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Plant Contributed Papers

P-1000

Effect of Desiccation Stress and Subsequent Recovery on the In Vitro Growth of the Epiphytic Orchid *Dendrophylax lindenii*. JAMESON COOPMAN and Michael Kane. Environmental Horticulture Department, University of Florida, Gainesville FL. Email: coopmanj@ufl.edu

The Ghost Orchid, *Dendrophylax lindenii* (Lindl.) Benth x. Rolfe, is an endangered leafless epiphytic orchid that is anecdotally considered to be difficult to grow and manage under greenhouse conditions. Successful growth supposedly requires still air and high humidity, but this may not be the case. Recent in vitro, greenhouse, and field observations suggest that the species may exhibit desiccation tolerance. To determine this, the desiccation tolerance and recoverability of *D. lindenii* was tested. Individual Ghost Orchid plants were randomly placed into sterile, filter-capped baby food jars within air tight chambers maintained at 10% relative humidity using a supersaturated potassium hydroxide salt solution for 1, 2, 3, or 4 weeks. At the end of the desiccation period, the orchids were recovered in vitro on P723 Orchid Sowing Medium + 30 g/L Banana Powder for 4 weeks. Data on root weight, number, and length, as well as number actively growing root tips were collected. Water potential values were collected for each desiccation exposure time. Preliminary results indicate that the Ghost Orchid exhibits high tolerance to desiccating conditions. After 4 weeks at 10% relative humidity, plant survival was 80%. Desiccating conditions overall led to a decrease in viable root number per plant. Of the surviving plants subjected to 4 weeks of desiccation, 63% remained alive after the recovery period and the number of viable roots increased demonstrating a resumption of active growth. Understanding the desiccation tolerance of *D. lindenii* to long periods of extreme water stress could have positive applications to enhance both conservation and horticulture production of this species. In horticulture, successful propagation could be increased through drier growth and maintenance conditions, and in conservation this desiccation tolerance could facilitate the direct field establishment of plants from culture, avoiding the time and expense of Stage IV greenhouse acclimatization.

P-1001

Soybean (*Glycine max*) Promoter Characterization Through CRISPRi. ANDIKA GUNADI and John J. Finer. Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH. Email: gunadi.9@osu.edu

Gene expression in all organisms is largely regulated by the promoter, which is located upstream of the gene coding region. The promoter contains a variety of regulatory elements that are recognized by DNA-binding proteins, leading to spatio-temporal regulation of gene expression. Promoters and promoter regulatory elements have typically been studied by evaluating the effects of either promoter truncation or regulatory element modulation on gene expression. In this study, CRISPR interference (CRISPRi) was evaluated as an additional tool for promoter characterization using a constitutive soybean (*Glycine max*) promoter (GmScreamM8). Through the use of a deactivated Cas9 protein (dCas9), promoter sequence-specific guide RNAs were designed to bind to the targeted promoter sequence, thereby competitively displacing any DNA-binding proteins required for transcription. DNA constructs, containing the native promoter fused with the *green fluorescent protein* (*gfp*) gene, as well as dCas9 and target-specific guide RNA cassettes were generated and co-introduced into lima bean (*Phaseolus lunatus*) cotyledon tissue using particle bombardment. The 6 different guide RNA targets consisted of 4 distinct sequences within the promoter, along with a sequence in the GFP coding sequence, and a sequence in the plasmid backbone. Transient GFP expression of 6 biological replicates per target was monitored for 66–140 hours post-bombardment using a semi-automated image capture platform. Based on image analysis, use of one of the guide RNAs to a target in the promoter region led to a clear reduction in gene expression while many of the guide RNAs were not effective in reducing promoter activity. Among the GFP-expressing cells in the lima bean cotyledons, the epidermal cells showed different expression patterns from the parenchyma cells, which seemed to be less responsive to CRISPRi. These findings represent the first documented promoter characterization using CRISPRi in plants.

P-1002

A CRISPR Toolkit for Plants. J. W. HOYLE[1], P. R. LaFayette[2], and W. A. Parrott[2]. [1]University of Georgia, Institute of Plant Breeding Genetics and Genomics 111 Riverbend Road Athens, GA 30602 and [2]University of Georgia, Department of Crop and Soil Sciences, 120 Carlton St., Athens, GA 30602. Email: JHoyle@uga.edu

CRISPR-mediated genome editing is the newest and cheapest way to create targeted gene knockouts or introductions that are valuable to plant breeding and gene discovery research. The technique utilizes endonuclease proteins like Cas9, guided by hair-pinned RNA sequences designed to target specific genomic regions. However, any given Cas9 lacks the target flexibility of a TALEN. To overcome this limitation, four different endonuclease proteins were adapted for use in plants. These are the Cas9 genes from *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Streptococcus thermophilus*, and the Cpf1 gene from *Acidaminococcus spp.* Each of these endonucleases targets different potential sequences in the genome, so having an assortment of endonucleases provides flexibility when choosing targets for genome editing. The *S. pyogenes* Cas9 has been the most widely used, but the others have their own distinguishing advantages. *S. aureus* Cas9 is the shortest Cas9 gene identified, and hence, is easier to use. Cpf1 from *Acidaminococcus* uses a significantly shorter RNA sequence that is particularly amenable to multiplexing, and *S. thermophilus* is a probiotic bacterium, which faces fewer regulatory hurdles. Accordingly, the endonucleases were cloned into an expression cassette made of plant-derived sequences, which includes a nuclear localization signal from the *Glycine max* E1 flowering gene. The relative efficiencies of each CRISPR Cas9/Cpf1 were compared by measuring their ability to inactivate a green fluorescent protein in hairy root cultures.

P-1003

An Approach Towards Generating Selectable Marker Free, Transgenic, Insecticidal Chickpea by Means of Plumular Meristem Transformation. GOURAB GHOSH and Shreeparna Ganguly. Post Graduate Department of Biotechnology, St. Xavier's College, University of Calcutta, Kolkata, INDIA. Email: gourab7@gmail.com

Chickpea is the third most important pulse crop, grown mainly in the semi-arid conditions. It is important for the cropping systems of resource-starved farmers in the developing countries. In spite of being an economically important crop, the production of chickpea has been low due to several biotic factors, specifically the lepidopteran pest, *Helicoverpa armigera* or pod borer causes devastating loses. Development of resistant varieties against pod borer by conventional breeding techniques have failed due to its narrow genetic base. For the last three decades scientists have resorted to genetically modify chickpea to produce *Bacillus thuringiensis* (Bt) endotoxin to combat pod borers. However, there is a growing public concern regarding the possible risks related to the expression of selectable marker gene in genetically modified (GM) food crops. This area of biosafety concern has never been touched upon during the generation of GM chickpea. To address the biosafety issue, this study is a step towards generating selectable marker free chickpea by means of an efficient plumular meristem transformation system. Chickpea seedlings were decapitated and their cotyledonary nodes and apical meristem were pricked, followed by co-cultivation in *Agrobacterium tumefaciens*. Two binary vectors were used for this study. One of them contained the *cry1Ac* gene and the *nptII* selectable marker flanked by *lox* sites, while the other contained the chimeric *cre* gene construct. Transformed chickpea plants were generated with these two independent vectors, with an efficiency of 90%. All T_1 *lox* plants were crossed with their T_1 *cre* counterparts. Excision of the *nptII* gene in a precise manner was observed in some of the T_2 hybrid progenies. Transgene integration was monitored by Southern hybridization and expression of *cry1Ac* was analyzed by western blot and ELISA. Transgenic events obtained, exhibited optimum expression of Cry1Ac protein. Keeping in mind the environmental safety assessment, this study has a great significance from agricultural and biotechnological points of view.

P-1004

Enhancement of Halotolerance in *Fremyella diplosiphon* by Electroporation-mediated Overexpression of Malate Dehydrogenase Gene. BEHNAM TABATABAI[1], AnithaChristy S. Arumanayagam[2], and Viji Sitther[1]. [1]Department of Biology, Morgan State University, Baltimore, MD and [2]Department of Pathology, Methodist Hospital Research Institute, Houston, TX. Email: betab1@morgan.edu, Corresponding author: viji.sitther@morgan.edu

Fremyella diplosiphon is a freshwater cyanobacterium that has great potential as a biofuel agent due to its ability to grow in low light intensity and acclimation to different wavelengths. Prior efforts to enhance salt tolerance in *F. diplosiphon* via heat mutagenesis have led to a mutant that thrives in 20 g L^{-1} NaCl. Further augmentation of halotolerance to 35 g L^{-1} NaCl which is the average salinity of seawater would be a desirable trait to exploit the organism for biofuel production in naturally available salt water systems. In the present study, we identified a homolog of the malate dehydrogenase (*mdh*)-genein *F. diplosiphon* using PCR screening and sequence analysis. The plasmid containing the *mdh* gene, designated

pGEM-7Zf-MDH, was cloned in an expression vector and transformed into the wild type. Electroporation-mediated overexpression of the gene resulted in a transformant designated HSF33 with a 20-fold increase in *mdh* transcript level. Physiological evaluation in BG11/HEPES medium and seawater adjusted to 35 g L^{-1} NaCl revealed that the transformant could thrive in high salinity, while a complete cessation of growth was observed in the wild type. Additionally, decrease in 630: 680 nm phycobiliprotein to chlorophyll *a* absorption peak ratio was observed solely in the wild type indicating that HSF33 maintains its photosynthetic pigment accumulation in seawater. Our results provide new insights into the role of the *mdh* gene in *F. diplosiphon* salt stress response enabling its cultivation in marine waters for large-scale biofuel production.

P-1005

Global Scale Investigation of Soybean MicroRNAs Responsive to Soybean Cyst Nematodes Infection. BIN TIAN[1], Shichen Wang[2], Charles D. Johnson[2], Guiliang Tang[3], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, 4024 Throckmorton Plant Sciences Center, 1712 Claflin Road, Manhattan, KS 66506; [2]Texas A&M AgriLife Genomics and Bioinformatics Service, Texas A&M University, College Station, TX, 77845; and [3]Department of Biological Sciences, Michigan Technological University, Dow Environmental Sciences and Engineering Building - Room 406, 1400 Townsend Drive, Houghton, MI 49931. Email: btian@ksu.edu, hnt@ksu.edu

The soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most important pests limiting soybean production worldwide. It is known that small RNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), play central roles in growth and development, defense against pathogens, and responses to environmental changes in plants. In this study, we sequenced 24 small RNA libraries including three biological replicates from two soybean cultivars (SCN susceptible KS4607; and SCN HG Type 7 resistant KS4313) that were grown under SCN-infested and noninfested soil at two different time points (SCN feeding establishment; and egg production). Several hundred conserved and putative novel miRNAs in soybean were identified from a total of 0.3 billion reads (average about 13.5 million reads for each sample) with the programs of Bowtie and miRDeep2 mapper. Differential expression analyses were carried out using DESEQ to evaluate significant differences among miRNAs involving in soybean and SCN interaction. Comparative analysis of miRNA profiling indicated several known miRNAs with similar expression pattern as previous reports, plus additional10 families of miRNA candidates might be specifically related to cultivar responses to SCN. Expression analysis by quantitative RT-PCR revealed correlations between miRNA and potential target genes. These findings suggest that miRNAs are likely to play important roles in soybean response to SCN. Recently, new technology such as short tandem target mimic (STTM) has been successfully applied to demonstrate many miRNA functions. The present work could provide a framework for miRNA function identification and the development of novel approaches for improving soybean SCN resistance in future studies.

P-1006

It's About Time; Rapid and Simple Maize Transformation. GEORGE HOERSTER, Keith Lowe, Mauricio La Rota, Craig Hastings, Ning Wang, Emily Wu, Ajith Anand, Maren Arling, Brian Lenderts, Mark Chamberlin, Todd Jones, and William Gordon-Kamm. DuPont/Pioneer, 8305 NW 62nd Ave., Johnston, IA 50131. Email: george.hoerster@pioneer.com, William.gordon-kamm@pioneer.com

Previously we demonstrated that ectopic expression of morphogenic genes BBM and WUS2 enhances maize transformation and extends transformation to normally recalcitrant inbreds. Gene excision was necessary since constitutive expression of these transcription factors resulted in difficulties in regeneration, plant phenotypic abnormalities and sterility. As an alternative to excision, we have found that carefully chosen developmentally regulated promoters still provided the stimulation of embryogenesis but did not produce negative pleiotropic effects in the plant. The resultant unique combination of promoters allows rapid direct ectopic embryo formation. These well-formed somatic embryos could be observed in as little as two days after Agrobacterium transfection, appear to be derived from single cells, were uniformly transformed and could be directly germinated into plants. This new transformation method is callus-free and appears to be genotype independent. Using this method, transgenic plants can be sent to the greenhouse in as little as one month. This method can potentially provide a rapid and efficient method for many academic labs to transform any maize inbred of interest, which previously was not possible.

P-1007

Under the Radar: Enhanced Legume Transformation by Altering the Host Receptor - *Agrobacterium* Effector Interactions. TIMOTHY M CHAPPELL[1], Brian H Kvitko[2], and Wayne Parrott[3]. [1]Institute of Plant Breeding, Genetics & Genomics, University of Georgia, Athens, GA; [2]Department of Plant Pathology, University of Georgia, Athens, GA; and [3]Department of Crop and Soil Sciences, University of Georgia, Athens, GA. Email: timchappell@uga.edu

While *Agrobacterium tumefaciens* is renowned for the ability to deliver DNA to a wide range of plants, several species still remain recalcitrant to transformation, including most soybean genotypes, because they recognize *Agrobacterium* as a pathogen. Avoiding the pathogen defense response in the host could facilitate transformation in recalcitrant species. A search for soybean homologs to known bacterial effector receptors identified several in the soybean reference genome, which was obtained from a genotype resistant to *Agrobacterium*. One of these effector receptors is absent in *Agrobacterium*-susceptible genotypes. Furthermore *Bradyrhizobium*, which is very closely related to *Agrobacterium*, nodulates soybean rather than triggering an immune response, presumably due to an insertion in its effector that is not present in the *Agrobacterium* homolog. Thus, while absence of this effector receptor in the host putatively leads to successful transformation by *Agrobacterium*, replacing the *Agrobacterium* effector with its homolog from *Bradyrhizobium* may permit it to avoid host recognition, and provide another strategy to transform receptor-containing varieties.

P-1008

Employing Linear Minimal DNA Expression Cassettes for Sugarcane Genetic Transformation Using Biolistics Approach. MUHAMMAD SOHAIL AKRAM[1,2] and Javed Iqbal[2]. [1]Department of Botany, Government College University (38000) Faisalabad, PAKISTAN and [2]School of Biological Sciences, University of the Punjab (54590), Lahore, PAKISTAN. Email: sohailakram79@gmail.com

Agrobacterium-mediated and particle bombardment (Biolistics) are the two commonly used methods of plant transformation. Particle bombardment, being a physical approach, is not restricted by a microbe-host contact; a requisite for Agrobacterium-mediated transformation. Moreover, vector back bone sequences are not necessary for delivering DNA constructs to target tissues using Biolistics (Altpeter *et al.*, 2005). Sugarcane is an important industrial crop widely cultivated in the world. We optimized a system for DNA delivery to sugarcane calli using the device PDS1000/He (BioRad). Circular and linear DNA configurations of the pCAMBIA1301 vector were used, separately coated onto gold particles, for expression of *gus* and *hpt*II genes in sugarcane calli. The number of GUS positive and hygromycin resistant calli revealed that both the configurations could be used for transient and stable genetic transformation of sugarcane. Molecular characterization (by PCR) confirmed the presence of both *gus* and *hpt*II genes in transformed calli. A recombinant vectors (C2Ac7–1-pG0029) was developed by ligating cry2Ac7 (of *Bacillus thuringiensis*) into the vector backbone of pG0029. Linear minimal expression cassette, harbouring cry2Ac7 under control of CaMV35S promoter along with *npt*II expression cassette, resulted in 1.7% transformation efficiency (with 78% escape rate) of sugarcane cv. HSF-240. Molecular characterization (PCR and Southern blotting) confirmed the integration of gene in 8 transgenic events while RT-PCR analysis confirmed expression of the transgene in 6 lines. The in vitro insect bioassays, conducted using 2nd instar larvae of sugarcane stem borer proved the toxicity of all 6 lines. We concluded that the linear minimal expression cassettes, without any vector backbone sequences, can effectively be employed for sugarcane genetic manipulation via PDS1000/He particle delivery system.

P-1009

Improving *Agrobacterium*-mediated Transformation of Cowpea (*Vigna unguiculata* L.). ZHIFEN ZHANG, Kathleen Monfero Marasigan, Yinping Guo, and Peggy Ozias-Akins. NESPAL/University of Georgia, 2356 Rainwater Rd, Tifton, GA 31793. Email: zhifen@uga.edu

Cowpea is an important dietary protein source for millions of people in Africa, South Asia, and South America. Use of genetic transformation can help to develop new cowpea varieties by introducing new genetic diversity or synthesizing new biological pathways. However, production of transgenic cowpea plants remains inefficient and inconsistent. This study aims to improve the cowpea transformation method so that biological engineering of this important crop will become facile. Two regeneration methods were evaluated. Use of cotyledonary-node explants from 4-d-old seedlings could produce 3.8 shoots per explant after 2-wk culture on a shoot induction medium (SIM) containing 1.67 mg/l 6-benzylaminopurine (BA). When seeds were germinated on media containing BA, cotyledon explants from 4-d-old seedlings could produce nearly twice as many shoots per explant as those without preconditioning. Shoot organogenesis was also obtained by using cotyledonary nodes from mature seeds imbibed in sterile water overnight, producing 3.3 shoots per explant after 2-wk culture on SIM. Sonication, vacuum infiltration, needle injection and incision with scalpels at the shoot-forming region were evaluated in order to enhance *Agrobacterium*-mediated transformation at the target tissue. Using cotyledonary nodes from imbibed seeds, sonication for 20 s and 40 s increased the number of explants showing transformed cells at the shoot-forming region, with over 45% of explants having cells or sectors expressing a fluorescent protein transgene at the target region. The increased number of transformed cells at the shoot-forming region is being tested for improved recovery of transgenic shoots. After over 1-mo selection on 5 mg/l phosphinothricin, 0–1.4% of explants were able to produce transgenic shoots, varying among

experiments. Efforts to improve shoot regeneration from transformed cells are continuing.

P-1010

Enhanced Production of Prenylated Stilbenoids and Elucidation of Their Biosynthetic Pathway in Hairy Root Cultures of Peanut. TIANHONG YANG[1], Lingling Fang[1], Keithanne Mockaitis[2], and Fabricio Medina-Bolivar[1,3]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR, 72467; [2]Pervasive Technology Institute and Department of Biology, Indiana University, Bloomington, IN, 47408; and [3]Department of Biological Sciences, Arkansas State University, Jonesboro,72467, AR. Email: tianhong.yang.1029@gmail.com

Peanut (*Arachis hypogaea*), a species from the Fabaceae (Leguminosae) family, is currently grown around world as an economically important oil and food crop. This species is capable of producing prenylated stilbenoids as phytoalexins to protect itself against various pathogens. In addition to their plant defense mechanisms, these prenylated stilbenoids are associated with a variety of biological activities which have attracted increasing interest in the field of medicine and nutrition for their potential applications in the prevention and treatment of human diseases. Despite their importance to plant and human health, the availability of prenylated stilbenoid is limited and their biosynthetic pathways remain to be elucidated. In this study, hairy root cultures of peanut were induced to produce peanut stilbenoids upon treatment with various elicitors. Co-treatment with methyl jasmonate (MeJA) and methyl-β-cyclodextrin (CD) led to sustained high levels of stilbenoids in the culture medium when compared to other elicitor treatments. Real-time quantitative PCR results showed that MeJA and CD had a synergistic effect on resveratrol synthase gene expression. This elicitor-controlled hairy root bioproduction system was further used to study the biosynthetic pathway of prenylated stilbenoids. Using metabolic inhibitors, it was demonstrated that the prenyl moiety on the prenylated stilbenoids derives from a plastidic pathway. Furthermore, the characterization, for the first time, of a membrane-bound stilbenoid-specific prenyltransferase activity from the microsomal fraction of peanut hairy roots was achieved. The characteristics of this enzyme provide important information for subsequent cloning and comprehensive definition of the prenyltransferase gene(s) of peanut.

P-1011

Development of a Protoplast System for Non-transgenic, Targeted Genome Editing in *S. tuberosum*. M. M. FOSSI[1,2,3], K. R. Amundson[1,2], S. N. Jinata[1], and L. Comai[1,2]. [1]University of California Davis, Genome Center, 451 Health Sciences Drive, Davis CA 95616; [2]University of California Davis, Plant Biology Department, 1 Shields Avenue, Davis, CA 95616; and [3]HM Clause, 9241 Mace Blvd., Davis, CA 95618. Email: mmfossi@ucdavis.edu

Genome editing technologies based on the CRISPR-Cas9 programmable nuclease are emerging as instrumental tools for research and plant breeding. Crops modified using this technology have recently been deemed non-regulated by U.S. regulatory agencies, provided that any transgenes used during editing are removed from the final product. For clonally propagated crops, such as potato (*Solanum tuberosum* Group Tuberosum), transgene removal remains particularly challenging and time-consuming. Non-transgenic genome editing by transfecting protoplasts with preassembled Cas9 ribonucleoprotein complexes offers a strategy for efficiently editing clonally propagated crops. To knock-out selected genes and to modify others in place, we have established a protoplast regeneration platform in *Solanum tuberosum.* This system should provide a rapid method of testing new guide RNAs and the ability to simultaneously introduce multiple edits in a crop genome. Here, we report on our protoplast isolation and plant regeneration from two potato clones: *S. tuberosum* Group Tuberosum *cv.* Desiree and *S. tuberosum* Group Phureja *cv.* DM1–3, as well as our gene target design strategy. We observe efficient protoplast isolation frequencies in both *S. tuberosum* Group Tuberosum cv. Desiree and in *S. tuberosum* Group Phureja cv. DM1–3. Contrary to 'DM1–3,' 'Desiree' successfully regenerates callus and plant shoots, making it an ideal candidate to further explore the potential of this genome editing technology for crop modification. We anticipate that edited lines produced from our pipeline will provide useful material for potato functional genomics.

P-1012

Development of *Agrobacterium*-mediated Transformation of *Physalis peruviana* and Application of CRISPR/Cas9 Genome Editing. JOYCE VAN ECK[1], Kerry Swartwood[1], Zachary H. Lemmon[2], Justin Dalrymple[2], and Zachary B. Lippman[2]. [1]The Boyce Thompson Institute, Ithaca, NY and [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Email: jv27@cornell.edu

The *Physalis* genus is a member of the *Solanaceae*. Of the edible *Physalis*, *Physalis peruviana,* native to the Andean region, is an underutilized crop that has been gaining popularity because of its high nutritional value and health-promoting compounds as well as its multiple uses that include fresh fruit, juice and jam. *P. peruviana*, also known as Cape gooseberry

and Pichuberry, has remained a minor crop in part due to its wild, unmanageable growth habit, tiny fruit, and other undesirable characteristics from a food crop production standpoint. The purpose of our work is to investigate the feasibility of applying CRISPR/Cas9 technology to in a sense domesticate *P. peruviana* by focusing on key domestication and improvement genes we have identified in tomato, a distant relative. In anticipation of utilizing CRISPR/Cas9, we developed an *Agrobacterium*-mediated transformation method based on infection of hypocotyl sections from 7-day-old seedlings with *A. tumefaciens* AGL1 that contained a construct with the GFP reporter and nptII genes. We found that selective regeneration medium that contained 200 mg/l kanamycin resulted in the highest transformation efficiency (27%). We performed RNA-seq and transcriptome assembly to identify homologous coding sequences to genes of interest identified through our work with tomato. To demonstrate the effectiveness of CRISPR/Cas9 in *P. peruviana*, which is an autotetraploid, we chose our first two targets based on experience with tomato. We targeted the tomato homolog of *ARGONAUTE7* (*SlAGO7*), which is required for the biogenesis of a class of small RNAs known as transacting short interfering RNAs, and subsequently targeted the homolog of *CLAVATA1* (*SlCLV1*), which is part of the meristem proliferation control complex. Mutation of these homologous genes in *P. peruviana* resulted in similar phenotypes as we observed in tomato. *P. peruviana* T0 plants recovered from these two transformations with CRISPR/Cas9 constructs were chimeric for *PpAGO7* and *PpCLV1* mutations and had the expected phenotypes of narrow leaves and flowers with more organs, respectively.

P-1013

Generation of Glyphosate Tolerant Cassava Plants Through CRISPR/Cas9-Mediated Gene Editing. RAJ DEEPIKA CHAUHAN[1], Aaron Hummel[2,3], Tomas Cermak[2], Colby Starker[2], Rebecca Bart[1], Daniel Voytas[2], and Nigel Taylor[1]. [1]Donald Danforth Plant Science Center, St. Louis, MO; [2]University of Minnesota, St. Paul, MN; and [3]Current address: KWS Gateway Research Center, BRDG Park, St. Louis, MO. Email: rchauhan@danforthcenter.org

Our goal is to generate non-transgenic glyphosate tolerant cassava. Cassava production is constrained by weed infestation, with most African farmers relying on labor-intensive hand weeding. The herbicide glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), preventing synthesis of chorismate-derived aromatic amino acids and their secondary metabolites. Development of glyphosate tolerant cassava could reduce labor inputs and increase storage root yields. As proof of concept, T-DNA carrying three mutation combinations of the *EPSPS* coding sequence that produce enzymes resistant to inhibition by glyphosate, were engineered into cassava. Transgenic plants showed high levels of tolerance to glyphosate, with the T102I/P106A (TIPA) allele proving most efficacious. A parallel CRISPR/Cas9 approach was undertaken in which gRNAs were used to target the native cassava *EPSPS* locus and insert a 4.2 kb sequence consisting of the 2x35S promoter plus coding sequence carrying the TIPA mutation. Molecular analysis of regenerated plants confirmed presence of repair template at the intended EPSPS site. Shikimate assays and greenhouse glyphosate applications confirmed functionality of the promoter-TIPA "knockin" within gene edited plants showing tolerance to glyphosate at levels equal to that generated by the conventional transgenic approach. It was observed that a strong constitutive promoter is required to provide maximum glyphosate tolerance. Native cassava promoters were identified by RNAseq analysis, screened as transcriptional fusions with the GUS marker gene, and confirmed to drive gene expression in a strong and constitutive manner. Transgenic cassava plants expressing the TIPA mutation under control of one such native cassava promoter showed tolerance to glyphosate application. Recovery of gene edited EPSPS TIPA variants driven by a strong constitutive native promoter is underway as the next step towards production of glyphosate tolerant cassava.

P-1014

Opportunities for Plant Engineering to Promote Sustainable Food Systems. LIZ SPECHT and Christie Lagally. The Good Food Institute, Washington, DC. Email: lizs@gfi.org

Plant engineering presents tremendous opportunities to increase both the acreage under cultivation and the functionality of diverse plant-based protein sources for incorporation into plant-based alternatives to animal products. It is well known that animal agriculture poses massive problems for public health, the environment, and animal welfare, yet plant-based meats currently comprise only 0.25% of the U.S. meat market. Greater innovation in plant-based food technology is needed to develop alternatives that compete with animal products on taste, price, convenience, and functionality. Most plant-based products currently on the market are made primarily of soy protein, wheat protein, or pea protein. Hundreds of other plant protein sources – including lentils, beans, quinoa, and other cereals – are virtually unexplored for their functionality within plant-based meat formulations. This is due in part to a lack of targeted breeding or engineering efforts for these crops, often constricting them to limited geographical distribution, sub-optimal yields and thus high cost, and overall limited and inconsistent supply. This reduces their attractiveness to

food scientists for exploration as novel protein ingredients in plant-based meat, egg, or dairy formulations. This talk will discuss how plant engineering approaches – coupled with advances in high-throughput digital phenotyping and genomic trait mapping – can improve upon these diverse plant protein crops on traits like robustness to biotic and abiotic stress, yield, palatability, and germplasm fidelity/stability. Furthermore, we will discuss engineering opportunities to improve the functionality of plant proteins themselves to specifically enhance their performance as plant-based meat, egg, or dairy alternatives.

P-1015

A Novel Approach to Cell Selection from *Taxus* Plant Cell Culture Via an Engineered Mammalian Caspase. MICHELLE MCKEE[1], Maureen Hill[2], Jeanne Hardy[2], and Susan Roberts[3]. [1]Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA; [2]Department of Chemistry, University of Massachusetts, Amherst, MA; and [3]Department of Chemical Engineering, Worcester Polytechnic Institute, Worcester, MA. Email: mcmckee@wpi.edu

Plant specialized metabolites provide a great diversity of valuable chemicals that can be used as flavorings, fragrances, pesticides and pharmaceuticals. One notable natural product is the chemotherapeutic agent paclitaxel (Taxol®), a drug commercially synthesized using *Taxus* plant cell culture. This production process is not optimal due to the inherent heterogeneity of plant culture which includes a large population of cells that do not produce and accumulate paclitaxel. Individual cells vary significantly in metabolic capacity, and there is not an effective method to cull cells due to their aggregated growth and inability to survive separately. We are investigating a new and exciting approach to select and propagate only those cells that synthesize paclitaxel. Our goal is to engineer a mammalian caspase protein to induce death in metabolically deficient cells by designing a caspase binding site which inhibits protease activity when bound by paclitaxel. This poster will highlight progress in several areas: (1) characterization of programmed cell death and necrosis in *Taxus* plant cell culture, (2) successful transient expression of mammalian caspase proteins in plant cells using particle bombardment and PEG mediated transformation, (3) quantification of caspase functionality within *Taxus* plant cells, and (4) robust assessment of protoplast transformation and viability via flow cytometry. This work serves as the foundation for future efforts towards caspase engineering and stable transformation to develop an efficient selection system and propagate superior cultures for use in paclitaxel synthesis bioprocesses.

P-1016

In Vitro Anther Cultures of *Camellia assamica* ssp. *assamica* (Masters) for Haploid Plant Production and Possibilities of Accumulation of Catechins, Caffeine and Theophylline in Them. RAKHI CHATURVEDI, Mishra Vijay Kumar, and Bajpai Ruchira. Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, INDIA. Email: rakhi_chaturvedi@yahoo.co.uk, rakhi_chaturvedi@iitg.ernet.in

Camellia assamica ssp. *assamica* (Masters) or Tea is a tropical, evergreen plant of the family Theaceae and is one of the most economically important beverage crops in the world. Tea is highly cross pollinated and cultivated taxa comprise of hybrids of the three main types, Assam type, China type and Cambod type. The genetic improvement of this tree species and development of homozygous lines (pure) is completely impossible, using the conventional methods, because of cross pollination as the rule. To overcome the inherent heterozygosity in tea, androgenic haploid embryos were produced via callusing of microspores at early-to-late uni-nucleate stages in anther cultures. The embryos germinated into complete plantlets. The chromosomal constitution of these in vitro plantlets was confirmed by cytological squash preparation from root-tips as 2n=X=15. The flow cytometric analysis of leaves from these in vitro plantlets reconfirmed the ploidy status as haploid. Following this, the in vitro regenerated calli, embryos, and leaves from parent plants (control) were subjected to extraction using various solvents, such as hot water, methanol, ethyl acetate and hexane. Isolation, estimation and quantification of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin gallate, caffeine and theophylline from in vitro androgenic cultures as well as from in vivo leaves was performed through HPLC and confirmed by Mass spectrometry. In general, hot water extracts from leaves of field grown parent plant (control) contained highest amount of metabolites but among the androgenic cultures, embryogenic cultures possessed high content of all the compounds when compared to that of dedifferentiated callus. The study is an endeavour not only to develop pure breed lines but also to yield desired metabolites at a constant rate, independent of seasonal and geographical variations.

P-1017

Applications of 3D Printing to Develop New Plant Tissue Culture Systems. A. MAXWELL P. JONES, Mukund Shukla, Amritpal Singh, Kevin Piunno, and Praveen Saxena. Gosling Research Institute for Plant Preservation, Department of Plant Agriculture, University of Guelph, Guelph, ON, CANADA. Email: amjones@uoguelph.ca

Due to the complex process of designing and manufacturing new plant tissue culture vessels through conventional means there have been limited efforts to innovate improved designs or develop systems to address specific research problems with limited commercial application. Additive manufacturing, or 3D printing as it is commonly known, allows users to design and produce low volume runs of custom objects in a reasonable time and low cost. This technology has recently been used to develop new plant tissue culture systems to address limitations in existing platforms. Specifically, a modular, stackable, culture vessel design with integrated LED lighting was designed, prototyped using 3D printing, and evaluated for plant growth. This system allows the fluence rate and spectrum of light to be controlled independently in each vessel to evaluate the effect of light quality on plant growth and morphogenesis. Independent control of lighting in each vessel will allow researchers to use appropriate experimental designs with proper replication to meet their statistical assumptions, which cannot be easily done using conventional systems. From a commercial perspective, this system provides a more uniform growth environment due to the independent light source for each vessel and is more space/energy efficient as the efficient LED lights as close to the plants and the vessels can be stacked directly upon one another. This system was evaluated by culturing several species in different spectra of light at similar fluence rates and evaluating their growth, morphology, and chlorophyll content. These experiments demonstrate that light spectra have important effects on plant growth and development in vitro, but the results were species specific. Overall, this system demonstrates the power of 3D printing to allow individual research groups to develop new culture systems that address research specific problems. In this talk, this and other culture systems being developed using 3D printing will be discussed.

P-1018

Developments and Progress in Southern California Cannabis Tissue Culture. NORMAN SENN. Knockout Genetics, Los Angeles, CA. Email: Normansenn80@gmail.com

Legalization of *Cannabis sativa* in California presents a challenge–the need to find strains that are suitable for commercial production. Legalization also provides an opportunity to find out more about what each strain has to offer therapeutically now that cannabis can be studied more openly. However, there is a significant cost associated with housing a large volume of strains. In addition, patients are becoming more concerned with biological and chemical contaminants. Since there are no approved pesticides for cannabis, growers are also particularly concerned with starting with clean clones. Cannabis tissue culture provides a superior technique for obtaining pathogen-free clones and producing many copies within a small space. This body of work presents preliminary results for nodal explants, meristem culture and shoot tip culture using an all in-one shooting and rooting media. Preliminary data indicates that meristem cultures come out more lush and green with a higher success rate than nodal explants.

P-1019

Highly Efficient In Vitro Multiplication of Commercially Important Black Pepper Cultivar (*Piper nigrum* cv. Srilanka). VIRENDRA M. VERMA. Micronesia Plant Propagation Research Center, Kosrae Agricultural Experiment Station, Cooperative Research and Extension, College of Micronesia-FSM, Kosrae, MICRONESIA. Email: vmv_vmv@hotmail.com

Black pepper (*Piper nigrum* L.) is a flowering vine of Piperaceae family, cultivated for its berries, which are usually dried and used as a spice and seasoning. Native to the humid jungles of the Malabar Coast of Southwestern India, black pepper is currently cultivated worldwide in the tropics. In Micronesia, it is gaining commercial importance as an important cash crop. A study was undertaken to develop an economically feasible, efficient, rapid and reproducible in vitro multiplication protocol for a local commercially important black pepper cultivar (*Piper nigrum* cv. Srilanka). Murashige and Skoog, 1962 medium (MS) was used throughout the study. Shoot meristems were used as explants for culture establishment. The cultures were initiated on MS medium augmented with various concentrations and combinations of cytokinins and auxins. To prevent browning of explants 100mgL^{-1} ascorbic acid was added to all media. Best culture initiation was observed on MS medium augmented with 5μM 6-benzylaminopurine (BAP). For further growth and subsequent multiplication, the established cultures were transferred on MS medium augmented with 1μM BAP. The number of multiple shoots produced from each explant after two subcultures varied from 8 to 20. A 16-h photoperiod with a temperature of 24°C day and night, light intensity of 40μmol m^{-2} s^{-1}, and 60% relative humidity were maintained for multiplication. Multiple shoots were transferred on MS medium augmented with 2μM indole-3-acetic acid for rooting. Plantlets were transferred to soil: vermiculate (1:1, *v*/v) mixture in 72-cell trays and acclimatized with 68% survival rate in 10 weeks. Fully acclimatized plants were planted in the field.

P-1020

Initiation of Cell Suspension Cultures from Axenic Leaf Explants of Lentisk (*Pistacia lentiscus* L.). Ayşe Hoşer[1], Elif Demir[1], Hilal Surmuş Asan[2], VEYSEL SÜZERER[3,4,5], Engin Tilkat[1], Abdulselam Ertaş[6], and Ahmet Onay[2]. [1]Department of Biology, Batman University, Batman, TURKEY; [2]Department of Biology, Dicle University, Diyarbakır, TURKEY; [3]Department of Medical Services and Techniques, Bingöl University, Bingöl, TURKEY; [4]Department of Biology, Division of Botany, İstanbul University, İstanbul, TURKEY; [5]Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, TURKEY; and [6]Department of Pharmacognosy, Dicle University, Diyarbakır, TURKEY. Email: beyso1985@gmail.com

Plant cell suspension cultures provide a valuable platform for the production of high-value secondary metabolites of commercial interest. The aim of this study was to describe how to initiate and maintain plant cell cultures starting from axenic leaf explants of lentisk obtained from in vitro germinated seedlings. Several factors including combinations of different concentrations of the two cytokinins (BAP, KIN, each at 1 and 0.5 mg/l) and an auxin (2,4-D, 1 mg/l), different shaking speeds (90, 95, 100 and 110 rpm), different light intensities (dark and light) and different temperatures (4, 25, 37°C) were examined. The most effective medium among the different BBD combinations was 1 mg/l BAP+1 mg/l 2,4-D in terms of packed cell volume (PCV, ml/l) fresh and dry weight (g/l) values. The highest PCV (54.00±3.67), fresh (17.50±0.90) and dry (5.25±0.26) weight values were obtained from cultures grown at 25°C, when the effect of different temperatures on the optimization of leaf suspension cultures was examined. The highest PCV, fresh and dry weight values were obtained from cultures that developed at a shaking rate of 95 rpm. It was also determined that the PCV, fresh and dry weight obtained from light conditions was higher than the cultures grown in the dark. Cell suspension cultures are regularly maintained by subculturing using 50 ml of initial inoculum growing in MS medium supplemented with 3% sucrose, 1 mg/l 2,4-D+1 mg/l BAP subcultured every 14 d. These finding suggests that dedifferentiated plant cell suspension cultures of lentisk may be convenient for the large-scale production of fine chemicals in bioreactors.

P-1021

Biotechnological Approaches to Cultivation of Some Relict Endemics. IRINA MITROFANOVA, Alexander Nikiforov, Nina Lesnikova-Sedoshenko, and Olga Mitrofanova. Department of Plant Developmental Biology, Biotechnology and Biosafety, FSBSI "The Order of the Red Banner Nikita Botanical Gardens – National Scientific Center RAS", Yalta, 298648, RUSSIAN FEDERATION. Email: irimitrofanova@yandex.ru

Currently, there is a need to preserve plant world biodiversity, especially rare and endangered species, in particular, relict endemics with biotechnological methods. Relict endemic plant species of the Crimean Mountains flora, characterized by a restricted ecotopolological attachment to the specific conditions of lithogenic landscapes have been first introduced in vitro. Among the studied species the rarest one was *Silene jailensis* N.I. Rubtzov (Caryophyllaceae). The total number of its specimens in four populations was not more than 500. This species is an obligate chasmophyte. The species *Lamium glaberrimum* (K. Koch) Taliev (Lamiaceae), *Scrophularia exilis* Popl. (Scrophylariaceae), *Sobolewskia sibirica* (Willd.) P.W. Ball (Brassicaceae) are attached to the talus slopes (obligate glyareophytes). The species *Heracleum ligusticifolium* M. Bieb. (Apiaceae), *Lagoseris callicephala* Juz. (Asteraceae) and *Lagoseris purpurea* L. (Asteraceae), *Valerianella falconida* Schvedtsch. (Valerianaceae) can grow and develop both on the fissured rock surfaces and talus colluvium. To overcome seed and fruit dormancy they were kept under the low positive temperatures (4 ± 1°C) for 4–8 weeks. For establishment of seed and fruit aseptic culture we used 70% ethanol, 1% solution of fungicide Thimerosal, 0.2–0.4% «Dez TAB» solution, 0.4% solution of cefotaxime antibiotic. Effect of MS and Monnier culture media composition on seed germination was studied and explants viability index was determined for each of the studied plant species. Mass *Silene jailensis* seed germination occurred in two steps - after 14 and 60 days of the culture. Number of viable plantlets was up to 95.83%. Seeds of *Lagoseris purpurea*, *Lagoseris callicephala* and *Valerianella falconida* germinated after 12–60 days of the culture, and their viability was 30.2%, 45.6% and 12.5%, respectively. In the species *Scrophularia exilis* the only single seedlings were obtained. On MS medium supplemented with BAP and NAA morphogenetic capacity of the studied species was realized through direct organogenesis.