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Plant Posters

P-2000

Secondary Metabolism of *Hypericum perforatum* Induced by *Agrobacterium rhizogenes*. E. R. SANTAREM, D. C. Zamban, L. M. Felix, and L. V. Astarita. Laboratory of Plant Biotechnology, Pontificia Universidade Catolica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil, 90619-900. Email: esantarem@pucrs.br

Hypericum perforatum is a perennial species well known for its medicinal properties. *Hypericum* extracts contain more than 25 bioactive compounds and hyperforin, hypericin, and flavonoids are reported as functional metabolites. The production of these compounds has been improved through in vitro elicitation with plant pathogens. In this study, we evaluated the response of *H. perforatum* plants to the infection with *Agrobacterium rhizogenes* and tested a procedure for the transformation with this pathogen. Adventitious shoots of *H. perforatum* from in vitro culture were infected with *A. rhizogenes* (strain K599) and transferred to a cocultivation medium supplemented with acetosyringone (100 μM). Bacterial growth was later inhibited with ticarcillin (200 mg L^{-1}). Shoots in the control treatment were inoculated with distilled water and cultured in the same conditions described above. The levels of induced phenolic compounds, quercetinic flavonoids, and hypericin and the activities of polyphenol oxidases (PPO) and peroxidases (POX) were evaluated. These parameters were analyzed at 0, 1, 15, and 30 d after infection. Plants inoculated with *A. rhizogenes* showed an increase in the production of phenolic compounds after 15 d associated to a dramatic decrease in the PPO and POX activities. On the other hand, there was a simultaneous decrease in the levels of flavonoids. Hypericin levels were higher in the infected shoots when compared to the control treatment. These changes on the metabolism may represent a defense strategy of *H. perforatum* against the infection by phyto-bacteria and therefore pose obstacles for *Agrobacterium*-mediated transformation. Despite the absence of hairy roots, the infection per se is an alternative for improving the in vitro production of secondary metabolites.

P-2001

Establishment and Rooting of In Vitro Microcuttings from Winter Buds of *Quercus kelloggii*. D. KITTERMAN, J. Johnson, and J. Bushoven. Department of Plant Science, California State University, Fresno CA 93740. Email: jbushoven@csufresno.edu

The California Black Oak (*Quercus kelloggii*) is native to California and Oregon mountains between 500 and 1,000 m. The stands of mature Black Oak in the Yosemite Valley have been identified as a sensitive plant community requiring protection and restoration. One specimen, directly adjacent to the historic 90-yr-old Ahwahnee Hotel, has been identified as an important piece to the cultural landscape of this National Historic Landmark. Unfortunately, this tree, estimated to be at least 150 yr old, exhibits multiple structural defects, and although efforts to preserve this specimen tree have been attempted, these were largely unsuccessful. The National Park Service subsequently recommended complete removal of this tree prior to winter storms, but not before attempts to vegetatively propagate this tree were undertaken. The utilization of vegetative rather than traditional seedling propagation was deemed important to preserve the historical value of this specimen tree. Successful propagation of *Quercus* spp. via rooted cuttings is inherently difficult to achieve, and therefore, little data is present to support such efforts. The objective of this study was to determine the feasibility of utilizing in vitro micro-cuttings to regenerate true clones of *Q. kelloggii* via winter bud culture. Similar studies in *Q. suber* have been moderately successful, although, as with many in vitro techniques, culture contamination is often a significant problem. Data from efforts made to establish aseptic in vitro cultures of *Q. kelloggii* through the manipulation of

explant preparation are reported here, along with preliminary steps toward establishing rhizogensisis from microcuttings.

P-2002

Aseptic Coculture of Native Plant-derived Calli with Native and Nonnative Seedlings Affects Growth in a Dose-Dependent and Contact-Independent Manner. C. POTENZA[1], K. Yeater[2], D. James[1], and J. Barrow[1]. [1]Jornada Experimental Range, USDA-ARS, Las Cruces, NM 80003 and [2]USDA-ARS, Southern Plains Area, Lubbock, TX 79415. Email: cpotenza@nmsu.edu

The endophyte transfer hypothesis states that some plants native to semiarid and other extreme environments contain cryptic, plant-specific, growth-promoting microbes that are structurally integrated into the plants. When callus is made from these plants under aseptic conditions, microbial endophytes persist in the tissue but do not emerge from the plant cells and cannot be cultured separately. The callus culture subsequently serves as a source of inoculum for the unculturable, associated cryptic microbes that, when placed in direct contact with nonhost seedlings, transfer to the targeted plant. The targeted plant then experiences increased growth and establishment above that of untreated control plants, due to the presence of the novel cryptic endophytes. To independently test the endophyte transfer hypothesis and its effect on plant growth, callus was developed from *Atriplex canescens*, a drought and salt tolerant shrub, *Sporobolis cryptandrus* (sand dropseed), a grass native to the desert southwest of the Americas, and *Nicotiana tabacum* (tobacco) and cocultured under aseptic conditions with native and non-native seedlings. Seedlings were then measured for increased growth and developmental changes based on the effect of callus coculture. Results showed effects from the native callus–seedling coculture that were dependent on initial callus size and length of time of coculture but relatively independent of the species of seedling or callus. Growth effects were present even when the seedlings and callus were grown in parallel with no direct physical contact. The results presented give evidence that the coculture growth enhancement caused by the callus coculture treatment method is due to one or more callus-secreted factors derived from either the plant or an associated endophyte.

P-2003

Iridoids Accumulation in Root Cultures of the Cancer Herb *Castilleja tenuiflora*. G. Rosas[1], A. Zamilpa[2], K. Bermudez[1], M. Rodríguez[1], and G. TREJO[1]. [1]Centro de Desarrollo de Productos Bióticos—IPN, Morelos, MÉXICO, 62731 and [2]Centro de Investigación Biomédica del Sur—IMSS. Morelos, MÉXICO, 62790. Email: gttapia@ipn.mx

Castilleja tenuiflora (Scrophulariaceae) is a Mexican medicinal plant largely prescribed to heal cancer, but little scientific research has been performed on this species. Glycoside iridoids such as aucubin, bartsioside, and geniposidic acid derivatives with antitumoral, cytotoxic, and immunostimulant activities have been identified in the aerial part. The aim of the present work was to establish organ cultures of *C. tenuiflora* and to compare its iridoid profile with that of the wild plant. To induce organogenesis, leaf explants from wild-grown plants were inoculated in Murashige and Skoog (MS) medium containing 10 μM auxins (2,4-dichlorophenoxy-acetic acid or naphthalene acetic acid) and cytokinins (kinetin or 6-benzylaminopurine). Cytokinins induced shoot formation while alpha-naphthalene acetic acid induced rhizogenic callus, which were used to establish root cultures in the hormone-free MS liquid medium. Root cultures reached the highest biomass (25.1±1 g/L) at 28 d with a growth index of 2.0. Spontaneous shoot formation was observed in 30–35-d-old root cultures; shoots were periodically excised and presented continuous multiplication and elongation during subsequent subculture. Chemical analysis (thin-layer chromatography, high-performance liquid chromatography, and ^{13}C nuclear magnetic resonance) confirmed that the iridoids aucubin and bartsioside are accumulated in the aerial part but also in the root of wild plants. Root cultures accumulated aucubin, bartsioside, and three iridoids of unknown identity, which were found in the culture medium, representing an advantage for eventual recovery of the products.

P-2004

Growth Patterns, Secreted Protein Profiles, and EST Transcripts from Sugar Beet (*Beta vulgaris* L.) Hairy-root Cultures. BRETT J. SAVARY[1,2], Prasanna Vasu[1], Daya Anandan[1], Ann C. Smigocki[3], and Alberto Nuñez[4]. [1]Arkansas Biosciences Institute and [2]College of Agriculture, Arkansas State University, State University, AR 72467; [3]Molecular Plant Pathology Laboratory, USDA-ARS, Beltsville, MD 20705; and [4]Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA 19038. Email: bsavary@astate.edu

Sugar beets (*Beta vulgaris* L.) are the major root crop in the USA, and they are the source of about half of all sucrose produced domestically. Plant improvement through breeding and biotechnology are critical for sustaining yield and profitability. We are using the *Agrobacterium rhizogenes*-transformed hairy-root culture system as a means to investigate cell wall-associated proteins and their genes, particularly those involved in cell wall modification and protection from pests and pathogens in the rhizosphere. Sugar beet hairy roots are an excellent in vitro experimental system since such roots express the primary growth phenotype (axial growth from an

apical meristem) and associated biochemical profiles. They therefore are representative of roots in seedlings prior to the induction of the secondary growth phenotype during development of the tap root (via radial growth from interstitial lateral meristematic zones). To study macromolecular components of the root cell wall and their corresponding genes, we are integrating biochemical, proteomic, and genomic tools to isolate, identify, and characterize the major proteins accumulated in root culture media and isolated directly from root cell walls. We will present our progress on developing sugar beet root cultures for these studies, including characterization of protein profiles by enzyme assay and mass spectrometry and the cloning of more than 150 root expressed sequence tags. These results will support rational molecular strategies to improve sugar beet plants through a better understanding of the fundamental cell wall biochemistry in roots.

P-2005

In Vitro Studies of Tropical Woody Species. S. BHATNAGAR, S. Chandrasekharan, D.Y. Xie, and Y. Hong. Temasek Life-Sciences Laboratory, 1 Research Link, National University of Singapore, SINGAPORE 117604, Email: hongy@tll.org.sg

With the ever increasing demand for wood and its products and diminishing harvestable forests, there is an urgent need for using molecular and biotechnological tools in woody plant research and improvement. We have developed micropropagation systems for three commercially important tropical trees namely *Paraserianthes falcataria*, *Gmelina arborea* and *Acacia mangium* which provide traditional wood products like timber and fiber, and are also potential sources of bioenergy. Elite *Gmelina* plants have been produced on an industrial level (~ 1,00,000 plants per year) through an efficient and cost-effective large-scale clonal propagation system. Young twig with 10 nodal segments (each bearing two axillary buds) was collected from an elite mature plantation tree. After surface sterilization, the nodal segments were multiplied on solid MS medium supplemented with cytokinins for a month under 30°C, 5000 Lux, 16 hour photoperiod. Upon transfer to half-strength MS medium supplemented with auxins, 90% of these shoots produced roots within a month. The *in-vitro* regenerated plants served as mother plants for further propagation, multiplying 8-10 folds per two-month-cycle. These plants consistently carry similar characteristics as parents, show high rates of survival, and grow rapidly in shorter time, producing more biomass than the seed derived plants. Genetic markers are being developed to assess global biodiversity in *Gmelina arborea*. A regeneration system through organogenesis, somatic embryogenesis and Agrobacterium-mediated transformation system has been developed for *Acacia mangium*. Studies are underway on flower and wood development and we target to genetically improve this important tree species, with gene flow concern properly addressed. With the large-scale, fast biomass production systems in one hand and molecular understanding of tree development in the other, we aim to contribute towards sustainable forestation.

P-2006

Phloem Specific Transgene Expression Driven by *AtSUC2* Gene Promoter in Transgenic Citrus Plants to Develop Citrus Greening Resistance. AHMAD OMAR, Manjul Dutt, Gary Barthe, Vladimir Orbovic, and Jude Grosser. University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850. Email: Omar71@ufl.edu

Citrus greening, also known as huanglongbing (HLB) or yellow dragon disease, is one of the most serious citrus diseases in the world. It is caused by different strains of *Candidatus liberibacter* (nonculturable bacteria) and greatly reduces production, destroys the economic value of the fruit, and kills trees. This pathogen is restricted to phloem tissue and is transmitted from infected trees to healthy ones by the Asian citrus psyllid (*Diaphorina citri*). All citrus species are susceptible to citrus greening irrespective of rootstock. Control of citrus greening in citrus has been very difficult because of the lack both of information about the culture of the pathogen and plant tolerance or resistance to the pathogen. Classic control relies on the eradication of the infected trees and the use of insecticide against the vectors. One strategy to improve citrus resistance to phloem diseases involves transgenic expression of antimicrobial peptide genes in the phloem. The companion cell-specific AtSUC2 promoter was used to target phloem-specific expression. We constructed a binary vector (pC1391AO1) with an expression cassette bearing the β-glucuronidase (GUS) reporter gene (*uidA*) under control of the *Arabidopsis* sucrose-H+ symporter gene (*AtSUC2*) promoter. Transgenic lines of Carrizo citrange and Duncan grapefruit were generated. Histological results of GUS activity indicate that the promoter of the *AtSUC2* gene is active in transgenic citrus and is capable of directing phloem-specific expression of the GUS reporter gene. The AtSUC2 promoter may be useful for engineering greening-resistant transgenic citrus.

P-2007

Genetic Manipulation for Enhancing Calcium Uptake in Lettuce. S. H. PARK[1], M. P. Elless[2], J. Park[1], W. Lim[1], and K. D. Hirschi[3]. [1]Department of Horticulture, Forestry and Recreation Resources, 2021 Throckmorton Plant Science,

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Increasing nutritionally available calcium (Ca^{2+}) in crops could enhance Ca^{2+} dietary intake and lower the incidence of osteoporosis. One approach to alter the Ca^{2+} content in plants is to directly engineer high expression of Ca^{2+} transporters in the edible portion of the plant. Previously, our results demonstrated that an *Arabidopsis* H^+/Ca^{2+} transporter *sCAX1*-expressing carrot, tomato, and potato plants showed a Ca^{2+} increase of up to threefold in tomato fruits, potato tubers, and carrot roots. The immediate question became whether *sCAX1* can also increase the Ca^{2+} content of lettuce leaves. In this paper, we demonstrate that lettuce plants expressing *sCAX1* under the control of the cell division cycle (cdc2a) promoter or the cauliflower mosaic virus 35S promoter contained up to 25% more Ca^{2+} than plants transformed with control vectors. Furthermore, *sCAX1* expression does not appear to alter lettuce growth, development, and yield. These results suggest that modulation of ion transporters could be an important means of increasing the Ca^{2+} content of agriculturally important crops. Further tests, designed to determine whether the increases in Ca^{2+} in the *sCAX1*-expressing lettuce plants might alter the taste, shelf-life, or texture of the lettuces, are continuing.

P-2008

Optimization of Transformation Efficiency in Flax. S. L. KELLY and M. C. Jordan. Agriculture and Agri-Food Canada, Cereal Research Center, 195 Dafoe Road, Winnipeg, Manitoba, R3T 2M9, CANADA. Email: kellys@agr.gc.ca

Canada is the world's top exporter of flax and generates 40% of world production, mainly for its oil. To further diversify the products and uses of flax, genetic modification through transformation would be desirable. In the past, efforts to generate transgenic flax from *Agrobacterium*-inoculated explants were hampered by the large number of plants regenerated that were not transformed and were identified as escapes. As such, our initial focus was to optimize conditions for plant regeneration and selection of transformants from hypocotyls and hypocotyl-derived calli (HDC) or cotyledon-derived calli from the cut ends of explants. HDC were identified as being more embryogenic and therefore more easily regenerated. The best conditions identified, using two genotypes of diverse genetic origins (Norlin, a Canadian, and GlenElg, an Australian, genotype), were then applied across a panel of nine newer Canadian cultivars to attempt to identify a more recently registered variety that was as good for transformation efficiency than Norlin, if not better. Prairie Grande, a newer variety, was identified as a potential candidate, along with Norlin and GlenElg, for further experimentation. Two transformation vectors in the *Agrobacterium tumefaciens* strain GV3101, one with a marker gene for kanamycin resistance (pORE T3) and one with the *pat* gene for Basta resistance (pORE T2), both driven by the $P_{ENTCUP2}$ promoter and with T_{NOS} termination site, were used to assess transformation efficiencies. Each cassette also included a B-glucuronidase (GUS) gene with a plant promoter (P_{HPL}) to assess transient expression (see Coutu et al., *Transgenic Res.*, 16: 771–781, 2007). Although transient expression with GUS was observed for both constructs, only pORE T3 maintained kanamycin expression for hypocotyls, and only pORE T2 maintained Basta expression for HDC. Results on the effect of the pretreatment and source of explants (HDC vs. hypocotyls), the length and conditions during cocultivation, and the selection conditions postcocultivation on transformation efficiencies will be presented.

P-2009

Base-by-Base Analysis of siRNA Production by a Plant Transgene. V. GABA, Y. M. Shiboleth, S. Singer, E. Kukurt, D. Liebmann, L. Maslenin, A. Rosner, and A. Gal-On. Department of Plant Pathology and Weed Science, ARO Volcani Center, Bet Dagan 50250, ISRAEL. Email: vpgaba@volcani.agri.gov.il

Tobacco plants (*Nicotiana tabacum* L.) were transformed with a construct containing a "hairpin" inverted repeat of 598 nucleotides of the Potato Virus Y (PVY) replicase gene. Such constructs efficiently produce double-stranded ribonucleic acid (dsRNA) and thereby confer virus resistance by a post-translational gene-silencing mechanism. Homozygous plants were resistant to a range of PVY strains. Small interfering RNA (siRNA) production could not be observed by Northern blot. The production of siRNA by the transgene was evaluated in noninoculated plants using a custom-designed chip for the detection of small RNA molecules. Probes of 25 bp were printed on the chip covering the sense and antisense PVY sequences. Each probe was moved 1 bp along the transgene from the previous probe so that siRNA production by the whole transgene could be mapped in both sense and antisense directions. Small RNA populations from transgenic and nontransgenic controls were differentially labeled and applied to the chip. siRNA was observed only in

the samples from the transgenic plant. siRNA peak intensities were 15–30 fold lower than that of major micro-RNA species (e.g., miRs 156, 157, 159, 166, 168, 319, 403, etc.). siRNA peaks were noted throughout the inverted repeat transgene. The transgene dsRNA was apparently cut at random: No rhythm in siRNA production (i.e., on a basis of 21, 22 or 24 bps) was observed. Generally, siRNA signal intensity increased with GC content, although this relationship was stronger for the siRNA against sense probes than against antisense probes. Effective siRNA molecules were GC enriched and greatly preferred G or C in the extreme 3′ position. Strand selection preference was found to be in disagreement with the “animal” model of Schwarz et al (*Cell* 115: 199, 2003). A model for strand selection in plants has yet to be determined.

P-2010

Wheat Virus Resistance Via Interference RNA. Luisa F. Cruz[1], John P. Fellers[2], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, and [2]USDA-ARS Plant Science and Entomology Research Unit, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506. Email: luisac@ksu.edu

Significant yield losses in wheat (*Triticum aesativum* L) occur throughout the major wheat-producing countries of the world due to viral diseases. Wheat Streak Mosaic Virus (WSMV) and Triticum Mosaic Virus (TriMV), a newly identified virus, are two of the major viruses in the Great Plains region of the USA. Cultural practices and mite vector control are the primary methods of disease management; however, they are not fully effective. Additionally, resistant varieties are deployed, although none of these varieties are totally effective. One biotech solution to this problem could be to use interference ribonucleic acid, a phenomenon recognized as a natural defense mechanism against viral infection. To this end, partial sequences of the coat proteins (CP) of both WSMV and TriMV were amplified independently via polymerase chain reaction, cloned in a pENTRTOPO cloning vector, and subsequently incorporated into a pANDAmini expression vector. Immature embryos of the wheat cultivars “Bobwhite” and “Fielder” were transformed via particle bombardment using the pANDAmini vectors bearing the inverted repeat of the CP sequences and pAHC20, which contains the bar gene for selection. After tissue culture, putative transformed plants have been generated. Our results of the transformation experiments, molecular and expression analysis, and future goals and milestones for this project will be discussed.

P-2011

Partial Characterization and Purification of Plant Derived Butyrylcholinesterase to Treat Organophosphate Poisoning. L. KANNAN, B. C. Geyer, P.-E. Garnaud, R. R. Woods, M. Muralidharan, I. Cherni and T. S. Mor. School of Life Sciences and Biodesign Institute, Arizona State University, Tempe, AZ 85287. Email: latha.kannan@asu.edu

Current medical intervention in the case of organophosphate (OP) nerve-agent poisoning involves a combination of drugs, such as the carbamate and pyridostigmine bromide. While these drugs can mitigate the immediate effects of OP poisoning, they cannot alleviate postexposure toxicity, and they have serious short- and long-term side effects. The use of human proteins as OP bioscavengers is emerging as a promising alternative for prophylaxis and postexposure treatment against chemical warfare nerve agents. The best-studied bioscavenger to date, meeting with considerable success in preclinical research, is human butyrylcholinesterases (BChE), which can be purified from outdated serum samples or as a recombinant form from transgenic goats. However, both of these options are supply limited, expensive, and prone to contamination by human pathogens and prions. The aim of this project is to produce larger quantities of BChE in tobacco plants. To this end, we are comparing several plant expression systems including stable transgenic lines, *Agrobacterium*-mediated transient expression, and two novel viral-based systems based on the tobacco mosaic virus (MagnICON) and bean yellow dwarf virus (EPEPE). Expression levels and the purification profile and biochemical analysis of the plant-produced protein will be presented.

P-2012

High *f1-v* Gene Expression in Transgenic Tomato After Spontaneous or P19-induced Reversion of Gene Silencing. M. LUCRECIA ALVAREZ, Heidi L. Pinyerd, Emel Topal, and Guy A. Cardineau. Center for Infectious Diseases and Vaccinology (CIDV), The Biodesign Institute at Arizona State University, 1001 South McAllister Avenue, Tempe, AZ 85287-5401. Email: lucrecia.alvarez@asu.edu

Ribonucleic acid (RNA) silencing is the suppression of gene expression based on sequence-specific targeting and degradation of RNA that includes post-transcriptional gene silencing (PTGS) in plants. RNA silencing in plants is involved in the response to viruses and transposable elements. The P19 protein from the tomato bushy stunt

virus is a viral suppressor of RNA silencing that blocks the spread of RNA silencing defenses allowing the virus to infect the entire plant. P19 prevents systemic RNA silencing by sequestering siRNA. As a part of a project to develop a plant-made plague vaccine, we expressed the *Yersinia pestis* F1-V antigen fusion protein in tomato. We discovered that in some plants, the expression of the *f1-v* gene was very low or undetectable in leaves and fruit via enzyme-linked immunosorbent assay, despite a high copy number of *f1-v*, based on Southern blot analysis. A possible explanation of these results is the phenomenon of PTGS since gene silencing is more likely to occur when transgenes are introduced at a high copy number. In this paper, we report two types of reversion of *f1-v* gene silencing in transgenic tomato plants: one is spontaneous, and the other is related to supertransformation and expression of the suppressor of gene-silencing P19. Two of the *f1-v* gene-silenced lines demonstrated a spontaneous reversion of *f1-v* gene silencing after a decrease in gene copy number as a consequence of segregation in second-generation progeny. However, in the majority of *f1-v* gene-silenced tomato plants, reversion of silencing was induced by stable expression of the *p19* gene driven by either a constitutive or an ethanol-inducible promoter. These results confirm the potential exploitation of P19 to substantially increase the expression of value-added proteins in plants.

P-2013

MPR649-684-Hep B Core Antigen Fusion Forms Virus-like Particles in Plants and is Immunogenic in Mice. I. CHERNI, A. Vassall, N. Matoba, and T. S. Mor. Center for Infectious Diseases and Vaccinology, Biodesign Institute at Arizona State University, Tempe, AZ 85287. Email: Irene.cherni@asu.edu

The HIV-1 envelope protein gp41 is essential to transmission of HIV virus across mucosal surfaces. The membrane proximal region (MPR649-684) of the gp41 is a short stretch of amino acids highly conserved among various HIV subtypes. It is known to harbor epitopes recognized by broadly neutralizing antibodies such as 2F5 and 4E10, and is the minimal portion required for binding the epithelial receptor of HIV-1, GalCer, rendering MPR as an attractive vaccine candidate. When administered alone, MPR is unable to elicit potent immune response and requires an alternative antigen display strategy in order to obtain a more robust immune response. We have created a translational fusion by tailoring external membrane proximal region of gp41 to capsid protein of hepatitis B virus. Viral expression vector harboring the transgene was used to initiate transient infection in tobacco leaves where the fusion accumulated to moderate levels, ~2% TSP. The MPR-HBc fusion was capable of forming virus-like particles as was confirmed by sucrose density gradient ultracentrifugation. Integrity of fusion components and MPR surface accessibility was confirmed by sandwich ELISA. Semi-purified MPR-HBc fusion elicited strong humoral immune response against MPR when injected intranasally into mice. We were able to demonstrate that fusing MPR of gp41 to hepatitis B core antigen does not hinder virus-like particle assembly in planta, renders MPR accessible to antibody binding, and potentiates anti-MPR immune response when injected into mice. Immunogenicity of our fusion needs further investigating to fully evaluate the efficacy of MPR-HBc fusion as a prospective vaccine candidate.

P-2014

Characterization of Lipoxygenase Gene Expression in Rice Using Real-time PCR and RNAi Silencing. M. ROY CHOWDHURY and G. C. Phillips. Molecular Biosciences Program, Arkansas Biosciences Institute and College of Agriculture, Arkansas State University, P.O. Box 1080, State University, AR 72467. Email: Moytri.chowdhury@smail.astate.edu

Rice grain deterioration during storage is attributed to lipid peroxidation by a bran-specific lipoxygenase (LOX) enzyme. We are using an ribonucleic acid interference (RNAi) approach to analyze the functions of members of the LOX gene family in rice and to specifically inhibit bran-specific LOX activity. A search suggested *r9-LOX1* to be the best candidate gene corresponding to a bran-specific LOX. A seed-specific LOX member, *L-2*, was identified. Both of these produce 9-LOX enzymes. *RCI-1*, coding for a 13-LOX, was used to represent plastid-specific LOX family members, which are implicated in defense signaling. Gene inserts of 230–250 bp were designed and individually cloned into the pANDA RNAi vector. *Agrobacterium tumefaciens* EHA105-mediated transformation of Taipei-309 rice with r9-LOX1 RNAi insert was achieved with 7% transformation efficiency. Real-time polymerase chain reaction (PCR) analysis indicated that the target gene *r9-LOX1* in transformed plants was downregulated by a factor of −4.8 compared to nontransgenic controls. Semiquantitative GC analysis showed a reduction in nonanal (formed during oxidation of linoleic acid by lipoxygenase) by 74.33% in the r9-LOX1 RNAi transgenic line; however, increased levels of acetic acid and hexanal (direct products of 13-LOX activity)

were detected. This suggests the potential for enzymatic compensation by members of this family. Real-time PCR analysis of these three lipoxygenase genes in control plant tissues and in the putative bran-specific LOX-null mutant line Daw Dam showed increased transcriptional activity by various LOX genes to possibly compensate for impairment of other family members. None of these three rice LOX genes were exclusively tissue-specific in their transcriptional expression.

P-2015

Investigation of Hawaiian Sugarcane Cultivars for Sugarcane Yellow Leaf Virus (ScYLV). ABDELALEIM ELSAYED and Ewald Komor. Department of Plant Physiology, University of Bayreuth, Bayreuth, GERMANY. Email: elsayed@uni-bayreuth.de

The yellow leaf syndrome (YLS) observed first in Hawaii 1989 is now known as the symptoms of sugarcane yellow leaf virus (ScYLV), a member of the family Luteoviridae. Virus particles were observed in the cytoplasm of phloem companion cells. The diagnosis of ScYLV depends on serological and molecular techniques such as tissue blot immunoassay (TBIA), double-antibody sandwich enzyme-linked immunosorbent assay, and reverse transcription–polymerase chain reaction (RT-PCR). Six sugarcane cultivars from Hawaii (H73-6110, H78-4153, H87-4319, H78-7750, and H87-4094) had been tested for ScYLV by TBIA. It was suggested that cultivars H78-4153, H78-7750, and H87-4319 were resistant to ScYLV, cultivars H87-4094, H73-6110, and H65-7052 were susceptible for ScYLV (Schenck and Lehrer 2000, Schenck et al. 2001, and Schenck 2007). In this study, we used RT-PCR to detect the low titer of ScYLV to determine if the so-called resistant cultivars were indeed resistant to ScYLV. The results showed that the cultivars assumed to be resistant for ScYLV (H78-4153, H78-7750, and H87-4319) show a clear presence of ScYLV. Moreover, the cultivars H78-4153 carried a higher level (titer) of ScYLV compared with cultivars H78-7750 and H87-4319, whereas the cultivar H87-4319 had a very low titer of ScYLV. Expectedly, the susceptible cultivars H65-7052 and H73-6110 showed a high expression of ScYLV. The results of RT-PCR indicated that these cultivars (H78-4153, H78-7750, and H87-4319) are generally infected but that virus titer might be below the detection threshold of the serological technique (TBIA). Therefore, the presence of ScYLV in the cultivars with a low amount of virus titer (H78-4153, H78-7750, and H87-4319) indicates that they should better be called tolerant for the virus in the sense that they allow a low replication rate for ScYLV due to genetics factors.

P-2016

Rapid and Large-scale Plant-based Production of Catalytic Nerve-agent Bioscavenger Paraoxonase-1. P. E. F. GARNAUD, L. Kannan, and T. S. Mor. Arizona State University, School of Life Sciences and Biodesign Institute, Tempe, AZ 85287. Email: pgarnaud@asu.edu

Human cholinesterases were shown to be effective for prophylactic and postexposure treatment against organophosphate (OP) nerve agents by covalently binding and removing the toxins. However, this therapeutic approach necessitates the availability of large amounts of these enzymes, which are required in stoichiometric rather than catalytic quantities. Mammalian-based production strategies for human cholinesterases, including the purification of outdated blood-banked human plasma and milk from transgenic goats, are supply limited, cost prohibitive, and bear risk of associated pathogen and prion contamination. In this paper, we explore alternatives to this stoichiometric bioscavenger approach in terms of both the nature of the bioscavenger and the production system. Paraoxonase-1 (PON-1) is a human serum enzyme with OP-hydrolyzing capacity and can therefore be used as a catalytic bioscavenger. In addition, plants offer a highly scalable, cost-effective, and safe means for PON-1 production. In this paper, we will compare several plant expression strategies for the production of PON-1 including viral-based transient expression systems and *Agrobacterium*-mediated stable plant transformation.

P-2017

Effect of Jasmonic Acid on In Vitro Tuberization of Yam (*Dioscorea cayenensis rotundata*). O. AMARIN KOUADIO, H. Dodo, and K. Konan. Food Biotechnology Laboratory, Department of Food and Animal Sciences, Alabama A&M University, Normal, AL 35762. Email: amarin_olivier@hotmail.com

Yams (*Dioscorea* spp.) are tuber crops used as staple food in tropical areas and mostly in Africa. However, the lack of planting material restrict yam production. The traditional method of planting using fragments of tubers or bulbils is inefficient. Tissue culture techniques can increase the multiplication of yam planting material for a rapid and large-scale production of yam tuber. This work was performed to develop an efficient in vitro tuberization method for yam. Yam microtubers could then be used as seeds to provide farmers with sufficient planting material.

In vitro nodal segments of two yam cultivars from the variety *D. cayenensis rotundata* (cv Kponan Fissa, cv Krengle IB 35) were first propagated on Murashige and Skoog (MS) medium and then transferred onto a second medium for tuberization. The tuberization medium was either the T medium or the basal MS medium supplemented with Jasmonic acid (0.1, 1.0, and 2.5 μM), or kinetin (2.5 μM). Tuberization was most responsive in the T medium compared to the MS medium. Jasmonic acid at 1.0 and 2.5 μM produced the highest frequency of tuberization with 66.67% and 65.64%, respectively. Microtubers appeared within 5 to 15 d following the transfer of the plantlets into the tuberization medium. Tuberization was cultivar dependant. The cultivar Kponan was the most responsive compared to cultivar Krengle. In vitro production of yam tubers could be an alternative way to provide farmers with sufficient planting material in large-scale yam production.

P-2018

Identification of RNA Interference Induced Soybean Cyst Nematode (*Heterodera glycines*) Resistance in *Glycines max* Using a Hairy Root Bioassay. JIARUI LI[1], Timothy C. Todd[1], William T. Schapaugh[2], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 and [2]Department of Agronomy, Kansas State University, Manhattan, KS 66506. Email: jli2@ksu.edu

With an annual loss of over one billion US dollars due to yield reductions, the soybean cyst nematode (SCN), *Heterodera glycines* is undoubtedly the most damaging pest of soybean. Current methods to control this pest are not totally successful in part due to new SCN biotypes emerging that can overcome current resistant varieties. To control SCN in soybean, our laboratory has been evaluating the use of small interfering ribonucleic acid (siRNA) expressed in the host plant directed toward specific nematode genes. In attempt to streamline our research, we evaluated the use of producing chimeric soybean seedlings expressing siRNAs against specific genes in transgenic hairy roots. Three separate nematode genes (*JL-F1*, *JL-A1*, and *JL-25*) were selected for this study. Gene fragments were amplified by polymerase chain reaction (PCR) using specific primers and were cloned into siRNA-expressing vectors. Using this hairy root system, the siRNA constructs of these three genes were independently transformed into soybean. The tap roots from 4-d-old seedlings (cultivars “Jack” and KS4607) were excised, and hairy roots were induced from hypocotyls via *Agrobacterium rhizogenes*-mediated transformation. Inoculated hypocotyls were cultured in a Murashige and Skoog-based medium containing an antibiotic selection. Transgenic roots were confirmed via PCR and Southern blot analysis. Transgene expression was surveyed by reverse transcription PCR. SCN bioassays resulted in up to 90% reduction in eggs g^{-1} root tissue, indicating that chimeric transgenic plants significantly suppressed the reproductive potential of *H. glycines*. Our results indicate that a chimeric hairy root-induced transgenic plant can be used to test nematode resistance.

P-2019

Antimicrobial Potential of the Medium Used for Growth of Transformed Hairy Roots of *Artemisia* Species. A. MANNAN[1,2], N. Shaheen[1], and B. Mirza[1]. [1]Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan and [2]Arkansas Bioscience Institute, Arkansas State University, State University, AR. Email: abdulmannan_ka@yahoo.com

Transformation of two *Artemisia* species (*A. dubia* and *A. indica*) was carried out using two *Agrobacterium rhizogenes* strains, LBA 9402 and 8196, to obtain hairy roots. The induction of hairy roots was higher in both *Artemisia* species when infected with LBA9402 as compared to 8196. After 30 d of culture of hairy roots on full- and half-strength MS medium, the media were tested for antibacterial and antifungal activities to determine if any antimicrobial activity is present in the spent root medium or not. Moderate/low activity was observed in the half-strength Murashige and Skoog medium from both *A. dubia* and *A. indica*. Using the agar well diffusion method, antibacterial activity was measured against *Micrococcus luteus* ATCC 10240, *Escherichia coli* ATCC 15224, *Salmonella setubal* ATCC 19196, *Pseudomonas picketii* ATCC 49129, *Bordetella bronchiseptica* ATCC 4617, and *Enterobacter aerogens* ATCC 13048. Some antifungal activity was also observed against *Mucor* species and *Fusarium solani*. These results suggest that hairy roots of *A. dubia* and *A. indica* release antibacterial and antifungal metabolites into their culture medium. Hairy roots of these species may thus be a source of such metabolites.

P-2020

Use of Serological and Molecular Methods to Determine Citrus Tristeza Virus (CTV) Resistance in Candidate Root-

stocks to Replace Sour Orange. AZZA H. MOHAMED, Ron H. Brlansky, and Jude W. Grosser. University of Florida, Department of Horticultural Sciences or Plant Pathology, Citrus Research and Education Center, Lake Alfred, FL 33850. Email: azza@ufl.edu

Citrus tristeza virus (CTV) is the most economically important virus affecting citrus worldwide. CTV is a phloem-limited, single-stranded ribonucleic acid virus belonging to the *Closterovius* and vectored by aphids. The purpose of this study is to screen new citrus rootstock candidates, created in vitro via protoplast fusion, for CTV quick-decline resistance. The hope is to replace sour orange rootstock, the most susceptible rootstock to CTV-induced quick decline. Candidate somatic hybrids rootstocks were screened using a top-working technique by grafting each of 80 selections, mostly mandarin+pummelo somatic hybrids but also including parental pummelos, along with sour orange onto CTV-infected trees. The virus infection was then detected by serological techniques including tissue blot immunoassay, double-antibody sandwich enzyme-linked immunosorbent assay, and Western analysis. Molecular techniques including multiple molecular markers analysis and heteroduplex mobility assay followed by the deoxyribonucleic acid sequencing of the amplified region were applied to detect the differential movement of CTV genotypes into to the test rootstocks. Based on these results, hybrids were classified into four different groups based on the different combinations of T30, T36, and VT CTV genotypes that moved into the newly grafted scion material. None of the used serological or molecular methods provided a reliable estimation of virus accumulation. Therefore, the quantification of the CTV titer was attempted by using reverse transcriptase quantitative real-time polymerase chain reaction (qRT-PCR). Based on the preliminary analysis of the qRT-PCR results, some of the tested rootstocks such as A+7-2-99-5 showed a very high quantity of CTV and disease symptoms, making this rootstock very susceptible to CTV infection. On the other hand, rootstocks such as Amb+5-1-99-3, 2247-OP-A2, and pummelo parent HBJL-5 showed a very low CTV titer and no disease symptoms, suggesting some resistance to CTV replication and quick decline disease. Many hybrids showed intermediate levels of CTV replication but no disease symptoms.

P-2021

Plant Produced Human Cholinesterases and Plant Cholinesterase(s). T. S. MOR[1], B. C. Geyer[1], M. Muralidharan[1], I. Cherni[1], S. P. Fletcher[1], T. Evron[2], and H. Soreq[2]. [1]School of Life Sciences and Biodesign Institute, P.O. Box 874501, Arizona State University, Tempe, AZ 85287-4501 and [2]The Department of Biological Chemistry, The Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, ISRAEL. Email: tsafrir.mor@asu.edu

Bioscavenging of organophosphate (OP) by human cholinesterases (ChEs) is emerging as a promising medical intervention for prophylaxis and postexposure treatment against chemical warfare nerve agents and pesticides, meeting considerable success in preclinical studies. ChEs are very efficient in sequestering OPs that become esterified to a serine residue at the active site. This covalent bond is very stable and in the case of certain OPs is further stabilized by subsequent “aging” reactions. With negligible turnover rate ChEs are effectively “single-use molecular sponges” requiring the application of stoichiometric rather than catalytic doses for effectiveness. Thus, a production system capable of supplying the forecasted demand for large amounts of active ChEs is needed. Several strategies for the production of ChEs were evaluated. Of the two ChEs in humans, only the serum enzyme butyrylcholinesterase can be obtained from natural sources, and large-scale purification efforts from outdated blood-banked human plasma were demonstrated. The expression in recombinant systems is the only way for producing the physiological target of OPs, acetylcholinesterase (AChE), which is abundant in muscle and nerve tissues but is normally absent from serum. Several mammalian-based recombinant production systems were described including engineered cell cultures and the milk of transgenic goats. As an alternative to these systems that are confronted with being supply restricted, of limited scalability, high costs, and risk of human pathogen contamination, we have introduced plants as a production system for human AChE. We carefully optimized the expression constructs and purification protocols and demonstrated that plant-derived AChE-R from *Nicotiana benthamiana* retains all of the catalytic properties of a mammalian-derived enzyme and furthermore that this AChE-R is capable of completely ameliorating all of the gross clinical symptoms and some of the long-term molecular consequences implicated in OP poisoning. The availability of plants overexpressing ChEs prompted us to investigate the intriguing possibility of the presence in plants of the signaling molecule acetylcholine and the enzymes involved in its metabolism. Supported in part by DARPA research contract N66001-01-C-8015 (to T.S.M. and H.S.), by US National Institutes of Health Center of Excellence Grant U54N5058183-01 (to T.S.M.), and by Israel Science Foundation Grant 618/02-1(to H.S.).

P-2022

Polyvinyl Pyrrolidone is not Effective for Improving Recovery of Cryopreserved Shoots from Two *Rubus* Cultivars. Magfrat Muminova[1] and BARBARA M. REED[2]. [1]3-22 Pushkin-Salar Street, Tashkent, 100000 Uzbekistan and [2]USDA-ARS NCGR, 33447 Peoria Rd, Corvallis, OR 97333-2521. Email: mmuminova@yahoo.com, Barbara.Reed@ars.usda.gov

The use of the antioxidant polyvinyl pyrrolidone as a pretreatment or recovery treatment for *Rubus* shoot cultures was tested with two blackberry cultivars. Plantlet growth was initially evaluated on a medium with 1 to 10% polyvinylpyrrolidone (PVP; 10,000 MW). Growth and multiplication of the plantlets were severely inhibited with 2.5% to 10% PVP in the medium. Following cryopreservation, recovery of plantlets pretreated for 48 h on 5% dimethyl sulfoxide medium with 1% or 2.5% PVP was not significantly different from the controls; however, plantlets pretreated with 5% or 10% PVP had significantly less regrowth. PVP in the recovery medium produced significantly less regrowth than the control medium in all cases and reduced recovery of plantlets after cryopreservation from 60% to less than 20%. These results indicate that PVP has no effect or is detrimental to the successful cryopreservation and subsequent recovery of *Rubus* shoot tips.

P-2023

Organogenesis from Cultured Petiole of Yam (*Dioscorea rotundata*). F. N. OGUNKUNLE, K. S. Konan, and H. W. Dodo. Food Biotechnology Laboratory, Alabama A&M University, Normal, AL 35762. Email: ngozianike@yahoo.com

The lack of efficient regeneration system for edible yam (*Dioscorea rotundata-cayenensis*) is a major setback to yam improvement through biotechnology. Edible yam serves as a staple food and plays an important role as a source of carbohydrate for more than 300 million people globally. Although more than 95% of annual yam production estimated at 48.7 million metric tons occur in sub-Saharan Africa, the crop is important in other regions of the world including Asia, West Indies, and North and Central America. Several factors make yam a good candidate for genetic improvement. It is vegetatively propagated requiring more than 20% of total tuber yield as planting material. Furthermore, more than 25% of annual yield is lost to diseases and pests, while 50% of total stock is lost during storage. Furthermore, yam is severely limiting in essential amino acids. Progress in classical yam breeding has been severely impeded as a result of biological constraints. The present difficulty in improving yam through classical breeding has motivated the search for an alternative. Biotechnology is an attractive potential solution and offers a novel strategy to yam improvement through the techniques of tissue culture and genetic engineering. Although yam is recalcitrant to regeneration, a rapid and efficient method for its regeneration through organogenesis has been developed for the first time. Adventitious shoots were induced from petiole segments cultured on Murashige and Skoog (MS) medium supplemented with various concentrations (0.1–5.5 μM) of cytokinins (kinetin, 6-benzylaminopurine, zeatin). The efficiency of regeneration, shoot elongation, and development were improved by adding putrescine to the medium. Regenerated shoots were rooted on phytohormone-free MS medium. The protocol will serve as a fundamental step to developing a transformation system for yam.

P-2024

Transient Expression of Ebola Recombinant Immune Complex in *Nicotiana benthamiana*. WARANYOO PHOOLCHAROEN, Chandana Uppalapati, Charles J. Arntzen, Qiang Chen, and Hugh S. Mason. Center for Infectious Diseases and Vaccinology (CIDV), The Biodesign Institute at Arizona State University, 1001 McAllister Avenue, Tempe, AZ 85287. Email: pang@asu.edu

The immune complex can stimulate both humoral immune response and cell-mediated immune response. There is a high potential to use the immune complex as the effective vaccine. The goal of this study is to transiently express Ebola virus monoclonal recombinant immune complex in *Nicotiana benthamiana*. A recombinant protein consisting of Ebola glycoprotein1 (GP1)-specific monoclonal antibody fused with Ebola GP1 was designed. *Agrobacterium* is used to transiently express the immune complex in *N. benthamiana*. The level of antibody expression and the presence of the recombinant immune complex can be detected by using enzyme-linked immunosorbent assay and c1q assay, respectively. After the Ebola immune complex is expressed, extracted, and purified from leaf material, it will be used to study the immune response in mice. If the Ebola immune complex can increase the immune response in mice and provide protection against challenge, this will provide a significant validation to the immune complex concept as a robust immunization strategy.

P-2025

Molecular Characterization of Horticultural Crops of the Humid Tropics. K. RAJMOHAN. Department of Plant

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Humid tropical region is characterized by diverse ecosystems and landscapes and has a long history of intensive agriculture, leading to great crop genetic diversity. A wide variety of fruits, vegetables, spices, and medicinal plants exist. The diversity within each species, including cultivars, landraces, and modern varieties, is substantial. Characterization of the crop genetic resources will help identify their essential features, useful for breeding programs. It is important for bioprospecting as well as germplasm conservation. Many of these genetic resources are endangered, and genetic erosion is serious. Molecular markers are increasingly being used to characterize crop genetic resources as they are capable for precise determination of genetic diversity. Unlike morphological traits, molecular markers are not influenced by the environment and represent neutral loci unaffected by selection. A number of molecular markers like restriction fragment length polymorphisms, random amplification of polymorphic deoxyribonucleic acids, amplified fragment length polymorphism polymerase chain reaction, microsatellites, and single nucleotide polymorphisms (SNPs) are available, differing in their informational content. They differ in requirements of time, money, and labor. Sampling strategies, data analysis, and determination of genetic relationships are important. Choosing the appropriate techniques is critical. A combination of techniques is often necessary to gather the desired information. Better understanding of genome helps the use of markers, closely linked to desirable traits, for characterizing genetic diversity. Increasing numbers of expressed sequence tags and SNPs help for more targeted sequence-based approaches. Estimating the genetic relationships between populations within a species is one of the major uses of molecular markers. Molecular marker information can be used in gene banks for sampling of materials and management of collections. Valuable genes can be identified for developing new varieties. Molecular characterization of a number of important horticultural crop varieties and cultivars of the humid tropics is discussed.

P-2026

Transgenic Tobacco BY-2 with cDNA of Human Calcitonin. M. T. SADDER. Plant Biotechnology Laboratory, Department of Horticulture and Crop Science, Faculty of Agriculture, University of Jordan, Amman, 11942, JORDAN. Email: sadderm@hotmail.com

This is the second study conducted as a series of experiments to promote the biopharming industry in Jordan. Plant cell culture was chosen for their advantages over other cell systems. The first selected protein was the human interferon-gamma. After a successful production of interferon (proved by polymerase chain reaction [PCR], reverse transcriptase–PCR, and specific monoclonal antibody), another human protein was selected (calcitonin). Calcitonin acts to reduce blood calcium, opposing the effects of parathyroid hormone, and oral calcitonin may have a chondroprotective role in osteoarthritiscalcitonin and has an analgesic effect on bone pain. However, calcitonin needs a post-translational modification, where the C terminus is amidated with a modifying enzyme. Calcitonin was amplified with *Pfu* polymerase and cloned directionally into a pCAMBIA binary vector, where the selection marker for bacteria is kanamycin and for plants is hygromycin. The *Agrobacterium tumefaciens* strain C58C1 (rifampicin and gentamicin) was used for transformation. The construct was electroporated into the *Agrobacterium* and screened with miniprep and PCR for the specific calcitonin fragment. The *Agrobacterium* harboring the binary vector was cocultured with BY-2 tobacco cells, and putative transformants were obtained. Two culture methods were used (cell suspension in shaking flasks or unshaken thin cell layer in small Petri dishes). Cell spreads from shaken flasks showed better growth over selection plates. Putative transformants are being screened with specific primers for calcitonin. We designed additional primers specific to the binary vector backbone to detect *Agrobacterium* that could have survived carbenicillin. The calcitonin-modifying gene was cloned into pET28a bacterial expression vector to be expressed in BL21(DE3) cells.

P-2027

Risk Assessment of Transgenic, Apomictic Forage and Turf Grass (*Paspalum notatum* Flugge). S. SANDHU[1], F. Altpeter[1], and A. Blount[2]. [1]Agronomy Department, PMCB, Genetics Institute, University of Florida—IFAS, Gainesville, FL 32611 and [2]Agronomy Department, North Florida Research and Education Center, University of Florida—IFAS, Marianna, FL 32446. Email: faltpeter@ifas.ufl.edu

The commercially important bahiagrass cultivar “Argentine” is an obligate apomict. Its asexual reproduction resulting in uniform seed progeny could reduce the risk of unintended gene dispersal by pollen. The primary objective of the present study was to investigate pollen-mediated gene transfer from apomictic tetraploid bahiagrass to sexual diploid bahiagrass using glufosinate resistance as a marker. Glufosinate-resistant, fertile transgenic bahiagrass was generated by biolistic gene transfer and used as pollen donor in a field trial (USDA-APHIS permit no. 05-365-01r) in Marianna, FL, with two replica-

tions. Primary transgenics and the apomictic seed progeny was characterized by Southern blot analysis, immunochromatographic assay, and glufosinate application. Open-pollinated seeds were harvested from wild-type, sexual-diploid bahiagrass surrounding the transgenic glufosinate-resistant bahiagrass at 1 m distance. Gene transfer frequency was determined by application of 0.14% glufosinate to germinated seedlings using the wild type as the negative control and the transgenic parent as the positive control. Very low hybridization frequency of less than 0.1% (26 hybrids out of 28,000 seedlings analyzed) was observed between the apomictic transgenic and wild-type bahiagrass. *Bar* gene integration and expression in the hybrids were confirmed by Southern blot and immunochromatographic analysis and herbicide application, respectively. All six of the so far analyzed hybrids have been confirmed as triploids by both flow cytometry and root-tip chromosome counting. Embryo sac analysis detected both apomictic and sexual embryo sacs, suggesting facultative apomixis. Fertility of hybrids will be evaluated. All triploid hybrids showed reduced vigor compared to diploid or tetraploid bahiagrass. The results suggests that using the apomictic bahiagrass cultivar Argentine as a target for bahiagrass transformation provides a high level of transgene containment compared to open-pollinating sexual-diploid turf and forage grasses.

P-2028

Plastid-transformed Tobacco Plants Express a Multiepitope DPT Fusion Protein Retaining the Antigenicity of the Three Components. R. E. SORIA-GUERRA[1], A. G. Alpuche-Solís[2], S. Rosales-Mendoza[1], R. López-Revilla[2], E. M. Bendik[1], and S. S. Korban[1]. [1]Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61821 and [2]División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, Camino a la Presa San José 2055, 78216 S. L. P., MEXICO. Email: rguerra@uiuc.edu

The Diphteria, Pertussis, and Tetanus (DPT) vaccine is widely used for the vaccination of infants and children worldwide. However, concerns over the safety of whole-cell pertussis vaccines have prompted the development of acellular vaccines that have contributed to high production costs. To produce a safer, inexpensive, and more efficient DPT vaccine, a plant-optimized synthetic DPT gene, containing immunoprotective exotoxin epitopes of *Corynebacterium diphteriae*, *Bordetella pertussis*, and *Clostridium tetani*, was transferred to tobacco plastomes using biolistic-mediated transformation. Several putative transformants were obtained, and the analysis of four lines by polymerase chain reaction and Southern blot confirmed the presence and integration of the expression cassette. Enzyme-linked immunosorbent assays of these transplastomic lines demonstrated that the tobacco-derived DPT protein was recognized by specific antibodies raised against each of the DPT toxins. This suggested that the three components present in the DPT polypeptide properly displayed the native epitopes. Protein quantitation using Western blot analysis revealed a DPT protein content of approximately 100-fold higher than that previously detected in transgenic tomato plants derived via nuclear transformation. This model would allow for the development of a new generation of DPT subunit vaccines.

P-2029

In Vitro Antioxidant Treatment Improves the Recovery of Cryopreserved Blackberry Shoot Tips. ESTHER E. UCHENDU[1] and Barbara M. Reed[2]. [1]Department of Horticulture, Oregon State University, Corvallis, OR 97331-7304 and [2]USDA ARS, National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333-2521. Email: uchendue@onid.oregonstate.edu; Barbara.Reed@ars.usda.gov

Cryopreservation imposes stress on plant cells and tissues resulting in increased oxidation and damage. This study evaluated the effect of the antioxidant compounds alpha tocopherol (vitamin E) and ascorbic acid (AA) added at critical points in the cryopreservation protocol. Shoot tips of in vitro grown *Rubus* (blackberry) cultivars (0.8–1 mm) were cryopreserved using the PVS2-vitrification technique. Pretreatment of shoot tips for 48 h on agar plates with 5% dimethyl sulfoxide and 15 mM vitamin E significantly ($P < 0.0001$) improved recovery, resulting in 58% regrowth compared to 35% for the controls. Vitamin E at 20 mM in the postcryopreservation rinsing solution significantly increased recovery from 45% for controls to 72%. There were no significant effects of vitamin E in the loading solution or the recovery medium. Treatments of 50 to 100 mM AA significantly improved regrowth when added to the loading solution (control 47%, AA 77–88%) or the rinsing solution (control 47%, AA 82–83%). In the standard recovery medium, AA was detrimental to regrowth of shoot tips. However, on the regrowth medium without iron, regrowth of shoot tips increased from 51% for controls to 85% with 50 mM AA. This study indicates that oxidation is an important factor in losses from cryopreservation and that the addition of antioxidants at critical points of the protocol can greatly improve regrowth following cryopreservation.

P-2030

Artemisinin Production from *Artemisia annua* Shoot Cultures. PAMELA J. WEATHERS[1,3], Melissa Towler[3], Chunzhao

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Artemisinin and its derivatives are the most potent antimalarial drugs in use. It is also highly effective against many other infectious diseases and a number of cancers. Unfortunately, the drug is in short supply, so there are major efforts aimed at developing technologies for overproduction of this important therapeutic. Shoots of rooted plantlets of *A. annua* have been reported to produce more artemisinin than plantlets lacking roots. In this paper, we share recent technology that we have developed using dimethyl sulfoxide (DMSO) on rooted shoots of *A. annua* to stimulate artemisinin production in Magenta boxes, in shake flask cultures and in bioreactors. Magenta GA7 boxes, 250-mL flasks or 4-L mist bioreactors were inoculated with clumps of *A. annua* shoots at equivalent mass per volume and grown at 25° C in low light (~2 μmol m^{-2} s^{-1}), for 2 wk in Murashige and Skoog (MS) multiplication medium (containing benzyladenine and naphthalene acetic acid). After cultures at least doubled in mass, they were shifted into MS rooting medium (no phytohormones) in each system for another week after which DMSO was added at 0.5% *v*/*v* concentration. Harvest was 1 wk later. Shoots from both rooted and unrooted cultures each with or without DMSO were weighed to obtain fresh weight, dried to obtain dry weight (DW), and then extracted and, using high-performance liquid chromatography, analyzed for artemisinin. Unrooted shoot cultures grown in shake flasks produced more DW than in either Magenta boxes or in the mist reactor; however, hyperhydration was also the highest in these cultures compared to the others. Shoots of the unrooted plantlets grown in the mist reactor produced two and five times more artemisinin (2.5 μg/g DW) than in shake flasks (1.5 μg/g DW) or Magenta boxes (0.8 μg/g DW), respectively. When shoots were stimulated to root and then subsequently also fed DMSO, the artemisinin level increased to 89 μg/g DW, which was twice that of rooted shoots (42 μg/g DW) without DMSO in the mist reactor. Growth was not significantly affected by addition of DMSO. DMSO had no effect on either growth or artemisinin levels in unrooted shoot cultures of *A. annua.* Together these preliminary results indicate that rooted shoot cultures of *A. annua* produce considerably more artemisinin in response to DMSO but only if rooted. Furthermore, it appears that *A. annua* can possibly be grown in large quantities in an inexpensive but scaleable reactor to overproduce artemisinin. Work is ongoing toward this end.

P-2031

Murine Interleukin 12 Production in Tobacco Hairy Roots in 3 Reactor Systems. PAMELA J. WEATHERS[1,3], Chunzhao Liu [1, 2], Giuliana Medrano[1], Maureen C. Dolan[1], Carole L. Cramer[1]. [1]Arkansas Biosciences Institute, Arkansas State University, State University, AR 72467-0639; [2]National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, CHINA; and [3]Worcester Polytechnic Institute, Worcester, MA 01609. Email: pweathers@astate.edu

Interleukin-12 (IL-12) is a complex glycoprotein that has proven challenging to produce in vitro in plants, usually with low yields and with only partial activity. Recently, Liu et al. (*J. Interferon Cytokine Res.*, 2008, in press) developed transgenic tobacco plants and hairy root culture lines that both showed high levels of fully functional, murine IL-12 (mIL-12) production. Purified plant-derived IL-12 showed equivalent bioactivity to animal cell-derived mIL-12 in both in vitro assays and in mouse vaccination studies. With an interest in scaling the production of this valued cytokine, we have compared the production yields of mIL-12 in hairy roots of *Nicotiana tabacum* (cultivar Xanthi) in three different culture systems: shake flasks, an airlift reactor, and a nutrient mist reactors. Using an inoculum level of 8 g fresh weight (FW)/L, hairy roots were inoculated into each culture system containing Gamborg's B5 medium with 3% sucrose and grown at 25° C, under continuous light (~2 μmol m^{-2} s^{-1}). After 14 d, roots were harvested and weighed (FW), and both culture media and roots were extracted to quantify respective mIL-12 production levels. We observed that root growth as FW was 80.4, 67.8, and 47.3 g/L in shake flasks, mist reactors, and airlift reactors, respectively. Using an anti-mIL-12 enzyme-linked immunosorbent assay, concentrations of the cytokine in the root tissue were measured as 76.2, 65.6, and 38.0 μg/g DW, respectively, while mIL-12 in the culture medium was 167.6, 49.9, and 1.30 μg/L. Together, these results are promising for the eventual scale-up of mIL-12 production. Further optimization should lead to greater increases in productivity to levels that are useful for yielding adequate amounts of product for research purposes.

P-2032

Genetic Mapping of Apomixis in *Pennisetum squamulatum* Using Retrotransposon-based Markers. HEQIANG HUO and Peggy J. Ozias-Akins. University of Georgia, Nespal, 2356 Rainwater Road, Tifton, GA 31794. Email: pumahuo@gmail.com

Pennisetum squamulatum reproduces by apospory, a type of apomictic reproduction where nongenerative nucellar cells develop into unreduced embryo sacs and embryos form without fertilization of the egg cells. Genetic mapping of this trait showed that apospory appeared to be transmitted as a single locus, but this "locus" actually is a large chromosomal block, which we named the apospory-specific genomic region (ASGR). The ASGR has been cytogenetically mapped by fluorescence in situ hybridization to a heteromorphic chromosome. The large physical size of the ASGR (>50 Mb), its high hemizygosity, and an abundance of repetitive elements including long terminal repeat (LTR) retrotransposons make it intractable for direct sequencing under the current circumstances. Additional molecular markers are required for high-resolution mapping and further characterization of the ASGR. In this study, the LTR regions of retrotransposons belonging to the family that is most abundant in the ASGR were isolated for the design of LTR-specific primers that were used for transposon display or sequence-specific amplification polymorphism (S-SAP). Both *Mse*I/*Eco*RI and *Mse*I/*Pst*I restriction enzyme combinations were tested. Twenty-seven out of 63 primer combinations showed ASGR-linked polymorphisms. Two hundred forty-eight fragments that fit a 1:1 ratio according to the Chi-square goodness-of-fit test ($P < 0.05$) were considered to be single-dose markers. One hundred seventy-four out of 248 markers (70%) show linkage with apomixis, and one potential marker shows linkage with sexual reproduction. The 248 markers plus one previous amplified fragment length polymorphism maker and one sequence characterized amplified region (SCAR) marker were used for genetic mapping. One fragment that shows no recombination in the F1 population was also recovered and sequenced. A new SCAR marker based on the sequence was confirmed to segregate with apomixis among F1 plants. BlastX analysis of the sequence of this fragment shows high similarity to a putative gypsy-type retrotransposon protein from rice (*Oryza sativa*). Its expression was observed in both ovary and leaf of *P. squamulatum*.

P-2033

Strawberry Transformation Using Kanamycin Selection. CHRISTOPHER PANTAZIS[1], Barry S. Flinn[1,2,3], Richard E. Veilleux[2], Jeremy Pattison[2], Craig Nessler[4], Vladimir Shulaev[5], and Yinghui Dan[1,2,3]. [1]Institute for Advanced Learning and Research, Danville, VA 24540 and [2]Departments of Horticulture, [3]Department of Forestry, [4]Department of Plant Pathology Physiology and Weed Science, and [5]Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Email: yinghui.dan@ialr.org, ydan@vt.edu

Strawberry is a member of the Rosaceae family that includes many valuable fruit and ornamental crops, with an annual crop value of approximately $7 billion. Genomic resources for Rosaceae species are rapidly becoming available with information from expressed sequence tag sequences, markers, linkage maps, and physical maps. The Rosaceae Genomic Executive Committee has promoted strawberry as a translational genomics model due to its unique biological features and transformability for fruit trait improvement. Our overall research goal is to use functional genomic and metabolic approaches to pursue high throughput gene discovery in a diploid strawberry, *Fragaria vesca*, as it has advantages of a fleshy fruit typical of most fruit crops, short life cycle (seed to seed in 12–16 wk), small genome size (206 Mbb/C), small plant size, and many seeds per plant. We have improved our current *Agrobacterium*-mediated strawberry transformation methods using both kanamycin and hygromycin selection. With our improved kanamycin transformation system, we are able to produce up to 98 independent kanamycin-resistant insertional mutant lines using a T-deoxyribonucleic acid construct carrying an AcDs Launchpad system from a single-transformation experiment involving inoculation of 22 leaf explants of Fv accession 551572. The parameters to improve the transformation systems will be described.

P-2034

Defense Metabolism of *Solanum tuberosum* L. in Response to Plant Pathogenic Bacteria. L. V. ASTARITA, V. D. Poiatti, and F. R. Dalmas. Laboratory of Plant Biotechnology, PUCRS, Porto Alegre, RS, CEP 90619-900, BRAZIL. Email: astarita@pucrs.br

The natural resistance of plants to disease is based on both preformed and induced mechanisms. This study evaluated the response of plants of *Solanum tuberosum* L inoculated with the pathogenic bacteria *Xanthomonas axonopodis* and *Ralstonia solanacearum* (incompatible) and *Erwinia carotovora* (compatible). Leaf inoculation was performed depending on the number and location of the leaves on the stem. Multiple-leaf inoculation was performed on basal, intermediate, and apical leaves and single inoculations on intermediate leaves. Phenolic compounds, including flavonoids, and the activities of polyphenol oxidases (PPO) and peroxidases (POX) were analyzed. Leaves inoculated with *X. axonopodis* and *R. solanacearum* showed hypersensitive response within 24 h postinoculation, while disease symptoms were observed on leaves inoculated with *E.*

carotovora. Regardless of the bacterial treatments, basal leaves presented higher PPO and POX activities and lower levels of total phenolic compounds and flavonoids when compared to apical leaves. However, basal and intermediate leaves inoculated with *R. solanacearum* and *X. axonopodis* showed an increase in total phenolic compounds and flavonoid levels. Multiple-leaf inoculations resulted in the highest levels of phenolic compounds and flavonoids, while single inoculations led to an increase in PPO activity. No differences were observed on POX activity between single and multiple-leaf inoculations. Plants inoculated with *E. carotovora* did not increase the defense mechanisms, such as enzyme activities and phenolic compounds. In the present study, resistance or susceptibility in *S. tuberosum* led to various defense responses that differed depending on leaf age, type of inoculation (single or multiple), and the plant–pathogen interaction.

P-2035

Characterizing Cholinesterase Like Enzyme in *A.thaliana*. M. MURALIDHARAN, A. Skalecka-Ball, and T. Mor. School of Life Sciences and The Biodesign Institute, Arizona State University, P.O. Box 4601, Tempe, AZ 85287. Email: minim@asu.edu

Acetylcholinesterase (AChE) is an enzyme that hydrolyzes the neurotransmitter acetylcholine in the neural synapse and neuromuscular junctions in mammals. There is evidence of non-neuronal functions of AChE including roles in development and stress physiology. The presence of AChE in other species such as plants and fungi has been determined indirectly by looking for AChE activity. Although a maize gene purported to be encoding for acetylcholinesterase was recently cloned (Sagane et al., *Plant Physiol.*, 138: 1359–1371, 2005), little is known about the function of AChE in plants. At3g26430 is the *Arabidopsis thaliana* ortholog of the maize gene, and it is currently annotated as lipase in The Institute for Genomic Research and The Arabidopsis Information Resource databases. We expressed the At3g26430 gene in a variety of systems including bacterial, yeast, plant, and mammalian expression systems. To establish its biochemical identity as a cholinesterase, we analyzed the ability of the enzyme to hydrolyze various esterase and lipase substrates and looked at its inhibition profile. Existing microarray data were mined to determine the expression patterns of this protein and suggest that the protein could be part of a plant defense mechanism. Its subcellular localization using green fluorescent protein fusions will be presented. We have created *A. thaliana* plants that are overexpressers and obtained knockouts for the gene to help us elucidate its function.

P-2036

Tobacco Plastid Transformation Using the Tobacco Anthranilate Synthase (ASA2) Selectable Marker Gene. P. BARONE[1], X-H. Zhang[2], and J. M. Widholm[1]. [1]Department of Crop Sciences, Edward R. Madigan Laboratory, University of Illinois, Urbana, IL and [2]Florida Atlantic University, Boca Raton, FL. Email: pib8@uiuc.edu

The feedback-insensitive anthranilate synthase (AS) alpha subunit (ASA2) gene encodes the tobacco tryptophan (Trp) biosynthesis control enzyme and imparts resistance to toxic Trp and indole analogs. The Trp and indole analogs are toxic because they are able to mimic the specific inhibitory effect (feedback effect) of Trp on the AS. A sensitive enzyme fails to discriminate completely between its normal feedback inhibitor, Trp, and an analog. If the ASA2 is expressed, then the cells are resistant to the analogs and can be selected for. We previously described a tobacco plant nuclear transformation system using 4-methylindole (4MI) 300 μM or 7-methyl-DL-tryptophan (7MT) 300 μM as selection agents with the ASA2 gene as a selectable marker (*Plant Cell Rep.*, 2008 Nov 30; DOI: 18060408). In this study, a modified ASA2 gene, encoding 556 amino acids without a putative transit peptide and containing 204 bp of 3′-noncoding region, was inserted into the tobacco plastid genome through site-specific insertion using accD and ORF184 genes as flanking sequences. The gene was under the control of the synthetic promoter Prrn (332 bp) to ensure efficient messenger ribonucleic acid transcription and translation initiation. Eleven transplastomic tobacco plants with normal phenotype and fertility were produced using either 7MT or 4MI as selectable agent. Southern and Northern blot analyses were carried out on them to confirm the presence and the expression of the Prrn-ASA2. The AS enzyme activity from the transformed plants was less sensitive to Trp inhibition than that from the wild type. Thus, ASA2 is a new selectable marker of plant origin for plastid transformation, a situation that leads to high level gene expressions with no silencing and in most species with no pollen transmission.

P-2037

Expression, Antigenicity, and Enzymatic Activity of the Cedar Allergen, Jun a 1, Using a Plant Viral Vector. ZUN LIU[1], Shikha Varshney[1], Randall Goldblum[2], and Christopher Kearney[1]. [1]Department of Biology, Baylor University, Waco, TX 76798-7388 and [2]Department of Pediatrics, Child Health Research Center, University of Texas Medical Branch, Galveston, TX. Email: zun_liu@baylor.edu

Mountain cedar (*Juniperus asheii*) pollen causes severe allergies in Texas and the central USA. Jun a 1 is the dominant allergen in this pollen and is homologous to pectate lyases. Repeated attempts to produce Jun a 1 in heterologous expression systems have proven unsatisfactory. We expressed Jun a 1 in *Nicotiana benthamiana* using an agroinfection-compatible tobacco mosaic virus vector. Expression resulted in severe necrosis within 6-10 days. Apoplastic fluid collected after vacuum-infiltration with buffer resulted in a 25% TSP yield of Jun a 1, with the other constituents mainly pathogenesis response proteins, as shown by MALDI-TOF and western blot. This protein preparation elicited some degree of patient IgE binding, but monoclonal antibody structure probing revealed some unfolded areas. A his203 to ala mutation which should remove the asp-his bridge covering the putative catalytic site did not affect plant necrosis. Removal of the N-terminus, also posited to be an inhibitory structure, led to genetic instability of the insert. Pectate lyase activity is currently being determined.

P-2038

Effect of Sucrose and Ventilation on Jojoba Plantlets in the Rooting Stage of Micropropagation. D. MILLS, I. Aviv, and A. Benzioni. The J. Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boker Campus 84993, ISRAEL. Email: mills@bgu.ac.il

The aim of this project was to study the effect of ventilation and sucrose concentration on the performance of jojoba (*Simmondsia chinensis*) plantlets in the rooting stage of micropropagation. Shoots taken for rooting were not exposed to ventilation in previous stages of propagation. Ventilation increased rooting and dry mass accumulation. Under ventilation, ambient water loss was lower due to improved stomata functioning and greater deposition of epicuticular wax. Ventilation did not affect CO_2 fixation and uptake of ^{36}Cl into roots but facilitated ^{36}Cl transport to the leaves. The increase in sucrose concentration enhanced rooting and dry mass accumulation. Under no sucrose, ambient water loss was the highest, while no difference was recorded in the range of 0.5 to 2.0%. Sucrose (1.0–2.0%) improved stomata functioning and facilitated deposition of epicuticular wax. Under 2% sucrose, plantlets had low CO_2 fixation but a better translocation level as compared to lower concentrations. In conclusion, sucrose and ventilation were found to be crucial for proper growth and rooting of jojoba shoots.

P-2039

Expression and Functional Analysis of an *Escherichia coli* Antigenic Fusion Protein in Transplastomic Tobacco Plants. S. ROSALES-MENDOZA[1], A. Alpuche-Solís[2], R. Soria-Guerra[1], A. Herrera-Díaz[2], and S. S. Korban[1]. [1]Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801 and [2]División de Biología Molecular, IPICYT, San Luis Potosí, SLP, 78216, MÉXICO. Email: qsrm@uiuc.edu

Enterotoxigenic *Escherichia coli* (ETEC) strains constitute important pathogens in developing countries, thus broad-spectrum vaccines against ETEC are needed. Although the heat-labile toxin B subunit (LTB) has been used in clinical trials, the induction of neutralizing antibodies against the poorly immunogenic heat-stable toxin (ST) would significantly improve the efficacy of formulations as most ETEC strains produce ST. In this study, a plant optimized synthetic gene encoding for an LTB–ST fusion protein has been introduced into plastids of tobacco leaf tissues using biolistic microprojectile bombardment with the aim of developing a single plant-based vaccine model against both toxins by means of using LTB as an ST carrier protein. For the expression of the LTB-ST fusion protein into tobacco chloroplasts, a transformation vector harboring a plant-optimized LTB–ST linked to the plastid 16S ribosomal ribonucleic acid gene promoter and the 5′ untranslated region from the phage T7 gene 10 was constructed. Transplastomic tobacco plants carrying the *LTB–ST* transgene were rescued and confirmed by both polymerase chain reaction and Southern blot analysis. The heterologous protein retained its oligomeric structure in a GM1-enzyme-linked immunosorbent assay and displayed antigenic determinants for both LTB and ST. Western blot analysis demonstrated the integrity of the fusion protein and the presence of the expected immunoreactive protein (17 KDa). Expression levels of the recombinant protein reached up to 2.36% of the total soluble protein. These findings suggested that these transplastomic tobacco plants were useful as a plant-based candidate vaccine model against ETEC strains producing LT and ST toxins.

P-2040

Improvement of European Plum (*Prunus domestica* L.) Genetic Transformation. LINING TIAN[1], Fatih A. Canli[2], Farida Meerja[3], Susan Sibbald[1], and Janny Lac[3]. [1]Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON,

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European plum (*Prunus domestica* L.) is an important fruit crop grown worldwide. Transformation has been reported for this plant, but the efficiency was very low, and transformation was only achieved in one or two varieties. Study and improvement of transformation is important for trait improvement for different varieties of this species. We studied different aspects of plum transformation, intending to improve transformation efficiency. Study showed that while some *Agrobacterium* strains could lead to successful plum transformation, some other *Agrobacterium* strain resulted in poor transformation. Such difference was consistent when different selectable markers were used. Use of different types of regeneration media can affect transformation efficiency. Thiol compounds, such as L-cysteine, sodium thiosulfate, dithiothreitol (DTT), which were reported to increase transformation in some species, had a negative effect on plum transformation. Based on the study, a reliable and effective transformation technology has been developed for European plum grown in Canada. The system is now routinely used to introduce new traits into European plum.

P-2041

Transformation of Barley (*Hordeum vulgare* L.) with Cytokinin Dehydrogenase Gene. SARKA VYROUBALOVA[1], Ludmila Ohnoutkova[2], and Petr Galuszka[1]. [1]Department of Biochemistry, Faculty of Science, Palacky University, Slechtitelu 11, 783 71, Olomouc, Czech Republic, and [2]Academy of Sciences of the CZECH REPUBLIC, Institute of Experimental Botany, Sokolovska 6, 77200, Olomouc, CZECH REPUBLIC. Email: sarka.vyroubalova@seznam.cz

Barley is one of the most important crops in the world's agriculture. Hence, it is today inevitable to improve or create new qualities of barley traits by genetic manipulation. We will present data concerning transformation of barley plants with gene involved to cytokinin deactivation using two methods: *Agrobacterium tumefaciens*-mediated transformation and particle bombardment of immature embryos. Cytokinin dehydrogenase is an enzyme responsible for the irreversible degradation of plant growth regulators—cytokinins. The main functions of cytokinins and their accurate equilibrium in plants are promotion of cell division and correct organ differentiation, followed by control of lateral buds growth, expansion of leaves, and delay of senescence. Genomic form of gene *gHvCKX2* (National Center for Biotechnology Information no. AF490591) encoding functional cytokinin dehydrogenase (CKX) from barley was used for the construction of different transformation vectors. Two different promoters, viral 35S and maize ubiquitin, together with two different selection genes, phosphinotricin and hygromycin resistance, were applied. Transgenic tobacco carrying the gene *gHvCKX2* under the 35S promoter showed a distinctive cytokinin-deficient phenotype with a dwarf overground part and an enormously enhanced root system. Unfortunately, regeneration frequency of barley calli was very weak. Overexpression of gene *gHvCKX2* during selection phases of transformants probably influenced the ability of proper regeneration. Therefore, the addition of different cytokinins, auxins, and specific inhibitors of CKX into the plant culture media has been tested to reduce effects of overexpressed gHvCKX2 during plant regeneration. Concurrently, tissue- and organ-specific barley promoters were cloned and functionally tested for ectopic expression of CKX genes in the root and aleurone layer of barley.

P-2042

Spent Medium Analysis for Hemerocallis Micropropagation on Liquid MS Medium. J. W. ADELBERG, M. P. Delgado, and J. P. Tomkins, Department of Horticulture, and Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634. Email: jadlbrg@clemson.edu

Culture vessels are closed with respect to mineral ions. The difference between the quantity of ions in media at initiation and in spent medium is held in plantlet tissues. Relationships were calculated for 1ml spent medium samples from a multivariate experiment involving 4 unrelated genotypes of daylilies, 2 explant densities, 2 sucrose concentrations, and 2 concentrations of BA in the presence and absence of ancymidol. Plantlet mass was measured following micropropagation and greenhouse growth. The genotype (Barbara Mitchell) with largest mass from the laboratory had the smallest plants in the greenhouse. Spent medium was analyzed by ICP. NO3- and $NH4^{+}$ concentrations were below the level of detection. Active uptake of 11 ions was evidenced by much greater concentrations of nutrients calculated for soma than in the medium. Luxuriant consumption likely

occurred at low explant densities where tissue ion content was greatest but unrelated to plant growth. In general, plants with the most growth (and uptake of water) had the lowest nutrient concentrations (on dry weight basis), and plants with the greatest ion concentrations had the least growth. K^+, Na^+, PO_4^{-3}, Cu^{+2}, and Fe^{+2} concentrations in the plantlets were in the ranges of well-fertilized daylily plants in modern production nurseries. However, Mn^{+2}, Mg^{+2}, Ca^{+2}, and B concentrations in the dry mass in Barbara Mitchell only was often below that defined as deficiency for these minerals, and the deficient plants were the ones that grew least in the laboratory (took up the least water). All other plantlets were in the normal range for daylily. Zn^{+2} and S did not reveal any strong trends other than the obvious active uptake. Growth regulators had a much less effect on nutrient use than genotype and plant density. Spent medium analysis indicated the genotype with the problems in the greenhouse was transferred ex vitro with low concentrations of several nutrients, and problems were not related to excessive growth or depletion of ions in the medium. Spent medium analysis is a useful tool to reveal some causes of what we often refer to as genotypic variation.

P-2043

Comparison of the Agronomic Performance of *Coffea arabica* L. Somatic Embryogenesis-derived Plants with Seed-produced Plants. J. C. Rezende[1], G. R. R. Almeida[2], M. Pasqual[3], A. C. R. Santos[4], and C. H. S. CARVALHO[5]. [1]EPAMIG, Caixa Postal 176, Lavras, MG, 37200-000, BRAZIL; [2]Minasul, Varginha, 37002-560, MG, BRAZIL; [3]UFLA, Dept. de Agricultura, Lavras, MG, 37200-000, BRAZIL; [4]Fundação Procafé, Alameda do Café, 1000, Varginha, 37026-400, BRAZIL; and [5]Embrapa Café, Alameda do Café, 1000, Varginha, MG, 37026-400, BRAZIL. Email: carlos.carvalho@embrapa.br

Micropropagation of plants has a great potential for commercial multiplication of superior heterozygous coffee plants, but it is still not fully elucidated if arabica coffee plants derived from somatic embryogenesis would show any important agronomic difference from plants traditionally produced by seeds. To help answer this question, a field trial was set in the southern region of the Minas Gerais state, Brazil, aiming to compare somatic embryogenesis-derived plants with plants obtained from seeds. A randomized block designed with ten replications and ten plants per plot of the Red Catuaí IAC 44 cultivar in F10 selfing generation was used. Somatic embryogenesis-derived plants were regenerated from callus with the aid of temporary immersion bioreactors. The following parameters were evaluated at the first harvest: yield, plant height, length of lateral branches, bean size, bean/fruit weight ratio, fruit maturation, and seed quality. Except for the length of lateral branches, in which somatic embryogenesis-derived plants were higher than seed-derived plants, all the other parameters evaluated were not statistically different for both propagation methods. A visual inspection did also not detected any significant abnormal phenotype for shape, color, and leaf size, plant architecture, blossoming time, and plant vigor and mortality. These findings clearly indicates that there is no significant constrain for the commercial use of arabica coffee plants produced by somatic embryogenesis.

P-2044

Improvement of Cryopreservation Technique for Long-term Storage of Shoot Tips of Ipomoea batatis. M. M. Jenderek, D. Skogerboe, and D. Ellis. National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, CO 80521. Email: maria.jenderek@ars.usda.gov

Roots of sweet potato (*Ipomoea batatis*) are an important food crop in subtropical and tropical regions. Being a vegetatively propagated crop, its genetic resources are predominantly preserved as field plantings and/or as tissue cultures. Cryopreservation is the most economic and reliable preservation method for many vegetatively propagated germplasm collections; however, long-term storage protocols developed are not applicable to all genotypes of a collection. Successful cryopreservation of selected sweet potato germplasm is published in literature; however, the survival rate reported could not be reproduced. Modifications developed in our laboratory to an original encapsulation–vitrification protocol by Hirai-Sakai significantly improved survival of 13 sweet potato germpalsm accessions exposed to liquid nitrogen. The modification included an 8-h dark incubation period of nodal sections prior to meristem excision, adjustments in the duration of post-rewarming treatment, and minor changes in culture medium recipes.

P-2045

Efficient In Vitro Bulblet Regeneration from Immature Embryos of Endemic and Endangered Geophytes *Muscari muscarimi* and *M. massayanum* and Ex Vitro Acclimatization. S. ÖZCAN[1], İ. Parmaksız[2], S. Mirici[3], D. Gürlek[1], S. Çöçü[1], O. B. Özcan[1], G. İpek[1], C. Sancak[1], S. Uranbey[1], B. Gürbüz[1], A. İpek[1], D. Doğan-Kalyoncu[1], C. Karaoglu[1], C. S. Sevimay[1], and N. Arslan[1]. [1]Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110,

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Habitat destruction has resulted in the disappearance of many plant species from the earth forever and many more face extinction. Likely, endemic and endangered *Muscari muscarimi* Medik. and *M. massayanum* Grunert belonging to the Liliaceae family and growing in natural habitats of Turkey are also threatened by complete extinction. Therefore, the collection of their bulbs from the natural habitats is forbidden in Turkey, in accordance with international agreements for the protection of endangered geophytes. Moreover, the natural propagation rate of these geophytes, like most geophytes, is relatively low, and it takes 4 or 5 yr to develop a flowering plant from seed. The propagation ratio of bulbs are also low as some bulbs do not develop new bulblets and some produce only one to two bulblets in a 3-yr period. This low propagation ratio inhibits large-scale cultivation of *M. muscarimi* and *M. massayanum*, which have a great potential in ornamental and perfume industry because of their attractive purple-white flowers with an intense musky fragrance. Besides conventional methods of propagation, endemic and threatened plants could efficiently be propagated and conserved with in vitro cultural methods, which have low impact on wild populations with a minimum of plant material. To develop an in vitro micropropagation system for *M. muscarimi* and *M. massayanum*, immature embryo explants were cultured on different nutrient media supplemented with various concentrations of plant growth regulators using different methods. Prolific shoot regeneration was obtained (more than 200 shoots per explants) from immature embryos on Murashige and Skoog (MS) medium supplemented with 4 mg/l benzylaminopurine (BAP) and 0.5 mg/l naphthalene-acetic acid (NAA). Individual shoots were separated and subcultured onto a MS medium containing various concentrations of jasmonic acid, BAP, NAA, sucrose, agar, and gelrite to develop shoots into bulblets. Best bulblet formation (100%) with a well-developed root system was achieved on a MS medium containing 5% sucrose, solidified with agar, and lacking growth regulators. Regenerated bulblets were transferred to soil with 100% success.

P-2046

Micropropagation of the Federally Endangered Autumn Buttercup (*Ranunculus aestivalis* L. Benson). V. C. PENCE[1], S. M. Charls[1], K. Lindsey[1], and S. Murray[2]. [1]Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 and [2]The Arboretum at Flagstaff, 4001 S. Woody Mountain Road, Flagstaff AZ 86001-8776. Email: valerie.pence@cincinnatizoo.org

The endangered Autumn buttercup, *Ranunculus aestivalis* L. Benson, is known from only one site in Utah. The size of the population has fluctuated, but a recent count found that it had declined to less than 30 plants. In an attempt to increase numbers, methods were developed for initiating and micropropagating in vitro cultures to provide plants to augment the declining population. Seeds were received from collaborators at The Arboretum at Flagstaff, surface sterilized, scarified, and placed into tubes of half-strength Murashige and Skoog medium with 1.5% sucrose and 0.22% Phytagel. Germination only occurred when the in vitro seeds were stratified (60 d at 4° C) and then moved to a 26° C incubator. Germination of a portion of the seeds occurred within 2–3 wk, but additional cold treatments were needed to stimulate further germination. Starting with 64 seeds, after four cold treatments, two seeds remain ungerminated. The shoots from in vitro seedlings were removed and transferred to Driver and Kuniyuki walnut medium plus 0.89 μM benzylaminopurine to initiate and propagate cultures. Lines from each seedling were maintained separately to maintain the genetic diversity of the original seeds. Propagated shoots produced roots on this same medium at a rate of more than 90% and have been acclimated to soil both at Center for Conservation and Research of Endangered Wildlife (CREW) and at The Arboretum at Flagstaff with a usual survival of more than 90%. In vitro propagated plants are now being used in a collaborative project between CREW, The Arboretum at Flagstaff, The Nature Conservancy, Utah Valley State College, and the US Fish and Wildlife Service, for producing, acclimating, outplanting, and monitoring plants at the Utah site. Thus far, more than 130 in vitro propagated plants of multiple genotypes have been planted, to augment the declining original population. [Protocol development was supported in part by grants from the Institute of Museum and Library Services in collaboration with the Center for Plant Conservation (St. Louis), while the outplanting project is supported in part by funding from the US Fish and Wildlife Service.]

P-2047

Micropropagation of the Federally Endangered Cumberland Sandwort (*Arenaria cumberlandensis* Wofford & Kral). V. C. PENCE, S. M Charls, B. L. Plair, and J. R. Clark. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

The Federally endangered Cumberland sandwort is located in only five counties of northern Tennessee and southern Kentucky. It grows in a moist, shaded habitat of sandstone rockhouses, or overhanging cliffs, and is threatened by hiking, rappelling, and illegal digging for Native American artifacts. To contribute to the recovery of this species, in vitro propagation protocols were developed to provide methods for increasing the number of plants in the wild. Seeds, from collaborators at the Missouri Botanical Garden, were surface-sterilized at Center for Conservation and Research of Endangered Wildlife and placed on half-strength Murashige and Skoog (MS) medium with 1.5% sucrose and 0.22% Phytagel for germination. The highest germination rate was observed when the in vitro cultured seeds were stratified for 2 mo at 4° C. Seedling shoots were isolated and cultured on MS medium with 3% sucrose, 2.2 μM benzylaminopurine (BAP), 0.27 μM naphthaleneacetic acid (NAA), and 0.22% Phytagel to initiate cultures. Once established, growth regulator levels were lowered to 0.44 μM BAP and 0.05 μM NAA, and each line initiated from a seed was maintained separately to maintain the genetic diversity of the seedlings. Cryopreservation procedures were also developed for shoot tips of *A. cumberlandensis*, using the encapsulation vitrification procedure. Survival of more than 60% was achieved, and the lines are being banked for long-term storage in liquid nitrogen. Although shoots rooted on the propagation medium, roots appeared more robust when initiated on woody plant medium with 3% sucrose, 2.1 μM indole-3-butyric acid and 0.8% agar. Plants could be acclimated to soil in clear plastic boxes with covers. Vents were added to the lids slowly to acclimate the plants to ambient humidity. Plants produced in this way have been used for an experimental outplanting at a site in southern Kentucky in collaboration with the US Forest Service, and the plants have shown good survival and have flowered and produced seedlings within 2 yr. [Protocol development for tissue culture and cryopreservation was funded in part by grants from the Institute of Museum and Library Services in collaboration with the Center for Plant Conservation (St. Louis).]

P-2048

Propagation and Acclimatization of the Cancer Herb *Castilleja tenuiflora*. G. Salcedo[1], E. Ventura[1], S. Evangelista[1], A. Zamilpa[2], and G. TREJO[1]. [1]Centro de Desarrollo de Productos Bióticos-IPN. Morelos, MÉXICO, 62731. [2]Centro de Investigación Biomédica del Sur-IMSS. Morelos, MÉXICO, 62790. Email: gttapia@ipn.mx

Castilleja tenuiflora (Scrophulariaceae) is a wild perennial herb highly recommended by Mexican traditional medicine for treating tumors and is source of iridoids with antitumoral, cytotoxic, and immunostimulant activities. *C. tenuiflora* grows in habitats exposed to disturbances such as fire and clearance, and it is indiscriminately harvested for commercialization. Because its sexual propagation through seeds is difficult, an in vitro procedure for propagation and acclimatization of plants was developed. Shoot induction from axillary buds of wild-grown plants using Murashige and Skoog full-strength medium with 0.2 mg/L benzylaminopurine (BAP)+0.1 mg/L naphthaleneacetic acid (NAA) was observed in 33%. Shoot multiplication and elongation were achieved in one step using 0.1 mg/L indole-3-butyric acid (IBA) and 0.25 mg/L BAP with a yield of four shoots per explant. Shoots of at least 1 cm in length were rooted with frequency 88% after increasing IBA to 1.0 mg/L and excluding BAP. Under these conditions, shoots were threefold longer than those developed in a free-hormone medium, rooting increased four times, and hyperhydricity was not observed. Shoot multiplicative capacity has been maintained in subsequent subculture for at least 14 mo. For acclimatization, rooted plantlets were removed from in vitro culture and maintained for 35 d in a hydroponic system. Then, they were transferred to a potting soil mixture of peat moss–agrolite–vermiculite (1:1:1; pH=5.8) under greenhouse conditions (55% relative humidity) where 91% of rooted plants were acclimated and flowers were produced. The propagation and acclimatization procedure, comprising 105 d, provides shoots amenable to rooting and transfer to soil. Micropropagated plants accumulated iridoids and appeared morphologically similar with the mother plants. This protocol should be useful for the conservation as well as mass propagation of *C. tenuiflora.*

P-2049

ARS-Media—A New Public Domain Software Package for the Calculation of Media Recipes Based on Ion-Specific Constraints. T. J. EVENS and R. P. Niedz. USDA-ARS, USHRL, 2001 S. Rock Rd., Ft. Pierce, FL 34945. Email: terence.evens@ars.usda.gov

Understanding the ion-specific effects of the mineral elements is a central theme of biology because these ions are fundamental to the composition and maintenance of life. However, experiments concerned with determining ion-specific effects are generally performed with salt, as opposed to ion, manipulations. This means that researchers have had to accept a covariance in the co-ion of the salt used to manipulate the ion(s) of interest. The result is that the effect of a single ion cannot be determined as it is confounded with the potential combined effects of the other ions that are co-varied. Because of this difficulty, the majority of research studies concerned with determining

ion-specific effects exhibit ion confounding. The software application ARS-Media utilizes a linear programming optimization algorithm to determine the combination of salts, acids, and bases that satisfies any given target solution of ions. ARS-Media therefore allows researchers to construct experimental designs that use ions, as opposed to salts, as individual factors and hence experimentally determine ion-specific effects on biological responses relating to ion type, concentration, and proportion.

P-2050

Regulating NH_4^+, NO_3^+, and K^+ concentration and proportions improves in vitro tissue growth. R. P. NIEDZ and T. J. Evens. USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: Randall.Niedz@ars.usda.gov

A mixture-amount experiment that simultaneously varied both the ratios and total ionic concentration (from 20 to 100 mM) of NH_4^+, NO_3^+, and K^+ was used to maximize sweet orange callus growth cv. “Hamlin.” These experiments were free of ion-confounding effects, i.e., ions added via pH adjustments and salt-derived counterions that are typically unaccounted for in experimental designs and analyses, and were used to quantify the effects and interactions of these nutrients on in vitro growth. The primary result was the identification of a region within the design space where callus fresh weight was approximately 175% greater than a Murashige and Skoog (MS) control (the standard mineral nutrient formulation used for this cell line). Media recipes selected from this region specified 37.5 mM total nitrogen with a 0.25:0.75 NH_4^+/NO_3^+ ratio as compared to MS with 60 mM total nitrogen with a 1:1 NH_4^+/NO_3^+ ratio. Using a multivariate, mixture-amount approach, it was possible to efficiently identify media compositions that provided the “best” growth and simultaneously quantify the main and interaction/blending effects, thus providing an empirical understanding of the “why” and “how” of ion-specific effects. These results illustrate the importance of designing experiments free of ion confounding when defining mineral nutrient formulations.

P-2051

Plant Expression of Chimeric Gag/gp41 Virus-like Particles as a Subunit Vaccine Against HIV-1. SARAH KESSANS, Tsafrir Mor, and Nobuyuki Matoba. School of Life Sciences and Biodesign Institute, Arizona State University, Tempe, AZ 85287. Email: Sarah.Kessans@asu.edu

The transmembrane human immunodeficiency virus (HIV) 1 envelope protein gp41 has been shown to play critical roles in the viral mucosal transmission and infection of CD4+ cells. Gag, on the other hand, is a structural protein configuring the enveloped virus particles and has been suggested to constitute a target of the cellular immunity potentially controlling the viral load. The goal of this project is to express chimeric HIV-like particles (VLPs) consisting of Gag and the deconstructed, critical membrane proximal domain of gp41 in plants toward an inexpensive subunit vaccine inducing a broad anti-HIV immune response. Using a polymerase chain reaction-based de novo gene synthesis method, a plant-optimized HIV-1 *gag* gene was constructed based on a primary subtype C R5 HIV-1 isolate. As a first step toward the chimeric VLPs, the Gag protein was expressed in *Nicotiana benthamiana* using a modified tobacco mosaic virus-based transient expression system. Western blotting using anti-p24 Abs demonstrated the expression of the 55-kDa Gag protein in the leaf tissue. Sucrose gradient sedimentation showed that the full-length Gag protein migrated at a density corresponding to that reported for Gag VLPs. Furthermore, the examination of leaf material and the extract in transmission electron microscopy showed potential budding as well as the formation of VLPs with a diameter of approximately 100 nm. These results suggest that plant cells can support the formation of HIV-1 Gag VLPs. Gag-based VLPs will be used as a base for displaying other components of the HIV-1 envelope.

P-2052

Plague Antigen Fusions with gp41 Membrane Proximal Region as HIV Vaccine Candidate. NAMRATA SHAH, N. Matoba, Howard Chang, John Hu, and T. S. Mor. Centre for Infectious Disease and Vaccinology, Biodesign Institute, School of Life Sciences, Arizona State University, Tempe, AZ, 85281. Email: namrata.shah@asu.edu

Epithelial transcytosis has recently been proposed as one of the major mechanisms for the mucosal transmission of human immunodeficiency virus (HIV)-1. The highly conserved membrane proximal region of gp41 of HIV (MPR) has been shown to mediate this process. The aim of this study is to create vaccines based on MPR, which can induce antibodies blocking the HIV mucosal transmission pathway. The protein comprised of Fraction 1 (F1) and V antigen of *Yersinia pestis* (F1-V) is known to elicit a strong immune response that can confer protection against a *Y. pestis* challenge in animal models and therefore considered to be a suitable immunogenic platform for

vaccine design. We therefore created a novel fusion protein where the $MPR_{649–684}$ is genetically linked to F1-V (F1-V–$MPR_{649–684}$) and expressed in *E. coli* and in plants using a virus-assisted transient expression system. The fusion protein expressed in *E. coli* was tested in BALB/c mice for its efficacy in inducing anti-MPR antibodies, either alone or in combination with another MPR fusion protein based on cholera toxin B subunit, CTB–MPR. Among the various immunization regimens tested, The groups cross-immunized with CTB–$MPR_{649–684}$, and F1-V–$MPR_{649–684}$ showed a significant anti-MPR response. Especially, mucosal immunization with CTB–MPR combined with systemic immunization with F1-V–MPR induced robust mucosal IgA as well as serum IgG responses to MPR. On the other hand, neither of the immunogens alone induced a significant anti-MPR antibody response. These results underscore the potential of prime-boost immunization based on heterologous carrier proteins to induce a robust antibody response to otherwise poorly immunogenic antigens. Future work points toward the analysis of the induced antibodies for their efficacy in blocking HIV transcytosis.

P-2053

Generation and Characterization of Transgenic Seashore Paspalum (*Paspalum vaginatum* Swartz). FREDY ALTPETER, Isaac Neibaur, Hangning Zhang, and Maria Gallo. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida—IFAS, Gainesville, FL 32611. Email: faltpeter@ifas.ufl.edu

Seashore paspalum (*Paspalum vaginatum* Swartz) is a salt-tolerant, fine-textured turfgrass used on golf courses in coastal, tropical, and subtropical regions. Targets for genetic engineering of seashore paspalum include improved disease and insect resistance. However, a genetic transformation protocol for seashore paspalum was lacking. Our objective was to optimize callus induction and plant regeneration and develop a genetic transformation protocol for this commercially important turfgrass species. A multifactorial experiment showed that the callus induction medium containing 3 mg L^{-1} dicamba and 1.0 mg L^{-1} benzylaminopurine (BAP) had a plant regeneration frequency that was 12 times higher than medium with 3 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) alone and ten times higher than the combination of 3 mg L^{-1} 2,4-D and 1.0 mg L^{-1} BAP. However, transgenic plants were regenerated on both 2,4-D- and dicamba-containing media following biolistic transfer of the hph-selectable marker gene and selection with hygromycin. Transgenic plants grew vigorously and did not show phenotypic differences compared to nontransformed controls. Stable transgene integration was confirmed by Southern blotting. Transgenic plants and their vegetative progeny stably expressed the transgene as indicated by reverse transcriptase–polymerase chain reaction.

P-2054

Producing transgenic Tifton-85 and TifEagle grasses using *Agrobacterium* vacuum infiltration of Stolon Nodes. S JOSHI[1] and M Gallo[2]. [1]Department of Agronomy, University of Florida, Gainesville, FL and [2]Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32610. Email: joshi76@ufl.edu

Tifton-85 and TifEagle are bermudagrass species belonging to the genus *Cynodon*. Until recently, many of these monocot species were difficult to genetically engineer as a result of their inherent limitations associated with resistance to *Agrobacterium* infection and their recalcitrance to in vitro regeneration. Furthermore, the initiation and maintenance of embryogenic callus in most of the monocots is genotype dependent, time consuming, and laborious. To avoid the callus culture, we describe a protocol that expedites a straightforward and callus-free transformation procedure for these two grass cultivars. An efficient in vitro regeneration system was established for both cultivars using different concentrations of kinetin and auxins. Stolon nodes were infected and cocultivated with *Agrobacterium tumefaciens* strains GV3101 or AGLO containing the antioxidant acetosyringone and the Cry 1Fa gene for fall armyworm resistance. Green shoots were directly produced from infected stolon nodes 4 to 6 wk after hygromycin selection. Rooted putative transgenic plantlets were then obtained 7 to 8 wk later. To date, 72 plants have been obtained using this procedure. The established plants are currently being screened for the transgene by polymerase chain reaction, and the transgenic nature of five plants has been demonstrated by Southern blot hybridization. Further research is focused on increasing the efficiency of transformation and evaluating expression of the transgene.

P-2055

Improving Turf Quality by Integration and Expression of *AT-GA-ox1* or *ATHB16* in Apomictic Bahiagrass (*Paspalum notatum* Flugge). P. LOMBA, F. Altpeter, M. Agharkar, H. Zhang, K. Kenworthy. 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 popular low-input turfgrass in the south-eastern USA. The quality of bahiagrass turf is compromised by the production of long seedheads and low turf density. The objective of the study is the comparative field evaluation of turf quality following the introduction of a gibberellin-catalyzing enzyme (*AT-GA-ox1*) or a repressor of cell expansion (*ATHB16*) into bahiagrass. *AT-GA-ox1* or *ATHB16* were subcloned under control of the ubiquitin promoter and cotransferred to the bahiagrass genome with the *nptII*-selectable marker by biolistic gene transfer (Agharkar et al. 2007). Transgene integration and expression was confirmed with Southern, Northern, and reverse transcriptase–polymerase chain reaction analysis, respectively. Transgenic bahiagrass and wild-type plants were established in 1×1-m plots under USDA-APHIS permit 06-219-01 r in a randomized block design with 24 replications and weekly mowed following establishment. Turf density was evaluated by counting number of tillers. The number of inflorescences per plot and the length of the inflorescence stems were also evaluated. Statistical analysis was performed according to the randomization structure using the analysis of variance procedure of SAS. Bahiagrass overexpressing *ATHB16* or *AT-GA-ox1* produced significantly more tillers than the wild type. Transgenic plants also showed decreased stem length and delayed flowering. Bioactive gibberellins have been proposed to suppress tiller bud outgrowth and enhance apical dominance in grasses (Lester et al. 1972). Increased tillering was reported following applications of Trinexapac-ethyl, inhibiting the synthesis of bioactive gibberellins (Ervin and Koski 1998). *ATHB16* represses cell elongation independent of GA signal transduction (Wang et al. 2003). Target genes downstream of *ATHB16* still have to be identified. A global gene expression profiling of transgenic bahiagrass overexpressing this transcription factor will shed light on this. Both transgenic strategies supported improved turf quality in bahiagrass. We currently evaluate the persistence of the transgenic plants under low-input conditions.

P-2056

Evaluation of Immature Embryos as Target Tissue for Oil Palm Transformation. G. K. A. PARVEEZ [1], A. M. Na'imatulapidah, A. Nur Hanin, and A. R. Omar. Malaysian Palm Oil Board, No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, MALAYSIA. Email: parveez@mpob.gov.my

Oil palm is an important economic perennial crop for Malaysia and its oil, palm oil, is one of the world's main source of vegetable oils and fats. Genetic engineering was identified as a tool for synthesizing high-value-added products in transgenic oil palm for ensuring the industry remain competitive in years to come. Genetic engineering requires reliable transformation and regeneration systems. Successful production of transgenic oil palm was achieved 10 yr ago using embryogenic calli as the target tissue. In this study, we evaluated the immature embryos as target tissue. Initially, the effectiveness of four antibiotics (kanamycin, geneticin G-418, paromomycin, and hygromycin) and herbicide Basta as a selection agent for oil palm immature embryos was evaluated. Bombardments were carried out on a minimum of five replicates with 25 immature embryos per plate. Bombardment were carried out at the following conditions: 1,100 psi rupture disk pressure, 75 mm stopping plate to target tissue distance, and 67.5 mmHg vacuum pressure. Bombarded immature embryos were cultured on hormone-free medium containing varying concentrations (10–2,000 mg/l) of the selection agents. The immature embryos were subcultured into fresh medium after 4 wk, and the growth of immature embryos were recorded every week up to 8 wk. The herbicide Basta and hygromycin proved to be the most effective as they could inhibit the growth of immature embryos at 20 mg/l. Paromomycin and geneticin G-418 requires 100 and 500 mg/l, respectively, for inhibition. Kanamycin is the least effective as it only inhibits 15% of the immature embryos grown at 2,000 mg/l, demonstrating a high endogenous resistance of oil palm immature embryos. Later, the immature embryos were bombarded (15 immature embryos per plate plates and with five replicates) and subjected to the selection at one of the following: (1) selection at immature embryos stage followed by direct germination (selection 1), (2) selection after the immature embryos were regenerated into calli and followed by whole plant regeneration (selection 2), or (3) selection when the immature embryos have been regenerated into embryogenic calli and followed by whole-plant regeneration (selection 3). Unbombarded immature embryos, either selected or unselected, were used as controls. Selection was carried out on kanamycin, hygromycin, and herbicide Basta, at different concentrations (kanamycin=50, 100, 200, and 300 mg/l; hygromycin and Basta=5, 10, 20, and 50 mg/l). The production of transgenic oil palm through selection 1 resulted in either chimeric plants or failure to germinate any plants. Selection 2 was not efficient as it only produce a few resistant colonies and one line of transgenic plant. Hygromycin and kanamycin failed to regenerate any transgenic plants. Finally, selection 3 also resulted in a few resistant colonies and one transgenic line regenerated for Basta. Positive polymerase chain reaction results were only obtained from selection 2 and selection 3 for Basta selection. It was concluded that transgenic oil could be effectively produced by bombarding embryogenic calli derived from immature embryos and selected on Basta.

P-2057

Tailoring Herbicide Treatments of Tomato Suspension Cultures to Produce ^{14}C-Carotenoids for Animal Metabolic Tracing. NANCY J. ENGELMANN[1], Randy B. Rogers[2], Mary Ann Lila[1,2], John W. Erdman, Jr.[1]. [1]Division of Nutritional Sciences and [2]Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801. Email: nengelma@uiuc.edu

The consumption of tomato food products is associated with a decreased risk of prostate cancer, and carotenoids are believed to play an integral role in this diet–disease relationship. Lycopene (LYC) is often credited as the main bioactive carotenoid in tomatoes, but two other prominent colorless carotenoids, phytoene (PE) and phytofluene (PF), may play an active role as well. The Utilization of ^{14}C-carotenoid tracers will facilitate building knowledge on LYC, PE, and PF metabolism and bioavailability, the basis for understanding disease prevention mechanisms. Norflurazon (NORF), and 2-(4-chlorophenyl-*thio*) triethylamine (CPTA) are bleaching herbicides, which lead to marked increases in PE and PF or LYC, respectively, by interrupting carotenogenesis. Cherry "VFNT" tomato cell cultures grown 14 d in Murashige and Skoog liquid media with growth regulators indole-3-acetic acid (5 mg/L) and all-*trans*-zeatin (2 mg/L) were treated with a combination (COMBO) of NORF (day 7, 0.75 mg/L) and CPTA (day 1, 0.075 g/L) or each herbicide alone. Analysis of concentrations of PE, PF, and LYC revealed that CPTA alone produced the highest combined total of the carotenoids (0.20, 0.19, and 1.62 mg/L), NORF alone produced the highest levels of PE (0.90, 0.05, and 0), and the COMBO resulted in the most uniform production across the three targeted carotenoids (0.40, 0.15, and 0.50 mg/L). The COMBO treatment was used to radiolabel these carotenoids by the addition of 363-uCi U-^{14}C-sucrose to 80-mL media inoculated with 4 mL of tomato cells with 8 mL spent media. Twelve labeled cultures were grown in an enclosed chamber system designed to aerate cultures six times per day and trap $^{14}CO_2$ in NaOH for the 14-d growth cycle. Of the initial ^{14}C-sucrose dose provided, 20% accumulated in the cells, 52% remained in the media, and 16% was lost to respiration. Using the methods described here, herbicide treatments can be tailored for the targeted production of radiolabeled carotenoids. Labeled cultures will be extracted for ^{14}C-carotenoids for use in carotenoid metabolism investigations. (Supported by NIH/NCI CA 112649-01A1)

P-2058

Effect of oxidative Stress on the Alkaloid Production in *Uncaria tomentosa* Root Cultures. A. HUERTA-HEREDIA[1], G. Trejo-Tapia[3], T. Ponce-Noyola[1], C. M. Cerda-García-Rojas[2], A. C. Ramos-Valdivia[1]. [1]Departamento de Biotecnología y Bioingeniería and [2]Departamento de Química, Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV-IPN), Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, MÉXICO, D. F. 07360, MÉXICO and [3]Centro de Desarrollo de Productos Bióticos del IPN (CEPROBI-IPN), P. O. Box 24, Yautepec 62730, Morelos, MÉXICO. Email: aramos@cinvestav.mx

Uncaria tomentosa (cat's claw), an indigenous plant from the Amazon rainforest, is the source of pentacyclic monoterpenoid oxindole alkaloids (PMOA) with immunomodulatory, cytotoxic, anti-acquired immune deficiency syndrome, and antileukemic activities. PMOA are obtained from the powered bark of more than 8-yr-old native plants. These highly oxidized alkaloids have been recently produced in cell suspension cultures of *U. tomentosa* in a stirred tank bioreactor. It was demonstrated that hydrodynamic stress in a bioreactor induced PMOA production via oxidative burst (Trejo-Tapia et al. 2007). In an attempt to further increase PMOA production, fast-growing and hormone-independent root cultures were established, and the effect of oxidative stress stimulators was investigated. Root cultures showed, by reverse-phase high-performance liquid chromatography (HPLC), the accumulation of PMOA (0.2–0.4 mg/gDW), mainly mitraphylline and pteropodine together with their isomers. Furthermore, the cell cultures produced a pentacyclic glucoindole alkaloid that was not previously detected in the wild plant. The structure of this compound, with hypotensive activity, was verified by mass spectrometry and 1D and 2D nuclear magnetic resonance spectroscopy as 3α-dihydrocadambine (DHC). As a result of the addition of oxidative stress inducers such as ethanol, H_2O_2, and a combination of buthionine sulfoximine (BSO) and jasmonic acid (JA), PMOA production was 2.7-, 1.7-, and 1.9-fold higher, respectively, in relation to the nonstressed shaker flask cultures. On the other hand, the addition of H_2O_2 or the addition of BSO and JA or H_2O_2 and horseradish peroxidase increased DHC accumulation. Furthermore, root cultures growing in an airlift reactor produced PMOA in similar amounts than those reached with oxidative stress inducers, although DHC accumulation was substantially reduced. (This research was supported by CONACYT grant 43228).

P-2059

In Vitro Callus Induction of Wild Wheat (*Triticum boeoticum*). N. A. HOVHANNISYAN and A. G. Yesayan. Department of Ecology and Nature Protection, Yerevan State University,

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Armenia is considered to be part of the center of origin for cereals including 13 wild wheat species and more than 360 cultivated varieties. Some of them are known to be drought or disease resistant and can be used in crop improvement programs. Nowadays, tissue culture techniques can be used for conservation and studying of cultivars and their wild relatives. The aim of this study was to induce callus formation and proliferation of *Triticum boeoticum* to determine the optimal media and growth conditions. The cultivated variety *Bezostaja-1* was used as a control. The surface-sterilized seeds were inoculated on agar-solidified Murashige and Skoog (MS) medium added with 1.5–4.0 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D). The callus induction rate was calculated as the number of explants with induced callus over the total number of explants plated×100. To obtain callus proliferation, a portions of calli obtained were transferred to MS media added with 2,4-D and 6-benzylaminopurine (BAP) in concentrations from 1.5 to 4.0 mgL^{-1}. Callus relative growth (RG) rate was calculated on the basis of the initial and final growth after 4 wk of subculturing. Callus cultures were cultivated in thermostat with temperature 26±2° C. A completely randomized design was used for all experiments. Each experiment was replicated three times with 100 samples in each replicate. Fisher's protected least significant difference analysis was used to separate means. A high frequency—76%—of callus induction was observed at 3.5 and 4 mgL^{-1} 2,4-D after a culture period of 4–5 wk. Good callus proliferation of *T. boeoticum* with RG 24.4 was obtained when the calli were subcultured on MS medium added with 4.0 mgL^{-1} 2,4-D and 1.5 mgL^{-1} BAP. The callus culture obtained can be used as a model for studying of breeding potentials of wild cereals in vitro.

P-2060

How to Measure and Report Growing Conditions for Experiments in Plant Tissue Culture Facilities. International Committee for Controlled Environment Guidelines. North American contact: C. KUBOTA, The University of Arizona, Department of Plant Sciences, Forbes Building, P.O. Box 210036, Tucson, AZ 85721. Email: ckubota@ag.arizona.edu

Conditions in controlled environment tissue culture facilities should be reported accurately, to allow replication of experiments and comparison of results among facilities and to avoid experimental artifacts from uncontrolled variables. This poster and its accompanying guidelines brochure (entitled "*Guidelines for measuring and reporting environmental parameters for experiments in plant tissue culture facilities*" and published by the International Committee for Controlled Environment Guidelines in March 2008) provide a recommended minimum for the amount, type, and format of information that should be measured and reported to meet these aims. The guidelines are the fruit of effort by an eight-member international subcommittee with members from the UK, France, USA, Australia, and Japan (see http://www.ceug.ac.uk/ICCEG.htm for details of the committee's membership) appointed by the 14-member International Committee for Controlled Environment Guidelines. They were initiated at the second international Controlled Environment meeting in 2004 at Brisbane, Australia, under the auspices of the North American Committee on Controlled Environment Technology and Use, the UK Controlled Environment Users' Group, and the Australasian Controlled Environment Working Group. For additional information, please visit: http://www.ceug.ac.uk/ and http://ncr101.montana.edu/.

P-2061

Initiation of Somatic Embryogenesis from Mature Zygotic Embryos of *Pinus oocarpa*. ALEJANDRA LARA[1,2], Ulrika Egertsdotter[1] and Barry Flinn[1,2]. [1]Forestry Department, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060 and [2]Institute for Advanced Learning and Research, Danville, VA 24540. Email: alarach@vt.edu

Somatic embryo initiation has been reported for different pine species. However, for most species, the percentage of initiation, subsequent conversion to mature embryos, and the production of seedlings is still low, reducing the applicability of this technique for commercial propagation. Enhanced embryo quality and quantity may be possible by mimicking natural embryo developmental conditions through a tissue culture medium and culture environmental modifications. To achieve this, a study of the mineral content of *P. oocarpa* seed tissues was conducted. The elemental composition was used for the development of a new tissue culture medium. This new medium, in combination with different hormone concentrations, was tested for effects on somatic embryo initiation from *P. oocarpa* mature zygotic embryos. Additionally, the somatic embryogenic process was investigated from mature seed of *Pinus oocarpa* under: (1) infiltration conditions (using liquid and condition media), (2) different 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine concentrations, and (3) different medium pH levels. We believe that this technology could be used in combination

with the traditional breeding of *P. oocarpa* to improve the qualities of the actual clones of hybrids or elite trees and thereby increase the raw material value and support economical growth in countries where a high percentage of the population depends on the forestry sector.

P-2062

Nitrogen Depletion is Related to Competence Acquisition to Root-to-Shoot Conversion in *Catasetum fimbriatum*. 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

Nitrogen compounds are important signals for the root growth maintenance and development. Roots of *Catasetum fimbriatum* stop growing when the plants are maintained in long-term cultures. Moreover, isolated root tips of this orchid are competent to their root apical meristem (RAM) conversion into buds, even when they are cultivated in a hormone-free medium. In this study, we investigated the root growth rate and the variations of protein content, ammonium levels, nitrate reductase (NR) activity, and morphological modifications in root tips of *C. fimbriatum* over the competence acquisition to the RAM conversion into buds. The results showed that this competence was increased in old root tips, and curiously, explants isolated from young plants did not show this competence and continued growing as roots. The morphological analyzes revealed that competence acquisition of old root tips was related to the root growth cessation, profound structural modifications in the RAM and a remarkable decrease in both fresh and dry weight. This process was correlated with the decrease in the analyzed nitrogen sources in the root tissue. Competent root tips showed lower NR activity, protein content, and ammonium levels; in agreement to this tendency, the ammonium content was decreased in the medium in which the old roots were cultivated. These results suggested that the competence to root-to-shoot conversion in *C. fimbriatum* depend on the root growth cessation and lost of the RAM structural features and function. A lack of nitrogen sources seems to influence the modification of the root tip organogenetic pattern in this species through the reduction in the root growth and, subsequently, the competence acquisition to the conversion of the RAM into buds. Supported by FAPESP.

P-2063

Expression of Ethylene Biosynthesis Genes in Barley Tissue Culture. N. TYAGI and L. S. Dahleen. Plant Sciences Dept. North Dakota State University and USDA-ARS, Fargo, ND 58105. Email: neerjatyagi@yahoo.co.in

The plant hormone ethylene influences green plant regeneration rates from barley callus cultures. Our studies have focused on the effects of short treatments of an ethylene inhibitor or an ethylene precursor on green plant regeneration from two barley cultivars and the expression patterns of two ethylene biosynthesis gene families, ACC (1-aminocyclopropane-1-carboxylicacid) synthase (ACS) and ACC oxidase (ACO), during different tissue culture stages. Calli from two barley cultivars, Morex and Golden Promise, were exposed to ten 1-wk treatments of ACC or aminoethoxyvinylglycine (AVG) during maintenance and regeneration stages of our culture system, and ethylene production was compared to untreated control samples. Results indicate that ACC and AVG have a differential effect on regeneration in the two cultivars. Both the tissue culture stage and the AVG and ACC treatments influenced the amount of ethylene produced from callus. The expression of ACO and ACS genes is being tested in callus collected from control samples during the same maintenance and regeneration stages. Preliminary results have identified at least one constitutive ACO gene and one putative differentially expressed gene.

P-2064

Inhibition of Pro-inflammatory Cytokines IL-6 and TNFα by *Ajuga turkestanica* Phytochemicals. DIANA M. CHENG[1], R. W. Johnson[2], I. Raskin[3] and M. A. Lila[1]. [1]Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign (UIUC), Urbana, IL 61801; [2]Department of Animal Sciences, UIUC; and [3]Biotech Center, Cook College, Rutgers University, New Brunswick, NJ 08901. Email: dcheng2@uiuc.edu

Upregulation of proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF) α have been associated with many age-related diseases, including Alzheimer's, Type II diabetes, and sarcopenia, and the loss of muscle mass due to aging. *Ajuga turkestanica* is a traditional medicinal plant from Central Asia used to treat

heart disease and muscle and stomachaches. This member of the family Lamiaceae produces a rich array of bioactive phytochemicals including phytoecdysteroids: plant-produced steroid-like compounds that confer metabolism-enhancing benefits. Proinflammatory cytokine inhibition was measured in vitro to evaluate the therapeutic potential of phytoecdysteroids and phytochemical mixtures of *A. turkestanica*. Bv-2 mouse microglial cells were dosed with 20-hydroxyecdysone, the most predominant phytoecdysteroid (10 and 100 μM), hairy root extracts of *A. turkestanica* (10 and 100 μg/mL), or wild-harvested shoot extracts of *A. turkestanica* (10 and 100 μg/mL). Cytokine production was stimulated with lipopolysaccharide (LPS) at 10 and 100 ng/mL. Wild-harvested shoot extracts inhibited IL-6 and TNFα production up to 70% and 52%, respectively, after treatment of the microglial cells with 10 ng/mL LPS. Hairy root extracts inhibited IL-6 and TNFα production up to 25% and 22%, respectively, but pure 20-hydroxyecdysone alone only provoked 7% and 18%, inhibition, repectively. These results suggest that the anti-inflammatory activity of *A. turkestanica* extracts is potentiated by interactions between the complex mixtures of phytochemicals inherent in this species and provide direction for in vivo studies of its potential therapeutic uses.

P-2065

Resveratrol Production in Transgenic Hairy Root Culture of Peanut, *Arachis hypogaea* L. YONG-KYUNG KIM[1], Sang-Un Park[2],*. [1] Department of Agricultural Biotechnology, Seoul National University, Seoul, KOREA and [2](*Corresponding author) Division of Plant Science & Resources, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, KOREA. Email: supark@cnu.ac.kr

Peanut (Arachis hypogea 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 (Arachis hypogaea L.) is one of the potent natural sources of resveratrol. trol is a potent chemical and studies show it has anti-inflammatory, antioxidant, anti-ective 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-1 kanamycin to select for transformants and 200 mg L-1 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 ß-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-2066

Oxidative Stress Induces the C_3-CAM Transition in Pineapple Plants Grown In Vitro. L. FRESCHI, M. A. Rodrigues, T. R. Semprebom, 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

The switch from C_3-photosynthesis to Crassulacean acid metabolism (CAM) in C_3-CAM facultative species can be triggered by a variety of environmental factors, such as high light intensity, salinity, and drought. A common convergence point of all these abiotic challenges is the generation of reactive oxygen species, which can induce alterations in the cellular redox state. Since changes in the redox balance can activate some plant stress responses, this study attempted to determine if oxidative stress is also involved in the regulation of CAM expression in pineapple plants grown in vitro. To achieve this, micropropagated pineapple plants, all performing C_3-photosynthesis, were subjected to CAM induction by water stress, and their leaf tissues were analyzed over 30 d for the levels of glutathione (GSH), ascorbate (ASC), and degree of CAM expression. Additionally, the effects of oxidative stress treatments, such as the application of menadione or hydrogen peroxide, were analyzed on the C_3-CAM transition. In all cases, the occurrence of C_3 or CAM photosynthesis was evaluated by measuring the nocturnal acid accumulation and activities

of phosphoenolpyruvate carboxylase (PEPCase) and malate dehydrogenase (MDH). The water stress-induced C_3-CAM transition in pineapple was followed by a marked accumulation of GSH and a slight depletion in the ASC pool. Moreover, plants subjected to oxidative stress treatments showed increases in nocturnal acid accumulation and activities of CAM enzymes. The CAM induction by oxidative stress was also accompanied by increases in GSH content and decreases in ASC levels. These results indicate that oxidative stress induces the C_3-CAM transition in pineapple, possibly by causing alterations in the cellular antioxidants content and redox status.

P-2067

Salicylic Acid Improved In Vitro Meristem Regeneration and Salt Tolerance in Two *Hibiscus* Species. HAMIDOU F. SAKHANOKHO[1] and Rowena Y. Kelley[2], [1]Southern Horticultural Laboratory, USDA-ARS, Poplarville, MS 39470 and [2]Department of Biochemistry and Molecular Biology, Mississippi State University, P. O. Box 9650, Mississippi State, MS 39762. Email: Hamidou.Sakhanokho@ars.usda.gov

Salicylic acid (SA) has been reported to induce abiotic stress, including salt tolerance in plants. The objective of this study was to determine whether application of various exogenous SA concentrations to in vitro grown meristem shoots could induce salt tolerance in two *Hibiscus* species. The effects of varying SA concentrations (0, 0.5, and 1 mM) on in vitro shoot apices of two *Hibiscus* species, *H. moscheutus* (cv "Luna Red") and *H. acetosella*, grown under various salt concentrations (0, 175, and 200 mM), were assessed with respect to shoot growth and multiplication, percentage of root formation, root elongation, plant survival rate, and proline accumulation. Application of exogenous SA, in particular 0.5 mM SA, had a beneficial effect on all these parameters in both species under saline and nonsaline conditions. Results obtained showed that *H. moscheutus* was more salt tolerant than *H. acetosella* and SA could be used to improve in vitro regeneration and induce salt tolerance in plants. These results also suggest that the approach described in this investigation could be incorporated in a breeding program aimed at a rapid screening and development of salt tolerant *Hibiscus* cultivars.

P-2068

In Vitro Cultivation of Colocynth (*Citrullus colocynthis* (L.) Schrad). S. GHAREMTROSSIAN. Department of Microbiology and Plant and Microbe Biotechnology, Yerevan State University, Yerevan, 0025, ARMENIA. Email: physiol@ysu.am

Colocynth is known in traditional and folk medicine as a source of substances with anticancer activity. Since the method of isolated cultures allows regulating of synthesis of secondary metabolites it would be useful to introduce this plant for cultivation in vitro. The objective of this work is to study in vitro callus formation and organogenesis of *Citrullus colocynthis*, to determine the optimal media and to select the appropriate explants. The seed germination took place after incubation of seeds in sulfuric acid for 15–20 min. Small segments of cotyledons, apical buds, hypocotyls, and roots from 20-d-old in vitro germinated seedlings were isolated and transferred on Murashige and Skoog (MS) medium with different set of phytohormones. Two medium were used for apical bud culture: MS with indole-3-acetic acid (IAA) and kinetin (KIN), 1.0 mg L^{-1} of each, and MS with double vitamin content and 2.0 mg L^{-1} 6-benzylaminopurine (BAP) and 0.1 mg L^{-1} naphtalene-acetic acide (NAA). The biomass of callus, which was formed in the presence of BAP and NAA, was greater than in the presence of IAA and KIN, though shoot generation and their length on the medium with IAA and KIN is more significant. Then shoots (2.0–2.5 cm length) were rooted most effectively in 1.0 mg L^{-1} IAA-supplemented MS medium.

P-2069

Germplasm Conservation in Apricot and Neem by Encapsulation-Refrigeration of Internodes from Micropropagated Shoots. I. M. G. Padilla, N. Alburquerque L. Burgos, and A. PIQUERAS. Plant Breeding Dept, CEBAS (CSIC), P.O. Box 164, 30100 Espinardo, Murcia, SPAIN. Email: piqueras@cebas.csic.es

Apricot and neen are tree species of agronomic and environmental interest that require the implementation of ex situ conservation programs by tissue culture. This approach is of clear interest the case of neem tree, which has recalcitrant seeds that only remain viable 2 mo after harvest. This condition is an important restriction for neem germplasm conservation and distribution. The aim of this work is to develop a methodology for the encapsulation of micropropagated internodes from apricot and neem that stored under refrigerated conditions could be used for medium- and long-term germplasm conservation. Nodal segments (0.5–1 cm) from micropropagated shoot cultures of the two species were used for the encapsulation experiments. The explants were included in an alginate matrix composed of Murashige and Skoog complete medium, 2.0 mg/l 2-morpholinojethanesulfonic acid and 3% alginate

at pH 5.7. The capsules included the explants and the alginate matrix were formed by the application of 70 mM $CaCl_2$ at 5, 10, 15, and 20 min. After the capsules were formed, their mechanical resistance and penetrability were measured to evaluate the impact of these parameters on shoot development in the encapsulated explants. The capsules were transferred to Quoirin and Lepoivre medium (apricot) and Driver and Kuniyuki walnut medium (neem) to allow shoot regeneration after encapsulation in the growth room at 24± 1° C and 16 h of photoperiod. The capsules showed significant differences in relation to compression and penetration in relation to the incubation time in the $CaCl_2$ solution. After 4 wk, the survival of the encapsulated material was 95% for neem and 85% for apricot. Encapsulated nodal segments of the two species were stored under refrigeration during 2 mo at 4, 8, and 12° C. After this period, a clear influence of temperature on viability could be observed in the case of neem capsules that only were able to resist at 12° C; however, apricot-encapsulated explants remained viable at the different temperatures. These experiments have provided a valuable basic methodological information that will be used to develop medium and long term germplasm conservation protocols in apricot and neem, two tree species of contrasting tolerance to low temperatures in the field.

P-2070

Callus Induction and Shoot Regeneration in Eggplant (*Solanum melongena L.*). E. ZAYOVA, V. Nikova, K. Ilieva, Ph. Philipov, and L. Krusteva. Departament of Plant Biotechnology, Institute of Genetics “Akad. D. Kostoff”, Tsarigradsko Shose, 13 km, Bulgarian Academy of Sciences, 1113 Sofia, BULGARIA. Email: e_l_y@abv.bg

The eggplant (*Solanum melongena* L.) is an economically important vegetable Solanaceous crop in Bulgaria. It is a good source of vitamins and minerals. This plant provides an unique system to study morphogenesis. The aim of the work was to be examined the effect of some phytohormones and their combinations on callus induction and plant regeneration in eggplant tissue cultures. Two eggplant breeding lines no. 3 and no. 4 were studied. Cotyledon and hypocotyl explants from 30-d-old seedling were the best explants for callus induction. The Murashige and Skoog (MS, 1962) medium supplemented with naphthaleneacetic acid (NAA)—2.0 mg/l and 6-benzylaminopurine (BAP)—0.5 mg/l proved very suitable for callus formation. Callus was obtained from 90.0% of cotyledon explants and from 63.3% of hypocotyl explants of line no. 3. When subcultured on MS medium with a lower concentration of NAA (0.05–0.1 mg/l), higher concentrations of BAP (1.0–3.0 mg/l), and 0.2 mg/l gibberellic acid (GA_3), the callus regenerated. It produced friable callus with numerous of shoot primordias. Callus induced from cotyledon segments was found to be more organogenic than callus from hypocotyl segments. Organogenic callus generated shoots when transferred onto a hormone-free MS medium. Callus from cotyledon explants of line no. 3 gave the maximum number of shoots (14.25 shoots per callus), while callus from the same explants of line no. 4 produced a mean of 9.72 shoots per callus. To elongate in vitro shoots, 0.4 mg/l GA_3 was added into culture medium. The regenerated plantlets were rooted on the MS basal medium.