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ANIMAL POSTER ABSTRACTS

CANCER BIOLOGY

A-3000

In Vitro Biomarkers of Tumor Promotion and Epigenetic Toxicity Altered by Endocrine Disruptors Methoxychlor and Vinclozolin. BRAD L. UPHAM[1], Rajus Chopra[1], Esha Kumar[1], Maxwell Mianecki[1], Lindsay Goodall[1], James Trosko[1], and Pavel Babica[2]. [1]Department of Pediatrics and Human Development and Food Safety and Toxicology Center, Michigan State University, East Lansing, MI and [2]Centre for Cyanobacteria and their Toxins, Institute of Botany, Czech Academy of Sciences, Lidicka 25/27, Brno, 65720, CZECH REPUBLIC. Email: upham@msu.edu

The pesticides, methoxychlor (insecticide) and vinclozolin (fungicide) are well-recognized endocrine- disrupting chemicals, which are known to induce reproductive toxicity and transgenerational epigenetic effects. We investigated the effects of methoxychlor and vinclozolin on established *in vitro* markers of epigenetic toxicity and tumor promotion, specifically inhibition of gap-junctional intercellular communication (GJIC) and activation of mitogen-activated protein kinases (MAPKs) in a F344 rat liver oval cell line (WB-F344). Both chemicals induced rapid, noncytotoxic dysregulation of GJIC with complete inhibition achieved at 25 M of methoxychlor and 250 M of vinclozolin after a 10-min exposure. Methoxychlor induced activation of ERK1/2 after a 10-min exposure and activation of p38 after a 30-min exposure. Vinclozolin activated both ERK1/2 and p38 after a 5-min exposure followed by return of their activity to a control level after 120 min. Both methoxychlor (30 min) and vinclozolin (10 min) decreased the number of connexin43 plaques in the plasma membrane. Although the activation of MAPKs has been implicated in the dysregulation of GJIC, inhibition of GJIC by methoxychlor and vinclozolin was independent of MAPK-ERK or MAPK-p38 activation. However, inhibition of phosphatidylcholine-specific phospholipase C (PC-PLC) prevented the dysregulation of GJIC by methoxychlor or vinclozolin, suggesting a PC-PLC dependent mechanism. In conclusion, our study identified a new *in vitro* mechanism of toxicity of methoxychlor and vinclozolin; dysregulation of GJIC and activation of MAPKs, which could be potentially involved in their transgenerational epigenetic *in vivo* effects and/or indicate their *in vivo* tumor promoting properties. Support: NIEHS grant #R01 ES013268-01A2 to Upham.

GENOMES/GENOMICS/BIOINFORMATICS

A-3002

MicroRNA Regulation of Neurodevelopmental Signaling Pathway During Glucocorticoid Treatment of the Mouse Eye. ZELJKA SMIT-McBRIDE, Alfred Yu, Sara Modjtahedi, and Lawrence S. Morse. Department of Ophthalmology and Visual Science, UC Davis School of Medicine, Davis, CA 95616. Email: zsmcbride@ucdavis.edu

Intravitreal administration of glucocorticoids Dexamethasone (Dex) and Triamcinolone (TAA) are commonly used in clinical practice to treat underlying inflammatory changes in a wide range of retinal pathologies. Despite their established therapeutic benefit, side effects such as cataract formation and ocular hypertension/glaucoma raise concerns. In previous work we identified the semaphorin signaling, as one of the main neurodevelopmental pathways affected by intravitreal application of glucocorticoids in the eye. In this study we focused on identifying differentially expressed microRNAs (master regulators of gene expression) targeting gene members of semaphorin signaling in retina. Intravitreal injections were performed transconjunctivally in anesthetized C57BL/6J mice. Each time point (1 week and 1 month) consisted of 3 groups of mice (control, Dex and TAA); and 3 biological replicates – total of 18 samples. Differential microRNA expression in retinal total RNA was determined using Affymetrix GeneChip miRNA microarrays and processing the data with Affymetrix 'miRNAQCtool' and Ingenuity Pathway Analysis (IPA) microRNA target filter analysis bioinformatics software. Bioinformatics data analysis paired microRNA differential expression data with mRNA differential expression data for the same set of samples. Following 1 week of Dex treatment we identified 9 differentially expressed miRNAs while with TAA treatment we identified 18 miRNAs targeting select genes in the semaphorin pathway. Currently we are analyzing the one month time point and these data will be presented. It is our hope that once the complexity of glucocorticoid receptor activation and

signaling is better understood clinical decision making could be greatly enhanced.

SILENT ABSTRACT
A-3003
Determination of ADP, Thrombin, and Aspirin Platelet Stimulation by Evaluation the Role of Phosphatidylserine as an Annexin V Binding Molecule. M. TONDAR[1], S. Yazdani[2], M. R. Aghanoori[3], Y.Yazdani[3], F. Ghasemvand[4], L. Heidary Rad[5], and N. Gholamazad[6]. [1]South Baylo University, N. Brookhurst St., No 1126, Anaheim Branch, 92801; [2]Lund University, Paradisgatan St., SE, SWEDEN; [3]Shiraz University of Medical Science, Meshkin Faam St., I.R., IRAN; [4]Pasteur Institute of Iran, Pasteur St., I.R., IRAN; [5]Norwegian University of Life Science, 1432 As, NO, NORWAY; and [6]University of Pune, Maharashtra 411007, IN, INDIA. Email: mahditondar.bio@gmail.com, abi10sya@student.lu.se

Phosphatidylserine (PS) as the main constituent of the plasma membrane (PM) and modulates the activity of enzymes involved in cellular signaling which accelerate apoptosis. PS externalization on PM outer leaflet due to decrease in the aminophospholipid translocase activity is a key feature for many apoptotic cells. Annexin V is a key element in phosphatidyl-L-serine recognition mediation. It can bind and polymerize PS through protein-protein interactions on membrane patches expressing PS. Our hypothesis is evaluating the role of ADP and thrombin as stimulators and aspirin as an inhibitor for increasing or decreasing of the PS externalization. Type II Phosphatidylserine Decarboxylase (PSD) encoded by *PISD* gene *(*22q12.2) catalyzes phosphatidylethanolamine (PE) formation by PS decarboxylation and converts PS into PE. FACS detected a population of platelets exposing PS bound to fluorescein-labeled annexin V (50µmol/L). RT-PCR evaluated *PISD* expression in activated platelets. FACS averagely showed: 55.09%, 59.99%, 65.98%, and 67.7% of population of ADP-activated platelets, and 64.64%, 70.2%, 78.61%, 83.11%, and 87.88% of population of thrombin-activated platelets respectively. On average, 43%, 40.01%, 39.6%, and 33.77% of aspirin-inhibited platelets in were exposing PS bound to fluorescein-labeled annexin V. RT-PCR indicated at least 32 and 41.1-43.5 fold increase in expression of *PISD* following by ADP and thrombin activation respectively. Following by PS exposure, its level in mitochondria would decrease, therefore it is expected that *PISD* expression decrease too. Contrary, *PSID* expression increased. In mammalian cells PE is synthesized in PS decarboxylation and CDP-ethanolamine pathways. PS is synthesized by PS synthase-1 and PS synthase-2 enzymes. Up regulation in any of CDP-ethanolamine, PS synthase-1, or PS synthase-2 is also expected which can be investigated in further experiments. It can be assumed that *PISD* over expression and PS externalization are significant signs of platelets apoptosis, and can potentially predispose platelets for phagocytosis.

EDUCATION POSTER ABSTRACT

HIGH SCHOOL SILENT ABSTRACT
E-3000
Effect of 1-methyl-4-phenylpyridnium on Dopamine Neuron Loss in LPS Mouse Model of Parkinson's Disease. BRINDA GUPTA. Rush University, Chicago, IL. Email: bgupta@imsa.edu

Parkinson's disease (PD) is the second most common neurological disorder, characterized by degeneration of dopamine (DA) neurons within the substantia nigra (SN). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is nontoxic, passes through blood-brain barrier (BBB), and produces murine PD models. The purpose of this experiment was to analyze effects of the MPTP toxic metabolite 1-methyl-4-phenylpyridnium (MPP+) on DA neurons in mice with prenatally injected lipopolysaccharide (LPS). LPS was hypothesized to compromise BBB, allowing tail-vein injected MPP+ to be taken up via the dopamine transporter, going into the neuronal synapses. Twenty-one C57/BL6 male mice were given saline or LPS prenatally (5 mg/kg). At eleven months, mice had MPP+ or LPS injected via the tail vein (120 µg/mL). All mice were perfused with 4% paraformaldehyde, fixed for 24 hours, and sliced into series of 40 µm sections. After, they were tyrosine hydroxylase-immunoreactive (TH-ir) stained and examined using StereoInvestigator to evaluate TH-ir neurons within the SN. Average DA count for saline/saline and saline/MPP+ mice was significantly higher than LPS/saline and LPS/MPP+. saline/saline, saline/MPP+, LPS/saline, and LPS/MPP+ mice had 10362.41, 10834.19, 8813.42 and 8387.11 DA neurons respectively, suggesting that LPS plays a role in DA degeneration. Toxins destroy DA neurons because they pass through compromised BBB, allowing for new PD pathogenesis.

PLANT POSTER ABSTRACTS

BIOFUELS
P-3000
Ploidy Levels of Potential Biodiesel Crops Determined by Flow Cytometry. L. RANGAN, S. Basak, and A. M. Ramesh. Department of Biotechnology, Indian Institute of Technology Guwahati, Assam 7810039, INDIA. Email: lrangan@iitg.ernet.in

Plant source as diesel that replaces fossil fuels is a topical subject and has gained prominence as "Biodiesel crops". Flow cytometry, with propidium iodide as the DNA stain, was used to estimate the genome size of 15 individual plants belonging to three biodiesel crops viz; *Jatropha curcas, Pongamia pinnata*, and *Mesua ferrea* collected from Assam, India. The flow cytometry analysis indicated that the mean *C* value of *J. curcas, P. pinnata*, and *M. ferrea* is 0.45, 1.32 and 0.59 pg with predicted genome size of about 197, 563 and 263 Mb respectively. Coefficient of variation in FCM analysis was within the limit of 5.0 % indicating that the results were reliable. The genome size of all the three species studied is relatively small although they show a strong plasticity to tolerate extremes of environmental conditions. The basic research conducted on the potential biodiesel crops in the current study at the cellular level is a prelude for the more advanced genomic and genetic research in future.

BIOTECHNOLOGY
P-3001
Pollen Viability and Longevity of Switchgrass (*Panicum virgatum* L.). Y. GE, C. Fu, H. Bhandari, J. Bouton, C. Brummer, and Z.-

Y. WANG. Forage Improvement Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401. Email: yge@noble.org, zywang@noble.org
Switchgrass is a wind-pollinated species that has been developed into a model herbaceous biofuel crop. Pollen plays an important role in seed production and gene flow in such outcrossing grasses. This study investigated pollen viability, pollen longevity and pollen size using different materials, including the tetraploid cultivar Alamo, the octoploid cultivar Cave-in-Rock and transgenic Alamo plants. Pollen grains were collected from field-grown Alamo and Cave-in-Rock plants, and greenhouse-grown transgenics. Pollen size was in the range of 42.5 to 54.0 mm; no significant difference was observed in average pollen size between transgenic and control plants. Increasing temperature and doses of UV-B irradiation negatively affected pollen viability and longevity, while relative humidity had only limited impact. In general, the half-life of Cave-in-Rock pollen was longer than that of Alamo pollen, and the difference was more evident at relatively low temperatures (18–24ºC). Weather conditions had a large impact on pollen longevity. Under sunny atmospheric conditions, pollen longevity of both cultivars decreased rapidly, with a half-life of less than 4.9 min and a complete loss of viability in 20 min. Under cloudy atmospheric conditions, the half-life of pollen was more than five-fold longer than under sunny conditions, and it took approximately 150 min to lose viability completely. No difference in pollen viability and longevity was found between transgenic and non-transgenic control plants, indicating that the knowledge obtained from the study of non-transgenic pollen can be applied to transgenic pollen. The baseline information obtained from this study will be useful for further biosafety studies of transgenic bioenergy crops.

P-3002
Degradation of Aflatoxin B_1 and Biological Control of *Aspergillus flavus* Using Soil Microbial Diversity. RAJESH KUMAR[1] and Ved Pal Singh[2]. [1]Department of Botany, Hindu College, University of Delhi, 110007, INDIA and [2]Applied Microbiology and Biotechnology Laboratory, Department of Botany, University of Delhi 110007. INDIA. Email: rajesh_bot@ yahoo.com

Many agriculture crops are being affected by the aflatoxins. Crops like maize, groundnut, wheat, and many other oil crops are severly affected by the aflatoxins. Aflatoxins are the notorious secondary metabolites, produced by *Aspergillus flavus*, which pose potential threat to both humans and animals health as well as the environment. Therefore, strategies are needed to degrade these toxins and to control the growth of toxigenic strain (CMI 102566) of *A. flavu*s. The observations indicated that two bacterial soil isolates degraded aflatoxin B_1 most efficiently and also inhibited the growth of toxigenic strain (CMI 102566) of *A. flavus*. On the basis of 16S rRNA gene sequence analysis, theses two bacteria have been identified as *Bacillus licheniformis* and *Bacillus cereus*. The growing culture, culture filtrate and the bacterial cell pellets were tested for their ability to degrade aflatoxins. The growing cultures of these bacteria were found to be most effective in degrading aflatoxin B_1 and B_2. The 24 h old culture of *B. licheniformis* degraded aflatoxin B_1 completely (100%), whereas *B. cereus* degraded this aflatoxin by 56.04%. Dual-culture assay indicated that both *B. licheniformis* and *B. cereus* inhibited the growth of *A. flavus* by 71.40%..

P-3003
New Gene Delivery System for Mitochondria and Chloroplast. TREVOR MACMILLAN[1], François Eudes[1], and Igor Kovalchuk[2]. [1]Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, AB T1J 4B1, CANADA and [2]University of Lethbridge, Department of Plant Biotechnology, University of Lethbridge 4401 University Drive Lethbridge, AB, T1K 3M4 CANADA. Email: macmillant@agr.gc.ca

Plant genetic engineering employs gene transfer technologies such as polyethylene glycol (PEG), electroporation, microinjection, particle bombardment, and *Agrobacterium* mediated gene delivery to create transgenic plants. All of these plant transformation methods have intrinsic limitations which affect transformation rates and rely upon random integration of exogenous DNA into the nuclear genome of plant cells. Transfection of the mitochondrial or plastid genomes of plant cells is an alternate solution to the complications associated with nuclear transformation. We have developed novel peptide nanocarriers called plant organelle targeting cell penetrating peptides (POTCPPs) to transfect nucleic acids into the mitochondrial or plastid organelles in cultured plant cells. POTCPPs are short peptides derived from protein sorting signal that possess similar physicochemical characteristics as cell penetrating peptides (CPPs). Candidate plant protein sequences were first screened using *in silico* analysis software. An organelle co-localization assay involving ***confocal*** microscopy was used to qualitatively assess 60 fluorescently labeled peptides' ability to transduce the outer plasma membrane and specifically target either the chloroplast or the mitochondria of triticale mesophyll protoplasts. Subsets of the initial candidate peptides were selected based on these tests and evaluated for their ability to deliver a DNA green fluorescent protein (GFP) reporter construct to the target organelles in protoplasts and microspores. Transient expression of the DNA GFP reporter construct was visually determined using ***confocal*** microscopy and quantitatively measured using reverse transcriptase polymerase chain reaction (RT-PCR). POTCPPs open new opportunities for the development of globally important food and industrial crops such as rice, wheat and corn with desirable natural traits such as resistance to biotic and abiotic stresses. These peptides may also have the potential to be used for the development of new mammalian therapeutic applications as well.

P-3004
Effect of 2, 4-D on *In Vitro* Regeneration of Callus in Sugarcane. KALPANA SENGAR and R. S. Sengar. Tissue Culture Lab, College of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut-250110 INDIA. Email: kalpana.sengar19@gmail.com

Sugarcane is a vegetative propagated plant thus its production offers continuing challenge to the development of high yielding high sugared and disease resistant clones. Sugarcane, belonging to the *Saccharum* (Poaceae) genus, is an important industrial crop and accounts for about 70% of sugar production all over the

world. Being vegetative propagated, hence it requires to be grown in the field to harvest mature cane tops, carrying meristematic tissues. Major impediments to traditional breeding of sugarcane are its complex genome, poor fertility, the long breeding cycle and recalcitrant nature. In the present studies stems of *in vitro* grown plants of four Sugarcane Varieties; Cos 99259, Cos 98259, Cos 96258 and Cos 767 were used for *in-vitro* regeneration. Inoculation of four varieties was done with various levels of 2,4-D, conc. of medium the segments were placed on regeneration medium containing a combination of casein hydrolysate (500 mg/l), kinetin (0.5 mg/l) and BAP (0.5 mg/l). We evaluated that lower levels of auxin speed up organogenesis and cytokinin too, supports it synergistically if applied in lower concentration (0.5 mg/l). It is inferred from these studies that continuous exposure of the explant tissues to reduced level of 2,4-D (0.25-0.5 mg/l) supplemented with casein hydrolysate (500 mg/l), kinetin (0.5 mg/l) and BAP (0.5 mg/l) in the presence of light, resulted in excellent shoot induction. Hence, auxin (2,4-D) is not only a significant determinant of callus induction but also of *in vitro* organogenesis in sugarcane.

P-3005

Evaluation of Biolistic and Agrobacterium-mediated Sugarcane Gene Transfer Methods. H. WU[1], F. S. Awan[1, 2], Q. Zeng[1, 3], T. Phipps[1], A. Vilarinho[1], J. McCuiston[4], W. Wang[4], K. Caffall[4], and F. Altpeter[1]. [1]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville, FL 32611; [2] Current address: Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, 38000, PAKISTAN; [3]Current address: Biochemistry and Biotechnology Department, Yunnan Agricultural University, Kunming 650201, CHINA; and [4]Syngenta Biotechnology Inc. Research Triangle Park, NC 27709. Email: altpeter@ufl.edu

Sugarcane (*Saccharum* spp. hybrids) is one of the most productive crops and is extensively utilized for table sugar or biofuel production. Both agrobacterium-mediated transformation or biolistic gene delivery have been successfully used for efficient gene transfer to sugarcane. For the biolistic gene transfer method we have previously employed various optimizations including reduced amounts of DNA and/or removal of the vector backbone prior to gene transfer. These modifications resulted in reduced copy number and backbone insertion of the transgenic loci as well as reduced somaclonal variation. Data from biolistic or agrobacterium mediated gene transfer of minimal *nptII* expression cassettes to sugarcane will be presented. Callus derived from 6 weeks culture of immature leaf whorl cross sections of commercial cultivar CP88-1762 was used as target for either biolistic gene transfer of the minimal *npt*II expression cassette or agrobacterium- mediated gene transfer of the *nptII* expression cassette in five independent experiments. Selection of transgenic events followed the same concentrations and types of selective agents for both transformation procedures. PCR amplification by *npt*II specific primers confirmed a total of 383 transgenic plants. The number of transgenic plants generated per tissue unit, variability from experiment to experiment, the time required from explant to transgenic plants in soil, the number of non-transgenic escapes, the frequency of complex or simple transgenic loci and the transgene expression level of simple and complex loci events for both agrobacterium-mediated and biolistic gene transfer will be presented.

CELLULAR AND MOLECULAR BIOLOGY

P-3006

Establishing the Tools to Explore the Use of a Plant-expressed Nontoxic Lectin, RTB, in Targeting Recombinant Therapeutic Protein Delivery to Animal Cells. M. JARRETT[1,2], J. Ayala[1], M. R. Fergus[1], C. L. Cramer[1], and M. C. Dolan[1]. [1]Arkansas Biosciences Institute, Arkansas State University, PO Box 639, State University, AR, 72467 and [2]Department of Chemistry and Physics, Arkansas State University, PO Box 419, State University, AR, 72467. Email: mjarrett@astate.edu

In developing new and effective strategies for therapeutic protein delivery with application to animal and human health, we have focused efforts on the nontoxic ricin B lectin (RTB) to bind and carry large molecules across animal cell membranes. Efforts to understand the mechanism of RTB cellular uptake are critical and require the development of proper tools. To this end, the fluorescent protein EGFP was expressed as a genetic fusion with RTB. EGFP provides a convenient model for studying the ability of RTB to deliver therapeutic payload with key advantages in intensity and stability compared to the use of conjugated fluorophores such as fluorescein. A plant-based, transient expression platform was used to produce RTB:EGFP fusion protein. The fusion protein retained both the lectin binding activity of RTB and the fluorescence of the EGFP. Purified recombinant protein was then used for *in vitro* cellular uptake and subsequent fluoroscopy experiments. RTB:EGFP recombinant protein exhibited distinct subcellular routing with a panoply of uptake kinetics that varied with cell type. The trafficking of RTB:EGFP in a variety of human and animal cells was tested that included human fibroblasts; lung and gut epithelial cells; murine leukemia cells; and fish gut and gill epithelial cells. These studies demonstrated the ability of RTB to rapidly and efficiently deliver large, complex proteins into multiple animal cell types. The importance of developing more targeted presentation of recombinant protein therapeutics and vaccine antigens, as well as the potential of RTB to contribute to this need, will be discussed.

DISEASE RESISTANCE

P-3007

Virus Induced Gene Silencing: A Host-induced Transient RNA Interference Approach as a Functional Genomic Tool for Cereal Rust Fungi. VINAY PANWAR[1], Brent McCallum[2], and Guus Bakkeren[1]. [1]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Center, Summerland, BC, V0H 1Z0, CANADA and [2]Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, MB, R3T 2M9, CANADA. Email: vinay.panwar@agr.gc.ca

Rust fungi are obligate biotrophic pathogens that present major constraints to wheat production worldwide. Three *Puccinia* species attack wheat causing stem, leaf and stripe rust, respectively. Despite their agricultural importance and available genome sequences, little progress has been made towards studying *Puccinia* pathogenicity and virulence genes because

efficient transformation and mutagenesis protocols are lacking. Recently, gene silencing induced by small interfering RNAs (RNA interference or RNAi) has emerged as a possible method to develop disease control measures against different plant pathogens and there is a great interest in applying RNAi to rust fungi. The ability to trigger RNAi in rust pathogens by in planta expression of parts of candidate fungal genes would offer tremendous applications for studying gene functions and identifying targets to develop resistance strategies against these devastating pathogens. We have developed a transient approach by expressing RNAi molecules in wheat targeting fungal genes using *Barley Stripe Mosaic Virus* (BSMV) mediated virus-induced gene silencing (VIGS) . We succeeded in inducing host-expressed in vivo silencing of candidate leaf rust fungus (*P. triticina*) pathogenicity genes leading to disease suppression. We show at a molecular level production of RNAi molecules and silencing of endogenous fungal target genes. Overall, our results indicates that the RNAi molecules can target homologs in the other rust species and may be used as potential agents to develop broad-range resistance against rust pathogens in cereals. Of interest are underlying mechanisms of timing and location of the generation and cross-species transfer of the "silencing molecules".

EMBRYOGENESIS/MICROPROPAGATION/ REGENERATION

P-3008

Encapsulation of Various Oil Palm Tissues for Synthetic Seed Production. A. H. TARMIZI and R. Zaiton. Malaysian Palm Oil Board (MPOB), 6, Persiaran Institusi, Bandar Baru Bangi, 43300 Selangor, MALAYSIA. Email: mizi@mpob.gov.my

Synthetic seed or artificial seed can be defined as the artificial encapsulation of somatic embryos, shoot buds, embryogenic aggregates or any tissue which has the ability to form a plant in *in vitro* or *ex vivo* conditions. At MPOB, attempts were made to encapsulate various tissues of oil palm *in vitro* cultures in sodium alginate for the production of synthetic seeds. Those selected tissues were zygotic embryos, embryogenic aggregates and shoot apices. All encapsulated tissues were successfully germinated on MS media under sterile conditions. Comparatively, shoots apices were observed to be the best tissue for synthetic seed production of oil palm *in vitro* cultures. They can be easily germinated and developed further into single plantlets. Rooting of the germinated synthetic seeds was further improved by using MPOB Fast Transfer Technique (MoFaTT) system. Hence, the development of oil palm synthetic seeds offers a convenient and practical means for long distance delivery of oil palm *in vitro* cultures.

P-3022

Use of Microplants and Associated Biotechnlogies to combat Climate Change in Jamaica, a Small Island Developing State (SIDS). SYLVIA MITCHELL. Medicinal Plant Research Group, The Biotechnology Centre, University of the West Indies, Mona, JAMAICA. Email: sylviamitchell.biotech@gmail.com

Climate change is exacerbating the situations under which small farmers produce crops in the tropics especially in Small Island Developing States (SIDS) such as Jamaica by changing the intensity and reliability of rainfall. In order to increase the resilience of farmers to these various challenges, research into developing new crops has been undertaken. These New Crops have been chosen because they had the following characteristics : they grow well in Jamaica, they are a Jamaican brand in demand in the market-place, have multiple-uses, and will benefit from clean planting material. Such new crops include native, indigenous and endemic plants traditionally wild-crafted from the forest such as ramoon, bitterwood, guinea-hen weed, black jointer, sarsaparilla, chainy root, search-mi-heart, medina, strong back, culinary herbs, spices and wicker. New Crops also include existing crops in need of revitalization and expansion such as pineapple, yam, turmeric and ginger. Research undertaken has included the development of macro- and micropropagation protocols, DNA fingerprinting, biochemical analysis and anti-microbial testing. Most of the explants were sucessfully initiated on BM supplemented with 0.5 mg/l BAP with leaf-of-life growing better on BM+0.1mg/lBAP and lemon grass, ginger and turmeric requiring 3.0 mg/l BAP. Multiplication media supplements ranged between 0.5-3.0 mg/l BAP while rooting occurred with hormone supplements of 3.0 mg/l BAP (ginger, turmeric), 0.5 mg/l IBA (chainy root, sarsaparilla), 1.5-2.0 mg/l IBA (aloe, bottle brush, lemon grass, tuna, wicker), 0.1 mg/l NAA (leaf-of-life), or 2.0 mg/l IBA (pineapple). All plantlets were successfully hardened in the same manner, in seedling bags filled with a 2:2:1 mix of soil:potting mix:sand and covered with a plastic cup for two weeks. Developmental activities have included field-testing of micropropagated plants by rural communities in the Cockpit Country and St. Catherine, establishment of rural hardening and demonstration plots, and the production of innovative machines for the production of biochar and primary processing. Biochar is a soil ameliorant found to increase crop yield while also sequestering carbon for a long period of time making it an important climate change mitigation strategy especially for the tropics. Harnessing the potential of these new crops will secure the future by giving our farmers the tools to sustainably benefit from Jamaica's vast biodiversity.

GENE TRANSFER TO PLANTS

P-3009

Microspore Transfection and the Production of Transgenic Doubled Haploid Plants Through Macroinjection of Nanocarrier: DNA Complexes into Immature Floral Buds. P. K. BHOWMIK, J. Dirpaul, J. Brost, P. L. Polowick, and A. M. R. Ferrie. National Research Council - Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, CANADA. Email: Pankaj.Bhowmik@nrc-cnrc.gc.ca

We have developed an efficient microspore transformation system that involves macroinjection of optimized nanocarrier :DNA complexes into immature donor floral buds. This has resulted in regeneration of transgenic doubled haploid *Brassica napus* (canola) plants. In addition to transient expression, molecular confirmation of reporter gene integration included PCR, Southern hybridization, and sequence analysis in both the primary regenerants and their offspring. The transfection method, carrier used, vector containing the gene of interest and the ratio of nanocarrier:DNA largely determine uptake and the ultimate efficiency of stable expression. Different types of

nanoparticles are able to interact non-covalently with DNA and can act as carriers for DNA and protein into plant cells. We have had success with four different classes of nanocarriers and two genotypes of *B.napus* and preliminary results that suggest this method could be applicable with a wide range of species. Efficient delivery of DNA to microspores and development of a routine, highly efficient doubled haploidy protocol are both equally important for successful utilization of this technology. The unique combination of efficient microspore transformation and successful regeneration of transgenic doubled haploid plants, homozygous in one generation, has the potential to have a significant impact on many basic and applied research areas as well as for the development of new traits.

P-3010

Evaluation of Four *Agrobacterium Tumefaciencs* Strains for the Genetic Transformation of Tomato (*Solanum lycopersicum* L) Cultivar Microtom. N. CEBALLOS[2], W. Lopez[1], D. Garcia[1], J. Narvaez[1], and M. L. Orozco-Cárdenas[1]. [1]University of California Riverside, Riverside, CA and [2]Universidad de Caldas, Manizales Caldas, COLOMBIA. Email: nelson.ceballos@ucaldas.edu.co, mlorozco@ucr.edu

Tomato microtom is considered as model plant for functional genomics. Microtom has several unique features as Arabidopsis, such as small size, that enables it to grow a high density plants/m^2, seed seating under fluorescent light and a short life cycle that allows for mature fruit to be harvested within 70-90 days after sowing. In addition tomato (*Solanum Lycopersicum* L.) is an economically important food worldwide. For these reasons, microtom becomes an important cultivar for understanding gene function through genetic engineering. Therefore, it is required to have a highly efficient reliable protocol for the *Agrobacterium*-mediated genetic transformation of a miniature dwarf tomato. Because the plant transformation efficiency is highly related with the virulence of the *Agrobacterium tumefaciens* and its capacity of gene transfer into the genome, four *Agrobacterium tumefaciencs* strains Aglo 1, EHA 105, GV3101, and MP90 mediated transformation of tomato microtom were compared. Each strain was transformed by electroporation with the binary vector pBI121, which contain the neomycin phosphotransferase *(nptII* gene), and the ß-galacturonidase (*GUS*) reporter gene. The presence of the *nptII* gene conferring resistance to kanamycin was confirmed by PCR, qPCR and southern blot analysis. The highest tomato transformation rate was 65% for the Agro strain GV3101 followed by EHA105 with 40%. In addition, GV3101 strain was also the most efficiency in terms of transfer both genes into the genome of the plant. This is the first study where both parameters, efficiency of transformation and gene transfer have been studied using four different *Agrobacterium* strains.

P-3011

Optimization of Various Parameters for Biolistic Delivery of Plasmid DNA in Omani Date Palm. S. A. FAROOQ, S. H. Al Rashdi, and T. T. Farook. Department of Biology, College of Science, Sultan Qaboos University, Al Khoud PC123, OMAN. Email: sfarook@squ.edu.om

Date palm (*Phoenix dactylifera* L.) is an economically important fruit tree of tropical desert regions. It is a perennial, dioecious species propagated traditionally by offshoots. Recently micropropagation of elite cultivars is taken up in large scale. How ever, the reports on genetic improvement and gene transformation are scanty. The aim of this study is to optimize various parameters for biolistic delivery of plasmid DNA into the embryogenic calli in 3 cultivars of Omani date palm. The callus cultures were initiated from the young offshoot meristem explants and the somatic embryogenesis was obtained using MS media with different levels of 2, 4-D and 2ip. The biolistic parameters such as helium pressure, type and size of micro carriers (tungsten or gold), target distance and DNA concentration were evaluated to identify the most suitable condition for date palm transformation. The plasmid DNA contained β-glucuronidase (uidA) and hygromycin phosphotransferase (hpt) genes along with the CaMV 35S promoter. Histochemical Gus assay was used to detect the transient expression of the uidA gene in transformed calli. This study concludes the best conditions for high efficiency DNA delivery with minimum damage to target tissue in date palm.

P-3012

Genetic Transformation of Soursop (*Annona muricata* L.) via *Agrobacterium tumefaciens*. D. GARCÍA[1], A. H. Alegría[2], J. Narváez[1], and M. L. Orozco-Cárdenas[1]. [1]University of California Riverside, Riverside, CA and [2]Universidad Tecnológica de Pereira, Pereira Risaralda, COLOMBIA. Email: dorag@ucr.edu, mlorozco@ucr.edu

Soursop, *Annona muricata L.*, is a tropical tree known for the economic value of the pulp from its fruit and its medicinal uses. The lack of breeding programs and propagation strategies to generate uniform plantations with trees of good vegetative growth characteristics and productivity has been a big constrain for the commercial cultivation of *A. muricata.* Clonal propagation through tissue culture and genetic engineering are promising technologies that represent alternatives to produce more stable genotypes and the propagation of trees. A protocol for *Agrobacterium*-mediated transformation of *Annona muricata* L. was developed using different media and growth regulators. The highest number of shoots (12 shoots per explant) and *in vitro* plant regeneration was achieved using hypocotyls from 30d-old seedlings planted in Murashige and Skoog (MS) media supplemented with 1mg/l of zeatina. For the establishment of a genetic transformation protocol, a GFP reporter gene and phosphinothricin (PAT) selection system was used, and different pre-culture and co-cultivation conditions with three *Agrobacterium tumefaciens* strains were tested. The best treatment was the explants inoculated with *A. tumefaciens EHA105*, pre- culture in MS media supplemented with 1mg/l of zeatina during two days and co-cultivation in MS media supplemented with 1mg/l of zeatina and acetosyringone 50µm for two days. GFP fluoresce was observed in shoots regenerated on PAT selective medium. The presence of the gene conferring resistance to phosphinothricin was confirmed by PCR. This is the first report which shows that *Agrobacterium tumefaciens* can infect and efficiently transform *Annona muricata* L.

IN VITRO TOOLS, TECHNIQUES, AND OPTIMIZATION

P-3013

In Vitro Hardening of *Manihot esculenta* Crantz (Cassava) Using the Triozole Ancymidol. ALICIA JESSAMY-BENJAMIN[1], Clare Bowen-O'Connor[1], and Judy Rouse-Miller[2]. [1]Dept. of Food Production, The University of the West Indies, St. Augustine, TRINIDAD AND TOBAGO and [2]Dept. of Life Sciences, The University of the West Indies, St. Augustine, TRINIDAD AND TOBAGO. Email: jessamy7@hotmail.com, Corresponding author: clare.boc@gmail.com

Survival of micropropagated cassava is low unless stringent, labor intensive, procedures for *ex vitro* hardening are employed. The effectiveness of the Ancymidol as a pretransplant medium component for *in vitro* conditioning of plantlets and on survival of conditioned compared to unconditioned plantlets during *ex vitro* acclimatization were investigated. Single node explants of two cassava cultivars were placed on plantlet regeneration medium supplemented with or without Ancymidol supplied at 3.43, 6.86 or 13.72 µM and incubated for 8 weeks before transplanting in peat:sand (2:1) and placed in a humidity chamber. Data (shoot height and fresh weight, and number of internodes, leaves, and roots) were collected to assess changes at the *in vitro* hardening stage and again at six weeks post transplanting. Leaf anatomy data was collected at the time of transplanting. Percentage survival data was collected at weekly intervals. Before transplanting, genotype differences were observed in response to Ancymidol treatments. White stick cassava cultivars were taller than MCol22 cassava cultivars across all treatments (P=0.027). Additionally, Ancymidol reduced shoot height compared to the control in all treatments for both cultivars (*P*=0.000). At the hardening stage, survival percentage decreased over time for both cultivars but was consistently higher for MCol22 cultivars (>75%) when compared to White Stick cultivars (<40%). Survival percentage differed significantly by genotype (*P*<0.001). Ancymidol improved survival for MCol22 compared to the control but not for Whitestick. The mesophyll tissue of the control and 13.72 µM treatment was disorganised with numerous airspaces however, there was no significant difference in width among the layers for each treatment and cultivar. Ancymidol treated shoots were more robust in stature than the control for all treatments with shorter shoots (*P*<0.001*)* and fewer internodes (*P*=0.015). Ancymidol was effective in reducing acclimation time and increasing survival percentage, however, the response is genotype specific.

P-3014

Elimination of *Columbian datura virus* (CDV) without Thermal Therapy from *Brugmansia x candida* Pers 'Creamsickle' by *In Vitro* Culture of Shoot Tips on Medium Containing Ribavirin. R. P. NIEDZ, S. E. Hyndman, D. O. Chellemi, and S. Adkins. USDA-Agricultural Research Service, US Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: randall.niedz@ars.usda.gov

Brugmansia x candida Pers 'Creamsickle' plants produced by *in vitro* treatment with ribavirin, and no thermal therapy, remained PCR-negative for *Columbian datura virus* (CDV) after one year. The plants were produced by establishing *B. x candida* 'Creamsickle' shoot cultures on autoclaved MS basal medium (Murashige and Skoog 1962), sucrose 30 g/L, myo-inositol 100 mg/L, thiamine HCl 1 mg/L, pyridoxine HCl 1 mg/L, nicotinic acid 1 mg/L, glycine 2 mg/L, BAP 1.1 µM, pH 5.7, and bacteriological agar (USB Corporation, Cleveland, Ohio, USA) 9.0 g/L with 15 ml of medium per 25×100 mm flat-bottomed glass culture tubes with polypropylene caps (Magenta Corporation, Chicago, Illinois, USA). The cultures were maintained in a growth room illuminated by cool-white fluorescent lamps (26 µmol m^{-2} s^{-1}), constant 27 °C, and a 16h photoperiod. Four weeks after initiation, the cultures were transferred to the same medium in polypropylene capped glass tubes except that the BAP concentration was reduced to 0.5 µM. *In vitro* derived shoots were excised and further dissected to 3-6 mm in length before transferring onto the same medium containing ribavirin at 0, 50, 62.5, 75, 87.5, or 100 mg/L; these shoots were cultured for 30 days. The ribavirin treated shoots were then transferred onto the multiplication medium without ribavirin for one subculture before being rooted *in vitro* on the same MS basal medium except with one half strength MS nitrogen salts and 3 µM IAA for four weeks followed by greenhouse acclimatization. *In vitro* derived plants that were free of CDV symptoms and tested PCR-negative one year after transfer to the greenhouse were produced over the entire range of 50-100 mg/L ribavirin tested. A single line from these PCR-negative plants was selected, CS2²B, for long-term assessment – this line remains symptom-free and ELISA-negative after 6 years.

MONOCOT TRANSFORMATION

P-3015

Evaluation of Minimal Inhibitory Concentration of Selection Agents for Effective Selection of Transformed Oil Palm. GHULAM KADIR AHMAD PARVEEZ, Fahisza A Rahman, Rafiqah Md Aman and Omar Abdul Rasid. Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board (MPOB), PO Box 10620, 50720 Kuala Lumpur, MALAYSIA. Email: parveez@mpob.gov.my

The effectiveness of three selection agent based on *bar* gene (herbicide Basta, biolaphos and glufosinate ammonium) was evaluated on different stages untransformed oil palm regeneration, i.e. embryogenic calli, embryoids, polyembroids, shooting and rooting. The effectiveness of the selection agents was determined by identifying the minimal concentration of the selection agent required to fully inhibit the growth of oil palm tissues. Bombarded oil palm tissues (without any DNA) were cultured on specific regeneration mediums containing varying concentrations (1- 100 mg/l) of the three selection agents. The tissues were subcultured into fresh medium after four (4) weeks and the growth of tissues were recorded every week up to six (6) months. The experiments were started by using 500mg of tissues for each treatment. Each treatment was carried out with 5 replications and was carried out twice. The weight and physical appearance of the tissues were checked monthly. The optimum concentration for selecting transformed tissues at each stage was determined and will be used in transformation experiments for constructs carrying bar gene besides other useful gene of interest. Detail results obtained and physical observation will be elaborated in the poster.

PLANT SECONDARY METABOLISM

P-3017

Shoots Culture of *Castilleja tenuiflora* Benth. in a Temporary Immersion Bioreactor as a Potential Source of Bioactive Compounds. B. P. MARTÍNEZ-BONFIL, R. Valdez-Tapia, A. R. López-Laredo, and G. Trejo-Tapia. Carretera Yautepec-Jojutla Km 6, calle CEPROBI No. 8, San Isidro, Yautepec, Morelos, 62731, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, MÉXICO. Email: bbonfil@ipn.mx, gttapia@ipn.mx

Castilleja tenuiflora Benth. (Orobanchaceae, cancer herb) is a hemiparasitic species of medicinal value that grows in pine-oak woods in Mexico. It accumulates secondary metabolites such as iridoids and phenolic compounds with a wide range of biological activities (anti-inflammatory, cytotoxic and antioxidant). As part of our interest in conservation and sustainable management of medicinal plant species, we established shoots culture of *C. tenuiflora* in a temporary immersion bioreactor, and evaluated the total content of phenolic compounds and flavonoids, and its antioxidant activity. Three-week-old *C. tenuiflora* in vitro shoots were cultured in a RITA® apparatus with B5 culture medium. Four immersion times, resulting from the combination of two frequencies (every 3 and 24 h) and two immersion durations (5 and 30 min) were examined. Both, immersion duration and frequency had a significant effect ($p<0.05$) on shoot multiplication rate (SMR). In condition RII (5 min immersion every 24 h) the highest SMR (6 shoots/explant) was obtained; vitrification was not observed and all the shoots formed roots. Shoots developed in RIV (30 min immersion every 24 h) presented the highest content of total phenolic compounds (243.96 ± 12.11 mg EAG/g dry weight) and flavonoids (365.23 ± 25.46 μg CE/g), which coincided with the highest free-radical scavenging against ABTS (476.99 ± 15.11 μmoles Trolox/g extract). The highest reducing power was observed in shoots from RI (5 min immersion every 3 h, 1042.24 ± 33.81 μmol Trolox/g extract). The shoots obtained by temporary immersion culture showed a 100% survival when transferred to potting mix. Culture in temporary immersion bioreactors represents a reliable and efficient methodology for shoot regeneration of *C. tenuiflora*.

P-3018

Nitrogen Deficiency Stimulates Phenylethanoid Glycosides and Anthocyanin Biosynthesis in *Castilleja tenuiflora* Benth. Cultured in a Temporary Immersion Bioreactor. B. P. MARTÍNEZ-BONFIL, V. Medina-Pérez, A. R. López-Laredo, G. Sepúlveda-Jiménez, and G. Trejo-Tapia. Carretera Yautepec-Jojutla Km 6, calle CEPROBI No. 8, San Isidro, Yautepec, Morelos, 62731, Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, MÉXICO. Email: bbonfil@ipn.mx, gttapia@ipn.mx

Castilleja tenuiflora Benth. (Orobanchaceae) accumulates the phenylethanoid glycosides (PhGs) verbascoside and isoverbascoside. These compounds have biological activities such as anti-inflammatory, cytotoxic and antioxidant. The biosynthesis of PhGs has not been studied in *Castilleja*; we hypothesized that their caffeoyl structure synthesized from phenylalanine via the enzyme phenylalanine ammonia-lyase (PAL). Nitrogen is an essential macronutrient for plant growth and development and as for other phenolic compounds its deficiency may result in the accumulation of PhGs. The aim of this study was to evaluate the effect of nitrogen deficiency (ND) on PhGs metabolism. *C. tenuiflora* shoots were cultured in temporary immersion bioreactors with B5 culture medium and 25.74 mM of N (control) and in B5 medium with 1.32 mM N (ND), without changing nitrate:ammonium ratio. ND affected negatively shoots growth (length, biomass and total chlorophyll content), multiplication rate and, inhibited root formation. PAL activity in shoots cultured under ND (1.45 μmol CAE h^{-1} mg^{-1} protein) was higher than that found in the control (0.36 μmol CAE h^{-1} mg^{-1} protein). The concentration of verbascoside under ND was 113.88±8.6 mg/g (dry basis) compared to 57.64±1.4 mg/g of the control (21 days of culture). Similarly, isoverbascoside accumulation was significantly higher under ND (36.41±2.3 vs 21.25±0.5 mg/g). Anthocyanins accumulation in the aerial part was evident at 9 days of culture under ND. POD activity was higher on the shoots cultured under ND, which suggested a stress condition. Nitrogen deficiency influenced negatively growth and development of *C. tenuiflora*. In response to nitrogen stress, *C. tenuiflora* shoots accumulated the PhGs verbascoside and isoverbascoside, whose biosynthetic pathway involves the participation of the enzyme PAL.

PLANT TRANSFORMATION

P-3019

Precise and Controlled Integration of Transgenes into the Chloroplast Genome of Soybean. T. M. KLEIN, A. Finch, A. Geinger, A. Bedarkar, and J. Yin. Pioneer/DuPont Agricultural Biotechnology, DuPont Experimental Station, Wilmington, DE 19880. Email: Ted.M.Klein@Pioneer.com

Methods that can provide precise and predictable transgene expression are extremely important for the commercial application of biotechnology to agriculture. Chloroplast transformation represents one of these methods. Although chloroplast transformation in soybean is now reproducible and efficient, mechanisms to control levels of gene expression as well as the ability to excise selectable marker genes are needed if the system is to be useful. We have tested three recombination systems to foster marker excision from the chloroplast genome: Cre-lox, Flp-frt and PhiC31-att. Our results show that all three systems can be used for marker excision in soybean. Although high-level transgene expression can be useful, the ability to control and modulate expression levels with the appropriate promoter and control elements is critical for achieving optimal expression levels. We show that expression can be controlled by insertions site (single copy vs inverted repeat region) and promoter.

P-3021

Characterization of *Zea mays* and *Glycine max* 4-hydroxy-phenylpyruvate Dioxygenase (HPPD) Cellular Localization Using a Fluorescent Reporter and Transient Expression in Leaf Sections. M. HECKERT, H. Albert, D. Siehl, Y. Dong, A. Madrigal, B. Lincoln-Cabatu, and L. Castle. Pioneer Hi-Bred International, Inc., 4010 Point Eden Way, Hayward, CA 94545-3721. Email: matt.heckert@pioneer.com

Herbicide tolerance strategies associated with the HPPD inhibitor class of herbicides may represent a new opportunity to broaden weed management options for growers. Further understanding of native HPPD subcellular localization could ultimately lend itself to trait efficacy. A series of plant expression vectors comprising relevant controls, *Z. mays* (corn) and *G. max* (soybean) HPPD N-terminal sequences were fused to Aequore coerulescens green fluorescent protein 1 (AcGFP 1) and delivered to leaf tissue of soy and maize by Agro-infiltration. Upon infection, free-hand sections were inspected to determine the subcellular accumulation patterns. These experiments confirmed that the Z. mays HPPD protein is chloroplast localized in monocots, but is found in both chloroplasts and the cytosol in dicot cells. In G. max, the native HPPD protein is localized to both the chloroplast and the cytosol.

SILENT ABSTRACT
P-3020
Differences in Proline Accumulation and Gene Expression Profiles of Salt-Tolerant Soybean Mutants. ÖZGE ÇELİK and Selin Gül Ünsal. Istanbul Kultur University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, 34156, Ataköy, Istanbul, TURKEY. Email: ocelik@iku.edu.tr
Proline is generally thought to have an important role in improvement of salt-tolerance of plants. The balance between synthesis, catabolism and transport determines the intracellular proline levels. In this study, we discussed the relation between free proline accumulation and the expression patterns of the genes which have role in proline metabolism (delta-1-pyrroline-5-carboxylate synthase, P5CS; pyrroline-5-carboxylate reductase, P5CR; pyrroline dehydrogenase, PDH and delta-1-pyrroline-5-carboxylate dehydrogenase,P5CDH) under 90 mM NaCl stress. Therefore, we used 3 M_2 generation soybean mutant plants (Ataem 150-68, Üstün 150-2 and Üstün 150-114) irradiated with 150 Gy gamma radiation dose and selected as salt-tolerant under *in vivo* and *in vitro* salt stress treatments. We observed 1.96, 1.14 and 2.43 fold more increases in free proline accumulation of mutant plant after 7 days of salt treatment, respectively. The expression analyses were performed by using specific primers designed for soybean gene regions. We observed increased expression patterns of four genes. According to the results of RT-PCR analyses, the proline production mechanisms of salt-tolerant mutants are differently affected from gamma irradiation. In mutants belonging to Üstün variety showed increased proline production via increased P5CR and PDH expression in mitochondria. 4.61 fold more increases in expression of P5CDH than PDH gene in Ataem 150-68 mutant gave rise to thought that the activation of ornithine pathway as an alternative mechanism under salinity stress.

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