In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-016-0034-0

Plant Contributed Papers

P-1000

Phloroglucinol Enhances Clonal Micropropagation of *Magnolia macrophylla* var. ashei. R. FOLGADO, T. La Val, S. C. Lahmeyer, and T. Thibault. The Huntington Library, Art Collections and Botanical Gardens, 1151 Oxford Rd., San Marino, CA 91108. Email: rakel.folgado@huntington.org

Magnolia macrophylla var. ashei is considered the most threatened *Magnolia* taxon in the USA (NatureServe 2015; Cicuzza et al. 2007), and its distribution is limited to ten counties in Florida (NatureServe, 2015). Indeed, approximately one half of the Magnoliaceae taxa are threatened worldwide according to the Red List of Magnoliaceae (IUCN, 2015). Tissue culture is an important tool to ensure the ex situ preservation of threatened Magnoliaceae when seed viability is low, and propagation from cuttings is difficult, as it is the case with this taxon (Riefler, 1984). Although some Magnoliaceae species and hybrids have been successfully micropropagated (Parris 2011; Zeng et al., 2002; Merkle and Wiecko, 1990), in vitro culture has not been widely utilized for conservation purposes for endangered species in this family. The proliferation of *Magnolia* shoots is difficult due to the oxidation of phenolic compounds during micropropagation (JunLi, 2007). Phloroglucinol (PG) has been recently shown to reduce browning in some woody plants (Kim et al., 2007; Agud et al., 2010), and it can act as a hormone synergist and as a precursor in the lignin biosynthesis pathway (Teixeira da Silva et al., 2013). An in vitro repository is being created at the Huntington Library, Art Collections and Botanical Gardens (San Marino, CA) for the ex situ conservation of Magnoliaceae biodiversity. Shoots (n=288) of *Magnolia acrophylla* var. ashei (provided by the Arnold Arboretum, USA) were cultured onto media with different levels of PG. The PG clearly prevented the blackening of the shoots after six weeks of culture, resulting in healthier plantlets when compared with the use of other antioxidants. The effect of salts composition and sugar concentration were evaluated to optimize shoot multiplication rate. The effect of PG is being tested now in several *Magnolia* spp.

P-1001

Production of Three New Grapefruit Like Cybrids Confirmed by Plant Mitochondrial Intron, Chloroplast and Nuclear DNA Markers to Improve Citrus Canker Resistance. AHMAD A. OMAR[1,2], Mayara Murata[1], Qibin Yu[1], Fred G. Gmitter, Jr.[1], Christine D. Chase[3], James H. Graham[1], and Jude W. Grosser[1]. [1]University of Florida, IFAS, CREC/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850; [2]Zagazig University, College of Agriculture, Biochemistry Department, Zagazig, 44511, EGYPT; and [3]University of Florida, Horticultural Sciences Department, IFAS, Gainesville, FL 32611. Email: omar71@ufl.edu

The production of cybrids, combining nucleus of a species with alien cytoplasmic organelles, is a valuable method used for improvement of various crops including *Citrus*. Moreover, this technology considers a non-GMO biotechnology strategy. In citrus, Cybrid plants can be produced as a by-product of somatic fusion. Host resistance is the most desirable strategy for control of citrus canker caused by *Xanthomonas citri* subsp. *citri*. By using cybridization approaches, the combination of cytoplasm and nucleus from different species, several putative cybrids were created through the fusion between nucellar callus cells of Meiwa kumquat (*Fortunella crassifolia* Swingle), a citrus canker resistant species and mesophyll cells in three Citrus combinations of commercial grapefruit (Marsh grapefruit, *Citrus paradisi* Macfadyen, Flame grapefruit, *Citrus paradisi* Macfadyen, and N11-11 somaclone grapefruit), a citrus canker susceptible. Kumquat produces non-commercial fruits, however, this species is highly resistant to citrus canker. In contrast, grapefruit is a very important fresh citrus cultivar that is highly susceptible to citrus canker. We regenerated putative cybrid-like grapefruit plants resembling mesophyll parent from the three combinations. Four mitochondrial (mt) introns, a mt ribosomal RNA spacer region, and four chloroplast (cp) DNA regions were selected to genotype the generated clones for cytoplasmic DNA polymorphisms between kumquat and grapefruit. Mt intron *nad*7i2, mt rRNA spacer and two cp DNA regions confirmed cybrids

Published online: 19 May 2016

with mt genome and cp genome originating from the nucellar callus parent kumquat. EST-SSR marker confirming that the nucleus genome in all the generated cybrids came from the grapefruit parent. These cybrids have the potential to provide citrus canker resistance in commercial grapefruit varieties and act as a valuable model for understanding the contribution of cytoplasmic organelles to plant disease resistance.

P-1002

Transgenic *Brassica juncea* Plants Expressing MsrA1, a Synthetic Cationic Antimicrobial Peptide, Exhibit Resistance to Fungal Phytopathogens. D. KUMAR[1], A. Rustagi[2], M. A. Yusuf[3], and N. B Sarin[4]. [1]Department of Bioscience and Biotechnology, Banasthali University, Rajasthan-304022, INDIA; [2]Department of Botany, Gargi College, University of Delhi, New Delhi-110049; [3]Department of Bioengineering, Integral University, Lucknow-226026 INDIA; and [4]School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, INDIA. Email: deepakinjnu@gmail.com

Cationic antimicrobial peptides (CAPs), which are part of innate defense mechanisms across the living kingdoms, have shown great potential in fighting against a broad spectrum of phytopathogens. Synthetic versions have been modeled on these natural peptides to incorporate desirable properties for use in agriculture. MsrA1 is a one such synthetic chimera of cecropin A and melittin CAPs with demonstrated antimicrobial properties. Here we describe the generation of transgenic *Brassica juncea* plants overexpressing the*msrA1* gene with an aim of conferring resistance against fungal pathogens. Different transgenic lines were generated and characterized for integration and expression of the transgene. Five independent transgenic lines were evaluated for their resistance to *Alternaria brassicae* and *Sclerotinia sclerotiorum*, two of the most dreaded fungal pathogens that often devastate *B. juncea* crops. In vitro assays showed inhibition by MsrA1 of *Alternaria* hyphae growth by 44-62%. As assessed by the number and size of lesions and the time taken for complete leaf necrosis, the *Alternaria* infection in the potted plants was delayed and restricted in the transgenic plants in comparison to the untransformed control plants. The disease protection against this pathogen varied from 68.8-85% in different transgenic lines. Similar results were obtained with *S. sclerotiorium* infection. Whereas the lesions were more severe and spread profusely in the untransformed control plants the transgenic plants resisted the infection. Number of sclerotia formed in the stem of untransformed control plants were significantly more and larger in size than those present in the transgenic plants. The disease protection was estimated to be 56-71.5%. We discuss the potential of engineering broad spectrum biotic stress tolerance by transgenic expression of CAPs in crop plants.

P-1003

Host-derived Gene Silencing of Pathogen Fitness Genes Improves Resistance to Soybean Cyst Nematodes in Soybean. BIN TIAN[1], J. Li[1], L. O. Vodkin[2], T. C. Todd[1], J. J. Finer[3], and H. N. Trick[1]. [1]Department of Plant Pathology, 1712 Claflin Road, Kansas State University, Manhattan, KS 66506; [2]Department of Crop Sciences, 1201 W. Gregory Drive, University of IL, Urbana, IL, 61801; and [3]Department of Horticulture and Crop Science OARDC, 1680 Madison Ave., The Ohio State University, Wooster, OH 44691. Email: btian@k-state.edu; hnt@k-state.edu

Soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most important pests to limit soybean production worldwide. Losses are estimated at 1 billion US dollars annually for the United States alone. RNA interference (RNAi) has become a powerful tool that can be used to silence many genes of interest. We report here that the expression of hairpin RNAi sequences derived from two SCN genes related to reproduction and fitness, HgY25 and HgPrp17, enhances resistance of soybean plants to SCN. Our previous studies using transgenic hairy root assays showed that transgenic composite plants expressing hairpin RNAi constructs targeting several SCN genes improved resistance against the nematodes. In current studies, bioassays performed on stable transgenic lines targeting SCN HgY25 and HgPrp17 fitness genes showed consistently significant reductions of 54% and 56% for eggs/g root, respectively. The analyses of T_2 to T_4 generations of transgenic soybeans by molecular detection and next generation sequencing confirmed the presence of specific short interfering RNAs complementary to target SCN genes. Targeted mRNAs of SCN eggs collected from the transgenic soybean lines were shown to be efficiently downregulated as confirmed by real time quantitative PCR. Based on the small RNA-seq data and bioassays, it is our hypothesis that a threshold of small interfering RNA molecules is required to significantly reduce SCN populations feeding on the host. Our results demonstrate that host-induced gene silencing of essential SCN fitness genes can be an effective strategy for enhancing resistance in crop plants.

P-1004

Evaluating Survival in Shoot Tips of Several Species Stored in Liquid Nitrogen for 4 to 16 Years. A. VANHOVE, M. Philpott, and V. C. Pence. Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH, 45220. Email: annecatherine.vanhove@cincinnatizoo.org

Cryopreservation has been utilized for several decades, but little is known about the long-term survival of the stored plant material especially when it is used as a tool in the *ex situ*-conservation of threatened and endangered species. The CryoBioBank® at CREW offers the opportunity to study the effects of long-term storage on cryopreserved samples. For this study, shoot tip samples of several species are being removed from the liquid nitrogen storage tanks and assessed for viability after 4-16 years in cryostorage. Shoot tip samples were removed from liquid nitrogen, thawed, placed on recovery medium, and evaluated for recovery at 4 weeks, using the same procedures as used at the time of banking. A preliminary analysis of data from four endangered, U.S. species (*Crotalaria avonensis*, *Deeringothamnus rugelii*, *D. pulchellus* and *Hedeoma todsenii*) shows at least some survival in 62% of all the samples. Of the tested variables, neither time in storage nor cryopreservation procedure explain any significant variation in the survival of the shoot tips, but species does explain significant variation. This suggests that the unique physiology of the species, rather than the length of storage, may be the most important factor in determining the survival of plant shoot tips following cryostorage. This highlights the importance of species-specific optimizations over broad generalizations in recommended storage techniques. These preliminary results show that long-term storage of shoot tips in liquid nitrogen is a viable tool in *ex situ* conservation. (This work supported in part by Institute of Museum and Library Services grant # LG-25-12-0595).

P-1005

Identification and Validation of Male Specific ISSR and SRAP Molecular Markers in Jojoba [*Simmondsia chinensis* (Link) Schneider] – An Economically Important Dioecious Crop. MONIKA HEIKRUJAM, Jatin Kumar, and Veena Agrawal. Department of Botany, University of Delhi, Delhi-110007 INDIA. Email:monikaheikrujam@gmail.com, Corresponding author: drveena_yahoo.co.in

Simmondsia chinensis (Jojoba) is dioecious crop cultivated commercially for its seeds which stores liquid wax esters of monounsaturated, straight-chain acids and alcohols. Being dioecious in nature, identification of sex in Jojoba prior flowering has been reckoned as a challenging problem. Molecular marker studies using Inter Simple Sequence Repeats (ISSR) and Sequence Related Amplified Polymorphism (SRAP) markers were carried out on male and female Jojoba genotypes in order to develop sex-linked markers. For ISSR analysis, out of 80 ISSR primers tested, only UBC-807, ISSR848 and VIS11 primers generated male specific bands of ~1200bp, ~1500 bp and ~1300 bp, respectively which were amplified in all the male genotypes and were absent in all the females. Two of the male-sex specific markers; UBC-807_{1200} and $VIS11_{1300}$ were further successfully converted into sequence tagged sites (STS) markers. $VIS11_{1317}$ marker was converted into an STS marker of size 584 bp while UBC-807_{1120} fragment was converted into an 800 bp STS marker, both specific to males. These STS markers were further validated on 200 populations, further confirming the results. The male specific STS markers thus generated were also found in only male flowers buds. For SRAP analysis, of the 225 combinations of SRAP primers used, only one primer combination "Em14/M10" amplified a band of 400 bp specific to only males. This marker was further validated on larger population comprising of 200 plants to ensure its reliability. Thus, the result obtained would be beneficial for Jojoba breeders in detection of sex at seedling stage which can be quite helpful for uprooting the male plants, thereby, saving resources like labor, water, fertilizers and space for highly desirable female plants.

P-1006

Analyzing Somatic Embryogenesis Gene Expression in Response to Tissue Culture Enhancer PLA1 Protein. TINA S. LAI[1], Katie Lagenski[2], and Wayne Curtis[1,2]. [1]Department of Plant Biology, Penn State University, University Park, PA 16802 and [2]Department of Chemical Engineering, Penn State University, University Park, PA 16802. Email: Tina.Lai@curtislab.org

It has been known for nearly half a century that 'conditioned media' aids tissue culture regeneration of plants. Advances in molecular biology and bioinformatics are now permitting unraveling the basis of these observations. Extracellular Phytocyanin-Like-Arabinogalactan-1 or *PLA1* protein has been shown to improve somatic embryogenesis (SE) in cotton (*Gossypium hirsutum*); Poon et al. (2012) applied *GhPLA1* proteins to tissue culture media for a two-fold increase in embryogenic calli production. Our work focuses on (1) production of orthologous PLA proteins and (2) investigation of their respective efficacies as a media additive towards enhanced regeneration of recalcitrant plants through SE. Specifically, we are evaluating PLA1 protein based on 1) observed effects on embryo production in *Theobroma cacao* SE tissue culture and 2) the effects of protein treatment on SE marker genes such as BBM, LEC1, LEC2, AGL15, and FUS3. Understanding how PLA1 interacts with SE genes can give insight into the complex orchestration of gene expression during the development of plant embryos. The generality of many elements surrounding the SE tissue culture process should allow improvement across all plants. GhPla1 protein was applied to *T. cacao* by dripping protein solution as a way to use less protein than media addition, while achieving the same enhancement effects. This is consistent with our goal to implement exposure in temporary immersion bioreactor propagation systems. Preliminary results showed embryos

were produced earlier in protein treated tissue compared to non-protein treated tissue and *GhPla1* treatment induced expression of SE transcription factor: *BBM*. Gene expression of *BBM* and other SE genes were analyzed via qPCR. In addition to *GhPla1*, we are developing *PLA1* proteins from a variety of plant species. *E. coli* will be used to recombinantly express *PLA1* proteins from*Theobroma cacao*, *Oryza sativa*, and *Dioscorea rotundata. PLA* proteins may prove to be a valuable addition to the in vitro toolbox (beyond plant hormones) for plant species that are difficult to transform or propagate.

P-1007

Susceptibility and Race Specific Wheat Genes Induced by Six *Puccinia triticina* Races. KERRI NEUGEBAUER[1], J. Fellers[2], and H. N. Trick[1]. [1]Kansas State University, Department of Plant Pathology, 4024 Throckmorton Hall, Manhattan, KS 66506 and [2]USDA-ARS-GMPRC, 4006 Throckmorton Hall, Manhattan, KS 66506. Email: kerrin@ksu.edu

Puccinia triticina, the casual agent of wheat leaf rust, is a devastating disease that can cause up to 40% yield loss. During fungal infection the host plant recognizes proteins, secreted effectors, and other molecules, which trigger a host defense response. Changes in the pathogen effectors and strong varietal selection pressure are responsible for the rapid development of new rust races. This study aims to understand how leaf rust races utilize wheat throughout infection by identifying wheat genes that are induced by individual races and genes that are induced by many races, which could be potential susceptibility genes. Six leaf rust races were evaluated on a single susceptible variety of wheat at six days post inoculation. RNA was sequenced and 63 wheat genes were identified that showed varying expression in response to the different leaf rust races. Out of the 63 genes, 54 wheat genes were characterized for expression patterns during the first seven days of infection using a time course study and real-time PCR. Race specific gene expression was found in two wheat genes that are affected by race shifts on Lr2A, Lr2C, and Lr17A. Potential susceptibility genes were also identified. Seven genes were selected for further investigation based on expression pattern and proposed function. The expression patterns were confirmed by conducting three biological replicates of real-time PCR data. RNAi was used to silence the seven selected wheat genes to further understand their role in leaf rust infection. Transgenic plants were inoculated and small changes in virulence were observed in two biological replicates. Gene expression data was obtained to show gene knock down in the transgenic plants.

P-1008

Improvement of Embryogenic Callus Production from Coconut (*Cocos nucifera* L.) Plumule Explant. QUANG THIEN NGUYEN[1,2], I. Antonova[1], J. P. R. Naranjo[1], E. A. B. Aitken[1], M. Foale[1], and S. W. Adkins[1]. [1]The University of Queensland, School of Agriculture and Food Science, St. Lucia, QLD 4072, AUSTRALIA and [2]School of Biotechnology, International University, Vietnam National University-HCM, Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, 70000, VIETNAM. Email: t.nguyen90@uq.edu.au

Commonly known as the 'Tree of Life', coconut (*Cocos nucifera* L.) is one of the most important palm crops in the world. However, its productivity is affected by age, natural disasters and a number of widespread lethal diseases. There is an urgent need to mass produce healthy seedlings for the replantation of affected areas. To do this, rapid multiplication of coconut by somatic embryogenesis is one such approach and has been attempted in the past few years with some degree of achievement, but as yet has not been optimised. Tissue browning due to the excessive production of phenolics during callogenesis is one of the major bottlenecks preventing optimization. The present study, demonstrates the use of 2-aminoindane-2-phosphonic acid (AIP), a competitive inhibitor of phenylalanine ammonia lyase, to prevent phenol production and increase somatic embryogenic callus production from plumule explants. Following the application of AIP (2 mM) a considerable drop (67.1%) in the total phenolic content was observed together with an increase of embryogenic structures on the callus masses (70.8%). Histological observation revealed that the reduction in phenolics was associated with a maximization of the number of emerging embryogenic structures. In addition, plumule age (1 to 4 wk after germination culture) was also shown to be an important factor. The highest percentage of embryogenic callus was observed when using plumule explants at 2 wk old. Primary callus was then thin-sectioned and subcultured to produce secondary friable callus which was more feasible to establish liquid-shake culture. This work provides critical insights for the advancement of coconut somatic embryogenesis through scale-up cell suspension culture.

P-1009

The Long-term Population Genetics of a Micropropagated Reintroduction of *Minuartia Cumberlandensis*, a Federally Endangered Perennial Plant. M. PHILPOTT[1,2] and V. C. Pence[1]. [1]Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220 and [2]University of Cincinnati, Department of Biological Sciences, 2600 Clifton Ave., Cincinnati, OH 45220. Email: philpome@mail.uc.edu

In vitro methods can be essential in the conservation and reintroduction of species that can't be propagated using traditional methods. However, the long-term genetic effects of using tissue culture in reintroductions are poorly understood. *Minuartia cumberlandensis* is a federally endangered plant found in Kentucky and Tennessee. In 2005, CREW and the US Forest Service planted an experimental outplanting of *M. cumberlandensis* in Daniel Boone National Forest (DBNF) using micropropagated plants to assess the viability of using tissue culture in future reintroductions. As of 2015, the outplanting had grown from an initial 63 micropropagated clones of 7 genotypes to over 150 individual plants. To assess how the population dynamics of the outplanting had changed over time, samples were collected in 2013 from the DBNF outplanting and two natural populations which served as the initial source for tissue culture material. Population genetic patterns were assessed using sequence-related amplified polymorphism (SRAP) markers. In addition, the original micropropagated material maintained in tissue culture was assessed to estimate how genetic diversity had changed over time. A preliminary analysis of the DBNF outplanting, the natural source population, and the initial tissue culture material using 4 SRAP marker combinations indicates that, as expected, there is more genetic diversity in the natural source population than the DBNF outplanting (Shannon's Information Index, I=0.52 vs. I=0.45). However, the genetic diversity in the DBNF outplanting has exceeded that of the original tissue culture material (I=0.45 vs. I=0.27). Methods are currently being developed to identify any sequence differences between the tissue culture genotypes and their long-term cryopreserved counterparts. This research represents the successful use of tissue culture in the conservation of a federally endangered species, and demonstrates the potential of tissue culture in restorations and reintroductions. (This work supported in part by IMLS grant # LG-25-12-0595)

P-1010

Metabolic Engineering of Sugarcane for Hyper-accumulation of Oil in Vegetative Biomass. SAROJ PARAJULI[1], Ratna Karan[1], Georgina Sanahuja[1], Hui Liu[2], John Shanklin[2], and Fredy Altpeter[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida, IFAS, Gainesville, FL and [2]Biosciences Dept., Brookhaven National Lab 463, 50 Bell Ave, Upton, NY 11973. Email: altpeter@ufl.edu

Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane has been proposed as a strategy to boost lipid yields per acre for biodiesel production. The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include: 1.) increasing fatty acid synthesis by expressing *WRI1*, a transcription activator of fatty acid biosynthetic genes, 2.) increasing fatty acid synthesis by RNAi suppression of tgd1which is involved in lipid reimport into the plastids from the cytoplasm, 3.) increasing TAG synthesis from diacyl-glycerol and acyl-CoA by over-expression of *DGAT1*, 4.) optimizing TAG storage by expression of oleosin which prevents the access of lipases to TAG storage compartments. Constitutive single or multiple gene expression/suppression cassettes were generated and co-delivered with the selectable *nptII* expression cassette by biolistic gene transfer into sugarcane callus. Plants were regenerated on geneticin containing culture medium and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Quantitative real PCR was performed to study the level of gene expression in transgenic TAG plants in different vegetative tissues. Plants were analyzed for TAG content by GC-MS. Line 327 with high expression of DAGAT, OLE and WRI accumulated TAG to 5.5% of its leaf dry weight and total lipids to almost 10% of its leaf dry weight. This is equivalent to 275-fold increase of TAG compared with non-transgenic sugarcane. Lines with low TAG accumulation co-expressed either fewer transgenes, or expressed the transgenes at lower levels. This research outcome will add value to the abundant sugarcane post-harvest residues for production of advanced biofuels.

P-1012

A Novel Micropropagation System for Moringa - A High Potential Nutritional and Pharmacologically Important Plant. SUDHERSAN CHELLAN, S. Jibi, J. Ashkanani, A. Al-Shatti, and S. Al-Melhem. Biotechnology Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, P. O. Box 24885, 13109, KUWAIT. Email: schellan@kisr.edu.kw

Moringa pterigosperma, commonly known as 'drumstick tree' or 'Moringa', is a perennial tree belonging to the family Moringaceae. It is a fast growing tree and is recognized worldwide for its high nutritional and pharmacological value. Due to the presence of high amount of minerals, amino acids, vitamins and growth factors essential for humans, its leaves, flowers and pods are used to control the malnutrition worldwide. It is commonly used to control several human diseases due to the presence of phytonutrients, antioxidants, antibacterial and antifungal properties. Propagation of this plant species is through seeds or vegetative cuttings. Micropropagation of moringa has been studied in our laboratory to meet the requirement of large number of healthy seedlings for the establishment of a commercial plantation. The literature review on

micropropagation of moringa indicated the utilization of several expensive growth hormones in the culture media for the in vitro growth and multiplication. Recently, while trying to introduce this species in Kuwait, the plants produced through the media containing growth regulators showed poor plant growth and multiplication in cultures which motivated us to develop an alternative protocol. As a result, a highly cost-effective, novel micropropagation protocol has been developed using a low-cost and simple culture medium without any growth hormones. In our new system, culture environmental factors were mainly standardized on plant regeneration, multiple shoot induction, shoot elongation and rooting as a substitute to the plant growth-hormones. Plants were multiplied at the rate of 1 X 50/10 days. Thousands of plants were successfully produced within 6 months duration from the culture initiation date in our laboratory. Plants produced through this new system were healthy and showed uniform growth in the greenhouse and field. This novel micropropagation system is economically feasible and could bring more income to the commercial producers of moringa.

P-1013

Strong Cytotoxic Potential and Anti-proliferative Effect of *Nardostachys jatamansi* D. C. Herbal Extract on Human Glioblastoma Cell Lines Manifested as Cell Cycle Arrest and Mitotic Catastrophe. HIMANSHI KAPOOR and Veena Agrawal. Medicinal Plant Biotechnology and Applied Research Laboratory, Department of Botany, University of Delhi, Delhi, INDIA. Email: Corresponding author: drveena_du@yahoo.co.in, Presenting author: himanshi.kapoor28@gmail.com

Glioblastoma is the primary cause of death due to brain tumours and requires an alternative approach for its management, despite the availability of multimodal treatment therapies (Meher et al., 2001, Taylor, 2010, Pan et al., 2015). Plant based medicines are beneficial as they have a synergistic and mult-itargeted action with minimum/no side effects (Amin et al., 2009, Millimouno et al., 2014). In the present investigation a strong bioefficacy of *Nardostachys jatamansi* D.C. rhizome extract (NJRE) on human Glioblastoma cell lines U87 MG and U373 MG has been seen. Bioassays conducted with NJRE revealed that the IC_{50} value as obtained through MTT assay was 33.73±3.5, 30.59±3.4 and 28.39±2.9 μg/mL for U87 MG and 36.9±8.8, 24.5±2.7 and 21.5±5.6 μg/mL for U373 MG after 24 h, 48 h and 72 h, respectively. NJRE at 30 μg/mL was able to induce DNA fragmentation in the cells, indicating apoptosis. Acridine orange and Ethidium bromide differential fluorescence staining revealed that the key process of cell death was apoptosis and 20 μg/mL of NJRE was able to induce early apoptosis, whereas higher doses exhibited complete apoptosis. NJRE also exhibited a cell cycle arrest at G_0/G_1phase at 60 μg/mL and 80 μg/mL as determined through flow cytometry. DAPI staining of the cells after NJRE treatment exhibited severe DNA damage at 60 μg/mL and 80 μg/mL along with excessive nucleation and mitotic catastrophe at 20 μg/mL and 40 μg/mL. These studies have proved that the plant extract has a strong cyto-toxic potential against Glioblstoma, the response being dose specific and time dependent. Incidentally, the extract was seen to be harmless to the normal cell line HEK. This is our first report proving strong anti-cancerous activity of the *Nardostachys jatamansi* rhizome extract against Glioblastoma.

P-1014

Meta-topolin Significantly Enhances De Novo Shoot Organogenesis and Plant Regeneration in Cassava, Sweetpotato and Soybean. RAJ DEEPIKA CHAUHAN and Nigel J. Taylor. Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132. Email: rchauhan@danforthcenter.org

Reliable plant regeneration is required for the application of effective genetic transformation and genome editing systems. In the tropical root crops cassava and sweetpotato, somatic embryogenesis is the preferred morphogenic process. However, many cultivars remain recalcitrant to the production of embryogenic tissues and/or to plant regeneration from somatic embryos. The effect of meta-topolin was studied in cassava and sweetpotato in place of benzylaminopurine (BAP) and other traditional cytokinins. In cassava, this relatively novel cytokinin was seen to induce shoot regeneration from somatic embryo-derived explants such as cotyledons and embryonic axis. Importantly, when employed at 1-6 μM, shoot regeneration was observed from non-embryo explants including the leaf, petiole and internode in a manner not previously reported for cassava. De novo shoot regeneration was observed at frequencies of over 80% from leaves in cultivar TME7, and has been shown to be effective in 10 additional African, American and Asian cassava varieties when combined with 2,4-D. A similar protocol employing meta-topolin has been adapted to sweetpotato and also enhances caulogenic potential in this crop, with the variety Jewel regenerating shoots at greater than 60% from root explants. Robust and phenotypically normal plants have been obtained in all cases. Effects of meta-topolin on the frequency of shoot regeneration from cotyledonary node explants of the soybean variety Jack have also been observed when it is combined with BAP. Enhanced shoot regeneration achieved with the use of meta-topolin increases the morphogenic potential of the three crop species investigated to date, and in the case of cassava, presents previously unavailable morphogenic pathways for development towards improved genetic transformation and gene editing technologies.

P-1015

Conservation of *Musa* sp. Seeds Via Cryopreservation. ERGUN KAYA[1], Fernanda Vidigal Duerte Souza[2], Muammer Ceylan[1], and Maria M. Jenderek[3]. [1]Mugla Sitki Kocman University, Faculty of Science, Molecular Biology and Genetics Department, 48000, Kotekli-Mugla-TURKEY; [2]Embrapa Cassava & Fruits, Caixa Postal 007, Cruz das Almas, BA 44380-000, BRAZIL; and [3]National Laboratory for Genetic Resources Preservation, USDA-ARS, Fort Collins, CO. Email: ergunkaya@mu.edu.tr

Fruits of bananas and plantains (*Musa* spp.) are essential for human nutrition and are the fourth most important crop in the world today (after rice, wheat, and maize). However, cultivated in over 100 countries in the tropical climate zone, the fruit production is globally threatened by diseases and pests. Since the establishment of the International Network for the Improvement of Banana and Plantain (INIBAP; in 1985), efforts were made to preserve the exisiting narrow pool of *Musa* spp. genetic resources and make them avaliable to breeding programs. Cryopreservation of seeds is one of feasible ways of preserving genetic diversity; this plant preservation method is used in many plant species. Our study tested the possibility of cryopreserving *Musa* seeds via their desiccation and direct immersion in liquid nitrogen (LN_2). Seeds of five *Musa* species were sterlized (20% commercial bleach for 10 min x two times), desiccated below 20% moisture content (MC) and were directly immeresed into liquid nitrogen (LN_2, -196^o C). After 24 h of LN_2 exposure, the excised from seeds embryos were geminated aseptically in vitro in dark condition. The highest embryo germination percents were observed for seeds of *Musa acuminata* Colla subsp. *burmanica* Simmonds (70.9% at 15.9% MC); *M. acuminata* Colla subsp. *zebrina* (Van Houtte) R.E. Nasution (69.3% at 16.1% MC); *M. ornata* W. Roxburgh (St. Lavender) (55.4% at 16.7% MC) and *M. velutına* H. Wendl. & Drude (Velvet Pink Banana) (77.2% at 15.3% MC). All seedlings originating from the cryopreserved seeds developed roots and acclimated to *ex vitro* conditions producing viable plants. Our study demonstrated that cryopreservation of *Musa* seeds is feasible and provided a conservation technique for seeds of selected geneotypes of banana and plantain.

P-1016

Iron and Zinc Biofortification of Cassava Storage Roots to Nutritionally Significant Levels. NIGEL J. TAYLOR[1], Narayanan Narayanan[1], Getu Beyene[1], Raj Deepika Chauhan[1], Eliana Gaitán-Solis[1], Dimuth Siritunga[2], Michael A. Grusak[3], and Paul Anderson[1]. [1]Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132; [2]Department of Biology, University of Puerto Rico, Mayagüez, PR; and [3]USDA-ARS, Department of Pediatrics, Baylor College of Medicine, Houston, TX. Email: ntaylor@danforthcenter.org

Although an excellent source of carbohydrate, cassava storage roots provide consumers with insufficient levels of micronutrients for a healthy diet. Transgenic technologies offer potential for crop plant biofortification, but their application has proved challenging. Efforts to enhance iron and zinc concentrations in cassava storage roots were attempted by over expression of the *Arabidopsis* vacuolar iron transporter (*AtVIT1*). In greenhouse conditions, expression of *AtVIT1* under control of the patatin promoter resulted in accumulation of 2-4 times higher iron concentration in storage roots compared with wild-type controls. When grown in the field, the same transgenic lines accumulated up to 10 times higher iron (110 mg/g vs 10 mg/g DW) in their storage roots, with no change in levels of zinc or other minerals. In a second approach, cassava was modified to co-express the *Arabidopsis* iron regulated transporter (*AtIRT1*) and Ferritin (*AtFER*). In the greenhouse, plants expressing *AtIRT1* + *AtFER* produced storage roots with up to 5 times higher iron concentration and 5 times higher zinc concentration compared to the controls. Storage roots of *AtIRT1* + *AtFER* co-expressing from field-grown plants harvested at 9 mo after planting showed 4-8 times higher iron (78 mg/g vs 9 mg/g DW), and 3-7 times higher zinc concentrations (81 mg/g vs 11 mg/g DW) compared to the non-transgenic controls. Both transgenic *VIT1* and *IRT1+FER* plants have proved to have root yields equivalent to the non-modified controls under greenhouse and field conditions. Impact of food processing on mineral retention and bioavailability in Fe and Zn enhanced storage roots is ongoing. Data will be shown describing how the increased concentrations of Fe and Zn could provide significant improvements in % Estimated Average Requirement (% EAR) of these minerals for consumers who utilize cassava as a staple component of their diet.

P-1017

Passage Through Somatic Embryogenesis Causes Loss of Resistance to Cassava Mosaic Disease in Regenerated Plants. NIGEL J TAYLOR[1],Getu Beyene[1], Raj Deepika Chauhan[1], Henry Wagaba[2], John Odipio[1,2], Theodore Moll[1], Titus Alicai[2], Douglas Miano[3], Mark Wilson[1], Haifeng Wang[4], Noah Fahlgren[1], Steven Jacobsen[4], James C. Carrington[1], and Rebecca Bart[1]. [1]Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132; [2]National Crops Resources Research Institute, Kampala, UGANDA; [3]Kenyan Agricultural and Livestock Research Organization, Nairobi, KENYA; and [4]Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA. Email: ntaylor@danforthcenter.org

Cassava mosaic disease (CMD) and Cassava brown streak disease (CBSD) are the most important biotic constraints to cassava production in Africa. Breeders utilize three sources of resistance to combat CMD: the dominant, mono-locus resistance CMD2; the polygenic recessive resistance CMD1; and the recently characterized CMD3. The molecular mechanisms underlying all three resistance types remain unknown. It was discovered that after transgenic modification of the CMD2-type cultivar TME 204, all regenerated plant lines had lost resistance to CMD. This response was uniform across 2000 plants exposed to vector-transmitted CMD under field trial conditions in Uganda and Kenya, and could be replicated in greenhouse-grown plants challenged with infectious clones of EACMV-KEe and ACMV-CM. Investigations have confirmed that integration of the T-DNA and associated transformation processes are not causal. Instead, loss of resistance has been confirmed to occur during induction of somatic embryos from leaf and meristem explants after 2-3 wk exposure to the auxin picloram. This phenomenon is consistent in all three different CMD2-type cultivars tested to date. Importantly, similar studies have confirmed that plants of CMD1- and CMD3-type cultivars do not lose resistance to CMD when regenerated through somatic embryogenesis. These discoveries pose challenges for the application of biotechnology to improve CMD2-type cassava cultivars that remain popular with farmers in East, West and Central Africa. However, it also provides unique opportunities to study and elucidate mechanisms underlying all three resistance types. Information will be presented describing development and application of genomic tools to address the loss of resistance to CMD. These include a PacBio genome sequence to the CMD2-type cultivar TME7, multiple RNA-seq datasets, small RNA profiles and a full methylome of resistant and susceptible cassava plants.

P-1018

Application of Somatic Embryogenesis to Hybrid Hemlocks for Use in Restoration Programs. CHANGHO AHN and Scott A. Merkle. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602. Email: dosanahn@uga.edu

The eastern North American hemlock species, eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) were important components of eastern forest ecosystems, but over the past few decades, they have been devastated by hemlock woolly adelgid (HWA). Interest in hybridizing these hemlocks with Asian hemlock species, Chinese hemlock (*T. chinensis*), Northern Japanese hemlock (*T. diversifolia*), and Southern Japanese hemlock (*T. sieboldii*), has recently increased, due to the Asian species' resistance to HWA. The objectives of the study were to: 1) initiate embryogenic cultures of hybrid hemlocks; 2) test different concentrations of abscisic acid (ABA), activated carbon (AC), and polyethylene glycol (PEG), for their potential to promote somatic embryo maturation; and 3) test the effect of light quality on somatic embryo germination and conversion. Embryogenic cultures were initiated on half-strength Litvay (LV) medium containing 9 μM 2, 4-dichlorophenoxyacetic acid and 4.5 μM 6-benzylaminopurine. ABA at 56 μM produced higher numbers of mature somatic embryos than 28 μM or 112 μM ABA, and 2 g l^{-1}AC improved mature somatic embryo production over medium with no AC. We also found that PEG 4000 was superior to PEG 6000 and that PEG 4000 ranging from 5 to 10 g l^{-1} improved somatic embryo production over no PEG, with 5 g l^{-1} producing the highest numbers of cotyledonary-stage somatic embryos. Of the three light quality treatments applied during germination (cool white fluorescent bulbs, red LEDs or blue LEDs), red LEDs significantly improved germination, conversion and other somatic seeding growth characters compared to the other light quality treatments. Application of these results can help improve hybrid hemlock somatic embryo production efficiency as well as somatic seedling quality. This system may also be useful for somatic embryo and somatic seedling production in other *Tsuga* species.

P-1019

Identification and Evaluation of Root Specific Promoters for Efficient Gene Expression in Citrus. M. DUTT and J. W. Grosser. Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: manjul@ufl.edu

Citrus is affected by a plethora of biotic and abiotic challenges with root borne disease and pests being especially difficult to control. *Candidatus* Liberibacter asiaticus (*C*Las), the causal organism behind the Huanglongbing (HLB) disease in the United States has also been reported to colonize roots prior to the appearance of foliar symptoms. We evaluated the activity of several putative root specific genes using the SYBR Green Real-time quantitative PCR detection system. RNA from citrus leaves, flowers, fruits, roots and stem were isolated from both healthy and HLB infected plants and template cDNA used in qPCR was obtained by the reverse transcription of this total RNA. We identified several sequences that was uniquely expressed in the citrus root with minimal or no expression in the aboveground tissues. To study the root specific pattern of transgene expression, a 5′-promoter fragment of citrus tonoplast intrinsic protein (Cic1867) gene was linked to the ß-glucuronidase (*gus*) reporter gene and transformed into tobacco and the citrus rootstock Carrizo citrange. This citrus promoter aligned well with AT4G17340.1; an *Arabidopsis* aquaporin-like tonoplast intrinsic protein 2;2 (TIP2;2). We also transformed tobacco and Carrizo citrange

with *gus* constructs driven by the 5′ flanking sequence of the tobacco RB7 gene and the tomato *SlREO* gene. Histochemical, fluorimetrical and qPCR analyses demonstrated that all three promoters were able to support the high level expression of the *gus* gene. GUS activity in tobacco plants transformed with the tobacco RB7 promoter was restricted to the roots while some transformed citrus plants demonstrated weak leaf activity in addition to strong root activity. The *SlREO* and theCic1867 were both root specific in their activity. This novel Cic1867 promoter can potentially be utilized to provide strong root specific gene expression in transgenic citrus plants to protect them from various diseases and pests affecting the root.

P-1020

Purple Is the New Orange - Next Generation Smart Fruits. K. DASGUPTA[1,2] and J. G. Thomson[1]. [1]USDA-ARS-WRRC, 800 Buchanan St, Albany, CA 94710 and [2]University of California, Davis, One Shields Ave, Davis, CA 95616. Email: kdasgupta@ucdavis.edu

Blood orange like citrus cultivars with more consistent purple or dark red appearance, increased health benefits from augmented antioxidants and modified flavor will be the next generation smart fruits. Currently blood and Cara cara orange varieties with differences in color, flavor and taste are in high demand and avialble only when they are in season. Modified varieties can be developed and grown more reliably in warm climates (without need for cold activation) by enhancing the expression of antioxidant pigments such as anthocyanin and promoting their presence in the fruit using plant genetic engineering. At this time very few promoters with fruit specificity are available from citrus. Thus, our objective is to express unique *MybA* transcription factors for anthocyanin accumulation in fruits instead of turning the whole plant purple/pink, which is energetically unfavorable for plant growth and development. We have characterized a collection of novel promoters that confer fruit specific patterns of expression in transgenic tomato plants. The candidate promoters were selected based on gene expression profiling data and the corresponding upstream promoter sequences were fused to a reporter gene for proof of concept experiments. Based on the above analysis, selected promoters were then used for *MybA* expressionto study the anthocyanin accumulation pattern in fruits. These promoters forms a novel molecular tool box that enable spatially-defined transgene expression that facilitate the expression within specific tissues. Further, utilization of *MybA* genes not only enables simple, versatile selection of transformed tissues through visualization, but will also increase the nutritional value of citrus plants. As the understanding of the potential health benefits conferred by anthocyanins has increased over the past decade, interest in consuming anthocyanin-rich purple oranges will surely grow in the market and the value of citrus industry will improve further.

P-1021

Morphoregulatory Role of Melatonin and Serotonin: Implications in Plant Conservation In Vitro. PRAVEEN K. SAXENA. Gosling Research Institute for Plant Preservation (GRIPP), Department of Plant Agriculture, University of Guelph, ON, CANADA N1G 2W1. Email: psaxena@uoguelph.ca

The indoleamines melatonin (N-acetyl-5-methoxytryptamine) and serotonin (5-hydroxytryptamine) are neurotransmitters ubiquitously present across all forms of life including plants. The high potential of melatonin and serotonin in detoxifying reactive oxygen species and sustaining myriads of biological functions has been demonstrated in simple unicellular as well as complex multicellular organisms including plants and animals. We have examined the role of melatonin and serotonin in the growth and development of a range of plant species during micropropagation and cryopreservation. Our results suggest that the application of these compounds as supplements to the nutritional and cryopreservation media significantly improves regenerative capacity of in vitro grown tissues following exposure to stresses that are caused by dehydration and cold temperatures. The alleviation of cold induced stresses and significant improvement in subsequent survival and growth of tissues and plants in the presence of melatonin and serotonin are accompanied by modulation in endogenous levels of melatonin, antioxidation enzyme activity and the expression of stress related genes. It is likely that the mechanisms of regulation of morphogenesis by melatonin and serotonin may be similar to those induced by the balance between cytokinins and auxins. They may also act through synergistic/antagonistic relationships with other plant growth regulators known to modulate morphogenesis in vitro. Regardless of the modes of action, this relatively novel class of plant growth regulators has far-reaching implications in the development of innovative approaches for long-term maintenance and conservation of endangered and cultivated plant species.

P-1022

Improving Photosynthetic Efficiency in Sugarcane by Expression of Pyruvate Orthophosphate Dikinase from *Miscanthus* x *giganteus*. RATNA KARAN[1], Baskaran Kannane[1], Nikhil Jaikumar[2], Kankshita Swaminathan[2], Liang Xie[2], Brandon James[2], Steve Moose[2], Fredy Altpeter[1], and Steve Long[2]. [1]Agronomy Department, University of Florida - IFAS, Gainesville, FL and [2]Department of Plant Biology, Department of Crop Sciences, University of Illinois, Urbana, IL 61801. Email: rkaran@ufl.edu

Sugarcane is a highly productive C4 crop used as main source for table sugar and an important feedstock for bioethanol production. Improving photosynthetic efficiency in sugarcane will further boost its economic value. Pyruvate orthophosphate dikinase (PPDK) regenerates the substrate phosphoenol pyruvate (PEP) for the initial carbon-fixation step and has been proposed as rate limiting enzyme in C4 photosynthesis. A recent study also suggests that *Miscanthus* x *giganteus* (*Mxg*) achieves cold tolerance by increasing the amount of PPDK rather than its intrinsic properties. We evaluated *MxgPPDK* expression for improvement of photosynthesis and biomass accumulation in transgenic sugarcane grown under replicated field conditions. Precultured cross-sections of immature sugarcane leaf whorls were used as target for biolistic transfer of *MxgPPDK4* genomic clone under its native regulatory sequences. An *npt*II expression cassette was co-transferred as selectable marker followed by selection of cultures with geneticin and regeneration of plants. The integration of intact *MxgPPDK4* transgenes was confirmed by PCR of sugarcane genomic DNA. Using qRT-PCR, PPDK transcript increased up to 1.5 times the level observed in non-transgenic control plants. PPDK enzyme activity and photosynthetic rates of these transgenic lines were significantly higher in *MxgPPDK4* transgenic lines compared to controls. Replicated field testing in single row plots displayed a trend to increased biomass production in *MxgPPDK4* lines. Our results indicate that elevating *MxgPPDK4* expression improves the photosynthetic efficiency of sugarcane and may have implications for improving biomass yield which needs to be confirmed with larger field trials.