In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-007-9027-3

Plant Contributed Papers

P-1000

Pigmented Maize Seed via Tissue-specific Expression of Anthocyanin Pathway Gene Transcription Factors. LIU Y SHEN, Joseph F. Petolino. Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268.
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A visual marker genetically linked to a trait of interest would enable the unequivocal identification of transgenic seed containing the trait and ensure that genetically-modified material could be easily differentiated from non-transgenic grain. Genes from maize that regulate the anthocyanin biosynthetic pathway, and are therefore responsible for directing the pattern of purple pigmentation, can be used to generate transgenic plants with unique, easily recognizable phenotypes. In the present study, a seed-specific maize globulin promoter was used to drive the expression of two transcription factor genes (Bp and C1) regulating the anthocyanin biosynthetic pathway in embryo and aleurone tissues resulting in maize seed with a distinct purple pigmentation. This report describes the construction of expression vectors containing these two genes and an herbicide resistance selectable marker, the generation and characterization of transgenic cultures, the induction of pigmentation in somatic embryos, the regeneration of transgenic plants with pigmented seed and the co-segregation of integrated DNA with seed pigmentation and herbicide resistance. The Mendelian co-segregation of seed pigmentation and herbicide resistance confirms the feasibility of using the expression of these genes in a ‘molecular stack’ for visual transgene identification.

P-1001

Evaluation of Viral Suppressors of Silencing to Stabilize Transgene Expression. TANIYA DHILLON, Joseph M. Chiera and John J. Finer. Department of Horticulture and Crop Science, OARDC/The Ohio State University, OH 44691. Email: finer.1@osu.edu

Gene expression is a highly regulated and complex process in eukaryotes. Over-expression of a transgene does not always result in an increase in protein levels, but may cause transcriptional or post-transcriptional gene silencing (RNA silencing). Plants use RNA silencing to suppress virus replication and spread in the plant. As a counter measure, some virus genes encode proteins that suppress the silencing mechanism of the plant. We evaluated six such viral suppressors of silencing for their ability to stabilize transgene expression. Various combinations of the *green fluorescent protein* (*gfp*) gene and silencing suppressors were introduced into lima bean (*Phaseolus lunatus* L.) cotyledons using particle bombardment. Post-introduction, GFP expression in the cotyledons was tracked over time with an automated image collection and analysis system. In this transient expression assay, we found that the silencing suppressors, HCPro and p19 stabilized GFP expression as 3′ *gfp* transcriptional fusions; p21 and gamma b stabilized GFP expression when co-introduced independently on separate plasmids from the *gfp* gene; however, AL2 and replicase did not show any stabilization of GFP expression. The 3′ *gfp* fusions of HCPro and p19 were bombarded into soybean (*Glycine max* (L.) Merrill) embryogenic tissue. Introduction of the *gfp* gene without any suppressor was used as a control. Variable GFP expression was observed in all stably transformed soybean clones. An abnormal downward-leaf-curling phenotype was observed in one p19 transgenic plant, suggesting that the p19 suppressor may not only affect the expression of the gene it is fused with, but also other genes *in-trans*.

P-1002

The Celery Mannose-6-phosphate Reductase Gene (*M6PR*): A Mannose-Dependent, Bifunctional Selectable Marker for Plant Transformation. GUO-QING SONG, Wayne H. Loescher, and Kenneth C. Sink. Department of Horticulture, Michigan State University, East Lansing, MI 48824. Email: songg@msu.edu

Isolation of friendly selectable marker genes (SMG) or development of novel strategies to produce marker-gene free crop plants are expected to meet one of the public’s concerns about the safety of genetically modified plants (GMP). Current single function SMG (*Si*-SMG) are mostly isolated from microbes and provide for either a positive or a negative selection mode. In contrast, we found that the celery mannose-6-phosphate reductase gene (*M6PR*) under the control of a CaMV 35S promoter can act as a mannose-

dependent, bifunctional-selectable marker gene (*bi*-SMG) acting in a positive or a negative selection mode depending on the plant species. Overexpression of *M6PR* enhanced mannose tolerance in *Arabidopsis thaliana* (L.) Heynh. and provided a positive selection for transgenic seeds. More importantly, *M6PR* expression was found to act as a negative SMG in *Nicotiana tabacum* apparently due to co-suppression of the phosphomannose isomerase or the mannose-6-phosphate reductase activity. The co-suppression increased mannose sensitivity in tobacco. Mannose at 30 g/L blanched leaf explants of transgenic tobacco with *M6PR* ($T0_{M6PR}$) in 3 weeks, in contrast, 30 mg/L or even 50 mg/L mannose did not inhibit shoot regeneration from leaf explants of wild type (WT) or transgenic plants with an antisense *M6PR* ($T0_{anti\text{-}M6PR}$). In addition, mannose at 25 g/L inhibited seed germination of transgenic tobacco with *M6PR*, but not that of WT and transgenic tobacco with the antisense *M6PR*. Either positive or negative selection using the celery *M6PR* is versatile for efficient production of concerns-free GMP.

P-1003

Protoplast/GFP Transformation System: Comparison between Endoplasmic Reticulum Targeting and Non-targeting GFP in Transgenic Citrus. A. A. OMAR and J. W. Grosser. University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: omar71@ufl.edu

Two green fluorescent protein (*GFP*) constructions, an endoplasmic reticulum targeted (*GFP*-ER) and a cytoplasmic targeted (Cy-*GFP*), were used to study expression and stability of *GFP* in citrus in order to improve transformation efficiency. Results using our citrus protoplast/*GFP* transformation system showed that *GFP-ER* had brighter fluorescence than the Cy-*GFP*. Although both of these constructs gave transient expression at the protoplast level, nontargeted *GFP* gave less stable expression than targeted *GFP* at the colony or plant level. Stable expression of *GFP* after four weeks of culture was observed in about 1.0% and 0.1% of the initial *GFP* positive cells in *GFP*-ER and Cy-*GFP* experiments, respectively. At the mature plant level, there were distinct fluorescent differences between shoots transformed with the two *GFP* constructs. Transgenic shoots containing the *GFP*-ER targeted (pARS108) were generally brighter than those derived from the cytoplasmic targeted construct (p524EGFP.1), and exhibited a uniform green fluorescence with minimal red fluorescence from chlorophyll. However, the fluorescence intensity varied among different parts of the plant, being higher in new leaves than in hardened off leaves, even when using the same construct. These results demonstrate that *GFP*-ER targeted gene expression in citrus tissues is more stable than the Cy-*GFP*. Screening for *GFP*-ER gene expression may prove useful to improve transformation efficiency and to facilitate detection of transformed citrus plants. Using the protoplast/*GFP* transformation method in citrus has an advantage over *Agrobacterium*-mediated transformation which requires an antibiotic resistance gene for selection and to kill residual *Agrobacterium*.

P-1004

Isolation and Characterization of a Soybean (*Glycine max*) Polyubiquitin (Gmubi) Promoter. R. A. Bouchard, J. M. Chiera and J. J. FINER. Department of Horticulture and Crop Science, Plant Molecular Biology and Biotechnology Program, OARDC/The Ohio State University, Wooster, OH, 44691. Email: finer.1@osu.edu

A DNA segment showing strong promoter activity was recovered by cloning a 912 bp region of soybean genomic DNA, 5′ to the ORF of a polyubiquitin gene. The 5′ portion of this *Glycine max* polyubiquitin (Gmubi) promoter is GC-rich and carries a TATA box, transcription initiation point, and cap sites. Similar to other polyubiquitin promoters, the Gmubi promoter also contains an AT-rich intron which splices directly to the ATG of the polyubiquitin ORF. Expression of a *gfp* reporter gene was evaluated with the full-length Gmubi promoter and an intron-less Gmupri (pre-intronic) version of the promoter. Constructions were evaluated using both transient expression analysis and in stably transformed soybean cultures and plants. In transient expression assays, the Gmubi promoter was 5-times stronger than a CaMV35S promoter, which was used as an expression standard. The intron-less Gmupri promoter was 2.5-times stronger than the CaMV35S promoter but showed only half of the activity of the full length Gmubi promoter. In stably-transformed soybean plants, the full-length Gmubi and intron-less Gmupri promoters supported strong GFP expression in seedling tissues and mature plants, with particularly high levels of expression in roots and vascular tissues. While plants containing Gmubi-driven GFP exhibited the highest overall levels of expression, plants containing the Gmupri construct also show substantial levels of expression in seedlings and plants, generally higher than seen in clones containing the CaMV35S-regulated GFP construct. The Gmubi promoter shows good promise for use in soybean, where high constitutive transgene expression is required from a native soybean promoter.

P-1005

Excision of a Selectable Marker Gene Mediated by Trans-activated FLP Recombinase in Tobacco Cells. D. GIDONI[1], B-H. Ben-Daniel[1], A. Mett[1], J. Feiler[1], I. Sobolev[1], N. Carmi[1] and U. Nir[2]. [1]Institute of Plant Sciences, A.R.O., The Volcani Center, ISRAEL and [2]Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, ISRAEL. Email: gidoni@volcani.agri.gov.il

Previous reports indicate the elimination of selectable marker genes from the plant genome, mediated by Cre, FLP and R recombinases, driven by constitutive, developmental or chemically regulated promoters. We report here on examination of an alternative, bi-molecular transactivation control system for FLP recombinase, based on *LacI-VP16* trans-activator/*Lac* operator-*FLP* chimeric fusions. The results indicate functional activity of the system in tobacco protoplasts. In these experiments, one plasmid expressed the chimeric LacI-VP16 transcriptional activator fusion protein under the constitutive CaMV-35S promoter (*35S*P) and a second plasmid harbored a chimeric *Lac* operator-TATA-*FLP* recombinase gene fusion. A recombination-reporter construct was designed to allow transcription of *gusA* after recombination-mediated excision of a *FRT*-bounded *NPTII* expression cassette, placed between *gusA* coding and *35S* promoter sequences. Recombination-mediated GUS expression, resulting from reporter gene excision product (*35S*P-*FRT-gusA*) was detected only after co-transfection with both the transactivator and operator-FLP plasmid constructs. Additionally, GUS expression was raised with the increasing amount of either transactivator or operator-*FLP* plasmid, indicating that the level of recombination depends on the level of transactivator and FLP products. By contrast, no GUS activity above background was observed in control experiments after transfections with either plasmid alone. Molecular PCR/Southern confirmation analyses are currently underway. The potential utility of this system in trans-elimination of both the selectable marker and recombinase, as well as other genes in hybrid plants will be discussed.

P-1006

A New Red Fluorescent Protein from Coral is Effective for Transgenic Plant Studies. Laura L Good, Mary Rudis, Mikhail V. Matz*, C. NEAL STEWART, JR. University of Tennessee, Knoxville, Knoxville, TX and *University of Texas at Austin, Austin, TX. Email: nealstewart@utk.edu

Fluorescent proteins (FPs), particularly green fluorescent protein (GFP), are widely used as tools in plant biotechnology. FPs have successfully been used as non-destructive markers of transgenicity, in protein tagging and trafficking studies as well as promoter analysis. Autofluorescence of plant samples in the GFP spectra limits detection in certain species and tissues. The discovery of FPs with altered spectra would allow dual imaging of fluorescent markers, and visualization in highly autofluorescent tissue such a mature leaf tissue. The red fluorescent protein gbr15, isolated from the clubbed finger coral (Porites porites), has an excitation maximum of 578 nm and emission maximum of 595 nm. A histidine tagged version of the coral protein was cloned into plant transformation vectors under the control of a constitutive CaMV 35S promoter. Transgenic plants were visualized with an epifluorescent stereoscope using a bandpass excitation filter (535/30nm) and a bandpass emission filter (600/50nm). Bright fluorescence was observed in both transgenic tobacco and Arabidopsis. Fluorescence intensity observed was comparable to that of the FP DsRed, whose excitation/emission is 558/583nm. Using the appropriate cutoff filters, gbr15 expressing lines can be clearly distinguished from non-transgenic control plants. High level expression was observed in inflorescences, with lower expression observed in other green tissues. Because of the unique spectral properties of gbr15, this protein may be a promising candidate for tagging, leaves, pollen, and roots.

P-1007

Improvement of Drought Tolerance in Canola Plants Expressing the Phosphotidylinositol Specific Phospholipase C2 (PLC-2) Gene. REDA E. A. MOGHAIEB, Swsan S. Youssef, and Ahmed M. El-Sharkawy. Department of Genetics and Genetic Engineering Research Center, Faculty of Agriculture, Cairo University, EGYPT. Email: moghaieb@yahoo.com

Phospholipase is a ubiquitous eukaryotic enzyme participating in various cellular processes and involved in signal transduction and vesicular trafficking and confer drought tolerance in plants through controlling the stomatal closure under drought stress. Phospholipase gene (PLC) was introduced into canola plant using an *Agrobacterium*-mediated gene-delivery system. The hypocotyl explants isolated from several canola genotypes were infected with the *Agrobacterium tumefaciens* LBA-4404 harboring the binary vector pBI-121 containing PLC-GUS gene fusion under the genetic control of 35S-promoter. Stable integration of the PLC gene into the regenerated plant genomes was confirmed by PCR and Southern blot analysis. GUS gene expression can be detected only in the transgenic plants. The turgor values of the PLC transgenic lines

increased with increasing drought stress period. PLC-gene product improves drought tolerance in canola plants by contributed to the maintenance of the osmotic potential of the cells. Thus leads to improve the maintenance of root function with respect to the uptake and supply of water to the shoot. In this way, the photosynthetic rate is improved through enhancement of cell membrane stability in oxidative conditions under drought stress.

P-1008

Development of a New Regeneration and Transformation System for Impatiens. YINGHUI DAN [1,2,3], Aaron Baxter[2] and Richard E. Veilleux[2]. [1]Institute for Advanced Learning and Research, 150 Slayton Avenue, Danville, VA 24540; [2]Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; and [3]Department of Forestry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Email: yinghui.dan@ialr.org, ydan@vt.edu

Impatiens (*Impatiens walleriana*) is one of the top selling floriculture crops, with an annual value of approximately 1.6 billion dollars in the U.S.A. in 2004. Impatiens Necrotic Spot Virus is one of the most destructive pathogens in the floriculture industry. The lack of Impatiens cultivars resistant to Necrotic Spot Virus restricts its production. Therefore, the development of the virus resistant Impatiens is important to solve the problem. The first goal of our project is to develop a new and efficient *Agrobacterium*-mediated Impatiens transformation system. We have firstly developed a new and efficient Impatiens plant regeneration system using cotyledonary meristem explants. With the regeneration system, 80% of explants produced a mass of buds/shoots per explant, up to 167 elongated shoots produced per explant and 100% of shoots produced good roots within 12 days under optimal conditions. Plants were regenerated whthin approximately 8 weeks. The regenerated plants were fertile and phenotypically normal. Secondly, we have developed a new Impatiens transformation method. Transgenic Impatiens plants were obtained about 10 to 12 weeks after inoculation and confirmed by biological assay and phenotype. Molecular analysis of the transgenic plants is undertaking. Development and optimization of the Impatiens regeneration and transformation methods will be discussed in this presentation.

P-1009

Application of In Vitro Techniques for Micropropagation and Protection of Endemic and Endangered Flowerbulbs of Turkey. S. ÖZCAN, I. Parmaksız[1], S. Mirici[2], S. Çöçü, C. Sancak, S. Uranbey, B. Gürbüz, A. İpek, D. Doğan-Kalyoncu, C. Karaoglu, C.S. Sevimay, and N. Arslan. Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110, Dışkapı, Ankara, TURKEY; [1]Department of Biology, Faculty of Science and Arts, University of Gaziosmanpaşa, Tokat, TURKEY; and [2]Faculty of Education, University of Akdeniz, Antalya, TURKEY. Email: özcan@agri.ankara.edu.tr

Flora of Turkey has approximately 3000 endemic plant species, in which beautiful flowering (geophytes) bulbous plants form an important part. Besides use as ornamental plants, flowerbulbs have great potential in perfume and pharmaceutical industry. Genera of Fritillaria, Ornithogalum, Muscari, Bellevalia, Tulipa, Galanthus, Sternbergia, Crocus, Arum and Biarum have important and critically endangered species with high export potential that enters into this group. Most of these are endangered and their collection from wild and export has been banned to conserve them. The natural propagation rate of most geophytes is relatively low, which often hampers the large-scale cultivation of these plants. Large scale production and conservation of these species could also be achieved by in vitro techniques. Therefore bulb scale and immature embryo explants of many endemic and endangered flowerbulbs species were collected from the wild flora and cultured on different nutrient media supplemented with various concentrations of plant growth regulators using different culture applications. Large numbers of bulblets were produced (over 100 bulblets/explants) from single immature embryos on nutrient media in most species tested after 12 months of culture initiation. Regenerated bulblets were then transferred to MSO medium to increase their sizes and for root formation. These bulblets were later kept at 5°C for 5 weeks and then transplanted to soil. Depends on the genera, in some species majority of bulblets were developed into full plants, whereas in many species bulblets were not developed in soil and finally they died. To increase the survival of the bulblets in soil we are now trying to understand the physiological stages of the bulblets in culture.

P-1010

Spontaneous and Induced Variation in Peanut Seed Protein. P. OZIAS-AKINS[1], L. Ramos[1], P. Faustinelli[1], Y. Chu[1], S. Maleki[2], J. Huntley[3], and J. Thelen[4]. [1]Department of Horticulture, The University of Georgia Tifton Campus, Tifton, GA 31793; [2]USDA-ARS-SRRC, New Orleans, LA 70124; [3]National Center for Genome Resources, Santa Fe, NM 87505; and [4]Department of Biochemistry, University of Missouri-Columbia, Columbia, MO 65211. Email: pozias@uga.edu

Ara h 2 is a peanut seed storage protein as well as a major human food allergen. Peanut (*Arachis hypogaea*) is a legume grown in subtropical and tropical regions for its nutritious, oil-rich seeds. The cultivated species is allotetraploid with

A- and B-subgenomes that most likely were derived from the diploid species, A. duranensis and A. ipaensis, respectively. The hybridization event that resulted in tetraploid peanut led to an evolutionary bottleneck with the consequence that relatively little genetic variation is available in the cultivated species. Although little variation in Ara h 2 isoforms may exist within A. hypogaea, wild relatives are likely to possess considerable polymorphism. Using the method of Ecotilling to analyze 30 accessions of A. duranensis, we have identified genetic polymorphisms in the A-genome ortholog of ara h 2 that result in altered isoforms of the protein. Some of these isoforms show different immunological reactivity compared with the wild type. Alternatively in tetraploid peanut, a quantitative reduction in the level of Ara h 2 using RNAi allowed both A- and B-subgenomic isoforms to be targeted. These transgenic materials are valuable for evaluation of immunological and plant developmental responses but may not be desirable for commercialization. Nevertheless, when RNAi-mediated variation is analyzed in parallel with naturally occurring molecular variation, the information produced can guide efforts to generate induced variation through mutagenesis of the tetraploid.

P-1011

Transformation of Faba Bean (*Vicia faba* L.): a Non-tissue Culture Based Approach for Generating Transgenic Plants.
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It is well known that legume in general and faba bean in particular are recalcitrant for in vitro regeneration. Improvement of faba bean using genetic engineering has been limited due to the difficulties in developing of an efficient and reproducible regeneration system. Plant regeneration capacity among three faba bean genotypes using the embryogenic axes as explants was compared. The embryogenic axis (E) explants showed higher organogenesis percentage (12–25%) compared with the junction regions between cotyledons and the embryos (JCE) (6–18%). This protocol was effective in the three *Vicia faba* genotypes. This technique gives a new prospective for the production of transgenic *Vicia faba* harboring economically important genes. *Agrobacterium*-mediated transformation was practiced to deliver defensin gene to faba bean. The embryogenic axis explants were infected with *Agrobacterium tumefaciens* strain LBA-4404 harboring AFP plasmid which contains defensin gene under the control of 35S promoter and NOS terminator and GUS-intron as a reporter gene. The integration of the defensin transgene into the genomes of faba bean plants was confirmed by PCR assay. *GUS* gene expression indicated that some of the putative shoots were transformed. The transformants were elongated, rooted and acclimatized in the green house condition.

P-1012

In Vitro Multiplication and Cryopreservation of *Hedychium Coronarium* a Rare Medicinal Plant of Central India.
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Hedychium Coronarium also known as ginger-lily or garden flower grows up to 2000-meter height in northeastern states of India. In central India it is mainly found in Maikal hills ranges near Amarkantak. The species contains important alkaloids in its rhizome and flowers. Conventionally, it is propagated through seeds but the seed germination is very poor (<25%). Other propagation methods of this species are difficult, only the rhizome is used for its vegetative propagation. Therefore an in vitro multiplication technology has been standardized through rhizomatic buds as an explant source. Extensive shoot multiplication was achieved when MS medium was supplemented with BAP 5.0 mg/lt and IAA 2.0 mg/lt. along with slight callus formation. On an average 8 to 9 young shoots were induced after second subculture. Cryopreservation technique using vitrification and encapsulation dehydration technique has also been standardized at −196°C in liquid nitrogen.

P-1013

Zinc Finger Nucleases, a New and Novel Method for Gene Targeting in Plants.
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Gene targeting is an essential tool of reverse genetics that allows studying gene function by creating mutations in the selected regions of the genome. Traditional methods of gene targeting based on Homologous Recombination (HR) were proven inefficient for plant species because of the strong dominance of random Non-Homologous End Joining (NHEJ) mechanism of transgene integration. Zinc Finger Nucleases (ZFNs) are a new breed of custom made enzymes that could introduce Double Strand genomic Breaks (DSBs) at specified genomic locations and thus lead to a variety of gene targeting

events, such as small insertions and deletions, T-DNA integration by NHEJ and increased frequency of transgene integration by HR. Despite the huge potential that ZFNs carry for gene targeting the difficulties with the design have been hindering their implementation. Here we describe a set of constructs and procedures we used to design of a set of four (two pairs) of ZFNs that will be use to break genomic DNA sequences upstream and downstream of Arabidopsis FtsH2 gene which will potentially lead to the removal or substitution of AtFtsH2. We started by de-novo assembly of DNA-binding regions of ZFNs from overlapping oligos containing modified sequences responsible for DNA triplet recognition and their insertion between a nuclear localization signal and the FokI endonuclease domain. Assembled ZFNs were expressed in E. coli cells and the cell lysates were directly used for in-vitro digestion analysis of palindromic target sequences. To confirm the activity of ZFNs in plant cells we used an in-planta activity test based on reconstruction of GUS expression following bombardment of a reporter and ZFN-expressing plasmids into mesophyll cells. Our successful design of FtsH2 ZFNs and a set of new procedures, plasmids and assays bring us one step closer to efficient implementation of ZFN-based technology for gene targeting in plant species.

P-1014

Novel Asexual Reproduction in Australian Sundews (*Drosera*; Droseraceae): Direct Tuberization on Detatched Leaves. DOUGLAS W. DARNOWSKI, Alan D. Tate, and Elizabeth Russell. Brooks Way Department of Biology, Indiana University Southeast, 4201 Grant Line Road, New Albany IN 47150. Email: ddarnows@ius.edu

Australian sundews include many types which are rarely, if ever, found outside Australia, and the rate of endemism for Australian tuberous sundew species is very high. Combined with threats due to habitat loss, new methods of propagation for Australian sundews are very valuable. In addition, an in-vitro method of tuber production would allow for precise studies of tuber formation in Australian members of the genus *Drosera*. We have created a method for the direct production of tubers on the leaves of one species, *D. peltata*, including optimized hormone treatments (treatment with 0.1 mg L-1 BA in 1/5 MS/B5 medium yields >50 tubers/leaf). These tubers are anatomically normal, based on the histology of fresh tissue, though smaller than tubers produced on soil in the greenhouse. They also begin to grow out very soon after formation, showing reduced dormancy. We will include data on our work to find treatments (osmotic, hormonal) to promote dormancy in leaf-derived tubers and on the application of this method of regeneration to other, morphologically distinct tuberous species (*D. stolonifera, D. gigantea*) from Australia. The ultimate goal is to derive a general method of tuber formation on leaves which could be used with very rare species such as *D. fimbriata*.

P-1015

Comparative Photoperiod Effects on In Vitro Seed Germination and Seedling Development in *Calopogon tuberosus* from Differing Geographic Sources. P. J. KAUTH and M. E. Kane. Environmental Horticulture Department, University of Florida, PO Box 110675, Gainesville, FL 32611. Email: pkauth@ufl.edu

Calopogon tuberosus var. *tuberosus* is a widespread terrestrial orchid distributed from Florida to Newfoundland, Canada, and west to Texas. *Calopogon tuberosus* grows in a variety of habitats including alkaline prairies, pine flatwoods, mesic roadsides, and acidic sphagnum bogs. With a wide latitudinal distribution and diverse habitats, the potential for ecotypic differentiation in *C. tuberosus* exists. The occurrence of ecotypes could be reflected in differing requirements for optimal seed germination and seedling development in vitro. To examine this, the effects of photoperiod on seed germination and seedling development of *C. tuberosus* seeds from wide geographical sources were studied. Seeds from South Florida, Central Florida, North Wisconsin, and four locations in South Carolina were collected. Seed germination and seedling development of surface sterilized seeds were analyzed weekly for 8 weeks under 8/16 h, 12/12 h, or 16/8 h L/D photoperiods at 24°C. After 8 weeks, seeds from the Central Florida population displayed the highest germination rate (60.2%) under a 8/16 h photoperiod. However, protocorm development was promoted in Central Florida seedlings cultured under a 12/12 h or 16/8 h photoperiods. Germination of South Florida seeds was also greatest in the 8/16 h photoperiod, but most seedlings did not develop past the imbibition stage. Germination in the Wisconsin population was less than 12% for all treatments, but seedling development was rapid with over 90% of germinated seeds developing to a leaf bearing stage. Germination from all South Carolina populations was less than 10%. Although the Wisconsin population exhibited low germination, seedling development was enhanced possibly due to the shorter growing season in northern latitudes.

P-1016

Culture and Storage Medium Iron Concentrations Influence In-Vitro Cold Storage Duration of Hops (*Humulus*) BARBARA M. REED, USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Rd, Corvallis, OR 97333-2521. Email: corbr@ars-grin.gov

The United States Department of Agriculture, Agricultural Research Service National Clonal Germplasm Repository (NCGR) at Corvallis, Oregon, preserves the global diversity of *Humulus* for the US Plant Germplasm System. To insure the safety of the collection, a subset of the field collection was established as virus-free in vitro cultures stored at 4°C. Our earlier studies showed that the iron formulation used in the storage medium affected storage duration. In this study four hop (*Humulus lupulus* L.) accessions were first cultured on MS medium with either standard MS iron or MS iron with 100 mg/L sequestrene iron 138 for 3 weeks before storage. Plants were stored in tissue culture bags on MS iron at 0, 0.5, 1, 2, 3, 4, and 5 X the normal concentration or on MS iron at 1X with 100 mg/L sequestrene 138 iron. There was a significant interaction of accession, pretreatment, and storage medium but not for pretreatment x storage medium. Initially the pretreatment medium produced no significant differences in plant condition ratings; however by 6 mo plantlets grown on 1X MS iron had significantly higher ratings than those on sequestrene iron. At 8 mo of storage the plants on storage medium with lower iron concentration (0–1X) had significantly higher ratings than did those on the high iron concentrations (2–5 X and sequestrene). Cultivar Vojvodina (Hum 155.003) declined quickly in storage while 'Cascade' (196.002) and 'USDA 21119' (410.002) showed significantly lower ratings with higher iron concentrations (>1X). 'Alpha Aroma' (627.002) had significantly better ratings with iron in the medium (>0), and there were no significant differences in ratings among the iron concentrations (0.5–5 X or sequestrene). The overall results indicate that each of these accessions should be grown on MS 1X iron and stored on MS 0.5 or 1X iron medium for the longest duration in cold storage.

P-1017

Histological Analysis of Callus Production and Plant Regeneration from Leaf Cultures of *Dieffenbachia* cv. Camouflage. X. SHEN, M. E. Kane and J. Chen. Department of Environmental Horticulture, University of Florida, PO Box 110675, Gainesville, FL 32611. Email: XIS300@UFL.EDU

Dieffenbachia is one of the most popular and high value ornamental tropical foliage plant crops. Traditional breeding is hampered by its naturally-occurring dichogamy and long breeding cycle. Selection of somaclonal variant plants generated in vitro indirectly from callus cultures is an alternative and more desirable means for new cultivar development. An indirect shoot organogenesis protocol was established for *Dieffenbachia* cv. Camouflage using leaf explants exercised from in vitro shoot cultures. Callus formation occurred on leaf explants cultured on MS medium supplemented with 5 μM TDZ and 1 μM 2,4-D after 8 weeks culture in dark and 4 weeks in a 16 h photoperiod. Adventitious shoot regeneration was achieved after calli were separated from leaf explants and cultured on MS medium supplemented with 40 μM 2iP and 2 μM IAA for 8 weeks under a 16 h photoperiod. Regenerated shoots readily rooted and acclimatized ex vitro (100% survival) in soilless medium in greenhouse conditions. Histological analysis verified that regeneration occurred via indirect shoot organogenesis and not somatic embryogenesis. Only unipolar structures (shoot or root meristems) were observed. Vascular connections were also observed between developing shoots and callus tissues which was another distinct feature of shoot organogenesis. Variation in leaf morphology and variegation patterns among the plants produced suggests that this *in vitro* shoot regeneration protocol has potential use for production of somaclonal variants and new *Dieffenbachia* cultivar development.

P-1018

Liquid Culture of *Hydrangea quercifolia* 'Sikes Dwarf' Produced Plantlets with Better ex vitro Growth than Plantlets from Agar. J. W. ADELBERG[1], J. Naylor-Adelberg[1] and M. Tascan[2]. [1]Department of Horticulture and [2]School of Materials Science and Engineering, Clemson University, Clemson SC, 29634 Email: jadlbrg@ clemson.edu

Liquid medium systems were developed that avoided hyperhydricity during micropropagation of *Hydrangea querciofolia* Bartr. 'Sikes Dwarf', a woody ornamental shrub. Plantlets grown on a rocker platform, intermittently immersed in thin-films of liquid medium became hyperhydric. Plantlets on saturated paper, floated above liquid on a sealed air raft were a mixture of both hyperhydric and non-hyperhydric types, and the use of vented caps was not effective in deterring hyperhydric growth. Plants grown on a paper-covered, needle-punched, polyester fleece saturated with liquid medium were non-hyperhydric. Direct comparisons were made between plantlets micropropagated in agar and paper-covered polyester fleece saturated with liquid medium. Plantlets grown on agar were shorter and used less sugar than those grown on liquid-fleece system. Nearly all plantlets from agar and liquid survived transfer to greenhouse, where they grew rapidly. There was great variation in plant size after six weeks of greenhouse growth, however, plants from liquid had more large leaves (midrib >3 cm) and had longer stems than plants from agar. When greenhouse plants were graded by size, greater numbers of larger plants came from liquid medium than agar. This method is being successfully applied to other varieties of hydrangeas, and non-related plants that are normally sensitive to hyperhydricity.

P-1019

Comparative Effects of Photoperiod and Culture Media on Asymbiotic Seed Germination and Seedling Development of *Vanda* Hybrids. T. R. JOHNSON and M. E. Kane. Environmental Horticulture, University of Florida, Gainesville, FL 32611. Email: timjohn@ufl.edu

The wholesale market for flowering potted orchids in the United States has consistently increased for the past 8 years. Between 2005 and 2006 this market has increased by $14 million. Sales of *Phalaenopsis* hybrids far exceed that of any orchid genera. This is due, in part, to research that has facilitated propagation and flowering of these hybrids. However, as consumers become more accustomed to displaying and growing orchids, demand for other closely related genera such as *Vanda* hybrids will increase. To assess the cultural requirements for the seed culture of *Vanda* interspecific and intergeneric hybrids, a comparative study of culture media and photoperiod on asymbiotic seed germination and seedling development was completed. Seeds from three different hybrids cultured on *Phyto*Technology Orchid Seed Sowing Medium (P723) under a 16 hour photoperiod developed to significantly more advanced stages than when cultured on other media. After 3 months culture, only seedlings of one hybrid, V. Motes Primerose × *Ascocenda* Tavivat, attained Stage 5 (two leaves and one or more roots present; total germination >85% under all treatments). By 12 weeks culture, two other hybrids screened displayed <60% germination regardless of treatment with no seedling developed past Stage 3 (first true leaf present). Overall, seedling growth and development of the *Vanda* hybrids screened was slow compared to that reported for *Phalaenopsis* seedlings. This may reflect past objectives in *Vanda* breeding programs where flower quality has been heavily selected for while plant vigor and other commercially desirable qualities have been largely ignored.

P-1020

Studies on the In Vitro Propagation of *Cymbidium aloifolium* (L.) Sw.—an Epiphytic Orchid. S. P. Rath[1], S. N. Patnaik[1], and N. R. NAYAK[2]. [1]Department of Botany, Utkal University, Bhubaneswar-751004, INDIA and [2]301C Plant Sciences Building, Department of Horticulture, University of Kentucky, 1405 Veterans Road, Lexington, KY 40546. Email: nayaknr@uky.edu

In vitro protocols were standardized for the propagation of *Cymbidium aloifolium* (L.) Sw. through culture of seeds, vegetative parts and encapsulated protocorm-like bodies (PLBs). Murashige and Skoog (MS) medium was found to be best medium for seed germination among the three mediums tested. Growth regulators like 1-napthaleneacetic acid (NAA) and Indole-3-acetic acid (IAA) found to be beneficial for seed germination and seedling growth. Vegetative parts like shoots, rhizomes and thin cross sections (TCS) of PLBs were cultured on MS medium supplemented with different concentrations and combinations of plant growth regulators. Thidiazuron (TDZ) at 1.0 mg/l was found to be most effective for producing highest number of shoots from shoots and TCS explants where as N^6-benzyladenine (BA) was suitable for rhizome explants. The PLBs developed from seeds were encapsulated with calcium alginate containing MS nutrients and successfully germinated on various plating substrates under in vitro and in vivo conditions. The encapsulated PLBs could be stored at 4°C for 45 days without affecting the germination potential. The plantlets regenerated from different explants could be successfully acclimated in the green house with a resulting survival rate 100%.

P-1021

Propagation and Cryopreservation of Todsen's Pennyroyal (*Hedeoma todsenii*) In Vitro. V. C. PENCE, S. M. Charls, B. L. Plair, K. Lindsey, G. D. Winget. Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

The federally endangered Todsen's pennyroyal (*Hedeoma todsenii* R.S. Irving, Lamiaceae) is found in only 18 populations in 3 clusters in the mountains of south central New Mexico. Since seeds are rarely produced, in vitro propagation methods have been developed to provide material for cryostorage and for reintroduction, if that becomes necessary. Cultures were initiated on MS medium with 0.5 mg/L BAP, 0.05 mg/L NAA, and 100 mg/L benlate and antibiotics from shoot cuttings sent to CREW and from shoot tips collected by in vitro collecting, both from plants growing in the collection at the Arboretum at Flagstaff. This has resulted in 12 lines representing 4 populations in culture. Shoot propagation rates are significantly higher on MS medium with 0.5 mg/L BAP than on medium with 0.1 mg/L BAP and 0.01 mg/L NAA, the two propagation media tested. Rooting occurs on these media, but occurred at a higher rate on MS medium with 0.5 mg/L IBA. Rooting also occurred at 0 and 2.0 mg/L IBA, although 10 mg/L IBA was inhibitory. Rooted plants have been acclimated to soil. Shoot tip cryopreservation protocols have also been developed using both the encapsulation dehydration and the encapsulation vitrification procedures. Survival ranged from 10–93% with experiment, but there was no correlation of survival with the cryopreservation procedure or with genetic line.RAPD analysis of the 12 lines indicates that there is less

diversity among lines established from plants that exist in close proximity in situ than among lines that are separated by more distance. Shoot tips from all 12 genetic lines have been banked in liquid nitrogen. This is particularly important, since this species does not produce enough seed for banking. These cryopreservation and propagation protocols and genetic analyses are all methods that can be used as tools to provide support for the long-term conservation of this species. This work has been supported in part by grants from the Institute of Museum and Library Services, in collaboration with the Arboretum at Flagstaff and the Center for Plant Conservation.