

## Plant Posters

### P-2000

Chilling and Freezing Tolerance of Transgenic Bahiagrass Over-expressing Structural and, or Regulatory Genes Involved in Stress Protection. W. Fouad, J. M. CELEDON, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL-32611. Email: faltpeter@ifas.ufl.edu

Bahiagrass is the predominant forage grass in the South-Eastern United States. However, freeze-damage and low bahiagrass forage production during colder winter months represent seasonal limitations for dairy and beef cattle production. Bahiagrass does not cold acclimate in response to low temperatures, unlike grasses from temperate regions. It is well established that expression of a large number of stress protective genes contributes to species specific differences in stress tolerance including chilling and freezing. We recently established a genetic transformation protocol for bahiagrass, which allows now the introduction of cold protective genes into bahiagrass. Over-expression of the DREB1A transcription activator of stress protective genes has improved abiotic stress tolerance of several plant species along with up-regulation of its target genes. However, it is unclear if over-expression of regulatory or structural stress protective genes, or the combination of both has a greater potential to improve cold tolerance in bahiagrass, since very limited sequence information is available from bahiagrass. In the current study we compared the chilling and freezing tolerance of transgenic bahiagrass over-expressing regulatory or structural stress protective genes, or the combination of both. Four different sets of transgenic bahiagrass were generated by biolistic gene transfer, expressing cold protective genes isolated from *Hordeum vulgare* and *Oryza sativa*. The first set expressed the OsDREB1A under the control of the stress inducible HVA1s promoter from barley. The second set expressed the HVA-OsDREB1A and two barley dehydrin genes Dhn5 and Dhn8 under the control of Dhn8 promoter. The third set expressed Dhn8-Dhn5 and Dhn8-Dhn8 to examine the inducible expressing of these genes without the effect of OsDREB1A. The last set constitutively expressed the two Dhn5 and Dhn8 under the control enhanced 35S promoter from cauliflower mosaic virus

(CaMV). Molecular and physiological data correlating transgene expression and plant performance under chilling and freezing stress will be presented.

### P-2001

Greenhouse Screening and Field Testing of Transgenic Grapevine for Fungal Resistance. S. A. DHEKNEY<sup>1</sup>, Z. T. Li<sup>1</sup>, M. Dutt<sup>2</sup>, T. W. Zimmerman<sup>3</sup>, and D. J. Gray<sup>1</sup>. <sup>1</sup>University of Florida/IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703-8504; <sup>2</sup>University of Florida/IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850; and <sup>3</sup>University of Virgin Islands Agricultural Experiment Research Station, RR1 Box 10,000, Kingshill, St. Croix, VI 00850. Email: sadanand@ifas.ufl.edu

An endogenous thaumatin-like protein (VVTL-1) gene was isolated from *Vitis vinifera* 'Chardonnay' and reengineered for constitutive expression via a CaMV 35S-derived bidirectional duplex promoter complex, which also controlled expression of an EGFP/NPT II fusion gene. Embryogenic cultures of *V. vinifera* 'Cabernet Franc', 'Merlot', 'Shiraz', 'Thompson Seedless' and *Vitis* hybrid 'Seyval Blanc' were transformed with *Agrobacterium* and transgenic plants regenerated. Transgenic plant lines were screened for resistance to powdery mildew (*Uncinula necator*) by comparing symptom development with the corresponding non-transgenic susceptible varieties and a resistant control variety. Among 71 transgenic 'Thompson Seedless' plant lines tested, 5 exhibited a 7–10 day delay in development of symptoms compared to susceptible control plants. The resistance of these lines was confirmed by repeated screening for three seasons. The presence of the VVTL-1 transgene and protein in resistant plant lines was confirmed by PCR and ELISA, respectively. The selected plant lines were vegetatively propagated to produce replicates and planted in field sites at the University of Florida and University of Virgin Islands. Some of these plant lines were grafted onto locally adapted rootstocks as well. Additional transgenic lines of *V. vinifera* 'Cabernet Franc', 'Merlot', 'Shiraz' and *Vitis* hybrid 'Seyval Blanc' are being screened and selected in the greenhouse for future field tests.

**P-2002**

Transplastomic Expression of the *E. coli panD* Enhances Photosynthesis and Biomass Accumulation in Response to High Temperature Stress. W. FOUAD and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL 32611. Email: faltpeter@ifas.ufl.edu

In plants, beta-alanine increases in response to high temperature and/or drought. Beta-alanine prevents protein aggregation and reactivates the thermally denatured enzyme in vitro. In prokaryotes, beta-alanine is a product of the alpha-decarboxylation of L-aspartate catalyzed by the *panD*-encoded L-aspartate-alpha-decarboxylase (AspDC).

In the current study, the *E. coli panD* gene, under the control of plastid *Prn* promoter and *rbcL* 3'UTR, was introduced into the tobacco chloroplast genome via homologous recombination following biolistic gene delivery. Site specific transgene integration into the chloroplast genome was confirmed by Southern blot analysis and PCR and its expression was verified by Northern blot and in vitro enzyme activity assays. Interestingly, transplastomic expression of *panD* resulted in more than three-fold AspDC in-vitro activities compared to earlier reported *panD* nuclear transformants. AspDC in-vitro activity showed significant elevation in response to high temperature stress. The *panD*-transplastomic plants displayed significantly higher levels of quantum yield of Photosystem II and Electron transport rate compared to wild type during and after recovery from high temperature stress. In contrast to wild type plants *panD*-transplastomic plants maintained their CO<sub>2</sub> assimilation rate following an extended period of high temperature stress. Following an extended period of high temperature stress, *panD* transplastomic plants accumulated 30–40% more biomass than wild type control.

In summary, chloroplast engineering of the  $\beta$ -alanine over-production by over-expression of the *E. coli panD* enhanced photosynthesis and biomass accumulation following high temperature stress.

**P-2003**

Screening of Transgenic Anthuriums for Bacterial Blight and Nematode Resistance. M. FITCH<sup>1</sup>, T. Leong<sup>2</sup>, H. Albert<sup>1</sup>, S. Schenck<sup>2</sup>, P. Moore<sup>1</sup>, H. McCafferty<sup>2</sup>, J. Zhu<sup>2</sup>, and D. Gonsalves<sup>3</sup>. <sup>1</sup>Pacific Basin Agricultural Research Center, ARS, USDA, 99-193 Aiea Hts. Dr., Aiea, HI 96701; <sup>2</sup>Hawaii Agriculture Research Center, 99-193 Aiea Hts. Dr., Aiea, HI 96701; and <sup>3</sup>Pacific Basin Agricultural Research Center, ARS, USDA, 99 Aupuni St., Hilo, HI 96720. Email: mfitch@pbarc.ars.usda.gov

Anthuriums exhibit limited resistance to bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* and to the nematodes *Radopholus simile* and *Meloidogyne javanica*. *Agrobacterium tumefaciens* transformation of embryogenic calli with strains LBA4404, EHA105, and AGLØ resulted in transgenic plants containing one of seven different gene constructs, *Arabidopsis* NPR1, attacin and cecropin from *Hyalophora cecropin*, T4 phage lysozyme, and attacin + T4 lysozyme for bacterial blight resistance, and cystatin and cystatin + cowpea trypsin inhibitor for nematode resistance. Approximately 600 'Marian Seefurth' and 42 'Midori' lines will be screened in bioassays for resistance to the pests under greenhouse conditions. While approval for permits are awaited, experiments are ongoing to use in vitro methods to screen rooted stem sections with aseptic cultures of *Radopholus*. Experiments showed decreased numbers of the nematode in transgenic cultures after 4 weeks compared to those on nontransformed controls. The experiments are being replicated. Bacterial resistance in vitro bioassays are being developed as well.

**P-2004**

Abiotic Stress Tolerance of Bahiagrass with Expression of *HvWRKY38* or *OsMYB4* Transcription Activators. X. XIONG, V. James, and F. Altpeter\*. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville, FL 32611. \*Email: faltpeter@ifas.ufl.edu

Bahiagrass (*Paspalum notatum* Flugge) is an important turf and forage grass in the south-eastern US and in subtropical regions around the world. The productivity and persistence of bahiagrass is limited by environmental stresses like drought, freezing and in salt affected areas. We isolated several transcription activators of genes involved in abiotic stress response and will present data on over-expression of the *HvWRKY38* and *OsMYB4* transcription activator in bahiagrass. Transcription factors, like *OsMYB4* are capable of activating the expression of multiple genes involved in protection against environmental stresses. Constitutive *HvWRKY38* or *OsMYB4* expression cassettes were successfully introduced into bahiagrass cv. Argentine via biolistic gene transfer as indicated by Southern blot or PCR analysis. Over-expression of these transcription factors was confirmed by RT-PCR and Northern Blot. Transgenic bahiagrass plants over-expressing *HvWRKY38* or *OsMYB4* are currently evaluated under controlled environment to determine their drought and cold tolerance. We will present physiological data on dehydration and freezing tolerance including biomass production and stress symptoms following severe dehydration, chilling or freez-

ing, photosynthetic efficiency during and after stress, membrane stability and metabolite accumulation in response to stress.

#### P-2005

Adventitious Shoot Regeneration and Genetic Transformation of *Prunus serotina* for Reproductive Sterility. XIAOMEI LIU<sup>1</sup> and Paula M. Pijut<sup>2</sup>. <sup>1</sup>Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St, West Lafayette, IN 47907 and <sup>2</sup>USDA Forest Service, Northern Research Station, HTIRC, 15 West State St., West Lafayette, IN 47907. Email: liu104@purdue.edu, ppijut@purdue.edu

Black cherry (*Prunus serotina* Ehrh.) is one of the most valuable hardwoods in the eastern United States and Canada. There has been an increase in demand for high quality black cherry wood and there is a need to establish plantations with improved black cherry. Genetically improved trees containing foreign genes will be subject to government regulatory guidelines because of the potential for dispersal of transgenic pollen, thus requiring the need for sterility. The objective of this research was to develop a reliable system for genetic engineering of reproductive sterility in black cherry. An improved method for adventitious shoot regeneration from leaves was established for three genotypes (F, # 3, and # 4; # 3 and # 4 are mature trees). The highest regeneration efficiency for F, # 3, and # 4 was 94%, 75% , and 58% respectively, obtained on WPM supplemented with 9.1  $\mu\text{M}$  TDZ plus 1.1  $\mu\text{M}$  NAA. The highest mean number of shoots was achieved on the same medium; 8.2 (F), 5.1 (# 3), and 4.7(# 4). The rooting efficiency of shoots was 87% (F), 29% (# 3), and 65% (# 4) by dipping shoots in 2.5 mM IBA. In vitro leaves were transformed using *Agrobacterium tumefaciens* strain AGL1 carrying an RNAi construct containing an *AGAMOUS* gene. Selection and regeneration of transformed cells and shoots was carried out for 12 weeks on a medium containing kanamycin. Shoot regeneration was achieved using WPM supplemented with 9.1  $\mu\text{M}$  TDZ, 1.1  $\mu\text{M}$  NAA, plus 10 mg/L kanamycin. Timentin (300 mg/L) was used after three days of co-culture to kill the *Agrobacterium*. Late selection was carried out on the same medium except kanamycin was increased to 15 mg/L. Transgenic black cherry shoots were achieved which have been confirmed by PCR. Three out of 118 shoots of genotype F were kanamycin resistant, but only one was confirmed positive by PCR. Six out of 154 shoots of genotype # 3 were kanamycin resistant, and PCR is underway to confirm these putative transgenic shoots.

#### P-2006

Promoters for Gene Expression in Developing Fibers of Cultured Cotton Ovules. J. L. ROBERTS. Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: jlroberts2@dow.com

Cotton fibers are single-celled hairs which develop on the seed coat. Fiber growth and differentiation occur in stages: fiber initiation, fiber elongation with primary wall deposition, and secondary wall deposition. Cotton fiber differentiation and growth can be conveniently observed in developing ovules cultured in vitro. Transgenic cotton ovules in which the GUS gene was driven by five different constitutive promoters were studied in culture for the ability to express GUS in the developing fiber. Three of these promoters effectively expressed GUS, and two did not. The Arabidopsis ubi10 and ubi11 ubiquitin promoters and the cassava vein mosaic virus (CsVMV) promoter were effective in directing GUS gene expression in fibers at all stages of wall development from 3 to 35 days in culture. These findings contribute to tools and methods for future studies of genes influencing cotton fiber development.

#### P-2007

Micropropagation of *Juglans nigra* L. in Liquid Culture. CHRISTIAN ROSCHKE<sup>1</sup> and Paula M. Pijut<sup>2</sup>. <sup>1</sup>Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907 and <sup>2</sup>USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907. Email: croschke@purdue.edu and ppijut@purdue.edu

Black walnut is a valuable hardwood tree species that has great future potential for growing in plantations. Continuous high-grade logging depletes quality trees from natural stands and the shift of public land into reserve programs reduces the market supply of walnut from these resources. Plantations will allow landowners the ability to meet the future market demand for black walnut wood. Rooting of black walnut via conventional stem cutting propagation is very difficult and has prevented the development of a clonal mass propagation system. In order to clonally propagate selected or genetically improved genotypes, a micropropagation, rooting, and acclimatization protocol is needed. Shoot cultures of black walnut from seedlings of selected genotypes were established on a semi-solid DKW medium containing 8.88  $\mu\text{M}$  BA, 0.005  $\mu\text{M}$  IBA, 200 mg l<sup>-1</sup> casein hydrolysate, and 2 ml l<sup>-1</sup> Plant Preservative Mixture<sup>®</sup> (PPM). Nodal explants were initially disinfected using a

treatment based on PPM to eliminate external and internal contamination, which is often a problem in establishment of in vitro cultures of black walnut. Nodal sections of in vitro shoots of a selected genotype (Purdue #295) were cultured in a liquid DKW medium containing 8.88  $\mu\text{M}$  BA, 0.005  $\mu\text{M}$  IBA, 200 mg l<sup>-1</sup> casein hydrolysate, and 2 ml l<sup>-1</sup> PPM, and incubated on a shaker at 100 rpm under a 16 h photoperiod with a light intensity of 55  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Buds broke and developed into clusters of buds, which elongated into microshoots once the cluster grew large enough to not be continuously submerged in the liquid medium. Buds removed from the clusters and placed into separate culture vessels developed into healthy, thick-stemmed microshoots. Buds cultured on semi-solid medium also developed into healthy microshoots. Microshoots from liquid medium are currently being tested for rooting ability.

### P-2008

Asymbiotic and Symbiotic Seed Germination of *Eulophia alta* (Orchidaceae)-Preliminary Evidence for Symbiotic Culture Efficiency. S. L. STEWART<sup>1</sup>, T. R. Johnson<sup>1</sup>, D. Dutra<sup>1</sup>, M. E. Kane<sup>1</sup>, and L. Richardson<sup>2</sup>. <sup>1</sup>Environmental Horticulture Department, University of Florida, Gainesville, FL 32611 and <sup>2</sup>Florida Panther National Wildlife Refuge, U.S. Fish and Wildlife Service, Naples, FL 34114. Email: slstewart@ufl.edu

Asymbiotic orchid seed germination is often considered the most efficient method of seedling production. However, asymbiotic germination methods do not account for the physiological role of orchid mycorrhizae in seed germination, seedling development, and plant nutrition, and therefore may not necessarily support the rapid germination, growth, and development of orchids in vitro. Symbiotic germination methods may represent a more efficient means of seedling production for some orchid species, especially those of conservation concern. Asymbiotic and symbiotic seed germination experiments using *Eulophia alta*, a terrestrial orchid from Florida, were designed to compare seed germination percentages under each in vitro germination method. Five asymbiotic germination media were screened for their effectiveness in supporting germination of *E. alta*-Knudson C, Malmgren Modified Terrestrial Orchid Medium, P723, 1/2-Murashige & Skoog, and Vacin & Went. Medium P723 supported both the highest final percent germination (87.9%) and most advanced seedling development (Stage 4; 32.7%) when compared to the other four media. Ten mycobionts were screened for their effectiveness in supporting the symbiotic germination of *E. alta* sown on oat meal agar. Only mycobiont Ealt-396 supported the in vitro symbiotic germination of this species (70.1%). Interestingly, seeds cultured

under symbiotic culture conditions not only germinated more rapidly than seeds cultured under asymbiotic conditions, but also developed to a leaf-bearing stage more rapidly. Given that asymbiotic and symbiotic culture conditions were identical, these findings support the notion that in vitro symbiotic seed germination methods may represent a more efficient method for the production of orchid seedlings in vitro.

### P-2009

In Vitro Production of Adaptogenic Phytoecdysteroids from *Ajuga turkestanica* Hairy Root Cultures. DIANA M. CHENG, R. B. Rogers, M. A. Lila, G. Yousef, and M. Grace. Department Natural Resources and Environmental Sciences, University of Illinois Urbana-Champaign, 1115 Plant Sciences Lab, 1201 S. Dorner Dr., Urbana, IL 61801. Email: dcheng2@uiuc.edu

In vitro shoot and leaf tissue established from wild-harvested *Ajuga turkestanica*, a source of adaptogenic phytoecdysteroids, was inoculated with *Agrobacterium rhizogenes* to induce growth of hairy roots. Precursors of phytoecdysteroids (mevalonic acid, cholesterol and acetate) were added on the day of subculture to elicit accumulation of phytoecdysteroids in hairy root cultures. Addition of 15 mg L<sup>-1</sup> and 150 mg L<sup>-1</sup> mevalonic acid increased the phytoecdysteroid 20-hydroxyecdysone (20E) from 10.3  $\mu\text{g mg}^{-1}$  (control) to 16.3 and 13.5  $\mu\text{g mg}^{-1}$ , respectively, in hairy root cultures. Addition of 15 mg L<sup>-1</sup> and 150 mg L<sup>-1</sup> cholesterol altered 20E content to 10.5 and 9.0  $\mu\text{g mg}^{-1}$ . Addition of 15 mg L<sup>-1</sup> and 150 mg L<sup>-1</sup> sodium acetate demonstrated an increase of 20E to 16.2 and 20.7  $\mu\text{g mg}^{-1}$ . Cyasterone and cyasterone 22-acetate were detected at 4.1 and 4.2  $\mu\text{g mg}^{-1}$  in control cultures. Cyasterone content increased up to 8.6  $\mu\text{g mg}^{-1}$  with the addition of 15 mg L<sup>-1</sup> sodium acetate to the media and cyasterone 22-acetate increased up to 6.8  $\mu\text{g mg}^{-1}$  with the addition of 15 mg L<sup>-1</sup> mevalonic acid to the media. Turkesterone content remained at approximately 1.0  $\mu\text{g mg}^{-1}$  in control cultures and in all treated cultures. The enhancement of phytoecdysteroid accumulation was demonstrated in hairy root cultures of *A. turkestanica*.

### P-2010

High-tech Production of Natural Anticancer Molecules from Plant Adventitious Roots through Bioreactor Culture. G. SIVAKUMAR<sup>1</sup> and L. Bacchetta<sup>2</sup>. <sup>1</sup>Arkansas Biosciences Institute, Arkansas University, Jonesboro, P.O. Box 639, State University, AR 72467 and <sup>2</sup>Biotech Genomics, ENEA, Casaccia, Via Anguillarese 301, 00060 Rome, ITALY. Email: sivakumar@libero.it

The enormous side-effect in the synthetic medicine, we try to find new promising natural stereoisomer anticancer molecules from plant biofactories. Several secondary metabolites of pharmaceutical interest are accumulated in plant roots. Natural vitamin E i.e., *RRR*- $\alpha$ -tocopherol and bioactive glucosinolate i.e., sulforaphane has been increasing interest for nutraceutical and pharmaceutical industry due to their anti-cancer effect on human health. The modification of cellular metabolism is of biotechnological and commercial significance because naturally occurring metabolic pathways are the source of diverse bioactive stereoisomer molecules used in fields ranging from medicine to nutraceuticals. The impossibility of obtaining above said anticancer molecules are equally high bioactive as well as toxic residue free through chemical synthesis, therefore, we established high-tech bioreactor technology to produce natural anticancer from adventitious root culture. The present study focussed on the higher production of the *RRR*- $\alpha$ -tocopherol from *Corylus avellana* and sulforaphane from *Brassica oleracea* in adventitious roots through bioreactor cultures by feeding the elicitor, methyl jasmonate (MeJA). An increase in the  $\alpha$ -tocopherol (197  $\mu$ g/g DW, four fold higher than control) content of adventitious roots was observed 5 days after treatment with MeJA 100  $\mu$ M/L i.e., 20 to 25 day of inoculation. HPLC analysis of the glucosinolate fraction indicated higher accumulation of the sulforaphane (271  $\mu$ g/g DW) in 40 d-old MeJA 150  $\mu$ M/L treated adventitious roots compared to control. Bioreactor cultured roots have the potential to serve as an alternative natural vitamin E and sulforaphane, which is pesticide residue free with reducing labours cost and year round production.

#### P-2011

In Vitro Chemoprotective Effect of Isolated Culture of *Nerium Oleander* L. N. A. HOVHANNISYAN, Department of Microbiology and Plant Biotechnology, Yerevan State University, Alec Manoogian str., 1, Yerevan, 375025, ARMENIA. Email: bionellbiotech@yahoo.com

*Nerium oleander* is evergreen shrub belonging to the family *Apocynaceae* and it is commonly used in folk medicine. Extracts of *N. oleander* are known for their cardiotoxic, anti-inflammatory, cytotoxic and antiviral activities. The cytotoxic and apoptosis-inducing properties of hot water extracts of *N. oleander* plant and callus tissue culture for human transformed cell lines in vitro have been specified by us early.

In the present study the chemoprotective effects of intact plant and callus tissue polysaccharide fractions on cyclophosphamide (CP)-induced chromosome damage were studied in human myeloid KCL - 22 cells. Pretreatment of KCL -22 cells before exposure to CP by *N. oleander* intact plant and callus tissue polysaccharide fractions at the dose

of 100  $\mu$ l/ml 30 min reduced the level of CP-induced micronuclei (MN). The results obtained show that under the experimental conditions used in this study *N. oleander* callus culture polysaccharide fractions can protect against CP-induced chromosome damage.

So, *N. oleander* callus culture may be used as an alternative source of substances with anticancer and chemoprotective activities.

#### P-2012

Development and Analysis of Peas with Reduced Raffinose Oligosaccharide Content. P. L. POLOWICK, D. S. Baliski, C. A. Bock and F. Georges. Plant Biotechnology Institute, National Research Council Canada, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, CANADA. Email: Patricia.Polowick@nrc-cnrc.gc.ca

The raffinose oligosaccharides (RFOs) are complex carbohydrates that accumulate during seed development. These oligosaccharides can only be digested anaerobically in the small intestine resulting in flatulence; this often limits legume consumption. As a proof of concept strategy, the objective of this project is to produce lines of legumes with reduced concentrations of these RFOs, and to determine any consequences on seed quality and viability. Two transgenic approaches are being investigated; the introduction of the catabolic enzyme  $\alpha$ -galactosidase and the down-regulation of galactinol synthase to restrict its participation in RFO synthesis. From a population of transgenic pea plants with the introduced  $\alpha$ -galactosidase gene, five independent lines with a single copy of the introduced line were chosen. The sugar content of ten T<sub>2</sub> seeds from each of the five segregating lines, in addition to untransformed and transformed controls, were analyzed via HPLC. Two of the five transgenic lines had significantly reduced levels of raffinose and stachyose. No abnormalities were observed in either seed development or germination. The T<sub>3</sub> generation was analyzed via HPLC using only a portion of the seed; the remainder of the seed was planted for molecular analysis of gene presence and expression. In addition, the individual seed tissues of cotyledon, embryo axis and seed coat were analyzed separately. These results will be discussed in detail.

#### P-2013

Cryopreservation of Tobacco Suspension. LIU Y SHEN. Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN, 46268. Email: lyshen@dow.com

A method for cryopreservation of tobacco (*Nicotiana tabacum* L.) cell suspension cultures that is easy, efficient

and displays a high recovery of viable cells has been developed. Slow freezing approach was used for the study. Growth conditions and cryoprotectants for cryopreservation and recovery etc were optimized for tobacco cell lines. Several types of cryoprotectants, including the addition of proline and mannitol, and the effect of residual cryoprotectant on recovery of cells were tested. It was observed that different cell lines appeared to exhibit differences in relative tolerance to freezing conditions. Moreover, cells from mid-log phase appeared to be most suitable for cryopreservation. Addition of proline to the culture medium increased recovery of an NT1 cell line. Several cell lines have been successfully cryopreserved and recovered.

#### P-2014

A Comparative Study of Three Cryopreservation Protocols for Effective Storage of Mint (*Mentha spp.*). ESTHER E. UCHENDU<sup>1</sup> and Barbara M. Reed<sup>2</sup>. <sup>1</sup>Department of Horticulture, ALS 4017, Oregon State University, Corvallis, OR 97331 and <sup>2</sup>United States Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333-2521. Email: uchendu@onid.orst.edu, corbr@ars-grin.gov

Four mint species [*Mentha x piperita citrata* (Ehrh.) Briq. (PI 557993); *M. canadensis* L., (PI 557613); *M. australis* R.Br. (PI 617498) and *M. cunninghamii* Benth. (PI 617481)] from the in vitro collections of the USDA-ARS National Clonal Germplasm Repository (NCGR), Corvallis, were cryopreserved using three standard protocols: controlled cooling (CC), encapsulation dehydration (ED), and PVS2 vitrification (VIT). All plants were cultured on MS medium with 0.5 mg l<sup>-1</sup> benzyladenine (BA), 0.1 mg l<sup>-1</sup> indole 3 butyric acid (IBA) and cold acclimated for 2 weeks before cryopreservation. Shoot tips were recovered on medium without IBA. All four genotypes responded well to the controlled cooling protocol. Regrowth following controlled cooling (93%) was significantly better ( $p < 0.05$ ) than for encapsulation dehydration (71%) or vitrification (73%). Recovery of *Mentha x piperita citrata* and *M. australis* showed significant differences among the three techniques with CC > VIT > ED. There were also significant differences in the recovery of *M. canadensis* and *M. cunninghamii* with CC and ED significantly better than VIT. Overall, regrowth of the genotypes was 60% to 90% for all but one treatment. These results indicate that controlled cooling was the most successful technique, however, recovery of shoot tips from VIT and ED was usually high enough that these techniques could also be used for cryogenic storage of mint germplasm.

#### P-2015

Asymbiotic Seed Germination of the Threatened Orchid *Bletia purpurea* in Florida. D. DUTRA<sup>1</sup>, S. L. Stewart<sup>1</sup>, P. J. Kauth<sup>1</sup>, T. R. Johnson<sup>1</sup>, N. Philman<sup>1</sup>, M. E. Kane<sup>1</sup>, and L. Richardson<sup>2</sup>. <sup>1</sup>Environmental Horticulture Department, University of Florida, Gainesville, FL 32611 and <sup>2</sup>Florida Panther National Wildlife Refuge, U.S. Fish and Wildlife Service, Naples, FL 34114. Email: ddutra@ufl.edu

*Bletia purpurea* (Lamarck) de Candolle is a threatened North America native orchid restricted to six counties in extreme southern Florida. *Bletia purpurea* occurs in open wet pinelands and prairie edges. Concerns about the decline of this species require that a propagation method for this species be developed. Asymbiotic seed germination represents an effective method of orchid production for species-level conservation. A protocol for the asymbiotic seed germination of *B. purpurea* was developed. Seeds were collected from the Florida Panther National Wildlife Refuge (Collier Co., FL), surfaced sterilized, and cultured on six basal media including Knudson C (KC), PhytoTechnology Orchid Seed Sowing Medium (P723), Malmgren Modified Terrestrial Orchid Medium (MM), Vacin & Went Modified Orchid Medium (VW), 1/2-strength Murashige & Skoog (1/2MS), and BM-1 Terrestrial Orchid Medium (BM). Germination plates were incubated in either 0/24 h or 16/8 h L/D photoperiod for 5 weeks. Germination (Stage 2 +; testa rupture) occurred regardless of photoperiod and medium treatments. However, advanced seedling development (Stage 6) only occurred on VW under a 16/8 h L/D photoperiod. By week 4 and 5, advanced seedling development (Stage 6; multiple leaves produced) occurred on VW in light (21.7% and 35.5%, respectively) compared to darkness (0% and 0%, respectively). In nature, *B. purpurea* grows in calcium rich soils. Since VW is a calcium rich germination medium (200 mg l<sup>-1</sup> Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), our findings may indicate that *B. purpurea* demonstrates a preference for high calcium availability during germination, growth, and development.

#### P-2016

Adventitious Shoot Regeneration of *Fraxinus pennsylvanica*. NINGXIA DU<sup>1</sup> and Paula M. Pijut<sup>2</sup>. <sup>1</sup>Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907 and <sup>2</sup>USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907, Email: ndu@purdue.edu, ppijut@fs.fed.us

A rapid regeneration protocol was developed from 7-day-old hypocotyls and cotyledons obtained from mature embryos of green ash (*Fraxinus pennsylvanica*), an important hardwood species in the eastern and Midwestern United States. The best regeneration medium for hypocotyls and cotyledons was MS medium supplemented with 13.3  $\mu\text{M}$  BA plus 4.5  $\mu\text{M}$  TDZ, and 22.2  $\mu\text{M}$  BA plus 2.3  $\mu\text{M}$  TDZ, respectively. Seventy-four percent of hypocotyl segments and 42% of cotyledon segments produced adventitious shoots, and the mean number of adventitious shoots induced per explant were 1.7 and 1.3, respectively. Adventitious shoots from hypocotyls and cotyledons were established as proliferating shoot cultures following transfer to MSB5 medium supplemented with 10  $\mu\text{M}$  BA plus 10  $\mu\text{M}$  TDZ. For in vitro rooting trials, a high rooting percentage (73–90%) for three stock genotypes was achieved on WPM with various combinations of IBA plus IAA under 10 days dark treatment followed by light treatment. Hormone-free WPM did not induce roots. The combination of 4.9  $\mu\text{M}$  IBA with different concentrations of IAA (2.9, 5.7, or 8.6  $\mu\text{M}$ ) had a significant effect on rooting percentage compared with only IBA in the media, but it did not have a significant effect on the number of roots, length of roots, or number of lateral roots produced. Significant clonal differences were also observed in response to in vitro rooting treatment. Rooted plants were successfully acclimatized to the greenhouse and are being overwintered for field planting. This regeneration system from hypocotyls and cotyledons provides a foundation for *Agrobacterium*-mediated genetic transformation of *Fraxinus pennsylvanica* for resistance to the emerald ash borer.

#### P-2017

In Vitro Propagation of Northern Red Oak (*Quercus rubra* L.). G. VENGADESAN<sup>1</sup> and Paula M. Pijut<sup>2</sup>. <sup>1</sup>Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907 and <sup>2</sup>USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., Lafayette, IN 47907. Email: vengi@purdue.edu, ppijut@purdue.edu

Northern red oak (NRO) is native to North America and widely distributed in the northeastern United States and southeastern Canada. Because of its good wood qualities it is one of the most important oaks in the timber and forest products industry. The tree is also grown for landscape purposes and the acorns are a major food source for several species of birds and mammals. Acorn production is highly variable (3–5 year intervals) and populations of NRO do

not produce acorns abundantly until they are 25 years of age. Acorns have poor viability and do not withstand long-term storage conditions. Clonal reproduction of NRO is desirable, in a tree improvement program, in order to provide improved planting stock for plantation forestry. In vitro propagation of red oak shoots was successful from cotyledonary node explants excised from 8-week-old in vitro grown seedlings. Initially, two shoots per explant were obtained on MS medium supplemented with 4.4  $\mu\text{M}$  BA, 0.23  $\mu\text{M}$  TDZ, and 500 mg/L casein hydrolysate (CH) after 4 weeks of culture. Although the regeneration response was low (10%), sub-culturing explants after harvesting shoots to fresh medium of the same composition significantly increased shoot bud regeneration (10 buds per explant), but the buds failed to elongate into shoots. Approximately 25% of the explants produced three shoots per explant on WPM supplemented with 4.4  $\mu\text{M}$  BA, 0.3  $\mu\text{M}$  GA<sub>3</sub>, and 500 mg/L CH after 4 weeks of culture. Sub-culturing the explants to fresh medium significantly increased shoot bud production (15 shoots per explant). Shoot elongation was achieved (4.0 cm) when shoots were cultured on WPM supplemented with 0.44  $\mu\text{M}$  BA plus 0.3  $\mu\text{M}$  GA<sub>3</sub>. In vitro regenerated shoots were rooted on WPM supplemented with 2.5  $\mu\text{M}$  IBA. BA and GA<sub>3</sub> produced a higher number of shoots in WPM, than BA and TDZ in MS medium. Lower concentrations of BA and GA<sub>3</sub> were required for shoot elongation. Shoot tip necrosis and dormancy was common on 4.4  $\mu\text{M}$  BA with both media. Each cotyledonary node yielded approximately 15 shoots within 12 weeks.

#### P-2018

Cloning Seedlings of *Moringa* Tree Species: A Method Adapted to Conservation of Biodiversity. B. STEINITZ<sup>1</sup>, Y. Tabib<sup>1</sup>, V. Gaba<sup>2</sup>, T. Gefen<sup>3</sup>, Y. Vaknin<sup>4</sup>. <sup>1</sup>Dept. of Vegetable Crops and Plant Genetics, <sup>2</sup>Dept of Plant Pathology and Weed Science, <sup>3</sup>Seed Research and Testing Lab, <sup>4</sup>Department of Agronomy and Natural Resources, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, ISRAEL. Email: steinitz@volcani.agri.gov.il

The Moringaceae comprises 13 tree species. *Moringa oleifera* is so far the single species cultivated, and grows in tropical and sub-tropical climate areas mainly in Asia, Africa and the Mediterranean. Cultivated trees are used for many purposes including production of nutritious food, animal feed, clarifying turbid water and as a source of phytomedicinal compounds. Other *Moringa* species have some comparable useful properties, but have not undergone domestication, are subjected to wild-harvest and are under extinction threats. Although various biotechnologies are

used to support endeavors to maintain plant biodiversity and alleviate extinction threats, *Moringa* species received no attention in this respect. The main objective of our work was to develop a simple procedure allowing vegetative cloning of seedlings of wild trees of *Moringa* spp.. The method utilized is a modification of a system previously employed for regeneration of tomato (*Lycopersicon* spp., Steinitz et al., Plant Cell Tissue Organ Culture 84:269, 2006). Propagation in vitro was achieved by shoot regeneration from the cotyledonary node of decapitated seedlings, followed by axillary shoot growth from single node shoot segments and finally rooting of excised shoots. All steps were accomplished on basal MS (Murashige and Skoog, Physiol. Plant 15:473, 1962) medium without plant growth regulators. The results will be discussed from the standpoint of asexual propagation sustaining biodiversity among individuals of a population of wild plants.

#### P-2019

Somatic Hybridization and Callus Production between ‘Autumn Royal Seedless’ (*Vitis vinifera* L.) and ‘Alachua’ (*V. rotundifolia* Michx.) Grapes. X. XU<sup>1</sup>, J. Lu<sup>1</sup>, and J. Grosser<sup>2</sup>. <sup>1</sup>Center for Viticulture and Small Fruit Research, 6505 Mahan Drive, Florida A&M University, Tallahassee, FL 32317 and <sup>2</sup>Citrus Research and Education Center, 700 Experiment Station Road, University of Florida, Lake Alfred, FL 33850. E-mail: xia.xu@famu.edu

Somatic hybridization is of great interest in plant breeding because it is a means of by-passing sexual incompatibility between species. An experiment was initiated to develop a protocol for somatic hybridization in grape, including isolation, fusion, cultivation and regeneration of protoplasts from somatic embryogenic cell suspension culture of ‘Autumn Royal Seedless’ (*Vitis vinifera* L.) and leaves of ‘Alachua’ (*V. rotundifolia* Michx.). The suspension culture and leaves were incubated in an isolation mixture overnight in dark on a rotary shaker (50 rpm). The mixture consists of 1% cellulase R-10, 0.2% pectolyase Y-23, 1% macer-ozyme R-10 with 24 mM CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.092 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.7 M mannitol, and 6.15 mM 2-(N-morpholino) ethanesulfonic acid, plus 0.1% polyvinylpyrrolidone (PVP). A high yield of viable protoplasts was isolated from both suspension cultures and leaves as indicated by fluorescein diacetate (FDA) staining and subsequent cell activities. 16.4% protoplasts of ‘Autumn Royal Seedless’ were fused together with leaf protoplasts of ‘Alachua’ when using 40% polyethylene glycol (PEG, MW 1,500) as aggregating/fusing after the fusion. Cell wall formation started 2–7 days and cell division began 4–14 days after cultivation of the fused protoplasts on EME : BH3 (1:1 v/v, Grosser and Gmitter, 1990) liquid medium. Double density of fused

protoplasts was placed on above medium solidified with 2.5 g/l phytigel for comparison. 36.7% of the fused cells divided on solid medium, which was 2.5% higher than that observed on liquid medium. However, calli were observed 5–10 days later on semi medium than on liquid culture. The callus formation from the fused protoplasts is the first important step towards developing interspecific somatic hybrids between the two grape species.

#### P-2020

Evaluation of Mineral Nutrient Symptoms During Micropropagation. N. D. BECKER and M. J. Bosela, Department of Biology, Indiana University-Purdue University at Fort Wayne, 2001 East Coliseum Blvd, Fort Wayne, IN, 46805-1499. E-mail: becknd01@ipfw.edu

Hydroponics is an invaluable tool for plant mineral nutrition research. Historically, hydroponics has been used to demonstrate inorganic nutrient requirements and to document deficiency symptoms. However, we are aware of few studies that have systematically evaluated the effects of mineral nutrient deficiencies on plant tissue cultures. Although mineral nutrient imbalances have been suggested to contribute to shoot tip necrosis and hyperhydricity, two of the most important limiting factors during shoot micropropagation, other media factors (cytokinin inclusion, gelling agent type and concentration, etc.) may have interacted synergistically with the nutrient deficiencies to cause the defects. We initiated this research to clarify the effects of mineral nutrient deficiencies during micropropagation. We have employed four plant species (aspen, tobacco, carnation, and tomato) using shoot cultures of aspen and seedling cultures of the other species. To ensure that our results are comparable with those from the plant mineral nutrition literature, we have used a standard hydroponic medium (Hoagland’s solution). Our experiments included both positive and negative controls as well as six experimental treatments each deficient in one of the following nutrients: calcium, iron, magnesium, nitrogen, phosphorus, and potassium. All of the media were prepared without hormones and each was supplemented with sucrose (10 g/L). Multiple gelling agents (agar, agarose, and gellan gum) were used to determine the significance of mineral nutrient contributions from each gelling agent. In initial tests with agar, shoot or leaf tip necrosis was observed for the calcium deficient solutions across all species. We did not observe hyperhydricity for any of the plants tested. However, in preliminary results from more recent tests with Gellan gum, we have begun to observe hyperhydricity for the calcium deficient treatments with tomato. More tests are ongoing and the results from these will be presented.

**P-2021**

Propagation and Germplasm Conservation of *Zeyheria montana*. A. M. S. PEREIRA<sup>1</sup>, B. W. Bertoni<sup>1</sup>, P. S. Pereira<sup>1</sup>, C. F. Damiao-Filho<sup>2</sup>, A. N. Salomao<sup>1</sup>, S. C. França<sup>1</sup>, R. M. Moraes<sup>3</sup>, and A. L. Cerdeira<sup>4</sup>. <sup>1</sup>University of Ribeiro Preto, Ribeiro Preto, SP, 14.096-380, BRAZIL; <sup>2</sup>Sao Paulo State University, Jaboticabal, SP, BRAZIL; <sup>3</sup>National Center for Natural Products Research, The University of Mississippi, University, MS; and <sup>4</sup>Brazilian Department of Agriculture, Embrapa/Environment, C.P. 69, Jaguariuna, SP, BRAZIL. Email: apereira@unaerp.br

Roots of *Zeyheria montana* Mart., a species native to the savanna (Cerrado) region of central Brazil, produce lapachol a naphthoquinone with anticancer properties and the precursor of  $\beta$ -lapachone, a novel drug candidate for preventive and adjuvant cancer therapies. This recent discovery on the potential prophylactic use of  $\beta$ -lapachone emphasizes the importance of this study on propagation and germplasm conservation of *Z. montana*. *Ex situ* procedures on seed germination and seed storage were conducted revealing that wing removal was a beneficial treatment for improving emergence and seedling survival. Being an orthodox seed, germplasm can be secured for long-term period using liquid nitrogen exposure. Further acknowledging the endangered status of *Z. montana*, germplasm in vitro techniques were used propagate and conserve elite plants. Multiple shoots were induced on Woody Plant media with supplemented 0.1 mg of thidiazuron (TDZ) per liter. Rooting was promoted on WP media containing 1 mg/L of naphthalene acetic acid (NAA). Plantlet acclimatization to ex-vitro condition was done at 70% success rate using different substrates. It was possible to store *Z. montana* cultures for six months on media containing 2% sucrose plus 4% sorbitol with or without spermidine.

**P-2022**

Development of an Efficient Micropropagation System for *Helleborus*. YINGHUI DAN<sup>1,2</sup> and R. E. Rothrock<sup>1</sup>. <sup>1</sup>Institute for Advanced Learning and Research, and <sup>2</sup>Departments of Horticulture and Forestry, Virginia Polytechnic Institute and State University, 150 Slayton Avenue, Danville, VA 24540. Email: yinghui.dan@ialr.org, ydan@vt.edu

Hellebores, the Genus *Helleborus* in the Family Ranunculaceae, are perennial flowering plants that are often grown in gardens for decorative purposes. The genus is increasing in commercial importance because of the evergreen nature of its species, its perennial winter and early spring blooming, and its use as a cut flower. It is also valued for its environmental adaptation such as frost, acid soil and

deer-resistance. *Helleborus* is one of the most popular shade plants in the U.S.A. and Virginia. The annual market value for *Helleborus* in Virginia is approximately \$1 million dollars. However, two major problems have highly restricted *Helleborus* production nationwide: the difficulty in its sexual and asexual propagation, and the long period (up to 24 months) of its natural seed dormancy. Due to the problems, Virginia can only produce 30% of the plants required for the \$1 million dollar market. Therefore we are developing an efficient micropropagation system, which enables a massive and rapid production of *Helleborus* to meet the current and future market demands in Virginia. Three elements of the micropagation system, which are 1) decontamination of field/greenhouse/growth room explant materials, 2) in-house *Helleborus* stock plant production to ensure clean and sufficient material supply and 3) tissue culture conditions for micro propagation, will be discussed in this poster.

**P-2023**

Shoot Induction from Nodal Explants of Herbaceous Peony. DAIKE TIAN, Ken M. Tilt, Fenny Dane, Jeff L. Sibley, and Floyd M. Woods. Auburn University, Department of Horticulture, 101 Funchess Hall, Auburn, AL 36849. Email: tiandai@auburn.edu

Nodal stem explants of eight cultivars of herbaceous peony (*Paeonia lactiflora*) were examined for their in vitro propagation potential. Shoots were induced successfully from meristematic region of nodal explants using an optimal concentration of BA, BA+TDZ, and TDZ in combination with or without GA<sub>3</sub> in half/full strength MS medium. GA<sub>3</sub> played a critical role on shoot elongation, but high concentrations of GA<sub>3</sub> resulted in narrow and weak shoots which are not useful for rooting. The shoot induction ability was dependent on PGR type and concentration, development stage and position of explants, and genotype. Callus was easily generated on all nodal explants within a two day period, but growth halted after two weeks of culture and resulted in browning due to phenolic production. The amount and quality of callus was dependent on genotype, development stage and position of explants, and depth of explants in contact with the medium. A challenge for commercial production of herbaceous peony is still the low rooting ability of shoots produced in tissue culture.

**P-2024**

Enhancement of Friable Embryogenic Callus Production and Plant Recovery by Tyrosine in a Range of Agronomically Important West African Cassava (*Manihot esculenta* Crantz) Landraces. B. B. HANKOUA, A. Mbanaso, R. Banda, C.

Fauquet, and N. Taylor. Department of Biological Sciences, Delaware State University, DE 19901. Email: bhankoua@desu.edu

The effect of tyrosine on the conversion of somatic embryos into high quality, proliferating friable embryogenic callus (FEC) and the FEC conversion into normal plants were evaluated. All cultivars were embryogenically competent in Murashige and Skoog-based medium supplemented with 50  $\mu\text{M}$  picloram. A significant difference was observed in the frequency of organized embryogenic structure (OES) induction from leaf explants between the 17 cassava cultivars. OES formation at 85% was highest in cv. TME1 and TME282 while TME127, TME117, TME2, TME1695 and TME8 was the least responsive with less than 20% of explants undergoing embryogenesis. Inclusion of the amino acid tyrosine at either 250  $\mu\text{M}$  or 500  $\mu\text{M}$  into OES induction medium had no positive effect on conversion of leaf lobes to OES in all the 17 cultivars. All cultivars formed FEC after cultured of OES on a Gresshoff and Doy based medium (FEC-IM). Inclusion of tyrosine at either 250  $\mu\text{M}$  or 500  $\mu\text{M}$  into FEC-IM significantly enhanced OES conversion into FEC in many but not all cultivars. The highest increase in FEC production was recorded with TME117 and TME1 at 1.5–3.0 fold increase with 250  $\mu\text{M}$  tyrosine. A linear decrease in the FEC production was observed in cv. 60444 and TME4 with increasing tyrosine concentration. No positive correlation was observed between the ability of a cultivar to form OES and FEC. Only TME3 and 60444 proliferated significantly in FEC-medium deprive of tyrosine. FECs induced from other cultivars were only prolific on tyrosine-based FEC-IM with the greatest response at 250  $\mu\text{M}$ . Recalcitrance to FEC production and proliferation in tyrosine-based media was pronounced in cv. TME1671 demonstrating the genotype-dependency of the tyrosine effect. Proliferation of FEC was significantly inhibited at 500  $\mu\text{M}$  with death of FEC tissues recorded in 60444. The number of maturing somatic embryos obtained from 0.5 g of FEC varied between 27 and 245 depending on the cultivar. Normal plantlets were recovered from germinated embryos at a frequency varying between 66–94% and they established at high frequency in the greenhouse at the Danforth Center.

#### P-2025

Effects of Auxin Transport on Competence Acquisition to Root Apical Meristem Conversion of *Catasetum fimbriatum* into Buds. M. A. RODRIGUES, L. Freschi, and G. B. Kerbauy. Laboratory of Plant Physiology, Department of Botany, University of São Paulo, P.O. Box 11461, CEP 05422-970, São Paulo, SP, BRAZIL. Email: auri@usp.br

The auxin and its polar movement have been shown to play a crucial role in many aspects of root development, including the establishment, patterning and differentiation of the root apical meristem (RAM). Root apices isolated from epiphytic orchids of the genera *Catasetum* represent an interesting model for studying signs that regulate RAM organization, since they are able to convert into buds when cultivated in vitro. It was previously shown that competence acquisition to this process was related to plant ageing, root growth stop and RAM morphological modification. RAMs of young plants did not present the mentioned competence. The aim of this work was to study the effects of auxin transport disturbance on RAM morphology of young *Catasetum fimbriatum* plants and its competence acquisition to convert into buds. To achieve this, young micropropagated plants of *C. fimbriatum* (30-day-old) were treated during 30 days with 10, 100 and 1000  $\mu\text{M}$  of NPA (N-1-naphthylphthalamic acid), a polar auxin transport inhibitor, and their root growth rate was observed weekly. Subsequently, RAM morphological and histological modifications were verified, as well its competence to convert into buds. The results showed that 10 and 100  $\mu\text{M}$  of NPA caused a decrease of 65% in root growth rate after the first week, besides remarkable meristematic differentiation. The treatment with 1000  $\mu\text{M}$  of NPA was totally inhibitory to root growth, caused stronger RAM differentiation, and permitted 79% of the isolated root tips to convert into buds. Control explants kept their normal RAM organization and did not show the aforesaid competence. The present results indicate that competence acquisition of *C. fimbriatum* RAMs to convert into buds was mediated by auxin transport disturbance, and that this kind of alteration can take place during the ageing of this species.

#### P-2026

The Effects of Magnetic Field on Germination of Soybean Seeds and on the Activity of Superoxide Dismutase and Catalase. Ö. ÇELİK<sup>1</sup>, Ç. Atak<sup>2</sup>, and N. Büyüksulu<sup>1</sup>. <sup>1</sup>Halic University, Faculty of Science and Arts, Molecular Biology and Genetics Department, Fındıkzade, 34280, Istanbul, TURKEY and <sup>2</sup>Istanbul Kültür University, Faculty of Science and Letters, Bakırköy, Istanbul, TURKEY. Email: ocelik@halic.edu.tr

Under magnetic field, the activity of superoxide dismutase (SOD) in vitro and in vivo and accompanying activities of catalase activity in vivo were investigated in soybean seeds. In plant cells, magnetic field creates a stress condition like other environmental stress factors do. To response to stress conditions, the occurred reactive oxygen species are scavenged by defense systems. In this study, two enzymes of the defense system, SOD and catalase activities are

investigated under magnetic field. SOD activities data were compared in vivo and in vitro systems. After soybean seeds were treated by various magnetic fields and time, the activities of superoxide dismutase and catalase were increased during germination. Thus, it is indicated that the function of defence enzymes in seedling was intensified due to the treatment of magnetic field.

#### P-2027

A Lab-to-soil Synthetic Seed System for Pomegranate. SOUMENDRA K. NAIK<sup>1</sup> and Pradeep K. Chand<sup>2</sup>. <sup>1</sup>Department of Biosciences and Biotechnology, Fakir Mohan University, Balasore-756019, Orissa, INDIA and <sup>2</sup>Plant Tissue and Cell Culture Facility, Department of Botany, Utkal University, Bhubanewar-751004, Orissa, INDIA. Email: sknuu@yahoo.com

Pomegranate (*Punica granatum* L.) is a medicinally important fruit tree of the tropics. We report the first successful attempt of the utilization of synthetic seed technology for pomegranate, which could be useful in germplasm distribution and exchange. Nodal segments from in vitro shoot cultures derived from mature nodal explants (source A) or axenic cotyledonary nodes (source B) were encapsulated in calcium alginate hydrogel containing Murashige and Skoog's (1962) medium (MS) supplemented with 4.44  $\mu\text{M}$  benzyladenine (BA) and 0.54  $\mu\text{M}$  naphthalene acetic acid (NAA). A combination of 3% sodium alginate and 100 mM calcium chloride was most suitable for formation of ideal synthetic seeds. Morphogenic response of encapsulated nodal segments on various planting media was evaluated. Encapsulated nodal segments of both the sources exhibited shoot development only in a few selected media. Of the planting media evaluated, % sprouting (shoot development) was the highest in MS medium augmented with 4.44  $\mu\text{M}$  BA and 0.54  $\mu\text{M}$  NAA and lowest in 1/2 MSS medium. One-step germination resulting in simultaneous shoot and root formation was possible only with encapsulated nodal segments of source B in MS, 1/2 MSS and natural soil + 1/2 MSS, of which MS was most effective. Encapsulated nodal segments stored at 4°C up to 30 days were capable of sprouting. Storage beyond 45 days led to a marked reduction in sprouting and after 60 days, sprouting failed to occur. Plants regenerated from the encapsulated nodal segments were hardened off and transferred to soil.

#### P-2028

Micropropagation of Endemic *Muscari mirum* Speta by Bulb Scale and Immature Embryo Explants. AYSE GUL NASIRCILAR, Semra Mirci, Ozgul Karaguzel,

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*Muscari mirum* Speta is an endangered endemic geophyte belonging to the Liliacea family. Because of this, in vitro production of this plant is important not only to protect gene pool but also for commercial production. Fresh bulbs and immature fruits of *Muscari mirum* Speta were collected in Fethiye, Muğla, a place where this plants naturally grown up in May. Bulbs were stored in the dark at 5°C for 6 weeks. Commercial bleach was used for surface sterilization of both explant types. Immature embryo and bulb scale explants consisting two or four scale segments were isolated and cultured on MS (Murashige-Skoog) medium supplemented with various combinations of BA (6-benzylaminopurine) and NAA ( $\alpha$ -naphthaleneacetic acid). Bulb scale explants produced higher number of bulblets than immature embryo explants. The highest bulblet formation ratio was obtained from two scale explants on MS medium supplemented with 4 mg/l BA and 0.25 mg/l NAA. Mean number of bulblets per explants was obtained 23.17 on this medium.

#### P-2029

Large-Scale Production of Pharmaceutical Proteins Using Plant Cell Suspension Cultures. S. LUKJAN, V. Srinivasan, G. Tous, S. Parekh, C. Swindell and M. Horn. Phyton Biotech Inc., East Windsor, NJ 08520. Email: Michael.Horn@phytonbiotech.com

The use of higher plant cell suspension cultures for the production of biopharmaceuticals has finally come of age. Phyton Biotech currently produces paclitaxel in plant cell fermenters up to 75 kL and has been a long-term supplier of this small molecule API Bristol-Myers Squibb's Taxol® oncology product. Our attention has now turned to the production of proteins using transgenic plant suspension cells. The cell lines are being carefully chosen from a large library of species for maximal growth, protein titer, scale-up ability, cryopreservation and glycosylation pattern. In addition, we are aggressively pursuing and developing glycol-engineering technology for products requiring such post-translational modification for efficacy or safety. We have also acquired rights for appending plant-specific glycosylation tail, which significantly extends the half-life of a pharmaceutical protein in the human body. Plant cells naturally have several advantages over other systems. By targeting the protein to the cell wall or different organelles, we can express any desired protein with different post-translational modification profiles. We have made significant

progress toward achieving our goal of 2g protein per liter of broth.

### P-2030

Overexpression of the Small Subunit of Geranyl diphosphate Synthase from Snapdragon Enhances the Production of Monoterpenes in Tobacco. I. ORLOVA<sup>1</sup>, D. Nagegowda<sup>1</sup>, E. Fridman<sup>2,3</sup>, E. Pichersky<sup>2</sup>, and N. Dudareva<sup>1</sup>. <sup>1</sup>Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, <sup>2</sup>Department of Molecular, Cellular, and Developmental Biology, University of Michigan, 830 North University Street, Ann Arbor, MI 48109-1048, and <sup>3</sup> Faculty of Agricultural, Food Quality and Environmental Sciences, Robert H. Smith Institute of Plant Sciences and Genetics, The Hebrew University of Jerusalem, Rehovot 76100, ISRAEL. Email: dudareva@purdue.edu, lel@umich.edu

Geranyl diphosphate (GPP) is the entry point leading to the synthesis of many monoterpene end products. It is the result of a condensation of dimethylallyl diphosphate and isopentenyl diphosphate in a reaction catalyzed by GPP synthase (GPPS). We have recently demonstrated that in snapdragon, GPPS is a heterodimer enzyme (Tholl et al. 2004). The large subunit is the catalytic subunit, while the small subunit is not catalytic but is responsible for regulating the formation of GPP and thus monoterpene formation. Snapdragon GPPS small subunit was overexpressed in tobacco under the control of the petal-specific *Lis* promoter. In contrast to wild-type tobacco plants, which produce only trace amounts of the monoterpene ocimene, transgenic plants over-expressing the snapdragon GPPS small subunit emitted 37.9 to 52 ng/g FW/hr in leaves and about 10 ng/fl/hr in flowers. However, the amount of sesquiterpenes emitted by leaves and flowers in transgenic tobacco was lower than that of the wild type. Transgenic plants with very high levels of expression of snapdragon GPPS small subunit developed bleached leaves and had delayed development and reduced stature. The amounts of carotenoids and chlorophyll in transgenic plants negatively correlated with the expression level of the introduced gene. The significant increases in monoterpene production observed in the transgenic tobacco plants suggest that the snapdragon GPPS small subunit formed a functional heterodimer with an endogenous partner, the nature of which is now under investigation.

### P-2031

Peroxisomal Metabolism of Propionic Acid and Isobutyric Acid in Plants. K. A. LUCAS, J. R. Filley, J. M. Erb, E. R. Graybill, and J. W. Hawes. Department of Chemistry and

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The sub-cellular sites of branched-chain amino acid metabolism in plants have been controversial, particularly with respect to valine catabolism. Potential enzymes for some steps in the valine catabolic pathway are clearly present in both mitochondria and peroxisomes, but the metabolic functions of these isoforms are not clear. The present study examined the possible function of these enzymes in metabolism of isobutyryl-CoA and propionyl-CoA, intermediates in the metabolism of valine and of odd-chain and branched-chain fatty acids. Using <sup>13</sup>C-NMR, accumulation of beta-hydroxypropionate from 2-<sup>13</sup>C-propionate was observed in seedlings of *Arabidopsis thaliana* and a range of other plants including both Monocots and Dicots. Examination of coding sequences and subcellular targeting elements indicated that the completed genome of *Arabidopsis thaliana* likely codes for all the enzymes necessary to convert valine to propionyl-CoA in mitochondria. However, *Arabidopsis* mitochondria may lack some of the key enzymes for metabolism of propionyl-CoA. Known peroxisomal enzymes may convert propionyl-CoA to beta-hydroxypropionate by a modified beta-oxidation pathway. The *chl1-3* mutation, creating a defect in a peroxisomal hydroxyacyl-CoA hydrolase, abolished the accumulation of beta-hydroxyisobutyrate from exogenous isobutyrate, but not the accumulation of beta-hydroxypropionate from exogenous propionate. The *chl1-3* mutant also displayed a dramatically increased sensitivity to the toxic effects of excess propionate and isobutyrate, but not of valine. <sup>13</sup>C-NMR analysis of *Arabidopsis* seedlings exposed to U-<sup>13</sup>C-valine did not show an accumulation of beta-hydroxypropionate. These data suggest that peroxisomal enzymes for a modified beta-oxidation of isobutyryl-CoA and propionyl-CoA could function for metabolism of substrates other than valine.

### P-2032

ETHE1, a GLX2-like Protein, is Essential for Embryo/Endosperm Growth and Development in *A. thaliana*. M. M. HOLDORF and C. A. Makaroff. Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056. E-mail: megs906@gmail.com

It has been shown that mutations in human ETHE1 are the cause of Ethylmalonic Encephalopathy (EE), a complex metabolic disease known to affect the brain, gastrointestinal tract, and peripheral vessels. ETHE1 shows the greatest similarity to glyoxalase II enzymes, but does not exhibit glyoxalase II activity. The *Arabidopsis* homolog of ETHE1 displays 58% amino acid identity with the human protein. *Arabidopsis* ETHE1 loss of function mutants showed ~25% seed abortion in *AtEthe1*<sup>+/-</sup>

sliques, suggesting that the mutation may cause embryo lethality. Analysis of seed development in *AtEthe1*<sup>+/-</sup> sliques confirmed this hypothesis and demonstrated a delay in both embryo and endosperm development and ultimately arrest of the embryo after the heart stage. A SEM analysis also identified alterations in the coat of *AtEthe1*<sup>+/-</sup> seeds. Our results demonstrate that ETHE1 is an essential enzyme in plants and are providing insights into the metabolic role(s) of this important enzyme.

### P-2033

Resveratrol Production in Transgenic Hairy Root Culture of Peanut, *Arachis hypogaea* L. YONG-KYUNG KIM and Sang-Un Park. Division of Plant Science & Resources, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, KOREA. E-mail: jg1015@hanmail.net

Peanut (*Arachis hypogaea* L.), belonging to the Leguminosae family, is an annual oil seed and a legume native to South America but now grown in diverse environments in whole world. Resveratrol (trans-3,5,4'-trihydroxystilbene) is present in a wide variety of plants and peanut (*Arachis hypogaea* L.) is one of the potent natural sources of resveratrol. Resveratrol is a potent chemical and studies show it has anti-inflammatory, antioxidant, anti-infective properties, and it has promising therapeutic activity in various cancers, including breast, prostate, and neuroblastoma. An efficient protocol for the establishment of transgenic peanut (*Arachis hypogaea* L.) root cultures using *Agrobacterium rhizogenes* 15834 is reported. To characterize the putative transgenic roots, explant tissues were co-cultivated with *A. rhizogenes* strain 15834 carrying the pBI121 binary vector. Except for the co-cultivation medium, all formulations included 50 mg L<sup>-1</sup> kanamycin to select for transformants and 200 mg L<sup>-1</sup> timentin to eliminate the *Agrobacterium*. Four weeks after infection, kanamycin-resistant roots appeared on 90% of explants maintained on hormone-free medium. Isolated hairy roots were propagated in liquid medium to promote rapid growth. Detection of the neomycin phosphotransferase gene, high levels of  $\beta$ -glucuronidase(GUS) transcripts and enzyme activity, and GUS histochemical localisation confirmed the integrative transformation of root cultures. Transgenic root culture of *Arachis hypogaea* L. is a simple, reliable and well-defined model system to investigate the molecular and metabolic regulation of resveratrol biosynthesis, and to evaluate the genetic engineering potential of this important plants.

### P-2034

Expression of a Pharmacologically Important Terpenoid Metabolic Pathway from *Artemisia annua* in *Solanum*

*lycopersicon*. PATRICK R. ARSENAULT<sup>1</sup>, Pamela J. Weathers<sup>1,2</sup>, Kristin L. Wobbe<sup>1</sup>. <sup>1</sup>Worcester Polytechnic Institute, Worcester, MA 01609 and <sup>2</sup>Arkansas Bioscience Institute, Arkansas State University, State University, AR 72467. Email: parsenau@wpi.edu

The malaria parasite remains a persistent threat to world health and artemisinin drugs from the shrub, *Artemisia annua*, are the sole therapeutic agents to which the parasite, *Plasmodium falciparum*, has not developed a resistance. However, production is low in planta and synthetic methods are not yet cost effective. This work was conducted in an effort to secure a cost-efficient and convenient method for artemisinin therapies to be produced. Three genes from the artemisinin biosynthetic pathway of *A. annua* were cloned into a plant expression vector under the control of fruit specific promoters of *S. lycopersicon* (tomato). These include farnesyl pyrophosphate synthase (FPS) that catalyzes the conversion of isopentyl diphosphate (IPP) into farnesyl diphosphate, amorpha diene synthase (ADS) that catalyzes the products of FPS into amorpha-4, 11- diene, and finally a cytochrome P450 (CYP71AV1) that catalyzes three oxidation steps resulting in the production of artemisinic acid. Furthermore, while transformation was conducted of the nuclear genome, protein products are targeted to plastids in an effort to shunt available carbon from other secondary metabolites. Levels of expression of the introduced genes and quantification of their products will be shown.

### P-2035

Artemisinin is Produced by Both the Mevalonate and Non-mevalonate Arms of the Terpenoid Biosynthetic Pathways and Is Increased by Inhibition of Sterol Biosynthesis. PAMELA J. WEATHERS<sup>1,2</sup>, Melissa J. Towler<sup>2</sup>. <sup>1</sup>Arkansas Bioscience Institute, State University, AR 72467 and <sup>2</sup>Dept. of Biology/Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609. Email: pweathers@astate.edu, eeyore@wpi.edu

Terpenoid biosynthesis in plants uses two isopentenyl diphosphate (IPP)-generating pathways: the cytosolic mevalonate pathway, and the plastidic non-mevalonate pathway. By using inhibitors specific to each pathway, one can determine which supplies the IPP to an end product. Artemisinin, a potent antimalarial sesquiterpene lactone, is produced in low quantities by the plant *Artemisia annua*. The source and regulation of the IPP used in its biosynthesis has not been completely characterized, so we chose to study the effects of inhibiting each of the two pathways leading to IPP on artemisinin production in plants. After growing seedlings (7–14 days post cotyledon)

in liquid culture with either mevinolin to inhibit the mevalonate pathway, or fosmidomycin to inhibit the non-mevalonate pathway, artemisinin was measured 7–14 d later. Artemisinin production was reduced by each inhibitor compared to controls, thus showing that IPP from both pathways is used in the production of artemisinin. The physiological effects of the inhibitors (chlorosis, stunted roots) were much more noticeable in the younger plants. To determine whether artemisinin and sterols are also coordinately controlled, we attempted to channel carbon away from sterols and into sesquiterpenes by growing seedlings in miconazole, an inhibitor of sterol biosynthesis, resulting in a significant increase in artemisinin compared to controls. Together these results show that artemisinin is biosynthesized from IPP pools originating in both the plastid and the cytosol, and that channeling of carbon can be directed away from competing sterol pathways and towards sesquiterpenes.

### P-2036

In Vitro Tissue Culture for Sustainable Use of Medicinal Plants. CHUNZHAO LIU<sup>1, 2</sup> and Pamela J. Weathers<sup>1,3</sup>. <sup>1</sup>Arkansas Biosciences Institute, Arkansas State University, State University, AR 72467-0639; <sup>2</sup>National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, CHINA; and <sup>3</sup>Worcester Polytechnic Institute, Worcester, MA 01609. Email: czliu@home.ipe.ac.cn

The increased interest in natural remedies has brought about the great challenge of maintaining a balance between the demands for plant-based medicines with the need to protect medicinal biodiversity. A series of problems with medicinal plant products have prompted the introduction of regulations to ensure their quality and safety. These problems have included contamination by biological and environmental pollutants, quantitative and qualitative variations of bioactive compounds, adulteration with misidentified species, and unsustainable harvest. The development of effective cultivation technologies that define plant yield in terms of both biomass and medicinally active phytochemicals is, thus, important for long-term sustained use and conservation of medicinal plants. Use of our in vitro-in vivo culture systems provides large quantities of plant tissues with optimized medicinal content. We will present recent work on 1) establishment of efficient in vitro tissue culture systems of elite medicinal species including *Rhodiola fastigiata*, *Saussurea involucrata*, *Hydrastis canadensis*, *Echinacea purpurea*, and *Artemisia annua* for the creation of germplasm banks of living medicinal plant tissues, mass-propagation, and long-term storage in a suspended physiological state; 2) design, manufacture and

microenvironment optimization of novel bioreactor systems including: modified airlift for hairy roots, temporary immersion for plantlets, turbine blade for immobilized callus, and nutrient mist for adventitious shoots and hairy roots for the production of high-quality plantlets and valuable phytochemicals.

### P-2037

Expression of *Arabidopsis* Phytochelatin Synthase (*AtPCS1*) in Indian Mustard (*Brassica juncea*) Enhances As and Cd Tolerance. K. GASIC and S. S. Korban. Department of Natural Resources and Environmental Sciences, University of Illinois, 1201 W. Gregory Dr. Urbana, IL 61801. Email: kgasic@uiuc.edu, korban@uiuc.edu

Phytochelatin (PCs), posttranslationally-synthesized peptides, play important roles in detoxification of heavy metals and metalloids in plants and other living organisms by chelating these substances and reducing their free concentration. This study reports on developing transgenic plants with increased tolerance for and accumulation of heavy metals and metalloids from soil by expressing an *Arabidopsis thaliana AtPCS1* gene, encoding phytochelatin synthase (PCS), in Indian mustard (*Brassica juncea* L.). Tolerance to and accumulation of cadmium (Cd) and arsenic (As) have been analyzed in Indian mustard plants expressing a FLAG-tagged *AtPCS1* gDNA (*pcs* lines), under its native promoter, and compared with wild-type plants. Significantly higher tolerance to Cd and As has been observed in transgenic Indian mustard plants when compared to wild-type plants. Cd-treated *pcs* plants had significantly higher concentrations of PCs and thiols in shoots than those of wild-type plants. However, shoots of wild-type plants accumulated significantly higher Cd levels than transgenic plants. Whereas, accumulation of As in transgenic plants was similar to that in wild type plants. Although phytochelatin synthase improves the ability of Indian mustard to tolerate higher levels of the heavy metal Cd and the metalloid As, it does not increase the accumulation potential of these metals in the above ground tissues of Indian mustard plants.

### P-2038

*Agrobacterium*-mediated Transformation of Immature Cotyledons of Two Soybean Genotypes. S. VIMOLMANGKANG, K. Gasic, and S. S. Korban. Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801. Email: svimolm2@uiuc.edu, kgasic@uiuc.edu, korban@uiuc.edu

Frequency of somatic embryogenesis and transformation of soybean [*Glycine max* (L.) Merr] cultivars Jack and Kunitz,

representing maturity groups II and III, respectively, were evaluated. Immature cotyledons, 5–6 mm and 7–8 mm in size, were co-cultivated with the binary *Agrobacterium* strain KYRT1 carrying a partially-disarmed virulence helper plasmid. Explants were then incubated, with their adaxial side in contact with the medium, on a hygromycin-containing selection medium. Both cultivars were found to be highly embryogenic, but this varied depending on the size of the explant. The embryogenic response of cv. Jack was significantly higher when explants of 7–8 mm in size were used; whereas, embryogenic response of cv. Kunitz was higher when explants were 5–6 mm in size. Confirmed transgenic lines from both cultivars were obtained and analyzed for presence and expression of the transgene. Overall, this study demonstrated the influence of the size of immature cotyledons on transformation and recovery of transgenic somatic embryos of different soybean cultivars.

#### P-2039

Production of Herbicide Resistant Sweetpotato Plants through *Agrobacterium*-mediated Method. K-M. KIM, H. J. Choi and H-Y. Lee. Subtropical Horticulture Research Center, Cheju National University, Jeju 690-756, KOREA. E-mail: kmkimus@hanmail.net

Herbicide-resistant sweetpotato plants were produced through *Agrobacterium*-mediated method using embryogenic calli derived from shoot apical meristems. Plant materials were infected with the plasmid vector containing the  $\beta$ -glucuronidase gene (*gusA*) and the herbicide-resistant gene (*bar*). Selection was carried out using phosphinothricin (PPT). Transgenic plants were screened by the histochemical GUS and chlorophenol red assays for initial confirmation. PCR and Southern-blot analyses revealed the presence of the introduced *bar* gene in the genome of the transgenic plants. Sweetpotato plants showed tolerance when sprayed with the herbicide Basta (900 mg/l–1 glufosinate). Hence, we report successful transformation of sweet potato using *bar* gene conferring herbicide resistance. *This work was supported by a grant (code #20050401-034-750-142-03-00) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.*

#### P-2040

*Agrobacterium rhizogenes* as a New Highly-efficient Stable Soybean Transformation Tool. M. J. KIM, Veena, R. Collier, and C. Taylor. Donald Danforth Plant Science Center, Saint Louis, MO 63132. Email: mkim@danforthcenter.org

*Agrobacterium tumefaciens* and biolistics are the most commonly used methods to insert foreign genes into

soybean (*Glycine max*). Soybean transformation has been limited by its poor transformability using *A. tumefaciens* and the cotyledonary-node method, typically with efficiencies between 3–5%. The greatest limiting step in transgenic soybean production is regeneration, but we set out to determine if we can make a substantial improvement in the efficiency of transformation by using other/variant strains of *Agrobacterium*. In our laboratory, we created a new disarmed strain of *Agrobacterium* using the highly virulent *A. rhizogenes* strain K599. We have shown previously that this new disarmed strain of *Agrobacterium* (we now call 18r12v) shows increased transformation efficiencies in tomato, tobacco, and *Arabidopsis*. We will present data on transformation efficiencies of the *A. rhizogenes* strain 18r12v and *A. tumefaciens* strain Chry5 in soybean (cv. Jack, Hutchinson, and Thorne) using the cot-node method of transformation with glufosinate as selection. Transformation with *A. rhizogenes* 18r12v resulted in 6-fold increase in transformation efficiency after 6 weeks of post-inoculation compared to *A. tumefaciens* strain Chry5. Glufosinate-resistant plantlets were isolated and their transgenic nature further confirmed using a GUS histochemical assay to detect the presence of the GUS enzyme encoded by the *uidA* gene that was linked to the *bar* selectable marker. At rooting, transformation efficiencies for the disarmed *A. rhizogenes* 18r12v were as high as 18%. Final transformation efficiencies are currently being determined for eight independent replicated experiments. Transgenic soybean plantlets were transferred to the greenhouse and were able to set seed. We are now in the process of performing Southern hybridization on the genomic DNA from those T1 plants to characterize transgene integration. The results of this study provide data that *A. rhizogenes* 18r12v is by far a more efficient transformer of soybean using the cot-node method resulting in an overall increase in soybean transformation rates.

#### P-2041

Sorghum Biotechnology Program at the University of Nebraska. NATALIA NERSESIAN<sup>1</sup>, Tejinder Mall<sup>1</sup>, Kaimei Xu<sup>1</sup>, Mike Irvin<sup>1</sup>, Arlene Howe<sup>2</sup>, Ismail Dweikat<sup>1</sup>, Mike Fromm<sup>1,3,4</sup>, Tom Clemente<sup>1,3,4</sup>. <sup>1</sup>Department of Agronomy, University of Nebraska-Lincoln, <sup>2</sup>Monsanto <sup>3</sup>Center of Biotechnology, University of Nebraska-Lincoln, <sup>4</sup>Plant Science Initiative, University of Nebraska-Lincoln 68588. Email: nnersesian2@unl.edu

An *Agrobacterium*-mediated transformation protocol for sorghum using *nptII* as the selectable marker gene has been successfully implemented with two sorghum genotypes, TX430 and C2-97. We are currently exploiting this system

to introduce novel input and output traits to the crop. With respect to the former, we are characterizing transgenic events carrying the cyanamide hydratase gene (*cah*) which provides tolerance to the herbicidal activity of the nitrogen fertilizer Ca-cyanamide, and transgenes reported to impart abiotic stress tolerance, an Arabidopsis glycine rich RNA binding protein, *atRZ-1a* and a rice Ca-dependent protein kinase, *OsCDPK-7*. As per the latter we are characterizing sorghum transformants harboring lysine insensitive genes, *dapA* and *lysCM4* from *Cornebacterium* and *E. coli*, respectively, designed to enhance lysine content in the seeds. Plant expression cassettes were assembled and subcloned into the binary plasmid pPZP212. The final binary plasmids were mobilized into *Agrobacterium tumefaciens* strain NTL4/pKPSF2. Primary transformants were characterized for *nptII* expression using a commercially available ELISA kit (Agdia). The *nptII* positive events were further characterized by Southern and Northern blot analyses. Selected lead events are being selfed to homozygosity and monitored in greenhouse and growth chamber conditions for tolerance to the commercial formulation of Ca-cyanamide, enhanced response to cold induced stress and altered amino acid profile in the seeds.

#### P-2042

Identification of RNA Interference Induced Soybean Cyst Nematode (*Heterodera glycines*) resistance in *Glycines max* Using a Hairy Root Bioassay. JIARUI LI, Timothy C. Todd, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Email: [hnt@ksu.edu](mailto:hnt@ksu.edu)

Soybean is the second largest crop in the United States, but annual losses related to the soybean cyst nematode (*Heterodera glycines*; SCN), the most damaging pest of soybean were estimated \$1 billion in the U.S. Current methods to control this pest are not totally successful partly because of new SCN biotypes are emerging that overcome resistant cultivars. Recently our laboratory has described an approach using RNA interference as a possible method for control of SCN in transgenic soybean. The current bottleneck in our evaluation of novel genetic sequences is the production of transgenic plants. To optimize our efforts we are evaluating the use of producing chimeric seedlings expressing siRNAs against specific genes in transgenic hairy roots. The tap roots from four-day old seedlings (cultivars 'Jack' and 'KS4704') were excised and hairy roots were induced from hypocotyls by *Agrobacterium rhizogenes* mediated transformation. Inoculated hypocotyls were selected in a MS-based medium containing either 200 mg/L kanamycin, or 15 mg/L hygromycin. Although both antibiotics produced transgenic hairy roots, selection

based on kanamycin appeared to be more effective. Three separate genes related to nematode reproduction or fitness were selected and amplified by PCR using degenerate primers and gene fragments were cloned into separate pANDA vectors using a Gateway cloning strategy. Using this hairy root system, the RNAi constructs of the three genes were transformed into soybean. Confirmation of transformation was attained by PCR and southern-blot analysis. Small interference RNA (siRNA) detection and SCN bioassays of transgenic roots will be discussed.

#### P-2043

Small Interfering RNAs (siRNA) to Control the Soybean Cyst Nematode (*Heterodera glycines* Ichinohe). WILLIAM R. DALL'ACQUA; Timothy C. Todd, Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Email: [wdacqua@ksu.edu](mailto:wdacqua@ksu.edu)

The soybean cyst nematode (*Heterodera glycines*) is an important pest in soybean production throughout the United States and around the world, causing annual yield losses amounting of billions of dollars worldwide. Although efforts to control soybean cyst nematode have traditionally been based on sanitation, conventional plant breeding and crop rotation, these methods have not been totally effective. However, new methods of control are now emerging and potentially bringing new ways to control plant parasitic nematodes. In this study, we utilize RNAi as a method for resistance against soybean cyst nematode by expressing double-stranded RNA (dsRNA) in roots, homologous to essential mRNA transcripts found in *Heterodera glycines*. Candidate genes that caused sterility and/or embryo lethality when knocked out in *Ceanorhabditis elegans* were selected from WormBase (<http://www.wormbase.org>) and their analogs were identified in the nematode EST's (Expressed Sequence Tags) (<http://www.nematode.net>) genetic data base using the NemaBlast search tool. High similarity sequences found were further selected for primers design. Sets of primers were designed to amplify the spliced 3'-end portion of each of the candidate genes based on genomic DNA from *Heterodera glycines*. The PCR products were sequenced, checked and used to design sense-antisense RNAi constructs. The sense-antisense constructs were sub-cloned into expression vectors for transformation into soybean. Transformation was accomplished via particle bombardment for one RNAi construct (MSPi), and via *Agrobacterium rhizogenes* for the other RNAi constructs. Soybean lines expressing the MSPi construct are still being evaluated by molecular analyses and bioassays. Data shows decreasing number of cyst, eggs, and juveniles in the MSPi lines when compared to non-transgenic susceptible line. Hairy roots produced from the other

constructs will also be checked for siRNAs molecules. Molecular analysis and bioassays of these will be discussed.

#### P-2044

Development of an In Planta Bioassay for the Evaluation of RNAi Constructs Directed Against Soybean Cyst Nematodes (*Heterodera glycines*). BRANDON L. VAN ALLEN, Jiarui Li, Timothy C. Todd, and Harold N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66502. Email: lbv6333@ksu.edu

The soybean cyst nematodes (SCN) are the primary biotic factor limiting soybean production in the United States. Recent yield loss estimates due to SCN in the U.S. exceeded 7.5 million metric tons and much of this loss was concentrated in the nation's principal north central soybean production region where 50–80% of fields are infested. Current methods of control of this plant parasite through rotation and the use of natural genetic resistance are not totally effective. Recently our laboratory has described an approach using RNA interference as a possible method for control of SCN in transgenic soybean. With our current soybean transformation protocols, it takes over nine months to develop transgenic soybean lines expressing these vectors. To expedite the analysis of new traits, we are evaluating novel in planta assay system for the expression of SCN-specific RNAi molecules. Our assay system is based on the production of hairy roots on soybean hypocotyls, regenerating the chimeric seedlings and using these seedlings for SCN bioassays. Two strains of *Agrobacterium rhizogenes*, R1000 and K599 with a binary vector containing the GFP and HPT genes in the T-DNA, were evaluated to produce hairy roots on soybean plants. Hairy roots were produced by injecting the *Agrobacterium* into the hypocotyls of 4 day old seedlings and, after co-cultivation, the hypocotyls were rooted on Hygromycin containing medium. GFP expression was also used to confirm the presence of hairy roots. The current transformation efficiency is 10%. Further molecular analysis, the progress made to date, and future applications of this technique will be discussed.

#### P-2045

Generation and Characterization of Bahiagrass (*Paspalum notatum* Flugge) Over-expressing a Gibberellin-catabolizing Enzyme. M. AGHARKAR, H. Zhang, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL 32611. Email: faltpeter@ifas.ufl.edu

Bahiagrass is a low input, drought tolerant and disease resistant warm season turfgrass used for residential lawns and along highways in the Southeastern US. Turf quality of bahiagrass is compromised by prolific seedhead production, open growth habit and light green color. Gibberellins are plant hormones involved in a number of processes including apical dominance and stem elongation. The objective of this study was to improve the turf quality of bahiagrass by over-expression of a gibberellin catabolizing enzyme, Gibberellin 2-oxidase (GA-2ox). *GA-2ox1* ORF was isolated from Arabidopsis and sub-cloned under the control of the constitutive ubiquitin or 35S promoters. Co-transfer of minimal, constitutive *nptII* and *GA-2ox1* expression cassettes without vector backbone into seed derived callus cultures from turf-type, apomictic bahiagrass (cv. 'Argentine') was carried out by biolistic gene transfer. Eight putative transgenic lines were regenerated from 600 bombarded calli. Transgenic nature of the regenerated plants and their seed progeny was indicated by NPTII ELISA (Agdia), PCR and RT-PCR. Southern blot analysis of plants regenerated from independent callus lines showed a simple and independent *GA-2ox1* integration pattern. Northern blot analysis allowed quantification of *GA-2ox1* expression levels and its correlation with phenotypic changes. Phenotypic characterization of the transgenic lines was carried out under controlled environment conditions in a hydroponics set up as well as in soil. Over-expression of *GA2-ox1* in bahiagrass resulted in transgenic plants with reduced height and increased tillering, while root length was not affected in most of the transgenic plants as indicated under hydroponics conditions. Data correlating *GA2-ox1* expression with plant height, tillering, seedhead emergence and seedhead length of plants grown in soil under controlled environment conditions will be presented.

#### P-2046

Development of a Novel Selectable Marker Gene for Transformation of Maize (*Zea mays* L.). N. L. ARNOLD, T. R. Wright, C. J. Clifford, L. L. Schulenberg, L. M. Rowland, A. C. Miller, C. M Dewes, J. M. Lira, A. Worden, S. E. Worden, M. A. Simpson, K. Y. Yau, P. Jayakumar, A. M. Palta, J. F. Petolino, and D. R. Pareddy. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268. Email: NLArnold@dow.com

Selectable marker genes are critical for genetic engineering of plants as they allow for selection of rare, transformed cells. Although dozens of selectable marker genes have been identified for research and commercial purposes over the past several decades (Miki and McHugh, 2004, J. Biotechnology, 193–232), efficient, non-antibiotic markers

are uncommon. Here, we describe a novel, herbicide-resistant selectable marker gene for transformation of maize. A proprietary selectable marker gene that confers resistance to various aryloxyphenoxypropionates (AOPPs or 'fops') has been identified, cloned, and tested for in vitro selection. This selectable marker gene was driven by the maize ubiquitin1 promoter and delivered into embryogenic suspension cultures and immature zygotic embryos of Hi-II via silicon carbide WHISKERS<sup>TM</sup> and *Agrobacterium tumefaciens*, respectively. R-haloxypop was used as the selection agent for transformation of both suspension cultures and immature embryos. Callus events isolated from selection were confirmed to be transgenic via PCR and Southern analyses. Fertile, transgenic plants were produced following regeneration on haloxypop-containing media. This gene provides a novel tool for efficient in vitro selection of maize, but can also be used for screening of transgenic plants in the greenhouse and field.

#### P-2047

Expression of a Synthetic  $\delta$ -endotoxin from *Bacillus thuringiensis* in Bahiagrass (*Paspalum notatum* var. Flugge) to Enhance Resistance against Fall Armyworm. G. LUCIANI, F. Altpeter, J. Yactayo-Chang, H. Zhang, M. Gallo, R. Meagher, and D. Wofford. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL 32611. Email: faltpeter@ifas.ufl.edu

Bahiagrass (*Paspalum notatum* var. Flugge) is the predominant forage grass in the southeastern US. Its popularity is a consequence of low maintenance requirements along with high tolerance to stress including drought, heat and overgrazing. However, bahiagrass is susceptible to pests such as fall armyworm (*Spodoptera frugiperda* J. E. Smith) which can cause significant economic losses. We recently established a bahiagrass transformation protocol which allows now the introduction of insect resistance genes. The objective of this study was to express an optimized *cry* gene encoding a  $\delta$ -endotoxin from *Bacillus thuringiensis* to enhance resistance against fall armyworm in bahiagrass.

An optimized *Bt cry* gene was synthesized and subcloned under the control of the constitutive *ubi1* promoter from maize. Transgenic lines were generated following biolistic gene transfer of the *nptII* gene and the *cry* gene expression cassettes into mature seed derived callus and regeneration of transgenic plants on media containing paramomycin. PCR and Southern blot analysis confirmed the presence and independent integration pattern of the synthetic *Bt cry* gene in six transgenic lines. RT-PCR analysis confirmed the transgene expression. Insect bioassays with three different transgenic lines in comparison with wildtype showed that neonate

survival rate was significantly lower if larvae were fed with leaves from transgenic lines. ELISA analysis for quantification of *Bt cry* protein in leaf extracts indicated a positive correlation of *Bt cry* protein expression and insect resistance.

Data on transgene integration patterns, transgene expression levels and insect resistance will be presented.

#### P-2048

Generation, Characterization and Risk Assessment of Transgenic, Herbicide Resistant Forage and Turf Grass (*Paspalum notatum* Flugge). S. SANDHU<sup>1</sup>, F. Altpeter<sup>1</sup>, and A. Blount<sup>2</sup>. <sup>1</sup>Agronomy Department, PMCB, Genetics Institute, University of Florida - IFAS, Gainesville, FL 32611 and <sup>2</sup>Agronomy Department, North Florida Research and Education Center, University of Florida - IFAS, Marianna, FL 32446. Email: faltpeter@ifas.ufl.edu

Bahiagrass (*Paspalum notatum* Flugge) is one of the most important forage and low-input turf grasses in the southeastern United States. However, its open growth habit facilitates weed encroachment and its low tolerance to commercially available herbicides complicates weed management. 'Argentine', a commercially important bahiagrass cultivar was chosen for genetic transformation to incorporate glufosinate herbicide resistance. Its apomictic mode of reproduction should allow production of uniform seed progeny and might reduce the risk of unintended gene dispersal by pollen.

To further investigate this, we co-transformed minimal unlinked *nptII* and *bar* expression constructs (MC's) into bahiagrass callus by biolistic gene transfer. The vector backbone was removed prior gene transfer to enhance co-expression and expression stability and eliminate the prokaryotic antibiotic resistance expression cassette. Twenty-one *nptII* expressing plants were confirmed by ELISA following selection of 300 bombarded calli on paramomycin containing media. Relatively simple integration patterns for the *nptII* gene, and higher copy numbers and more complex integration patterns for the non-selected *bar* gene were detected by Southern blot analysis. Consistent with earlier reports the co-integration and co-expression frequency of the unlinked MC's was higher than 90%. Integration of quantitative ELISA-, Southern blot- and herbicide resistance data under greenhouse and field conditions indicated that MC's support high level expression of the *bar* gene and resistance to high glufosinate application rates. Molecular analysis of the seed progeny of the transgenic lines, will also be presented. Glufosinate resistance in apomictic 'Argentine' bahiagrass was used as a marker to study pollen-mediated intraspecific gene flow from transgenic apomictic 'Argentine' bahiagrass to wild type diploids under field and greenhouse conditions. Data

on herbicide resistance of seed progeny, intraspecific gene transfer frequencies, ploidy, fertility and transgene integration patterns of gene transfer events will be presented.

#### P-2049

Comparative Field Evaluation of Turf-type Bahiagrass with Over-expression of a Gibberellin Catabolizing Enzyme or a Repressor of Cell Expansion. P. N. LOMBA, F. Altpeter, M. Agharkar, H. Zhang, K. Kenworthy, and T. Sinclair. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL 32611. E-mail: faltpeter@ifas.ufl.edu

Bahiagrass (*Paspalum notatum* Flugge) is a low-input turfgrass widely grown in the southeastern United States and other subtropical regions around the world. Bahiagrass' popularity is attributed to its strong persistence, supported by its drought and heat tolerance and resistance against most insects and diseases. However, its turf quality is compromised by prolific production of long seedheads, and its open growth habit. Improvement of the predominant turf-type bahiagrass cultivar 'Argentine' by conventional breeding is very difficult due to its apomictic reproduction mode. A genetic transformation protocol was recently established for Argentine bahiagrass and offers now the opportunity to improve its turf quality. Two transgenic strategies were explored for improving Bahiagrass' turf quality. Desired phenotypes with reduced height and increased tillering were observed under controlled environmental conditions following reduction of bioactive gibberellic acid, by over-expression of a gibberellin-catabolizing enzyme, GA 2-oxidase1 from *Arabidopsis* (AT-GA-ox1). The second strategy involving the over-expression of a repressor of cell expansion, *ATHB16* from *Arabidopsis* resulted in phenotypes with more narrow leaves in addition to dwarfing and more vegetative tillers, as observed under controlled environment conditions. Lines over-expressing *ATHB16* or *AT-GA-ox1* were further evaluated under two different mowing regimes and three different irrigation treatments in four replications under field conditions at the PSREC in Citra Fl. Data on establishment, irrigation requirements, turf density, clipping weight, chlorophyll content, seed head production and length, long-term expression and stability of transgenes, and persistence in comparison to wildtype Argentine bahiagrass and St. Augustine grass will be presented.

#### P-2050

Somatic Embryogenesis and Genetic Transformation in *Capsicum baccatum* (Willd.) Eshbaugh. SUBHASH KARAMPURI, Venkataiah Peddaboina, Christopher Thamidala, and

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A plant regeneration protocol via somatic embryogenesis was achieved in cotyledon and leaf explants of *Capsicum baccatum* PI 260434, when cultured on MS medium supplemented with various concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D, 0.5 - 5.0 mg l<sup>-1</sup>) in combination with Kinetin (Kin, 0.5 mg l<sup>-1</sup>) and 3% sucrose. The range of somatic embryogenesis frequency depends upon the 2,4-D concentration in combination with Kin. The best response was observed on MS medium containing 2,4-D at 2.0 mg l<sup>-1</sup> along with Kin at 5.0 mg l<sup>-1</sup>. Leaf explants are more pronounced in somatic embryogenesis than cotyledon explants. Various stages were observed during the development of somatic embryos, including globular, heart-shaped, torpedo-shaped, and early cotyledonary stages. Maturation and germination of somatic embryos was achieved by transferring isolated somatic embryos to MS medium containing various concentrations of Benzyl adenine (BA), among various treatments, BA at 1.0 mg l<sup>-1</sup> found to be best for germination of maximum number (14%) of somatic embryos into complete plantlets. The somatic embryogenesis a protocol was used for *Agrobacterium*-mediated genetic transformation of *C. baccatum*. *A. tumefaciens* strain LBA4404, harbouring pCAMBIA-1301 carries the hygromycin phosphotransferase (hpt) gene and  $\beta$ -glucuronidase (gus), was used for co-cultivation with cotyledon and leaf explants. Co-culturing of explants with *Agrobacterium* for 30 minutes, followed by co-cultivation with 0.1 mM acetosyringone for 3 days was found to be optimum for maximum transformation efficiency. Transient *GUS*  $\beta$ -glucuronidase) gene expression was used to monitor T-DNA delivery into the target cells. A very high frequency (65%) of *GUS* gene expression was obtained following *Agrobacterium*-mediated gene transfer into regenerative explants. The standardized protocol would be useful for *Agrobacterium*-mediated genetic transformation of *C. baccatum* with genes of agronomic importance.

#### P-2051

Spectral Evaluation of Red Fluorescent Protein Variants. ANN GOULDING, Suresh Shrestha, and Sapna K. Deo. Department of Chemistry and Chemical Biology, Indiana University - Purdue University, Indianapolis, Indianapolis, IN 46202. Email: agouldin@iupui.edu

A red fluorescent protein DsRed, originally isolated from the *Discosoma* species of coral reef has become a useful reporter for biosensing studies. Its red emission is an advantage because of low cellular auto-fluorescence in this

region of the spectrum, but this protein is tetrameric in nature and has tendency to form aggregates. Many genetic modifications have been performed on DsRed to overcome its drawbacks, including the commercial availability of a monomeric form (DsRed-monomer), which does not form aggregates. The relationship between structure and function of fluorescent proteins has been explored by site directed mutagenesis; however a second avenue of study is also available, this second approach involves the incorporation of non-natural amino acid analogues into the protein by a system of forced biochemical incorporation. In this work two such analogues of tyrosine have been incorporated into a DsRed monomer; 3-amino-L-tyrosin and 3-fluoro-L-tyrosine. Tyrosine analogues were chosen due to the role of tyrosine in the formation and structure of the protein's chromophore. The incorporation of these analogues appears to effect the overall conjugated electron system of chromophore, responsible for the observed fluorescence of DsRed. Fluorescence excitation and emission spectra were recorded of both native DsRed and the two mutants, no shift in excitation was observed for either of the mutants. However, red and blue shifts were seen for the emission spectra of the amino- and fluoro-tyrosine analogues, respectively. The CD spectra and UV-visible spectra of mutants and native protein were obtained. The spectra were comparable indicating no effect of non-natural amino acids on the overall structure of the protein.

#### P-2052

The Comparison of Two Novel Constitutive Promoters for the Transgene Expression of a Selectable Marker in Maize. M. E. WELTER, B. C. Rubin-Wilson, J. W. Bing, R. C. Blue, K. N. Curlee, K. S. Franklin, T. L. Strange Moynahan. Dow AgroSciences, Indianapolis, IN 46268. Email: mwelter@dow.com

Only a small number of cells are integratively transformed in plant tissues or plant explants with current plant transformation methodologies. Thus, it is crucial to be able to selectively propagate the transgenic cells. The most widely used method for screening of such cell populations is to express a selectable marker gene driven by a strong, constitutive promoter. The choice of the selectable marker and the expression level appear to be critical for optimal recovery of stable transgenic events. A minimum level of selection is required to inhibit the growth of non-transgenic cells thereby restricting the generation of escapes (non-transgenic isolates); however, the selection level should not be so high such that the recovery of stable transgenic events is in any way jeopardized. The rice actin-1 promoter has been characterized as a strong constitutive promoter. The present

study compared the efficacy of two alternative constitutive promoters (actin depolymerization factor [ADF] and aldolase both cloned from maize) to rice actin-1 relative to the ability to drive the selectable marker gene *pat*. Transgenic maize tissues were recovered from suspension cultures following WHISKER<sup>TM</sup>-mediated transformation and Herbiace<sup>TM</sup>-resistant plants were regenerated. In vitro selection efficiency and field tolerance were evaluated for transgenic events comprising the various promoters driving the *pat* gene. Both ADF and aldolase were shown to drive sufficient levels of expression of the *pat* gene in terms of transgenic tissue recovery and field tolerance.

#### P-2053

Zinc Finger Nuclease-Mediated Homologous Recombination in Tobacco Cell Cultures. LISA BAKER<sup>1</sup>, Charles Cai<sup>1</sup>, Robbi Garrison<sup>1</sup>, Jeffrey Miller<sup>2</sup>, Fyodor Urnov<sup>2</sup>, and Joseph Petolino<sup>1</sup>. <sup>1</sup>Dow AgroSciences, LLC, Indianapolis, IN and <sup>2</sup>Sangamo Biosciences, San Diego, CA. Email: lwbaker@dow.com

Gene targeting via homologous recombination has been demonstrated to occur at relatively low frequencies in plant cells. Recently, substantial increases in the frequency of homologous recombination have been observed following the induction of double stranded breaks in the host cell DNA followed by the apparent stimulation of cellular repair mechanisms. Strategies to achieve targeted DNA double stranded breaks have been developed by fusing sequence-dependent zinc finger DNA-binding proteins with sequence-independent nuclease domains derived from Type IIS restriction endonucleases. In the present study, engineered zinc finger proteins fused to nuclease domains, so-called 'zinc finger nucleases', were used to drive homologous recombination at a specific, engineered target in tobacco cell cultures.

#### P-2054

Evaluation of Phosphomannose Isomerase Gene as Alternative to Antibiotic Resistance for Vitis Gene Transfer. I. Vaccari, V. Poletti, and L. MARTINELLI. IASMA Research Center, Genetics and Molecular Biology Department, Via E. Mach, 1 - 38010 San Michele all'Adige (TN), ITALY. Email: Lucia.Martinelli@iasma.it

Positive selection with phosphomannose isomerase (PMI) gene (Positech, Syngenta's courtesy) seems a promising strategy to transfer exogenes in plants. In grapes, however, the use of PMI as alternative to antibiotic resistance needs to be fully exploited and literature reports ambiguous results (Kieffer *et al.*, 2004, *Vitis* 43: 35–39). The most crucial aspect concerns with the possibility to clearly appreciate the

effect of mannose on the cultures related with their ability to metabolize mannose. Thus, before setting up gene transfer assays, a meticulous preliminary work is needed for assessing this aspect. Embryogenic calli of *V. vinifera* (cvs. Chardonnay, Brachetto) and the rootstock 110 Richter were subcultured respectively in the presence of mannose (treatment), sucrose (control), or no carbohydrates in the media. Efficiencies of callus proliferation and embryo differentiation were evaluated. Whereas calli on carbohydrate-free media rapidly turned brown and died, both treatments and controls showed a similar growth rate and appearance, and several months were necessary for appreciating the differences. As for the callus propagation rate, after 8 months of subcultures on mannose, calli turned brown and growth resulted less efficient. Moreover, a different behavior was noted among the genotypes, resulting Chardonnay and Brachetto more sensitive than the rootstock. As for the plant germination efficiency, isolated somatic embryos induced to conversion on mannose were unable to complete a canonic germination since aberrations such as deformations and vitrification were observed. Gene transfer assays were started to make a comparison with cultures expressing PMI with the wild types. This research is supported by the Autonomous Province of Trento, Project EcoGenEtic.Com.

#### P-2055

Using a Double Layer System to Improve Elongation in In Vitro Micropropagated Plants+. F. Serrano, M. Cano, A. Marco, A. Piqueras, and J. L. CASAS. Unidad de Biotecnología Vegetal. Instituto Universitario de Investigación CIBIO. Universidad de Alicante. Carretera de San Vicente del Raspeig, s/n. E-03690 San Vicente del Raspeig, Alicante, SPAIN. E-mail: jl.casas@ua.es

A double layer (DL) system was tested in order to improve elongation on three different in vitro cultured species, *Sorbus aria*, *Tetraclinis articulata* and *Thymus moroderi*. Explants consisting in apical segments of each species were cultured in solid medium. After three days, 2 ml of liquid medium (consisting in half-strength Knop solution containing 50 g l<sup>-1</sup> sucrose and 3 or 6 g l<sup>-1</sup> activated charcoal (AC)) were added or not (control) to the explants. Length measurements were taken at 0, 4 and 8 weeks. A length increase was observed with the application of DL in all the species. After 8 weeks, the best results were achieved with the addition of 6 g l<sup>-1</sup> AC in *T. moroderi* and *S. aria*, with an increase of 75% and 81%, respectively. In the case of *T. articulata*, there were no differences between the results obtained with the addition of 3 and 6 g l<sup>-1</sup> of AC in the DL system. However, it showed the higher length increment after 8 weeks with 142% and 135%, respectively. Shoot length

increase (cm)\* Time (weeks) Control DL + 3 g/l AC DL + 6 g/l AC *Sorbus aria* 0-4 0,66 ± 0,59 1,29 ± 0,71 1,33 ± 0,79 4-8 0,31 ± 0,59 0,33 ± 0,52 0,52 ± 1,08 0-8 0,97 ± 1,04 1,62 ± 0,99 1,81 ± 1,46 *Tetraclinis articulata* 0-4 0,41 ± 0,20 0,57 ± 0,30 0,71 ± 0,41 4-8 0,26 ± 0,18 1,05 ± 0,10 0,90 ± 0,55 0-8 0,67 ± 0,27 1,62 ± 0,77 1,58 ± 0,72 *Thymus moroderi* 0-4 2,04 ± 0,51 2,16 ± 0,63 2,90 ± 0,95 4-8 3,02 ± 0,72 3,35 ± 1,73 5,96 ± 1,29 0-8 5,06 ± 1,66 5,52 ± 2,05 8,86 ± 1,92 \*The shoot length increase was calculated as the difference between the length at the end of the time interval and its initial length. According to the results obtained from three different species, it can be deduced that: 1) the DL system is a good method to improve the elongation of in vitro cultured plants; 2) in some species, the amount of AC could play an important role in the efficiency of this system. +This work has been partially funded by INTERREG IIB MEDOCC Programme. Project: IMPACT OF CLIMATE CHANGE ON THE MEDITERRANEAN FLORA AND CONSERVATION ACTIONS (SEMCLIMED).

#### P-2056

Influence of Water Stress-induced Abscisic Acid Accumulation on the C<sub>3</sub>-CAM Transition in Pineapple Plants. L. FRESCHI, M. A. Rodrigues, E. Purgatto, and H. Mercier. Laboratory of Plant Physiology, Department of Botany, University of São Paulo, P.O. Box 11461, CEP 05422-970, São Paulo, SP, BRAZIL. Email: freschi@usp.br

Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that minimizes water loss in plants from xeric environments. The degree of expression of this photosynthetic pathway can be strongly modulated, especially in facultative CAM species, which can shift between C<sub>3</sub> and CAM photosynthesis in response to changes in environmental conditions. In our laboratory, we have shown that in vitro grown pineapple (*Ananas comosus*) plants can function as either CAM or C<sub>3</sub> plants, depending on temperature or water availability. However, little is known about the hormonal stimuli involved in this alteration of photosynthetic metabolism. Thus, in the present work, a possible relationship between endogenous levels of abscisic acid (ABA) and the induction of CAM by water stress in pineapple plants was investigated. To achieve this, 3-month-old micropropagated pineapple plants were exposed to water stress obtained by adding 30% polyethylene glycol in the growth medium, and analyzed over 30 days for endogenous ABA levels and degree of CAM expression. The extent of CAM expression was evaluated by measuring nocturnal acid accumulation and the activities of phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH) and phosphoenolpyruvate

carboxykinase (PEPCK). These analyses showed that water stress led to a 12-fold increase in ABA content within the first 7 d of treatment, which preceded the rise in acid accumulation and activities of CAM enzymes, suggesting that water stress-induced ABA accumulation could trigger CAM induction in pineapple plants. To test whether this CAM induction requires ABA accumulation, we also investigated the effects of pre-treatment of pineapple plants exposed to water stress with the ABA biosynthesis inhibitor fluridone. Although this pre-treatment completely suppressed the accumulation of ABA, it did not affect CAM induction by water stress. Altogether, these results provide evidence that water stress induction of CAM in pineapple plants seems to occur via both ABA-dependent and ABA-independent signal transduction pathways.

#### P-2057

Effect of Promoters on *Bt* Transgene Expression in *Sorghum bicolor* (Moench.). V. GIRIJASHANKAR<sup>1</sup>, V. Swathi Sree<sup>1</sup>, M. Lakshmi Narasu<sup>1</sup> and N. Seetarama<sup>2</sup>. <sup>1</sup>Center for Biotechnology, IST, Jawaharlal Nehru Technological University (J.N.T.U.), Kukatpally, Hyderabad-500072, Andhra Pradesh, INDIA and <sup>2</sup>Director, National Research Center for Sorghum (NRCS), Rajendranagar, Hyderabad, Andhra Pradesh, INDIA. Email: vgirija\_shank@yahoo.com

Transgenic sorghum plants expressing synthetic *Bt* genes, under the control of a wound-inducible (maize *proteinase inhibitor* gene regulatory region (*mpiC1*)) and a constitutive

promoter (maize *polyubiquitin1* gene promoter (*ubi1*)) were produced and analyzed. Initially, three different sets of transgenic plants were developed separately carrying synthetic *mpiC1-cry1Ac*, *ubi-cry1Ac* and *ubi-cry1Ab* via biolistic method of gene transfer. PCR and Southern blot analysis confirmed that a total of nine T<sub>0</sub> plants were found to carry the insecticidal gene *cry* in which five plants were from *mpiC1-cry1Ac* series and two each from *ubi-cry1Ac* and *ubi-cry1Ab* series. Multiple copies of each *Bt* transgene were found integrated into the genome of sorghum plants as revealed from Southern blot analysis. The amount of  $\delta$ -endotoxin produced was quantified using ELISA technique. In response to mechanical wounding, the T<sub>1</sub> generation of transgenic plants carrying *mpiC1-cry1Ac* gene expressed a maximum of 8 ng of Cry protein per gram of leaf tissue at 12 h after wounding. Whereas, the T<sub>0</sub> plants with *ubi-cry1Ab* and *ubi-cry1Ac* genes expressed the Bt toxin up to 2 and 0.55 ng/gm of leaf tissue, respectively. From this transformation work in sorghum, it was evident that *mpiC1*, a wound-inducible-promoter is 14.55-folds stronger in expressing the transgene *cry1Ac* than the so far known constitutive promoter *ubi1*. Among the two *Bt* genes (differing by approximately 5% in nucleotide sequence), *cry1Ab* is better in transgene expression in sorghum by 3.64-folds than *cry1Ac*. Contrary to the in vitro promoter comparison studies carried so far, our results for the first time show that the transgene expression in the greenhouse established sorghum plants (produced by biolistic transformation) was in the order of *mpiC1cry1Ac* > *ubicry1Ab* > *ubicry1Ac*. However, further studies are needed to confirm the better expression of transgenes by *mpiC1* promoter.