Plantlets were regenerated from somatic embryogenic callus and organogenic callus when transferred to hormone free MS culture medium. Plantlets regenerated from organogenesis method and somatic embryogenesis method produced adventitious roots in hormone free MS culture medium. The plantlets produced through organogenesis method produced male and female flower buds within 4 wk while plantlets produced by somatic embryogenesis took longer time to produce flowers in vitro. Nodal segments containing male and female flowers isolated from the plantlets raised from MS medium with 1 mg/l BA cultured separately on hormone-free MS culture medium produced more number of male and female flowers separately. In vitro pollinated female flowers produced mini cucumber with viable seeds and un-pollinated female flowers produced parthenocarpic mini cucumbers. This study will help in transgenic plant production in cucumber.

P-2062

High Throughput Agrobacterium-mediated Switchgrass Transformation. R. Li and R. Qu. Dept. of Crop Science, North Carolina State University, Raleigh, NC 27695-7287. Email: rongda_qu@ncsu.edu

Switchgrass is one of the most important biomass/bioenergy crops. For its improvement as a feedstock through biotechnological approach, we have developed a high throughput Agrobacterium-mediated transformation system for cv. Alamo and two new elite cultivars, Performer and Colony. Highly regenerable and transformation-competent embryogenic calli were identified and used for genetic transformation. GFP reporter gene was employed to identify transformation events at early stages and to guide modifications at various stages for improvement of transformation efficiency. The modifications included infection under vacuum, co-cultivation at desiccation conditions, resting between co-cultivation and selection, and supplement of L-proline in the callus culture and selection media. Transformation efficiency over 90% was routinely achieved for Performer, and around 50% for Alamo and Colony. The new system substantially improved switchgrass transformation efficiency and will significantly contribute to the genetic improvement of this important biofuel feedstock via biotechnological approach.

P-2063

Efficient Transformation of African Upland and Lowland Rice Varieties for Improved Nitrogen Use Efficiency. L. Wu, M. Kamakeeaina, C. Downs, J. Wallace, L. Getchel, R. Bishop, J. Goodstal, H. Voelker, Y. Lu, and J. van Boxtel. Arcadia Biosciences Inc., 202 Cousteau Place, Davis CA 95616. Email: liying.wu@arcadiabio.com, michelle.kamakeeaina@arcadiabio.com

In an international collaborative effort between the African Agricultural Technology Foundation (AATF), the Public International Property Resource for Agriculture (PIPRA), and Arcadia Biosciences, we have been generating transgenic African rice varieties, with the aim of improving Nitrogen Use Efficiency. Upland varieties Nerica-1 and Nerica-4, and lowland variety Nerica-L-19 were efficiently transformed through Agrobacterium-mediated co-transformation. Implemented modifications to the standard protocol for transformation of mature embryos resulted in higher transformation efficiencies for all 3 varieties. Specific controlled growing conditions for plants will be discussed. Molecular characterization of the plants with regard to co-transformation efficiency, presence of the gene, copy number, vector backbone insertion, and out segregation of the selectable marker showed data as expected. Candidate lines will soon be tested under field conditions for improved Nitrogen Use Efficiency.

P-2064

Plant Tissue Cultures of Swertia japonica and Their Chemical Constituents. HIROKO KAWAKAMI, Kojiro Hara, Masashi Komine, and Yoshikazu Yamamoto. Kaidobata-Nishi 241-438, Shimoshinjo-Nakano, Akita City, Akita, 010-0195, Akita Prefectural University, Graduate School of Bioresource Sciences, JAPAN. Email: m12e004@akita-pu.ac.jp

Swertia japonica Makino is a Japanese folk medicine as used to improve the stomach disease and hair-growth problem. Swertiamarin is one of the main constituents of S. japonica and a bitter seco-iridid glucoside. To product their pharmacological compounds in a large-scale culture, we have found the optimal conditions for the induction of S. japonica cultures and analyzed their chemical constituents. First, to investigate effects of various phytohormones, sterilized stems of S. japonica were placed on Murashige Skoog’s medium (MS) containing combination of auxin [2,4-dichlorophenoxyacetic acid (2,4-D), naphthalenacetic acid (NAA) or indolebutyric acid (IBA)] and cytokinin [kinetin (KIN), 6-benzyladenine (BA) or thidiazuron (TDZ)]. The callus induction rate was the highest value (89%) on MS containing NAA and KIN. Next, to investigate effects of various basal media and to decrease the contamination, seedlings of S. japonica were placed on MS, Gamborg’s B5 medium (B5) or Woody Plant medium (WP) containing combination of auxin (NAA) and cytokinin (KIN or TDZ) with 3% sucrose. The callus induction rate was the highest value (80%) on WP containing NAA and TDZ, and we
Transformation of Cowpea (Vigna unguiculata L.) by Agrobacterium tumefaciens carrying cry Genes. M. AASIM, K. M. Khawar, S. F. Ozcan, M. Yildiz, C. Sancak, and S. Ozcan. Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110 Ankara, TURKEY. Email: ozcansebahattin@gmail.com, mshazim@gmail.com

Cowpea (Vigna unguiculata L.) is an important food grain legume crop and consumed in many countries of the under developed world as a staple food in fresh and dry form. The study reports efficient and rapid Agrobacterium tumefaciens mediated genetic transformation of cowpea cultivars using plumular apices as explants. Plumular apices were inoculated for 25 min with Agrobacterium tumefaciens strain LBA4404 harboring insect resistance cry 1AB and NPT-II genes followed by co-cultivation on MS medium containing 1.0 mg/l BA with 0.1 mg/l NAA for 24 h. The putative transgenic plant shoots were selected on selection medium containing 500 mg/l bacteriostatic augmentin and 50 mg/l kanamycin. After 25 d, putative transgenic shoots rooted on MS selection medium containing 0.50 mg/l IBA, 500 mg/L augmentin and 50 mg/l kanamycin. Rooted plantlets were acclimatized and grown in the greenhouse for successful flowering and seed set. The plants and seeds from T1 generation were successfully confirmed by PCR analysis using NPT-II primers. DNA samples from To and T1 were subjected to Southern analysis and bioassay for further confirmation of the results.

P-2067

Genetic Transformation of the Commercially Important Potato Chipping var. Atlantic and Expression of the Snowdrop Lily Lectin. I. S. CURTIS and T. E. Mirkov. Texas AgriLife Research, Weslaco, TX 78596. Email: iscurtis@ag.tamu.edu

Potato (Solanum tuberosum L. subsp. tuberosum), is the most widely grown root crop in the world with China, India, Ukraine and USA being the major producers. Pests and diseases have drastically affected global production. Numerous protocols have been developed to generate transgenic potato plants but these have been largely targeting culinary varieties instead of chipping types, which are of major economical importance to the potato chip industry. We have developed an Agrobacterium-mediated gene transfer system for one of America’s most important potato chipping variety Atlantic, which is susceptible to many diseases in the field. Several factors which can influence transformation efficiency such as explant type, Agrobacterium strain, antibiotic concentration for selecting transgenic plant output. In this study, Agrobacterium strains carrying pBinGUS or pBinGUS with the snowdrop lily lectin (Galanthus nivalis agglutinin, GNA) were used. Leaf discs were more amenable to regenerate transformed shoots (approx. 60%) compared to petioles and stem internodes. A. tumefaciens strain EHA105 (48%) was more efficient in producing transgenic plants compared to LBA4404 (37%). GUS positive

found high redifferentiation of the adventitious root on B5 or WP containing NAA and KIN. Finally, we analyzed the chemical constituents of its cultures by HPLC with a photodiode-array detector. We found that the adventitious root contained the swertiamarin for the first time. We also investigate the chemical constitutions of liquid cultures.

P-2066

Silencing of Ricin Production in Castor (Ricinus communis L.) by Genetic Modification. DANIEL J. BARNES1, Nancy A. Reichert2, and Brian S. Baldwin3. 1Dept. of Mol. Bio., Biochem., Ent., and Plant Path., Mississippi State University, Starkville, MS 39762; 2Dept. of Biology, Mississippi State University, Starkville, MS 39762; and 3Dept. of Plant and Soil Sciences, Mississippi State University, Starkville, MS 39762. Email: djb70@msstate.edu

The castor plant (Ricinus communis L.) bears seed that contain high quantities of a significant chemurgic oil. Unfortunately, the seed also contains a potent toxin, ricin. Castor was freely cultivated in the United States until the 1970’s when price fluctuations and increasing concern over the presence of ricin halted all domestic production. Since then, all of the castor oil used domestically has been imported, and the international price for castor oil can fluctuate by as much as 25% annually. The objective of this project is to silence the genes responsible for the production of the ricin protein in order to produce a ricin-free castor cultivar that can be utilized to revitalize the domestic production of castor oil. Castor is notoriously recalcitrant to many tissue culture techniques so, tissue culture protocols must first be optimized to generate viable tissue for transformation. Next several transformation protocols will be tested to find the most efficient method for introducing foreign DNA into castor tissue. Finally, mass screening must be performed in order to identify individuals in which ricin production has been completely silenced. By removing the concern over ricin content in castor cultivation, it is hoped that domestic castor oil production, potentially a multi-million dollar enterprise, can be restarted.
Co-expressing of Two Barley Dehydrin Genes Confer Tolerance to Chilling and Freezing Stress in Bahiagrass. WALID FOUAD1,2, J. Celedon1, and F. Altpeter1. 1Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611 and 2Current address: Biology Department, Biotechnology Program, The American University in Cairo, New Cairo 11835, EGYPT. Email: altpeter@ufl.edu

Bahiagrass is the predominant pasture grass in the southeastern United States. However, freeze-damage and low forage production represent seasonal limitations for beef cattle producers during the late fall and early spring. Unlike grasses from temperate regions, bahiagrass does not cold acclimate in response to low temperatures. Genetic engineering for improved cold tolerance will complement the traditional breeding efforts and improve the environmental adaptation of bahiagrass. Earlier reports suggested that dehydrin facilitates plant cold acclimation by acting as a radical-scavenging protein to protect membrane systems under cold stress. Two barley dehydrin genes Dhn5 and Dhn8 under transcriptional control of the cold inducible Dhn8 promoter or under constitutive 35S promoter were co-introduced into bahiagrass by biolistic gene transfer. Transgenic lines were evaluated for biomass production and chlorophyll fluorescence during chilling stress and after recovery from chilling and freezing. Transgenic bahiagrass expressing H. vulgare Dhn5 and Dhn8 under transcriptional control of the cold inducible promoter (Dhn8) displayed enhanced biomass production during chilling and recovery from chilling and freezing stress. Molecular and physiological data correlating transgene expression and plant performance under chilling and freezing stress will be presented.

P-2069

Co-expressing of Two Barley Dehydrin Genes Confer Tolerance to Chilling and Freezing Stress in Bahiagrass. WALID FOUAD1,2, J. Celedon1, and F. Altpeter1. 1Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611 and 2Current address: Biology Department, Biotechnology Program, The American University in Cairo, New Cairo 11835, EGYPT. Email: altpeter@ufl.edu

Bahiagrass is the predominant pasture grass in the southeastern United States. However, freeze-damage and low forage production represent seasonal limitations for beef cattle producers during the late fall and early spring. Unlike grasses from temperate regions, bahiagrass does not cold acclimate in response to low temperatures. Genetic engineering for improved cold tolerance will complement the traditional breeding efforts and improve the environmental adaptation of bahiagrass. Earlier reports suggested that dehydrin facilitates plant cold acclimation by acting as a radical-scavenging protein to protect membrane systems under cold stress. Two barley dehydrin genes Dhn5 and Dhn8 under transcriptional control of the cold inducible Dhn8 promoter or under constitutive 35S promoter were co-introduced into bahiagrass by biolistic gene transfer. Transgenic lines were evaluated for biomass production and chlorophyll fluorescence during chilling stress and after recovery from chilling and freezing. Transgenic bahiagrass expressing H. vulgare Dhn5 and Dhn8 under transcriptional control of the cold inducible promoter (Dhn8) displayed enhanced biomass production during chilling and recovery from chilling and freezing stress. Molecular and physiological data correlating transgene expression and plant performance under chilling and freezing stress will be presented.

P-2068

Cre-lox-mediated Site-specific Gene Integration in Maize (Zea mays). J. THOMAS and V. Srivastava. University of Arkansas, 115 Plant Science BLDG, Crop, Soil, and Environmental Science Dept., Fayetteville, AR 72719. Email: junnderwo@uark.edu, vibhas@uark.edu

Cre-lox-mediated DNA integration system generates a precise single-copy integration of foreign DNA cassette. As a result, the incorporated genes express consistently over successive generations in the gene-dosage dependent manner. The objective of this study was to develop a Cre-lox-mediated site-specific integration system for maize (Zea mays L.) using particle bombardment to deliver the foreign gene construct. Emybrogenic calli derived from two separate founder lines (each in Hi-II background) were bombarded with the donor construct containing the GUS gene as the gene-of-interest. The founder lines contain a unique lox site for site-specific integration of the GUS gene via Cre-lox recombination. Cre activity was provided by co-bombardment of cre gene. Site-specific integration of the donor construct is expected to generate resistance to geneticin. Thus, geneticin resistant calli were scored as putative transformants. So far, 3–4 geneticin resistant lines have been scored from each founder line. Molecular analysis of these lines will be conducted to determine the success of site-specific gene integration.

P-2070

The Role of Antioxidant Vitamin E Supplementation In Brassica juncea Plants: Regulation and Function Under Abiotic Stress. DEEPAK KUMAR1,2, Mohd. Aslam Yusuf1, Preeti Singh1, Meryam Sardar2, and Neera Bhalla Sarin1. 1School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA and 2Department of Biosciences, Jamia Millia Islamia, New Delhi-110025, INDIA. Email: deepakinjnu@gmail.com

Tocopherols (Vitamin E) are essential nutrients in mammalian diets and are exclusively synthesized by oxygen evolving phototrophs, including some cyanobacteria and all green algae and plants. They are very potent antioxidants protecting polyunsaturated fatty acids in the membranes from peroxidation and their higher dietary intakes are, thus, implicated in protection against various diseases including cancer. Out of the four types of tocopherols (α, β, γ, and δ), which differ only in the number and position of methyl substituents on the chromanol ring, α-Tocopherol has the highest biological activity, but constitutes only a small fraction of the total tocopherol pool in most of the oil seed crops, the major sources of vitamin E. To increase the human intake of this vitamin we successfully fortified transgenic Brassica juncea plants with α-tocopherol (~6 fold increase as compared to the control plants) by overexpression of γ-tocopherol methyl transferase which catalyzes a rate limiting step in the α-tocopherol biosynthetic pathway.
Further, to better understand the roles of different tocopherol forms in plants, which have not been studied exhaustively, we compared the physiological and photosynthetic performance of α-tocopherol enriched transgenic and untransformed control B. juncea plants under conditions of abiotic stresses. We found that abiotic stress induced by NaCl (salinity), CdCl₂ (heavy metal), and mannitol (drought) resulted in an increase in the total tocopherol levels in both the control and transgenic plants. Comparisons of seed germination, shoot growth, leaf disc senescence and measurement of antioxidant enzymes showed that transgenic B. juncea plants had enhanced tolerance to the induced stress. Analysis of the chlorophyll a fluorescence rise kinetics, from the initial "O" level to the "P" (peak) level, showed that there were differential effects of the applied stress on different sites of the photosynthetic machinery. These effects were found to be alleviated in the transgenic plants. These results provide newer insights into the protective role of α-tocopherol in plants exposed to abiotic stress. The α-tocopherol enriched oilseed crops could, therefore, serve a dual purpose, that of increasing the natural α-tocopherol content in human diets and helping the plants to better cope with the abiotic stress conditions.

P-2071

The Role of Rice Plasma Membrane Intrinsic Proteins in Boron Transport. KAREEM A. MOSA1-2, Kundan Kumar1, Sudesh Chhikara1, and Om Parkash Dhankher1. 1Dept. of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, MA 01003 and 2Dept. of Biotechnology, Faculty of Agriculture, Al-Azhar University, Cairo 11517, EGYPT. Email: kmosa@psis.umass.edu, parkash@psis.umass.edu

Boron, the essential metalloid, is a micronutrient required for plant growth and development. The main functions of boron relate to cell wall strength and development, maintaining membrane function, and supporting metabolic activities. Boron occurs primarily (96%) as boric acid, B(OH)₃, at the near-neutral pH found in most biological fluids. Both boron deficiency and toxicity severely affect the plant development and limit crop production worldwide. It has been reported that members of the Nodulin 26-like Intrinsic Proteins (NIPs) subfamily of aquaporins, NIP5:1 and NIP6:1, act as a channel that facilitate boron transport. Recently, members of the Plasma Membrane Intrinsic Proteins (PIPs) subfamily of aquaporins have also been shown to transport boron in barley. Here, we show that members of rice PIP subfamily are involved in boron transport. RT-PCR analysis of four rice PIP genes (designated as OsAQP9-1, OsAQP9-9, OsAQP9-10, and OsAQP9-16) showed that these genes were differentially regulated under boron deficiency and toxicity. Boron transport activity of rice PIPs were investigated using yeast complementation assays. Expression of rice PIP candidate genes in HD9 yeast strain resulted in an increased boron sensitivity and accumulation. To further characterize the boron transport function of rice PIPs, we overexpressed these four OsPIP genes in Arabidopsis thaliana. The transgenic Arabidopsis plants showed significantly higher tolerance towards boron toxicity. The overexpression lines attained 2- to 3-fold more biomass. Further, these plants accumulated higher levels of boron in roots whereas there was no difference in the level of boron in shoots. These results suggest that rice PIPs may involve in efflux of boron from cytoplasm to apoplastic region of the cell. Further studies for boron efflux and transport of other metalloids are in progress. Taken together; our preliminary data suggests a distinct role of specific rice PIP genes in boron transport in plants. These genes may prove highly beneficial in developing crops for enhanced tolerance to boron and other metalloids.

P-2072

In Vitro Salt Tolerance Selection and Multiplication of Giant Swamp Taro (Cyrtosperma merkusi (Hassk.) Schott) and Soft Taro (Colocasia esculenta (L.) Schott). VIREDRA M. VERMA. Micronesia Plant Propagation Research Center, Agricultural Experiment Station and Cooperative Extension, College of Micronesia-FSM, Kosrae, MICRONESIA. Email: vmv_vmv@hotmail.com

In Vitro techniques are being increasingly applied to supplement conventional methods of vegetative propagation. The benefits include mass multiplication, production of disease free stock and stress tolerant variants, and long term storage of viable germplasm. In vitro techniques not only help in successful multiplication of the elite plantlets for raising tuber crops but also provide a means for the conservation of germplasm in an inexpensive way. Giant swamp taro and soft taro are considered 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 and disease-free elite seedlings are major bottleneck in agricultural production. Therefore, a study was undertaken to develop efficient in vitro protocols for salt tolerance selection and mass multiplication of giant swamp taro (Cyrtosperma merkusi (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 were used for in vitro selection of salt tolerant germplasm. In vitro selected germplasm will be further screened ex vitro for salt tolerance in the greenhouse conditions to study a correlation between in vitro and ex vitro selection methods for taro.

P-2073

Physiological Changes During the Initiation of In Vitro Cultures of Saffron Intercalary Meristems for Micropropagation, Changes in Some Components of the Antioxidant Enzymatic System and the Levels of Endogenous Growth Regulators. A. PIQUERAS\textsuperscript{2}, M. Palacios\textsuperscript{1}, M. J. Clemente\textsuperscript{2}, G. Barba\textsuperscript{2}, H. Cantero\textsuperscript{3}, F. Perez\textsuperscript{3}, J. A. Hernandez\textsuperscript{2} and J. A. Fernandez\textsuperscript{1}. \textsuperscript{1}Biotechnology Dept. IDR (UCLM), Albacete SPAIN, and \textsuperscript{2}Depts. Fruit tree breeding and \textsuperscript{3}Plant Nutrition, CEBAS (CSIC), 30100 Espinardo, Murcia, SPAIN. Email: piqueras@cebas.csic.es

The physiological changes involved in the adaptation of the explants to in vitro conditions and further development of micropropagated cultures could offer practical information on important aspects required for the quality control of the initial stages saffron micropropagation. Saffron is one of the most important spices for which an effective clonal micropropagation protocol has not been fully developed so far. To achieve this objective, our reserach group has carried out preliminary research on the micropropagation of saffron by the culture of intercalary meristems from sprouting shoots. To improve our knowledge of the physiological alterations during stages I and II of the micropropagation process, the activities of several antioxidant enzymes and the levels of plant growth regulators have been studied. The plant material used in this research have been the initial explants (1) (sprouting shoots used as the source of the intercalary meristems), clean explants after two subcultures with activated intercalary meristems (2) and clusters of micropropagated shoots already established and after 18 mo in culture (3). The changes in the antioxidant enzymatic system have been related to both developmental processes and stress situations in vitro. During the initial stage of saffron shoots cultures a significant increase in the activity of catalase (CAT) and superoxide dismutase (SOD) could be observed with a concomitant decrease in the activity of ascorbate peroxidase (APX) and glutathione reductase (GR). These modulations od the antioxidant enzymatic activities reflect the stress imposed to the initial explants by the environmental conditions in vitro to which they became eventually adapted. The successive subcultures complete the multiplication stage and the cultures proliferate and develop new shoots with a reduced stress for the established cultures fully adapted to the in vitro environment. The activity of peroxidase showed a progressive increase through the three stages studied and could indicate the intense meristematic activity of the cultures in multiplication. In relation to the levels of endogenous plant regulation, a progressive decrease in ACC (aminocyclopentane carboxylic acid) the ethylene precursor and ABA (abscisic acid) were observed as the cultures progress to the multiplication stage and could be interpreted as a reduction in the stress in the cultures in coincidence to the previous observations for the antioxidant enzymatic system. The auxin indolacetic acid (IAA) and the cytokinin zeatin (Z) showed a progressive decreased that could be related to the increased concentration of the citokinin benzyladenine incorporated to the culture medium and required to support the multiplication of the proliferating cultures.

P-2074

Optimization of Two-dimensional Gel Electrophoresis for Total Protein of Microstrobilus in Ginkgo biloba L. YUHAN SUN, Yun Li, Nina Yang, Yaru Wang, and Cunquan Yuan. National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, P. R. CHINA. Email: syh831008@163.com, yunli63@163.com (Corresponding Author)

The use of 2n pollen is a kind of time-saving breeding of triploid efficient way (Triploid can be obtained from crossing 1n ootid with 2n pollen). Induction of 2n pollen is very difficult in Ginkgo biloba L.. With the funding support of NSFC (30771747 and 30471412), we have studied on induction of 2n pollen for 6 yr. The result showed that 2n pollen could be induced by colchicine in G. biloba, but the mutation rate of 2n pollen (the biggest mutation rate was only about 7%) was lower than other plants which 2n pollen can be induced. Based on inducing mechanism of 2n pollen, the protein is one of important influencing factors. For improving mutation rate of 2n pollen and studying on the effects of protein of G. biloba’s microstrobilus on the process which 2n pollens were induced by colchicine, it needs a protein analysis technology system of G. biloba’s microstrobilus. In this experiment, a two-dimensional gel electrophoresis (2D-PAGE) system was established for protein study of G. biloba’s microstrobilus. The study optimized two-dimensional gel electrophoresis techniques for total protein of microstrobilus from extract method of total protein, pH Range of IEF, composition of protein lysis buffer, loading dosage of the protein and gel concentration of SDS-PAGE, etc. The results showed that the system containing TCA/acetone/phenol method for extracting total protein, pH 4–7 for IEF, protein lysis buffer

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optimized two-dimensional electrophoresis technology following study in G. biloba system will provide the research foundation for the following study in G. biloba’s microstrobilus.

**P-2075**

Analysis of Mutate Biological Effects of Spaceflight on Seeds of Robinia pseudoacacia. YUN LI, Cunquan Yuan, Chao Lu, Yuhan Sun, and Peng Sun. National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, P. R. CHINA. Email: yunli63@163.com

To investigate the biological mutate effects of spaceflight on R. pseudoacacia seeds and make attempts to cultivate new varieties, 1000 seeds were loaded into Shijian No.8 Recoverable Breeding Satellite of China in 2006 for 15-d space flight (From 9,9,2006,15 pm to 9,24,2006,10:42 am, had flown 236 Circles). The spaceflight seeds together with another 1000 seeds which were non-loaded as ground control were planted in 2007 and investigated every year. The results showed: 1. The germination rate and seedling rate were increased 4.5% and 3.8% respectively after spaceflight. 2. 4-yr consecutive growth data were employed. The average height and base diameter of space mutagenesis plants were lower than that of the ground control. The height and base diameter of 1-yr-old seedlings which derived from space mutagenesis were 19.2% and 14.6% lower than those of control, while 22.0% and 24.1% lower of 2-yr-old seedlings, 13.1% and 22.4% lower of 3-yr-old seedlings, and 7.0% and 16.4% lower of 4-yr-old seedlings than those of control respectively. Meanwhile, the average base diameter growth were also lower than ground control in all 4 yr. However, the gap between treatment and control is narrowing in both height and base diameter on the older seedlings. 3. Measurement of changes in antioxidative enzymes parameters. The superoxide dismutase (SOD) activity and content of the malondialdehyde (MDA) of 4-yr-old seedlings were 35.4% and 19.6% lower than the control respectively, while the peroxidase (POD) activity was 14.1% higher. 4. Assessment of spaceflight effects on photosynthetic characteristics. The net photosynthetic (Pn) and stomatal conductance (Gs) together with transpiration rate (Tr) were significantly decreased after spaceflight, the decreasing amplitude were 10.6%, 17.3%, and 18.1% respectively, but the intercellular carbon dioxide concentration (Ci) increased 4.7%. 5. One excellent mutant with straight trunk and fast-growing traits was obtained. Other mutants which had phenotype characters as golden yellow color leaf, wrinkled or wavy or cracking leaves edge shape, wedge leaf shape, large leaves, or thornless of thorn were also observed. More work is being conducted to investigate the molecular variation mechanism and select the stable mutants with favorable traits in order to develop new cultivars.

**P-2076**

Development of an Agrobacterium tumefaciens-mediated Transformation Method for Setaria viridis (Green Millet). J. VAN ECK, K. Swartwood, and T. Brutnell. The Boyce Thompson Institute, Ithaca, NY14853. Email: jv27@cornell.edu

*Setaria viridis* (green millet), which is the weedy relative of the cultivated *S. italic* (foxtail millet) and close relative of several major grasses used as feed and biofuels, is being developed as a model system to study, namely, C4 photosynthesis. Several characteristics make it attractive as a model including short stature (<10 cm at flowering), short-generation time (6 wk from seed to seed), and relatively small genome (~510 Mb). An important part in development of a model is having an efficient transformation method to enable high-throughput functional genomic studies. We developed an *Agrobacterium tumefaciens* mediated-transformation method based on infection of callus derived from mature seeds. The seed coats are removed from the seeds before they are disinfected and cultured on a callus induction medium (CIM). Approximately 90% of the seeds that germinate produce callus. After several rounds of sectioning into 2–3 mm pieces followed by transfer to fresh CIM, the callus is ready for infection about 6–8 wk from the time the seeds are cultured. It takes approximately 9 wk from the time of infection with *Agrobacterium* strain AGL1 until well-rooted transformants can be transferred to soil. Seeds are ready for harvest in 6–7 wk following transfer to the greenhouse. To date, the transformation efficiency (percent of callus that gives rise to transformants) is 25%. Currently, we are using the hygromycin phosphotransferase gene as the selectable marker and hygromycin as the selection agent. In addition, studies are underway with a second selectable marker gene, *bar*, which confers resistance to the herbicide bialaphos. The first gene functions studies were initiated recently and results will be presented.