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LATE SUBMISSION ABSTRACTS

The following abstracts will be included in an upcoming issue of *In Vitro Cellular and Developmental Biology*:

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ANIMAL POSTER ABSTRACTS

IN VITRO ANIMAL CELL SCIENCES

A-3000

Evaluation of Cytokine Activity of Three Human Tissue Constructs for *In Vitro* Sulfur Mustard Research. Adele L. Miller, Offie E. Clark, Clinton L. Cario, Eric W. Nealley, Robert K. Kan, and WILLIAM J. SMITH. Research Division U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010. Email: William.j.smith3@us.army.mil

Sulfur mustard (2,2'-dichlorodiethyl sulfide, HD) is a cytotoxic chemical warfare agent known for its vesicating properties. Prior research has focused on the use of isolated cell cultures or whole animals to study the effects of HD injury. Commercially available, multicellular tissue models are of interest as they can serve to bridge research gaps between cell and organismal studies. To evaluate this possibility, we analyzed the cytokine activity of three tissue constructs after HD exposure. The models chosen, EpiDermFT™, EpiAirway™, (MatTek Corp.) and the Corneal Epithelial Cell Model™ (Lonza), represent the tissues of the skin, bronchial epithelium and cornea respectively. These constructs correspond to the principal target sites for *in vivo* toxicity in

response to HD exposure. Cytokines were measured by Luminex technology using Biosource Human Cytokine Inflammatory 10-plex kits. Media samples were collected at 0, 2, 4, 6, 8, and 24 hours following exposure to 1-300 μM concentrations of HD. EpiDermFT™ showed changes in the expression of GM-CSF, IL-6 and IL-8. The EpiAirway™ model displayed alterations in IL-6 production. The Corneal Epithelial Cell Model™ demonstrated increased activity of GM-CSF, IL-6, IL-8 and TNF-α. Control samples of all models underwent histological examination. The 3-D structure of only one model, EpiDermFT™ resembled intact epithelial tissue. The data obtained from this study confirm the ability of these tissue constructs to initiate inflammatory cascades due to HD. Further research will enable us to evaluate and select the appropriate tissue models for routine incorporation in future HD studies.

A-3001

The Protective Effect of Capsaicin Analog Treatment on Human Bronchial Cells Exposed in Culture to Sulfur Mustard (HD). Offie E. Clark¹, Eric W. Nealley¹, Adele L. Miller¹, Clinton L. Cario¹, Young-Sik Jung², and WILLIAM J. SMITH¹. ¹Research Division U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), 3100

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Sulfur mustard (2, 2'-dichlorodiethyl sulfide, HD) is a cytotoxic alkylating agent with mutagenic and vesicating properties. Respiratory cytotoxicity is a major component of HD exposure. Our research focus is to determine the mechanism of toxicity of HD for the purpose of developing medical countermeasures to this chemical threat agent. Capsaicin (8-Methyl-N-vanillyl-trans-6-nonenamide) is known to block neurogenic inflammatory pathways and the capsaicin analogs octyl homovanillamide and retro-ovaniol have some ability to prevent HD damage in the mouse ear vesicant model. We have evaluated the ability of these capsaicin analogs to prevent HD-induced changes in cultured normal human bronchial epithelial (NHBE) cells. KR-24095 a water-soluble capsaicin analog, was obtained from the Korea Research Institute of Chemical Technology (KRICT). NHBE were pretreated with each of these three analogs 15 minutes prior to HD exposure. Inflammatory cytokines were measured in the tissue culture media 24 hr. post exposure via Luminex technology using Biosource Human Cytokine Inflammatory 10-plex kits. Cellular viabilities were determined 24 hr. after HD exposure via vital dye (propidium iodide, PI) uptake as measured by flow cytometry. Morphological changes were documented via light microscopy. HD at 100 μ M caused increased uptake of PI. Retro-ovaniol, while preserving good cellular morphology, failed to prevent HD-induced vital dye uptake. KR-24095 markedly reduced the uptake of vital dye following HD exposure. Octyl homovanillamide was the least effective of the compounds in preventing PI uptake. The test compounds did not prevent HD-induced release of inflammatory cytokines into the cellular media. KR-24095 a water soluble analog has shown increased therapeutic effects against the cytotoxicity of HD. We recommend additional studies with KR-24095, to determine its further value as a potential HD prophylactic. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

A-3002

Lipid-based Supplements Enhance Cell Proliferation in Low-serum Media. A. Maughan and G. W. REESE. Caisson Laboratories, Inc. North Logan, UT 84341. Email: Gordon@caissonlabs.com

The addition of fetal bovine serum (FBS) to cell culture media revolutionized animal cell culture procedures and enabled major new industrial and pharmaceutical applications. However, concerns regarding potential prion (transmissible spongiform encephalopathies) and other viral contaminants have forced cell culture industries to consider serum-free alternatives. Furthermore, serum prices are volatile and are rapidly increasing due to rising agricultural commodity prices. In an effort to meet these challenges, Caisson Laboratories, Inc. is actively researching reduced-serum and serum-free alternatives to standard culture procedures. The latter generally call for media containing 10% FBS. We have developed a lipid-based supplement that supports cell growth and proliferation in media containing as low as 0.5% FBS. Commercial media, e.g. RPMI, DMEM, DME/F12 containing our lipid-based supplement and 2% FBS performed comparable to media supplemented with 10% FBS and no lipid-based supplement when MDCK, MRC-5, VERO and CHO cells were grown. Importantly, cells cultured in media containing our reduced-serum supplement have not experienced the same types of disassociation-enzyme-based cell damage common to many serum-free formulations.

A-3003

Application of Neuronal Progenitors to Study Pathomechanisms of Autism In Vitro. BOZENA MAZUR-KOLECKA, Ira L. Cohen, Edmund C. Jenkins, W. Ted Brown, and Janusz Frackowiak. NYS

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Altered behavior in autism may result from abnormal brain development during embryonic and postnatal life. Neurogenesis is regulated by neurotrophins, neuropeptides, regulatory proteins and neurotransmitters, the levels of which may be altered in the brain, CSF, and blood in autism. We have found differences between maturation of human neuronal progenitor (NP) triggered by sera from children with autism versus controls. Now we propose that neurons developing in an "autistic environment" are more susceptible to oxidative stress that is a risk factor in autism. We studied the effect of oxidative stress on proliferation and neuronal development of human NP stimulated with sera from normal children and with autism aged up to 5 years and between 7-14 years. Oxidative stress was induced with 200 μ M FeCl₂ at the beginning or during NP differentiation. Proliferation was tested by the BrdU incorporation assay (10 μ g/ml for last 2 hours of culture). Neuronal development was estimated by expression of immature (Sox2) and mature (NeuN) neuronal markers, by immunoblotting. Results showed that proliferation of NPs and expression of neuronal marker NeuN in culture depend on the age and diagnosis of autism of serum donors, and the stage of NP differentiation during exposure to oxidative stress. Oxidative stress applied to cultures of NP stimulated by sera from young children with autism reduced neuronal maturation. Results suggest that an "autistic environment" during neuronal development affects the vulnerability of NP to oxidative stress. In conclusion, we propose that a cell culture model can be used to study the effect of internal and external risk factors and may reflect altered neurogenesis in autism. Sponsored by NYS OMRDD; March of Dimes #12-FY03-42, and the NYS Legislative Grant, #M40438.

A-3004

Induction of Intracellular and Extracellular Deposition of Amyloid- β Peptide in Cell Culture Models of Vascular Amyloidosis- β in Three Species. J. FRACKOWIAK¹, B. Ranasinghe¹, D. Dickson², B. Mazur-Kolecka¹. ¹NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314 and ²Mayo Clinic, Jacksonville, FL 32224. Email: Janusz.Frackowiak@omr.state.ny.us

Amyloid- β deposits in the brain parenchyma and in brain blood vessel walls are the main neuropathological features in Down's syndrome (DS) and Alzheimer's disease (AD). The mechanisms that induce a focal deposition of amyloid- β peptide (A β), inside cells and extracellularly, remain unclear. It has been shown that neurons and vascular smooth muscle cells produce A β in the brain and in culture, and are the cells involved in formation of amyloid- β deposits. Vascular amyloidosis can be also detected in aged dogs and in human APP-transgenic mice. Hence, we compared amyloid- β deposition and response to oxidative stress in three cell culture models of cerebrovascular amyloidosis- β : smooth muscle cells isolated from leptomeningeal blood vessels from humans, dogs and APP-transgenic mice. Exposure of cells to ferrous iron (between 100 μ M to 600 μ M) for the periods of 24 to 48 hours, reduced secretion of A β into medium and enhanced intracellular A β accumulation. This process of A β accumulation continued in the absence of iron. Culture of cells embedded in extracellular matrix for up to 12 days led to deposition of granular A β -immunopositive material both intracellularly and extracellularly. This deposition of insoluble A β in the matrix could be significantly increased by oxidative modification of matrix with iron ions. Ultrastructural studies revealed aggregates of non-fibrillar material in the cells and in the extracellular matrix, consistent with formation seeds of fibrillization in culture. The data indicate that focal oxidative stress is a common mechanism that triggers intra- and extra-cellular deposition of vascular amyloid- β in AD and DS, and in animals with vascular amyloidosis- β . Supported in part with funds from the NYS/OMRDD.

A-3005

Characterizing the Cholinergic Anti-inflammatory Pathway in Skeletal Muscles. J. P. DELISLE, B. C. Geyer, and T. S. Mor. School of Life Sciences, The Biodesign Institute at Arizona State University, P. O. Box 874501 Tempe, AZ 85287. Email: josie.delisle@asu.edu

One of the ways the immune and nervous systems interact is through the cholinergic anti-inflammatory pathway. The cholinergic anti-inflammatory pathway attenuates macrophage activation by suppressing the production of pro-inflammatory cytokines such as TNF- α via vagus nerve-mediated acetylcholine (ACh) release. ACh binds to the $\alpha 7$ subunit of the macrophage nicotinic ACh receptor, with activation of this receptor resulting in JAK2-STAT3 activation and subsequent immunosuppression. However, the mechanism by which this pathway may function in other tissue types is still not known. In this study, we have shown that nicotine treatment of both mouse quadriceps muscles and cultured C2C12 skeletal muscle cells resulted in upregulated expression of the tristetraprolin (TTP) transcript. TTP is an anti-inflammatory RNA-binding protein that destabilizes mRNAs for pro-inflammatory cytokines such as TNF- α and IL-6. We further demonstrate that nicotine, an ACh-receptor agonist, suppressed the expression of the chemokines MCP-1 and KC in a dose-dependent manner in C2C12 cells. Quantitative RT-PCR on nicotine-treated C2C12 also showed suppression in MCP-1 transcript accumulation. However, further studies are needed to elucidate the molecular mechanism by which nicotine activates TTP, how TTP interacts with chemokine transcripts like MCP-1 and the specific transcription factors involved in this mechanism.

A-3006

Evaluation of EP-1, a Cell Line from *Anguilla japonica*, to Study the Life Cycle of the Microsporidian *Heterosporis anguillarum*. S. R. MONAGHAN^{1,3}, C. F. Lo², N. C. Bols¹, and L. E. J. Lee³. ¹Department of Biology, University of Waterloo, 200 University Avenue, Waterloo, Ontario CANADA N2L 3G1; ²Department of Zoology, National Taiwan University, Taipei, Taiwan 107, ROC; and ³Department of Biology, Wilfrid Laurier University, Waterloo, Ontario, CANADA. Email: llee@wlu.ca

An epithelial cell line from the Japanese eel (EP-1), persistently infected with *Heterosporis anguillarum* (Ha), is being evaluated to study the parasite's life cycle. This cell line is a convenient *in vitro* system for following the changing relationships between the host fish cells and the complex life stages of the microsporidian. Recent reports of *Heterosporis* sp. infections in fish within the Great Lakes have caused major concerns and prompted their inclusion as one of 25 Priority Invasive Species by the Great Lakes Panel on Aquatic Nuisance Species. A watch for these pathogens is in effect, however, there is no standardized test for their identification. Thus, this research evaluates EP-1 as a model system for the study of *Heterosporis*. Warm temperatures (21 to 32°C) favored EP-1 proliferation as well as of Ha, as detected by Giemsa staining. Methods to visualize and quantify all developmental stages of the parasite are being developed. In addition to Giemsa, Ha spores and sporophorocysts could be seen in fixed cultures with the fluorochrome, Calcofluor white (CF), which is known to bind chitin, and in living cultures with acridine orange (AO). After 3 days in culture, spores were seen as round or oval structures that were pink in Giemsa, white with CF, and red with AO. Sporophorocysts (SPC) were seen as light pink with Giemsa, a loci of white with CF and red with AO. Often EP-1 developed into giant cells with multiple nuclei. These giant cells were most often associated with SPCs, suggesting that either cytokinesis was impaired by SPCs or giant cells more easily contained SPCs. Chemotherapeutic treatment of EP-1 with Norfloxacin, Ofloxacin, Albendazole and Amphotericin B decreased the number of spores in a dose dependent manner but none completely cleared infection with a

range of concentrations from 1 to 15 $\mu\text{g} / \text{ml}$. Preliminary transmission electron micrographs provide some evidence of mature spores in EP-1 but will require further study and optimization for conclusive results. This *in vitro* study of *Heterosporis* could provide in depth information on their life stages and mechanisms of transmission as well as conditions that may limit or enhance their growth. The latter is especially important as warm temperatures appear to favour Ha proliferation causing concerns for fish infection in the Great Lakes due to global warming trends.

SILENT ABSTRACTS

A-3007

Functional Domain Mutations in Serum- and Glucocorticoid-Inducible Kinase Enhance Channel Activity of Human Cystic Fibrosis Transmembrane Conductance Regulator. J. D. SATO, M. C. Chapline, C. Lewarchik, J. VanderHeide, T. LaCasse, R. A. Frizzell, and B. A. Stanton. Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672. Email: dsato@mdibl.org

Serum- and glucocorticoid-inducible kinase (SGK1) regulates ENaC in frog and mammalian cells, and recent research suggests that SGK may be generally involved in regulating ion transport. SGK1 is activated by phosphorylation, and steady state levels of SGK1 are regulated by rapid transcription and protein turnover. SGK1 transcript levels are upregulated by serum, dexamethasone and environmental stresses such as hypertonicity. SGK1 is ubiquitinated by the E3 ubiquitin ligase Nedd4-2 and marked for degradation by the 26S proteasome. Six lysine residues within the N-terminal 60 amino acids of SGK1 are modified by ubiquitination. Deletion of amino acids 1-60 inhibits polyubiquitination and increases SGK1 stability. Human SGK1 was reported to stimulate CFTR-mediated Cl⁻ currents in *Xenopus* oocytes. We have confirmed that observation and further found that deletion of the C-terminal PDZ domain-interacting sequence SFL further enhanced the ability of SGK1 to stimulate CFTR activity in oocytes. In addition, a constitutively activating mutation (S422D) and the deletion of amino acids 1-60 (ΔN60) containing polyubiquitination sites also rendered SGK1 more effective than wild-type SGK1 in stimulating CFTR currents in oocytes. The stimulation of CFTR currents by S422D-SGK1 and SFL-SGK1 was also enhanced when isoproterenol was used as a $\beta 2$ -adrenergic receptor agonist. Kinase-dead K127N-SGK1 had no effect. Thus, two SGK1 mutants that were more effective than wild-type SGK1 in stimulating CFTR currents in the presence of IBMX also displayed stimulatory activity with the $\beta 2$ -adrenergic receptor agonist isoproterenol. Together these results demonstrate that mutations in known functional domains of SGK1 that render it constitutively active (S422D), unable to be polyubiquitinated (ΔN60) or unable to interact with PDZ domain-containing binding partners (ΔSFL) create novel forms of SGK1 that apparently stimulate CFTR activity more strongly than wild-type SGK1. This stimulatory activity of SGK1 is potentiated by $\beta 2$ -adrenergic receptors, which co-localize with CFTR in epithelial cell membranes where they promote cAMP-dependent activation of CFTR by protein kinase A. Our findings suggest that small molecule drugs that bind wild-type SGK1 and mimic the mutants described here could potentially be used to increase CFTR function in polarized airway epithelial cells from cystic fibrosis patients.

A-3008

Epithelial-mesenchymal Transition of Human Adult Pancreatic Ductal Cells maintained In Vitro. M. Fanjul^{1a}, V. Gmyr², G. Ratovo^{1b}, C. Sengenès³, J. Kerr-Conte², and E. Hollande^{1b}. ¹INSERM U.858, Equipes 16^(a) et 13^(b), Université Paul Sabatier, 38 rue des 36 Ponts, F-31400 Toulouse ; ²INSERM U.859, Laboratoire de Thérapie Cellulaire du Diabète, Faculté de Médecine, 1 Place de Verdun, F-59045 Lille Cedex ; and ³INSERM U.858, Equipe AVENIR1, I2MR, 1 avenue J. Poulhès, F-31432 Toulouse Cedex, FRANCE. Email : holland@ciict.fr

The identification of a pancreatic stem cell is of major importance for cell therapy of diabetes. Possible progenitor cells have been hypothesized to reside within the ductal pancreatic epithelium. In this study we have investigated gene expression, and ultrastructural modifications in human ductal cells in culture. This study was performed using the islet-depleted exocrine fraction isolated from pancreata harvested from brain dead donors. This fraction was composed of clusters of acini associated with intercalated ducts. Exocrine cells were first maintained in suspension cultures for 8 to 17 days, and then placed in tissue culture-treated flasks. Suspension culture led to the formation of spheroids, following the degeneration of the majority of acinar cells. Spheroids were composed of a large population (82%) of cells expressing both ductal CK7, CK19, CA II and pancreatic progenitor marker, PDX-1. No coexpression of vimentin was observed. These cells showed no proliferative activity. Electron microscopy analysis showed an ultrastructure of undifferentiated cells. Monolayer culture led to a population of cells largely with an epithelial morphology expressing CK7, CK19 and PDX1. A coexpression of CK7/vimentin and CK19/vimentin was observed in approximately 60% of cells. After trypsinization, the totality of cells showed a fibroblast-like morphology with a high capacity to proliferate. They expressed CK7, CK19, PDX-1 as well as vimentin and α -SMA, considered as markers of mesenchymal cells. Flow cytometry analysis for surface markers showed expression of CD13, CD29, CD44, CD49b, CD90, CD105, CD166, but not CD16, CD34 or CD45, confirming their mesenchymal phenotype. Identical expression profiles were obtained up until the 6th passage. These results taken together suggested the emergence in culture of a population of epithelial cells of ductal phenotype, capable of undergoing an epithelial-mesenchymal transition upon losing their 3D conformation. This epithelial-mesenchymal transition may be related to the dedifferentiation process of ductal cells toward the pancreatic progenitor/stem cell.

EDUCATION POSTER ABSTRACTS

EDUCATION

E-3000

Plant Tissue Culture Technique for the Secondary Classroom. C. A. HARRISON¹, M. Egnin², and S. Traore². ¹Booker T. Washington High School, Tuskegee, AL and ²Tuskegee University, Dept. of Plant Biotechnology. Email: cahar313@aol.com; megnin@tuskegee.edu

Booker T. Washington High School genetics class students had the opportunity to investigate selected aspects within the field of plant tissue culture through applicability of lettuce media for the cloning of mustard and carrot plants using lettuce as control. Due to the unavailability of media specific to carrot and mustard, lettuce media was utilized for the set experiment. The students performed in-vitro germination of plantlets from seeds on MS media. Leaf explants from 4-day old plantlets were cultured on Carolina Biological Lettuce shoot initiation media with auxin and cytokinin for 5-6 weeks. Newly regenerated plantlets were transferred to root initiation media with reduced auxin for 3-4 weeks for production of strong rooting for soil establishment. The experiment showed 100% germination of all seeds on the MS media. On the shoot initiation media, lettuce produced the greater amount of calli, with about 75% calli production of the mustard and 50% calli production of carrot. On the root initiation media, all plants rooted, however, the rooting system of the carrot was not as strong as the lettuce and mustard. Students surmised that mustard results were better than those of carrots due to the closeness of lettuce and mustard on the phylogenetic tree of species. Observations of all developmental stages showed that the lettuce media could have a broad range of application. Supported by USDA/CSREES and NASA.

HIGH SCHOOL SILENT ABSTRACT

E-3001

The Effects of Trimethiazole on the Density of Green Algae Cells. SHILPA KALLURU and GRETCHEN MILLS. Nicolet High School,

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Presently antibiotics all around the world are being dumped into bodies of water and affect marine life, such as algae. With a diminished amount of algae, the 40-50% of oxygen in the atmosphere we currently acquire from these organisms would also be reduced. This experiment was designed to study the effects of antibiotics on the density of green algae cells as measured by light absorption. The amount of the antibiotic Trimethiazole is the independent variable and the density of green algae cells is the dependent variable. The hypothesis for this experiment is that if the amount of Trimethiazole is increased, then the density of algae cells will also increase. The experimental groups consist of 0.3 grams, 0.6 grams and 1.2 grams. Before any antibiotics were added, an initial algae count was measured. This was determined in a spectrometer by the amount of light absorbed by 6 ml of algae in each of the five cuvettes. One week after the antibiotics were added and again at two weeks, this procedure was repeated. The results of this experiment revealed that Trimethiazole promoted algae growth up to a certain point. This peak was most evident after two weeks. In conclusion, as more antibiotics are dumped into bodies of water, algae cultures decrease.

PLANT POSTER ABSTRACTS

BIOFUELS

P-3000

Development and Validation of a Tobacco Transient Expression System for Expression and Characterization of Plant Produced Cellulases. KASI AZHAKANANDAM, Stacy Miles, Mary-Dell Chilton, Andrew DeBrecht, Myoung Kim, Sergio Arellano, Paul Oeller, Scott Betts, AND Simon Warner. Syngenta Biotechnology, Inc., Research Triangle Park, NC 27709. Email: kasi.azhakanandam@syngenta.com

A major obstacle to the commercial development of enzymatic conversion of biomass to ethanol is the vast quantity of cellulases required. Efforts to address this problem have focused on engineering more efficient cellulases. A complementary approach is the engineering of crop plants to express cellulases to high levels. To facilitate the development of cellulase-expressing crops, we are developing a viral transient expression system for tobacco to enable the rapid *in planta* screening of DNA vectors encoding cellulases. We have expressed a fungal cellobiohydrolase I (CBH1) gene in both stably transformed (transgenic) and transiently transformed (agroinfiltrated) tobacco leaves. Transgenic tobacco events show accumulation of active CBH1 in leaf tissue when the enzyme is targeted to the ER or vacuole. Results from the transient system with the same gene show a similar pattern of protein accumulation as seen in the transgenic plants. Analysis of extracts of agroinfiltrated leaf tissue shows the presence of CBH1 activity on both soluble and insoluble (cellulosic) substrates. The rapid expression of CBH1 in tobacco leaves will shorten considerably the time required to evaluate properties such as subcellular targeting, protein stability, and enzyme functionality. The transient system thus allows for screening of high numbers of different cellulase genes and expression vector components in less than 1 week, compared to months for transgenic events.

P-3001

Accumulation of Ricinoleic Acid in Seeds of Transgenic Soybean. SOOYOUNG PARK¹, Mark Smith², and Tom Clemente^{1,2,3}. ¹Center for Plant Science Innovation, University of Nebraska-Lincoln; ²NRC Plant Biotechnology Institute, Saskatoon; and ³Department of Agronomy & Horticulture, University of Nebraska-Lincoln, Lincoln, NE. 68588. Email: pminky@msn.com

Castor bean (*Ricinus communis*) oil is highly versatile industrial oil used in an array of applications including plastics, foams, surfactants and biofuel. However, supply of castor oil is limited due to a number of

negative characteristics, including presence of the highly toxic molecule, ricin, a by-product of castor bean meal, which limits its potential for domestic production. An avenue to circumvent the need for domestic production of castor bean is to produce the ricinoleic acid, which composes approximately 90% of the fatty acid profile of castor oil, in heterologous system, such as soybean. To this end, we introduced into soybean the castor oleate 12 hydroxylase gene under the control of the seed-specific phaseolin promoter. Fatty acid analysis of seeds carrying this transgene either displayed a mid-oleic acid phenotype (30% to 70% 18:1) without production of ricinoleic acid, or an elevated oleic acid phenotype (25% to 30% 18:1) with a concomitant production of ricinoleic acid ranging from trace levels up to 12%. In 2007, a field trial was conducted with one of the ricinoleic acid producing event designated 521-24. Yield estimates from the harvest under dryland conditions were 45bu/ac, with estimates of total protein and oil of 45.5% and 20%, respectively, based on NIR analysis. Ricinoleic acid levels from the harvest averaged 12.1%.

BIOTECHNOLOGY

P-3002

What is the Optimal Size of Elastin-like Polypeptide Fusions for the Enhanced Accumulation of Recombinant Proteins in Plants and their Subsequent Purification? ANDREW J. CONLEY¹, Jussi J. Joensuu², Anthony M. Jevnikar³, and Jim E. Brandle². ¹Department of Biology, University of Western Ontario, London, ON N6A5B7, CANADA; ²Southern Crop Protection and Food Research Center, Agriculture and AgriFood Canada, London, ON N5V4T3, CANADA; and ³Transplantation Immunology Group, Lawson Health Research Institute, London, ON N6A4V2, CANADA. Email: conleya@agr.gc.ca

The demand for recombinant proteins for medical and industrial use is expanding rapidly and plants are now recognized as an efficient, inexpensive means of production. Although the accumulation of recombinant proteins in transgenic plants can be low, we have previously demonstrated that fusions with an elastin-like polypeptide (ELP) tag can significantly enhance the accumulation of a range of different recombinant proteins in plant leaves. ELPs are biopolymers with a repeating pentapeptide sequence (VGVPG)_n that undergo a thermally-triggered inverse reversible phase transition, thus allowing them to be selectively purified from cellular extracts. The objective of this research is to determine the optimal ELP size for the enhanced accumulation of recombinant proteins in plants and their subsequent purification. ELP tags of various sizes were constructed and fused to erythropoietin (EPO), interleukin-10 (IL-10), green fluorescent protein (GFP) and a single chain variable fragment (scFv) and then transiently expressed into tobacco leaves. The data suggests that smaller ELP tags result in higher accumulation levels of their respective fusion partners while inverse-transition cycling (ITC) purification works better with larger ELP tags. ELP fusions with 30 pentapeptide repeats seem to provide the best compromise between the positive effects of small ELPs on recombinant protein accumulation when balanced against the beneficial effects of larger ELPs on recombinant protein recovery during ITC purification. Finally, the effect of ELP size on EPO's biological activity will be evaluated. This work will help elucidate ELP's mechanism of action, so that it can be further optimized for the enhanced accumulation and purification of recombinant proteins in plants.

P-3003

Excellence Through StewardshipSM. Advancing Best Practices in Agricultural Biotechnology (ETS), a New Program of The Biotechnology Industry Organization (BIO). THOMAS CURRIER¹, Sharon Bomer² and Michael Wach². ¹Bayer CropScience, Research Triangle Park, NC 27709 and ²Food and Agriculture Department, Biotechnology Industry Organization, Washington, DC 20024. Email: mwach@bio.org

Product stewardship is the responsible management of a product from inception through discontinuation. Ensuring quality control and responsible management is central to developing and commercializing new biotech crop varieties so that seeds sold to farmers will produce crops with the desired characteristics, yield, and harvest quality. Plant product integrity is essential if agricultural biotechnology is to provide more and better food, feed, and fiber to consumer markets. The Biotechnology Industry Organization (BIO) has launched a new program, *Excellence Through StewardshipSM. Advancing Best Practices in Agricultural Biotechnology* (ETS), the first industry-coordinated effort to address product stewardship and quality management. ETS continues BIO's commitment to enhancing regulatory compliance and product quality for consumers. The program provides guidance for quality management systems for the full life cycle of biotech plants, from gene discovery to product discontinuation. It is intended to promote the responsible management of agricultural biotechnology, the continued adoption of plant biotechnology globally, and the enhanced value of biotech-derived plant products in the marketplace. The program's three main components include: -Adoption of product stewardship systems and quality management Principles and Management Practices to maintain plant product integrity. -Publication of a Quality Management Program Guide for those involved in agricultural biotechnology research and development to use in understanding and implementing best practices. -Independent, third-party Audits designed to verify implementation of stewardship programs and confirm quality management systems.

P-3004

RNAi Suppression of 4-coumarate-CoA ligase to Improve Forage Quality of Bahiagrass. W. M. FOUAD, L. Martin, and F. Altpeter. University of Florida – IFAS, Agronomy Department, Plant Molecular and Cellular Biology, Laboratory of Plant Molecular Physiology, 2191 McCarty Hall, Gainesville, FL 32611. Email: faltpeter@ifas.ufl.edu

Bahiagrass is one of the most important warm season forage grasses. In Florida alone it is grown on more than 5 million acres most of these acres support the beef cattle industry. However, the high lignin content in the bahiagrass biomass significantly reduces its forage quality. A key enzyme in the lignin biosynthetic pathway is the 4-coumarate-CoA ligase (4CL). Reduction of 4CL and other lignin biosynthetic enzymes in various plant species resulted in reduced lignin content that was accompanied with increased digestibility of forage biomass. We cloned four 4CL cDNAs from tetraploid bahiagrass cv. 'Argentine'. An RNAi construct targeting a highly conserved domain in two out of the four genes was constructed using 200 bp of the coding sequences. The 4CL-RNAi construct under the constitutive ϵ 35S promoter or under xylem specific promoter was introduced to bahiagrass callus by biolistic gene transfer. Following regeneration of plants their transgenic nature was confirmed using PCR. Significant reduction of 4CL gene expression was detected in several transgenic lines by Northern blot analysis. Quantitative RT-PCR is currently used to precisely quantify the level of 4CL suppression. Data correlating the 4CL gene expression level with the lignin content will be presented.

P-3005

Accumulation Pattern of Methionine Rich β -Zein Protein in *Medicago sativa* (Alfalfa) and the Related Model Legume *M. truncatula* in Relation to Their Free Methionine Pools. F. OMAR HOLGUIN, Suman Bagga, and Champa Sengupta-Gopalan. Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003. Email: csgopala@nmsu.edu

Alfalfa (*Medicago sativa*) is an important forage legume providing quality protein and is low in methionine (Met), an important amino acid. A genetic engineering approach to increase the Met content of alfalfa is to express genes encoding for high Met protein. Seed storage proteins of corn, the β -(15kD) and δ -(10 and 18kD) zeins, are very high in Met and are ideal candidates for introducing into alfalfa. The β -

zein gene engineered behind the CaMV 35S promoter was introduced in alfalfa and *Medicago truncatula*, a model legume. Our analysis of these transformants shows a ~10 fold higher level of accumulation of β -zein protein and transcript in the leaves of *M. truncatula* plants when compared to alfalfa β -zein expressors. Our hypothesis is that the two *Medicago* species differ with regards to the amino acid composition and in the rate of synthesis of Met rich proteins. A metabolomic approach was used to create a model on the effects of methionine synthesis and accumulation in legumes. Comparative metabolic profiling of soluble primary and secondary polar metabolites in *M. truncatula* and alfalfa was carried out by GC-MS, UPLC and LC-MS. These analyses revealed a number of metabolic characteristics which are discretely associated with the fate of Met between species. Particular interest was focused on those metabolites associated with methionine biosynthetic pathway. These differences reveal that improving the Met content in a forage legume will require increased Met levels at the cost of decreased levels of SAM to allow for the availability of Met for protein synthesis in *M. sativa*. An understanding of the basis for the differences between *M. sativa* and the model legume, *M. truncatula*, with regards to the accumulation of the Met-rich β -zein protein will allow us to increase the Met-containing proteins in *M. sativa* using genetic engineering approaches.

P-3006

Genetic Markers for Cultivar Identification in Potato in Relation to Insect Tolerance as Revealed by RAPD Analysis. SANAA A. M. IBRAHIM¹, Reda E. A. Moghaieb², and K. Fujita³. ¹Plant Protection Institute, Agriculture Research Center, Giza, EGYPT; ²Department of Genetics, Cairo University, EGYPT; and ³Graduate School of Biosphere Sciences, Hiroshima University, JAPAN. Email: sanareda@yahoo.com

In Egypt losses of agricultural products by mole cricket are abundant. However the genetic information on tolerance to mole cricket is limited, therefore in the present study the yield of four potato cultivars (Alfa, Scort, Desrea and Diamond) were compared under natural mole cricket infestation. The tuber yield decreased by this insect infestation, and the reduction differed significantly among cultivars. The cultivar Alfa proved to be the one of choice under the soil that has infection with mole cricket. The infestation of potato by mole cricket reduced the carbohydrate concentration in the infected compared to the non-infected tubers. For RAPD analysis, seven arbitrary 10-mer primers were used. A total of 62 scoreable bands was detected of which 30 bands were polymorphic (48.4 %). Based on the data obtained by RAPD analysis, it was possible to discriminate between the four potato genotypes used in the present study. The genotype-specific marker indicates that six markers distinguish the cultivar Alfa (OPB-09-800, OPB-09-650, OPD-04-1970, OPD-04-475, OPD-04-317, OPE-13-890), three markers distinguish Diamond (OPA-06-1250, OPD-04-1000, OPD-04-600) one marker for Scort (OPD-04-580) and one marker for Desrea (OPA-15-2190). The cultivar-specific markers represented 17.7% of the total markers and 36.6 % of the polymorphic markers. These markers can help in marker-assisted selection breeding programs for improving mole cricket tolerance in potato plants.

P-3007

Plant Recombinant Antibodies for the Prevention of Foot-and-Mouth Disease Virus. J. J. JOENSUU¹, K. Brown¹, A. J. Conley¹, A. Clavijo², R. Menassa¹ and J. E. Brandle¹. ¹Agriculture and Agri-Food Canada, London, ON, CANADA, N5V 4T3 and ²Canadian Food Inspection Agency, Winnipeg, MB, CANADA R3E 3M4. Email: joensuu@agr.gc.ca

Foot-and-mouth disease (FMD) is the most significant constraint to international trade in live animals and animal products. While current whole-virus-based vaccines are protective against FMD, there are several limitations restricting their usefulness as prophylactic treatment or to control outbreaks: (1) current measures to tell infected from vaccinated animals are inadequate, (2) vaccinated animals can develop sub-clinical

carrier state during outbreaks, and (3) vaccination cannot always protect susceptible animals fast enough. As a consequence most Western countries do not vaccinate against FMD and outbreaks are controlled by mass culling of all infected and suspected animals. Passive immunization, using a mixture of low-cost recombinant antibodies, is an attractive alternative to protect susceptible animals in infection zones. Our research has focused on developing a cost-effective and scalable production system of recombinant anti-FMDV antibodies in plants. Virus-neutralizing monoclonal antibodies specific for the three major FMDV serotypes were developed. The genes corresponding to antibody variable regions were identified and cloned to generate recombinant single chain antibody fragments (scFvs). Vector design was optimized to facilitate high accumulation of scFvs in our biosafe low-alkaloid tobacco platform. Novel purification methods for recombinant scFvs were established and plant-made scFvs were shown to bind FMDV in vitro. Virus neutralization and small animal trials will be carried out in the near future to demonstrate passive immunization.

P-3008

Promoter Screening Using *Pteris vittata* Protoplasts. B. L. JOYCE and C. N. Stewart. Department of Plant Sciences, The University of Tennessee, Knoxville, TN 37996. Email: bjoyce3@utk.edu

Ferns are used for many different reasons including floriculture, horticulture, medicine, and phytoremediation. Specifically of interest for phytoremediation is the Chinese brake fern, *Pteris vittata*. Hosts of monocot and dicot crop plants have been studied and stably transformed to improve their agronomic traits, but to date there have been no reports of stable fern transformation. To improve *Pteris vittata* using transgenic approaches: 1) a tissue culture must be established, 2) vectors containing a promoter driving the gene of interest must be constructed, 3) the cloned vectors must be inserted into fern tissue that can be recovered. Recently, a tissue culture system has been developed for *Pteris vittata*, but transformation with biolistic bombardment and *Agrobacterium tumefaciens* has not been successful. To address this issue, a protoplast protocol was optimized to extract protoplasts from calli, gametophytes, and sporophytes of *Pteris vittata*. Protoplasts were PEG transfected with several well-known monocot and dicot promoters, as well as a the ribulose-1,5-bisphosphate carboxylase/oxygenase, RUBISCO, promoter from *Pteris vittata*. Due to high levels of autofluorescence, promoter strength was compared using the MUG fluorometric assay to detect beta-glucuronidase, GUS, activity.

P-3009

Complex Patterns of DNA Integration via Ectopic Recombination. C. MELAMED-BESSUDO, M. Liebermann-Lazarovich, and A. A. Levy. The Weizmann Institute of Science, Plant Sciences Department, Rehovot 76100, ISRAEL. Email: cathy.bessudo@weizmann.ac.il

In the past we have reported on a new assay of gene targeting based on the activation of the GFP reporter in seeds of Arabidopsis upon homologous integration between a T-DNA vector and the homologous Cruciferin endogenous locus. Here, we report on a similar assay, but using mRFP, that gives stronger fluorescence signals. Using this system we found complex patterns of DNA integration that involve homologous recombination between the vector and the target on one side, and non-homologous integration at the other end of the vector. Using Southern blot analysis, we found that the target gene was unchanged. However, the vector had invaded the homologous target and synthesized a stretch of DNA of at least 10Kb upstream to the region of homology. On the other side of the integration, we couldn't find sequence homology to the target and moreover the T-DNA right border was integrated at this side. At present we are mapping 8 different such complex integration events. Preliminary sequencing of inverse PCR products, together with the Southern blot analysis suggest a model whereby there is a one sided homologous invasion, followed by end elongation of the invading vector and copying of long stretches of DNA. The newly synthesized DNA, rather than being assimilated into

the target, becomes dissociated and integrates at an ectopic site, thus generating duplications of large DNA segments.

P-3010

Endoplasmic Reticulum Expression of Green Florescent Protein (GFP) in Octaploid Strawberry. HESHAM ORABY and James Hancock. Plant Breeding and Genetics Program, Michigan State University, East Lansing, MI 48824. Email: orabyhes@msu.edu

The cultivated strawberry (*Fragaria × ananassa*) is one of the most valuable fruit crops. It is grown in most arable regions of the world. Cultivated strawberry is octoploid (2n=8x=56). This complex genetic background is problematic in the development of genetic markers, functional genomics and transformation. However, transformation of octoploid strawberry has been reported and various protocols have been established. Yet, the transformation efficiency and stability varied among cultivars. In this study, an efficient shoot regeneration protocol, developed by Folta et al (2006), involving organogenesis from leaf and petiole tissues of octoploid strawberry (genotype LF9) was used. Two green fluorescent protein (GFP) constructs (endoplasmic reticulum targeted GFP and cytoplasmic non-targeted GFP), under the control of the constitutive CaMV 35S promoter, were delivered via *Agrobacterium*-mediated transformation system to study expression and stability of GFP in transgenic strawberry plants. Several transgenic events were recovered from both constructs. The results reported low regeneration efficiency from cytoplasmic non-targeted GFP with reduced bright fluorescent intensity compared with endoplasmic reticulum targeted GFP at very early leaf stage. As the leaf grows, the cytoplasmic non-targeted GFP showed lesser stable expression. The fluorescence level of the GFP in the endoplasmic reticulum was nearly stable by leaf growth. This can be due to the post-translational maturation of GFP, or less mild phototoxic effects of GFP which are seen with high levels of expression. Molecular characterization of the transgenic events is ongoing. It could be concluded that targeting protein to the endoplasmic reticulum may result more stable gene expression, improved transformation efficiency and recovery of transgenic plants.

P-3011

Development of Soybean Oil Low in Palmitic Acid and Elevated in Stearic and Oleic Acids. HYUNWOO PARK^{1,2}, George Graef¹, Mike Fromm^{1,2,3}, and Tom Clemente^{1,2,3}. ¹Department of Agronomy & Horticulture, University of Nebraska-Lincoln; ²Center for Plant Science Innovation, University of Nebraska-Lincoln; and ³Center for Biotechnology, University of Nebraska-Lincoln, 68588. Email: dnahyun@bigred.unl.edu

Most commodity soybean oil has a fatty acid profile of app. 10% palmitic acid, 4% stearic acid, 17% oleic acid, 55% linoleic acid, and 10% linolenic acid. Oil high in oleic acid, and elevated in stearic acid, with a concomitant reduction in palmitic acid and polyunsaturated fatty acids offers improved functionality for both food and industrial applications. To produce soybean oil with such a fatty acid profile we stacked a transgene that carries a silencing element designed to simultaneously down-regulate in a seed-specific fashion a palmitoyl thioesterase (FatB) gene and a delta 12 desaturase gene, with a stearyl ACP thioesterase seed-specific transgenic cassette, via sexual crossing. The parent that carries the silencing transgene was derived from a soybean event designated 335-13 which produces an oil high in oleic acid (>85%) and low in palmitic acid (<4%). This event, 335-13, was crossed to soybean plants that inherited the stearyl ACP thioesterase gene from a set of transgenic events that displayed stearic acid levels ranging from 8% up to 15%. F₁ seed from these crosses have generated fatty acid profiles with stearic acid ranging from 5% to 12%, and oleic acid levels ranging from 65% to 79%.

P-3012

Production of High Omega-3 Fatty Acid Soybean Oil as a Feedstock for Aquaculture. FAREHA RAZVI¹, Pat Tenopir^{1,2}, Robert Weber³, Steve

Weirer⁴, Tim O'Keefe⁵, Michael Cremer⁵, Bridgett Owen⁵, George Graef², Jennica Lowell⁶, Neil Sims⁵, and Tom Clemente^{1,2,7}. ¹Center for Plant Science Innovation, University of Nebraska-Lincoln; ²Department of Agronomy & Horticulture, University of Nebraska-Lincoln; ³Industrial Agricultural Products Center, University of Nebraska-Lincoln; ⁴Department Food Science and Technology, University of Nebraska-Lincoln; ⁵US Soybean Export Council; ⁶Kona-Blue Water Farms; and ⁷Center for Biotechnology, University of Nebraska-Lincoln 68588. Email: frazvi2@unlnotes.unl.edu

The identification and development of sustainable sources of protein and lipid will be required to maintain a plentiful and cost-effective supply of feed for the rapidly expanding aquaculture industry. Currently aquaculture feed relies upon both fishmeal and fish oil to meet the dietary needs of farm raised fish. As a means to address replacement of fish oil as a lipid source for the aquaculture industry, we report here on the development of novel soybeans that have potential to serve as a sustainable lipid source for farm raised fish. In soybean accumulation of high levels of stearidonic acid, a relatively rare omega-3 fatty acid, can be accomplished by co-expression of a delta 6 and delta 15 desaturase genes. To examine the capacity of a stearidonic acid producing soybean to synthesize the very long polyunsaturated omega-3 fatty acid eicosapentaenoic acid (EPA), a fatty acid elongase gene from zebra fish and a mammalian delta 5 desaturase gene were pyramided with the other desaturase genes. Soybean seed carrying this four gene stack displayed EPA accumulation up to 8% of the seed storage lipids. Studies are currently ongoing to evaluate the agronomics of this soybean germplasm and utility of both the stearidonic acid and EPA soybean oil as a dietary source of lipids for aquaculture.

P-3013

Constitutive Overexpression of Maize Sucrose Phosphate Synthetase (SPS) in *Medicago Sativa* (alfalfa): Effects on Nitrogen Fixation and Assimilation in the Nodules. MARK SEGER¹, Jose Luis Ortega² and Champa Sengupta-Gopalan^{1,2}. ¹Graduate Program in Molecular Biology and ²Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003. Email: csgopala@nmsu.edu

Nitrogen assimilation requires the availability of C skeletons on which to load the nitrogen. Attempts to improve plant performance by introducing a soybean cytosolic glutamine synthetase (GS_c) gene in alfalfa have not been successful. GS is regulated at multiple steps and the key to the regulation is the C/N status. To impact the C/N status, we have attempted to check how increasing the sucrose levels would affect GS activity. Sucrose-phosphate synthase (SPS), a key enzyme controlling the flux of carbon into sucrose biosynthesis, converts UDP-Glucose and Fructose 6P to Sucrose 6P, which is subsequently hydrolyzed by Sucrose phosphate phosphatase (SPP) to release sucrose. Sucrose produced in photosynthetic tissues is the main carbohydrate transported into the heterotrophic tissues including the root nodule, where sucrose is metabolized initially by sucrose synthase (SuSy). Since the nodule is the site of nitrogen fixation and assimilation, one of our objectives is to determine how the constitutive increase of SPS will affect the interactions between the C and N metabolic pathways in both the leaves and the nodules. We have transformed alfalfa with the maize SPS gene driven by the CaMV35S promoter. We will present data on SPS activity and the effect of modulating SPS activity on N fixation and assimilation in the nodules.

P-3014

Expression of a *De Novo* Methionine-Rich Protein MB16 in Transgenic Soybeans. Y. ZHANG¹, M. A. Hefford², and D. H. Simmonds¹. ¹Agriculture and Agri-Food Canada, Eastern Cereals and Oilseed Research Centre, Ottawa, ON K1A 0C6, CANADA and ²Centre for Biologics Research, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, ON K1A 0L2, CANADA. Email: simmondsdh@agr.gc.ca

To improve soybean seed nutritional quality, a synthetic gene, MB16 was introduced into the soybean genome to boost seed methionine content. MB1 is an 11 kDa *de novo* protein enriched in the essential amino acids methionine, threonine, lysine and leucine, which was first designed to express in rumen bacteria [M. Beauregard, Mary Hefford et al, *Biotechnology*, 13, 974-981]. A plant expression cassette, MB1 gene driven by a constitutive promoter CaMV 35S was first integrated into the soybean genome. MB1 protein was expressed in transgenic soybean leaves but was undetectable in seeds on western blots. For efficient seed expression, constructs were designed using the soybean codon bias, with and without the KDEL ER target sequence, and -conglycinin or cruciferin seed specific promoters to drive MB16 (modified MB1 gene with improved protein digestibility). Homozygous lines, with single locus integration, were identified for several transgenic events. Southern and western analyses show the same transgene integration patterns and MB16 protein expression through to the T5 generation. Quantitative RT-PCR, used to analyse gene expression during seed development, showed that the transcript reached a peak level in growing seed (size 5-6 mm), remained at peak level to the full-sized green seed and was significantly reduced in maturing yellow seed. Transformed events carrying constructs with the rumen bacteria codon preference showed the same transcription pattern as those with the soybean codon preference, but the transcription levels were lower at each seed development stage. The strongest western blot signals were detected in full-sized green seed while a weak signal occurred in yellow and dry seed in transgenic lines carrying constructs without the KDEL sequence.

CELLULAR IMMUNOLOGY

P-3015

Anti-inflammatory and Antioxidant Activities of *C. vulgare* L. D. R. BURK, A. R. Pah, L. C. Lopez, and S. M. Daskalova. The Biodesign Institute, Arizona State University, Tempe, AZ 85287. Email: drburk@asu.edu

Aqueous extract of *Clinopodium vulgare* L., a widely distributed medicinal plant belonging to family Lamiaceae, was tested for antiinflammatory activity using lipopolysaccharide (LPS)-stimulated murine macrophages as a model system. RAW 264.7 cells exposed to a 24 h treatment with 1 ug/ml LPS in the presence of a range of extract concentrations (125– 1500 ug/ml) exhibited dose-dependent reduced levels of LPS-induced nitric oxide (NO) production as evidenced by the measured nitrite concentrations in the medium using Griess reagent system. Consistent with this observation, the extract inhibited protein expression levels of the inducible nitric oxide synthase (iNOS) in a dose-dependent manner without affecting cell viability. The levels of IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-10, GM-CSF, IFN-gamma and TNF-alpha monitored by Bio-Plex suspension array system were not significantly altered. The extract showed only a slight inhibitory effect on IL-1beta and IL-10 synthesis at the highest concentrations tested. However, it demonstrated significant radical scavenging efficiency evidenced by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and a strong inhibitory effect on xanthine oxidase (EC 1.1.3.22) activity. Oxidative stress plays a prominent role in the pathogenesis of many diseases including rheumatoid arthritis, atherosclerosis, diabetes and cancer, and in this context *C. vulgare* L. might become a good source for isolation and identification of potent natural antioxidants.

DICOT TRANSFORMATION

P-3016

Development of NUE Test System in Arabidopsis. MICHELLE MEDINA, G. Theodoris, C. Manning, G. Fazio, P. Gallawa, N. Omelu, A. Palamo, J. van Bostel, Z. Lu, and J. Kridl. Arcadia Biosciences, 202 Cousteau Place, Suite 200, Davis, CA, 95618. Email: michelle.medina@arcadiabio.com

Use of nitrogen fertilizer has been necessary for sustaining high agricultural yields required to feed an increasing world population. However, overuse of nitrogen fertilizer has damaging environmental consequences and is costly to farmers. Increasing nitrogen efficiency (NUE) in plants reduces the amount of nitrogen fertilizer lost by farmers due to volatilization into the air and leaching into soil and waterways. We have successfully developed canola plants (*Brassica napus*) with increased nitrogen use efficiency using an alanine aminotransferase (AlaAT) gene. Greenhouse screens show increase in biomass and plants in the field show yield amounts equivalent to controls, with as much as 50-60% less nitrogen fertilizer applied. In order to screen more genes for NUE phenotypes we are developing a system for screening biomass differences on sub-optimal nitrogen concentrations in Arabidopsis. The system is being used to test AlaAT as well as other potential NUE genes.

EDIBLE VACCINES

P-3017

Cloning, Characterization and Expression of Synthetic Tumor Reducing Peptide Gene in Sweetpotato for Use as Therapeutic Drugs Against Cancer. SY TRAORE, Marceline Egnin, Frieda Sanders, Edith Powell, and Jesse Jaynes. George Washington Carver Agricultural Experiment Station, Tuskegee University, Milbank Hall, Lab 104, Tuskegee, AL 36088. Email: megnin@tuskegee.edu; sytraore@gmail.com

The CDC estimates that every year, over 10 million people develop cancer with 7.6 million cancer-related deaths (13% of all deaths). Despite the extensive application of established cancer therapies and the new wave of biotherapies, deaths from cancer are projected to continue to rise to an estimated 9 million people in 2015. In many developed countries, cancer is the main cause of death among 15 - 64 year-olds. The need to enlarge the therapeutic arsenal against cancer and to increase the production of existing and underdeveloped cancer drugs, as well as to decrease production costs, are driving forces for the continuous improvement of productive platforms for recombinant cancer drugs. Expression of pharmaceuticals in transgenic plants is molecularly and posttranscriptionally a convenient and inexpensive source for these therapeutic molecules. Thus, a series of novel synthetic tumor reducing peptides able to suppress abnormal cell growth in many type of cancers has been recently developed at Tuskegee University (Dr Jesse Jaynes). Plant-optimized synthetic genes encoding these peptides (*JC* series) have been *de novo* designed and constructed. This study focuses on the cloning of these peptide genes and their expression in Simon-1 (a sweetpotato medicinal cultivar) as an inexpensive alternative to current systems of production and delivery of cancer drugs. The genes were driven by a double 35S promoter with a translational enhancer at the 5' and *nos* terminator at the 3' end and cloned into the right border of the binary plasmid pGPTV-Kan carrying a *nptII* gene coding for kanamycin selectable marker at the left border of the T-DNA and each mobilized in *E. coli*. Positive colonies were selected on SOC containing antibiotics. Recombinant plasmid pGPTV-*JC-15N* and pGPTV-*JC-15ND* series were confirmed through restriction digest and agarose gel electrophoresis analyses. Each confirmed recombinant plasmids was mobilized in disarmed *Agrobacterium tumefaciens* strains EHA101 and EHA105, according to the "Freeze and Thaw" method using CaCl₂. Transformed colonies were selected on Yep plate containing antibiotics. *Agrobacterium* strains carrying the recombinant plasmids were utilized in sweetpotato cultivar Simon-1 transformation. Research supported by NIH, GWCAES, and USDA.

EMBRYOGENESIS/REGENERATION/

MICROPROPAGATION

P-3018

Optimization of Somatic Embryogenesis of Selected African Cassava Cultivars for Transgenic Technologies. MALA P. JAYATILLEKE, Claude M. Fauquet, and Nigel J. Taylor. Donald Danforth Plant Science Center, 975, North Warson Rd. St. Louis MO 63132. Email: mjayatilleke@danforthcenter.org

Optimization of the production of somatic embryos from African cultivars is an important prerequisite for an improved transformation protocol for cassava (*Manihot esculenta* Crantz). Embryogenic tissues developed from in vitro leaf explants are being utilized for the production of friable embryogenic callus which is the target tissue for transgene insertion and transgenic plant production. Wounding of leaf explants subsequently cultured on Murashige and Skoog semi-solid basal medium supplemented with 50 μ M picloram was found to increase production of somatic embryos in all tested cultivars (cv. 60444 71% to 87%, Bukalasa 79% to 88%, Aladu 71% to 89%, Serere 81 to 92%, Ebwanatereka 79 to 88% and Tereka 73 to 85%). When cultured in liquid of the same type explants showed even higher somatic embryo production for Serere (98%), Tereka (93%), Ebwanatereka (90%) and Aladu (85%). Smaller sized leaf lobes (<2mm) gave 100% embryo production for all the cultivars. However, Selection of leaf explants (1mm-10mm) and wounding of leaf lobes (4mm to 10mm) are now being successfully utilized to produce high quality somatic embryos of African cassava cultivars.

P-3019

In Vitro Plant Regeneration of Pecan [*Carya illinoensis* (Wangenh) K. Koch]. N. N. RENUKDAS, M. Manoharan, and J. O. Garner. Department of Agriculture, University of Arkansas at Pine Bluff, Pine Bluff, AR 71601. Email: renukdas@uapb.edu

The pecan, *Carya illinoensis* [(Wangenh) K. Koch] is a member of the family Juglandaceae and is an economically important nut crop. It is a hardwood tree that produces an edible nut with high commercial value. Propagation of pecan is done primarily by budding or grafting of improved cultivars onto seedling rootstocks. However, these methods suffer disadvantages such as considerable time and expense and poor transplanting survival of the plants. Germination of pecan seed, though genetic variability is concern, is an other option, and commonly done by stratification or kept in cold storage at high humidity through the winter and planted in the spring of the year. Tissue culture techniques offer great potential for regenerating pecan plants *in vitro*. Embryos of the varieties, Desirable, Stuart, Sumner, Cape Fear, Kiowa, Farley, Forkert, and Success were isolated and cultured on MS media containing different combinations of BAP (0.44 to 18 μ M) and IBA (5 μ M) for root and shoot induction. Pecan plants were efficiently regenerated on MS basal medium with 18 μ M BAP and 5 μ M IBA. Multiple shoots were obtained in varieties Kiowa and Farley. After the induction of shoot and tap root, the pecan plants were subcultured to hormone free MS medium for further elongation. Fully developed pecan plants were transferred first to peat pellets and then to greenhouse. This regeneration method is simple, rapid and may be used as an alternate for conventional seed germination.

GENE TRANSFER TO PLANTS

P-3021

High-efficiency *Agrobacterium*-mediated Transformation of *Nicotiana africana* Merxm. SERGEI KRASNYANSKI and George Allen. North Carolina State University, Plant Transformation Lab, Campus Box 7550, Partners II Building, Room 1200, Raleigh, NC 27695-7550. Email: sfkrasny@ncsu.edu

Nicotiana africana Merxm., a tobacco species discovered relatively recently in Africa, can be used as a source of nuclear-encoded resistance to potato Y-virus, and for production of plants with cytoplasmic male sterility following crosses with *Nicotiana tabacum*. Unfortunately data on the efficiency for regeneration and transformation of *N. africana* are very scarce. To our knowledge, there are only two reports showing plant regeneration and only one report on genetic transformation of *N. africana* (Rakosy-Tican and Menczel, 1998; Belorukova et al., 2004; Shcherbak et al., 2004). That single publication on transformation unfortunately does not provide any conclusive data on transformation efficiency and indicates that a very high percentage of putative transformants were actually escapes. We have developed an optimized

transformation and regeneration protocol for *N. africana*. A soluble modified red-shifted GFP (rsGFP) reporter transgene was used to transform *N. africana* leaf explants using *Agrobacterium* strain GV3101. Based upon the number of transformed plants per total number of explants used in experiments, 100% transformation efficiency has been achieved. Escapes have been practically eliminated by decreasing the size of leaf explants from commonly used 5 x 5 mm to 1-2 mm wide and 4-5 mm long.

P-3022

Expression of the Rice OsCDPK-7 in Sorghum Produces Local Lesion Formation in Leaves. TEJINDER KUMAR MALL¹, Ismail Dweikat¹, Natalya Neresian², Kaimei Xu², and Tom Clemente^{1,2,3}. ¹Department of Agronomy & Horticulture, University of Nebraska-Lincoln, 68583; ²Center for Biotechnology, University of Nebraska-Lincoln, 68588; and ³Center for Plant Science Innovation, University of Nebraska-Lincoln, 68588. Email: tejinderkumar@rediffmail.com

Sorghum is a crop of tropical origin and thus is very sensitive to low temperature. In an attempt to augment cold tolerance in sorghum, we introduced the rice Ca-dependent protein kinase 7 (OsCDPK7), which has been shown to impart cold tolerance to rice when constitutively ectopically expressed. We assembled two binary vectors that carried expression cassettes under the control of maize ubiquitin promoter, designated pPTN751 and pPTN780. The former carries the wild type OsCDPK7, while the later harbors an OsCDPK7 gene with series of mutations that abolishes kinase activity. A minimum of 8 independent transgenic sorghum events were derived via *Agrobacterium*-mediated transformation from each binary vector. In the majority of the events derived from the pPTN751 transformations, progenies displayed local lesions formation in leaves. This phenotype segregated with the transgene, while the transgenic progenies derived from the pPTN780 transformants grew normally. We are currently designing a set of studies to gain insight in the cellular differences between sorghum expressing the functional and non-functional versions of the OsCDPK7. The data generated from this study indicates that constitutive modulation of the CDPK signal transduction pathway in sorghum can trigger a localized cell death response.

P-3023

Evaluation of a Herbicide Resistant Trait Conferred by the Bar Gene Driven by Four Distinct Promoters in Transgenic Blueberry Plants. GUO-QING SONG, Kenneth C. Sink, Peter W. Callow, Rebecca Baughan, and James F. Hancock. Plant Biotechnology Resource and Outreach Center, Department of Horticulture, Michigan State University, East Lansing, MI 48824. Email: songg@msu.edu

Four chimeric bialaphos resistance (*bar*) genes driven by different promoters were evaluated for production of herbicide-resistant blueberry plants cv. Legacy (73.4% *Vaccinium corymbosum* and 25% *Vaccinium darrowi*) through *Agrobacterium tumefaciens*-mediated transformation. When the *bars* were used as selectable marker genes, different promoters yielded different transformation frequencies. Three chimeric *bar* genes with the promoter nopaline synthase (*nos*), cauliflower mosaic virus (CaMV) 35S, or CaMV 34S, yielded transgenic plants; whereas, a synthetic (Aocs)₃AmasPmas promoter did not lead to successful regeneration of transgenic plants. In addition, herbicide resistance in *bar* expressing plants was influenced by the promoter strength. Under controlled environmental conditions, three month-old plants from six single-copy transgenic events with either 35S::*bar* or nos::*bar*, as well as those non-transgenic plants, were sprayed with herbicide glufosinate ammonium (GS) at five levels (0, 750, 1500, 3000 and 6000 mg·L⁻¹). Evaluations on leaf damage two weeks after spraying indicated that all transgenic plants exhibited much higher herbicide resistance than non-transgenic plants. Additionally, the transgenic plants with the 35S::*bar* showed a higher herbicide resistance than those with the nos::*bar*. After application of 6000 mg·L⁻¹ GS, over 90% of the leaves from plants with the 35S::*bar* and 19.5%-51.5% of the leaves

from plants with the *nos::bar* showed no symptom of herbicide damage; whereas, only 5% of leaves from the non-transgenic had no damage. One year-old, field-grown plants from four transgenic events with the *nos::bar* were evaluated for herbicide resistance after spraying with 750 mg·L⁻¹ GS. Transgenic plants survived with variations in the level of foliar damage; in contrast, all non-transgenic plants died. This study is the first investigation of different promoters for engineering transgenic blueberry plants.

GENOMES/GENOMICS/BIOINFORMATICS

P-3024

Fe Bioavailability in Staple Crops of Developing Countries. MAGNOLIA ARIZA-NIETO¹, Ross M. Welch², and Raymond P. Glahn². ¹Food Science, Cornell University, Ithaca NY 14853 and ²Robert W. Holley Center for Agriculture and Health, USDA-ARS, Ithaca NY 148533. Email: magnolia_ariza_nieto@hotmail.com

The development of staple food crops enriched in bioavailable micronutrients (iron, zinc & provitamine A carotenoids) could help reduce micronutrient malnutrition among the poor in the developing world in a sustainable way. Using an *in vitro* digestion/Caco-2 model, we studied iron bioavailability in edible portions of different genotypes of rice, wheat, maize, beans, sweetpotato, potato and cassava. We also determined the concentrations of both enhancers (ascorbate, inulin and carotenoids) and inhibitors (phytate and polyphenols) of iron bioavailability in these crops. The edible parts were cooked to simulate traditional food preparation techniques before consumption. The results indicate that stage of seed maturity, cooking method and the genotype eaten are all factors affecting Fe bioavailability in staple food crops. HarvestPlus, USDA and Cornell University supported this research.

P-3025

Comparative Gene Expression Profiling and the Physiological Role of t-Zeatin Riboside (ZR) Between Invitro- and Hydroponic-grown Sweetpotato During Storage Root Initiation and Enlargement. M. EGNIN¹, D. Mortley¹, F. Sanders¹, S. Traore¹, H. Gao², J. S. Jack¹, and Tarek Radwan¹. ¹College of Agricultural, Environmental & Natural Sciences, Tuskegee University, Tuskegee AL 36088 and ²Auburn University, AL. Email: megnin@tuskegee.edu

Comparative molecular and physiological analyses between *in vitro* and hydroponic-grown sweetpotato were performed to assess the applicability of microstorage root to gene expression profiling and t-zeatin levels during storage root development, a most important physiological feature that makes it unique is the development of the edible storage roots. The process of storage root development is not well understood at the molecular level. The development of biochemical markers and high-throughput expression profiling techniques has provided a mechanism to gain insight into genomes at the hormonal, RNA and DNA level. For assessment of changes in t-Zeatin riboside (ZR) and gene expression levels using cDNA-AFLP, 4 sweetpotato cultivars [TU82-155, J6/66, and NCC-58 and PI 318846-3 (D-3)] differing in the thickening potential of storage roots were studied over a 42-day and 90-day periods (4-7 sequential harvests at 4-7-day intervals) using the nutrient film (NFT) and *in vitro* techniques, respectively. Variable ZR levels from 5-70 pmol/ml were obtained amongst all cultivars tested and between the two techniques. However, ZR accumulation in *in vitro* and NFT samples followed the same patterns in storage roots of D-3 (low), TU-82-155 and J6/66 ZR (consistently higher) and NCC-58 (slight fluctuations). ZR levels in fibrous roots were lower than the storage root. Four selective primer combinations were tested resulting in 60 Transcript Derived Fragments (TDF) between NFT and *in vitro* samples exhibiting the same profile in four different expression patterns, including up-regulated, decreased expression, transient expression and constitutive expression detected in all cultivars. The time point for peak expressions were delayed with *in vitro*-grown when compared to NFT. Storage root initiation was

confirmed between 10-14 days in NFT but delayed to 24-30 days post initial culture *in vitro*. Both types of culture revealed the same developmental pattern indicating that *in vitro* microstorage initiation methodologies could be utilized for assessment of physiological analyses and provide a quantitative method to measure specific transcripts within a cDNA such that sweetpotato in nutrient film technique. *Research project supported by NASA and USDA/CSRRRES.*

P-3026

Phylogenetic Analysis of *Saccharum officinarum* L. and Related Species Based on Simple Sequence Repeats of Chloroplast Genome. D. M. MELOTTO-PASSARIN¹, E. V. Tamarussi¹, K. Dressano¹, and H. Carrer¹. ¹Pádua Dias Av., 11, Laboratório de Biologia Molecular, Departamento de Ciências Biológicas, ESALQ/USP – CEP: 13418-900 – Piracicaba, SP, BRASIL. Email: dmmelott@esalq.usp.br

Microsatellites, or simple sequence repeats (SSRs), are the most widely applied class of molecular markers used in genetic studies, with applications in many fields including genetic conservation, population genetics and molecular breeding. The availability of chloroplast genome (cpDNA) sequences of sugarcane (*Saccharum officinarum* L.) and related Poaceae species *Agrostis stolonifera*, *Hordeum vulgare* subsp. *vulgare*, *Oryza nivara*, *Oryza sativa* indica cultivar-group, *Oryza sativa* japonica cultivar-group, *Sorghum bicolor*, *Triticum aestivum*, *Zea mays* and *Zea mays* cv. B73 allowed us to understand the organization of SSRs in their genetic and intergenic regions. This information was used to gain new insights into phylogenetic relationships among these species. We identified 208 SSRs in the sugarcane cpDNA. In general, mononucleotide repeats appeared to be the most abundant SSRs in studied species, but we also identified di-, tri-, tetra-, penta- and hexanucleotide repeats. About 48 of the chloroplast SSRs were observed in the genetic region. Multiple alignments of DNA sequences of SSRs made the identification of nucleotide variability possible and the phylogeny was estimated by maximum parsimony. Results confirmed polymorphism in the SSRs of the studied Poaceae species. Our study showed that the cpDNA database can be exploited for the development of SSR markers that can amplify *Saccharum* spp. and related genus for comparative mapping and biotechnological approaches.

P-3027

Transferability of Soybean (*Glycine Max*) SSR Markers in Peanut Genomic DNA (*Arachis hypogaea* L.). FRIEDA ELENA SANDERS, He Guohao, Limin Gong, Marcelline Egnin, and Desmond Morley. George Washington Carver Agricultural Experiment Station, Tuskegee University, Milbank Hall, Lab 108, Tuskegee, AL 36088. Email: hguohao@tuskegee.edu, friedasanders@aol.com

Peanut, an important oil and food crop, is ranked as the third major oilseed crop in the world, next to soybean and cotton. Legume crops have been the focus of intensive genetic studies since the time of Gregory Mendel, to improve the yield, quality, resistance factors and extend their geographical range. Some legume species' genetic systems have been well studied and characterized by biochemical and physical markers, cytogenetic analysis, chemically and physically induced mutations, and DNA marker-based genetic linkage maps. Although, peanut crops have a large genome size of about 2800 megabases; its genetic base is very narrow based on previous studies. Simple sequence repeats (SSRs) are useful DNA markers in plant genetic research, because of their ability to reveal polymorphism. However, they are not fully exploited in the peanut because of the cost and labor intensiveness required to develop and utilize in genetic research. Many studies have shown that some DNA markers are transferable among related species due to the conserved regions in their genomes. There is a pressing need in peanut genomic research to fully utilize the progress of other well-characterized legumes such as soybean to advance peanut genomic research. This study investigated the transferability of soybean SSR markers to the peanut because of their related membership in the same family as well as the availability of soybean SSRs. Eight hundred and

sixty-eight soybean SSR primer pairs were used to amplify peanut genomic DNA from varieties C20 and C3424. PCR products were resolved on 2% agarose gel, and the banding pattern was analyzed using Quantity One gel imaging software. The results showed that 22.9% (199) of soybean SSR primer pairs tested in this study could amplify peanut genomic DNA. These transferable markers will be screened for polymorphism between parental DNAs. Then these polymorphic markers will be added into a peanut genetic linkage map. Thus, these transferable markers will benefit peanut genomic research by providing additional DNA markers in peanut and allow for comparative mapping between peanut and soybean crops. Research supported by USDA-ARS, NASA, NSF.

P-3037

Marker-free Site-specific Gene Integration Technology. M. A. AKBUDAK and V. Srivastava. Department of Crop, Soil, & Environmental Sciences, University of Arkansas, Fayetteville, AR 72701. Email: aydin@uark.edu

Site Specific Recombination systems, such as FLP/*FRT* and Cre/*lox*, function efficiently in plant cells by carrying recombination between introduced *FRT* or *lox* sites, respectively. This has led to the development of two major applications: marker-gene deletion, and site-specific gene integration. Both recombination systems have been successfully used to delete marker genes. However, only Cre/*lox* has so far been successfully used in transgene integration into a previously introduced *lox* site in plant genome. There are several advantages of site-specific gene integration over random integration. Most significantly, transgenic plants developed by Cre/*lox*-mediated gene integration are precise integrants and express transgene consistently through successive generations. As a next step towards practical implementation of this technology, a molecular strategy was developed for generating marker-free site-specific gene integration. This strategy relies on Cre/*lox*-mediated gene integration followed by FLP/*FRT*-mediated marker excision. The molecular strategy is designed to generate an integration locus consisting of strategically-placed *FRT* sites to remove marker genes. To test this strategy, a rice line containing Cre/*lox* elements and a heat-shock-FLP gene was developed. The molecular analysis of this line revealed that FLP gene was duly induced by heat treatment and expressed at very low levels at RT. Subsequent bombardment of this target line with a donor plasmid generated a site-specific integration structure, which was stable through tissue culture, meaning basal FLP activity did not prematurely delete marker gene. Heat treatment of the resulting integrant line was expected to induce FLP activity and carryout deletion of *FRT*-flanked marker genes. Initial experiment on heat treatment and locus analysis suggested that induced FLP activity was not sufficient to catalyze *FRT* x *FRT* recombination. As an alternative approach, an ubiquitin promoter controlled FLP gene was introduced into the integrant line. The integrant locus structure before and after FLP-*FRT* recombination will be presented as a proof of feasibility of the marker-free site-specific gene integration technology.

PLANT SECONDARY METABOLISM

P-3028

Gelling Agent Effects on the Mineral Nutrition of Plant Tissue Cultures Assessed Using *In Vitro* Hydroponics Techniques. M. J. BOSELA and Kirk Bradtmiller. Department of Biology, Indiana University-Purdue University at Fort Wayne, 2001 E. Coliseum Boulevard, Fort Wayne, IN 46805. Email: boselam@ipfw.edu

Gelling agents are added to culture media to modify their physical characteristics, but since all of the gelling agents that are routinely used for plant tissue culture (agar, gellan gum, carrageenan) are extracted from living organisms, either red algae or fermented bacteria, they contain mineral nutrient impurities that may contribute positively to the level of plant-available nutrients in a medium. However, for gelling agents, such as gellan gum and carrageenan, that are negatively-charged, electrostatic interactions may adversely affect the availability of cationic

minerals (calcium, magnesium, potassium, etc.). Two types of reactions are possible. The cations may cross-link the gelling agents during gel formation or they may be adsorbed to 'free' acidic residues following gelation. The gelling agent in agar (agarose) is uncharged but agar also contains agaropectin, and in some cases also alginate, both of which are negatively charged and thus may be susceptible to similar charge-based interactions with mineral ions in the medium. We employed a hydroponics approach to directly assess the effects of gelling agents on the mineral nutrition of plant tissue cultures. Using a standard hydroponics medium (Hoagland's Solution) both positive and negative controls and six experimental media, each deficient in one of the following essential nutrients (calcium, nitrogen, phosphorous, magnesium potassium, and iron), were prepared and data was collected on the degree of plant growth inhibition as well as on the type and severity of visual deficiency symptoms (chlorosis, etc.). To date, we have evaluated four plant species (aspen, carnation, tobacco, and tomato), two types of tissue culture (seedling vs. shoot cultures), and three gelling agents (agar, gellan gum, and agarose). As a generalization, deficiency symptoms developed more rapidly and were more severe on agarose media than for agar or gellan gum, but for some of the nutrients other patterns were observed. Data from mineral analyses of the gelling agents will be used to interpret the results.

P-3029

Salicin Metabolism: Understanding Salicin Synthesis and Transport by Precursor Feeding and Gene Expression Studies. R. S. PAYYAVULA, S. A. Harding, and C. J. Tsai. Biotech Research Centre, School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931. Email: rspayyav@mtu.edu

Phenylpropanoid derived phenolic glycosides (PGs) and condensed tannins (CTs) are two major groups of secondary metabolites in *Populus*. Although biosynthesis of PG is less understood than that of CT, salicin, a simple PG, has been proposed as a putative precursor for the synthesis of higher-order PGs, such as salicortin, tremulacin and tremuloidin. The objectives of this study are to understand salicin synthesis and transport using a cell culture system. Salicin and higher-order PGs are not synthesized in cell cultures grown under standard conditions, but can be induced upon feeding with putative precursors. Feeding with salicyl alcohol, salicylaldehyde and helicin, but not benzoic acid, benzyl alcohol, benzaldehyde, salicylic acid, and *O*-coumaric acid, led to synthesis of salicin and isosalicin in the cells. Highest levels of glycosylated products were observed by salicyl alcohol feeding at the early log-phase, with a concomitant reduction in CT levels by 30%. Molecular analysis was conducted to identify candidate genes involved in salicin synthesis and transport. EST microarray analysis showed that expression of 973 non-redundant genes was significantly altered (false discovery rate 0.05) in salicyl alcohol-fed cultures compared to unfed cultures. The most highly up- and down-regulated genes belong to the functional category of defense and secondary metabolism, respectively. Q-PCR expression analysis confirmed up-regulation of specific sucrose transporters, invertases and glucosyl transferases. Q-PCR also revealed down-regulation of phenylpropanoid and flavonoid pathway genes, consistent with reduced CT accumulation upon salicyl alcohol feeding. Of the vacuolar localized transporters, an ATP-binding cassette transporter was the most up-regulated and might play a role in salicin uptake into the vacuole. The induction of a suite of defense genes suggests that salicyl alcohol may play a role in stress signaling. PG synthesis with concomitant CT reduction supports the view that PGs and CTs show metabolic reciprocity in their abundances.

PLANT TISSUE CULTURE

P-3030

Physiology of BY2 Cells During the Suspension Culture Cycle. JEAN ROBERTS, Erica Snodderley, Anthony Snyder, Rob Peterson, Karl Schnelle, and Robbi Garrison, Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: jlroberts2@dow.com

The well-established BY2 tobacco suspension cell culture provides a model system for plant cell physiology and a platform for production of useful foreign proteins. The behavior of BY2 cells in suspension over a weekly culture cycle was mapped. The accumulation of cell mass was monitored by several measures (fresh weight, dry weight, packed cell volume, cell number). Carbon nutrient drawdown was monitored by HPLC. Changes in medium osmolarity, conductivity and pH were observed. Observations were systematically collected on cell size and morphology as well as cell cluster size. Results of these studies indicate that several stress phenomena accompany changes in nutrient levels and medium osmolarity during a weekly culture cycle. Transition from carbon sufficiency to autophagy can be observed during entry to stationary phase. Subsequent subculture into fresh medium results in medium acidification, suggestive of osmotic stress. These insights can contribute to more informed handling of plant cells in culture.

PLANT TRANSFORMATION

P-3031

Transformation of Oil Palm Using *Agrobacterium tumefaciens*. DAYANG IZAWATI ABANG MASLI, Ghulam Kadir Ahmad Parveez, and Abdul Masani Mat Yunus. Advanced Biotechnology and Breeding Centre (ABBC), Biology Division, Malaysian Palm Oil Board (MPOB), N0 6, Persiaran Institusi Bandar Baru Bangi, 43000 Kajang, Selangor, MALAYSIA. Email: izawati@mpob.gov.my

Oil palm (*Elaeis guineensis* Jacq.) is the most important economic crop for Malaysia. In 2006, palm oil and palm kernel oil were the most traded oils in the global oils and fats market with a market share of 56.2%. Nevertheless, oil palm industry still faces several challenges such as labour shortage and limited land for expansion. Genetic engineering is envisaged to be a potential tool that could be used to overcome these challenges. The concept of using *Agrobacterium* as a tool to create transgenic plants is promising for monocotyledonous plants as it has been demonstrated in a number of crops such as rice, maize, banana, wheat, barley, and sorghum. In this work, we investigated the possibility of using *Agrobacterium*-mediated method to transform oil palm. Embryogenic suspension callus was chosen as target tissues due to its highly regenerative characteristic. In addition, modifications of the transformation method including treatment of the target tissues with acetosyringone, plasmolysis medium and physical injury via biolistic were applied during oil palm transformation process. The main reasons for such modifications are to activate virulence system and to initiate and increase the infecting probability in order to improve DNA transfer and transformation efficiencies. *Agrobacterium* LBA 4404 harboring *bar* (*phosphinothricin acetyltransferase*) gene was used as the transformation vector to produce transgenic oil palm resistant to herbicide Basta. The integration of transgene was confirmed by PCR, Dot blot, Southern blot and leaf painting analysis. Therefore, it is conclusively demonstrated here that oil palm can be transformed using *Agrobacterium*-mediated method.

P-3032

Expression of Betaine Aldehyde Dehydrogenase Gene in Transgenic Canola Contributes to the Maintenance of Osmotic Potential Under Salt Stress. REDA E. A. MOGHAIEB¹, Sawsan S. Youssef¹, Hirofume Saneoka², K. Fujita², and Ahmed M. El-Sharkawy². ¹Department of Genetics, Faculty of Agriculture, Cairo University, EGYPT and ² Graduate School of Biosphere Sciences, Hiroshima University, JAPAN. Email: Moghaieb@yahoo.com

In order to allow the biosynthesis of betaine and to examine the enhancement of stress tolerance that results from the biosynthesis of this compatible solute, the genetically engineered canola plants with the ability to synthesize glycine betaine were established by introducing the betaine aldehyde dehydrogenase gene (*BADH-1*) from sorghum plants. The hypocotyl explants derived from three canola genotypes were inoculated with the *Agrobacterium tumefaciens* strain LBA4404 which harbor the recombinant pBI-121. The data indicate that embryonic calli

were formed within two weeks in the presence of 1 mg l⁻¹ 2, 4-D. Adventitious shoots emerged from the embryonic callus in the presence of 4.5 mg l⁻¹ BA. The cultivars showed a varied response to shoot regeneration. The resulted putative transgenic plantlets were able to grow under kanamycin containing medium. The stable integration of the *NPT-II* gene into the plant genomes was tested by PCR using *NPT-II*-specific primers. The *GUS* gene expression can be detected only in the transgenic plants. The stable integration of the *BADH* gene into the canola genome was confirmed by PCR and Southern blotting analysis. The expression of *BADH* could be detected in the canola by RT-PCR. The osmotic potential (ψ_s) of the (T₂) transgenic plants was sufficient to allow water uptake under salt stress conditions. The accumulation of betaine was detected by ¹H-NMR analysis. The accumulation of betaine in the transgenic plants contributed to improvement of salt tolerance through the maintenance of the osmotic potential of the cells.

P-3033

Tissue-specific and Developmental Regulation of Floral Genes from Citrus. XUYEN THI NGO, Javier Narvaez, Lynn J. Pilletteri, Janet Giles, Carol Lovatt, and Martha L. Orozco-Cárdenas. Department of Botany and Plant Sciences, Plant Transformation Research Center, University of California Riverside, CA 92521. Email: xuyenn@ucr.edu

TERMINAL FLOWER 1 (TFL1) has been shown to be important for floral timing in *Arabidopsis* and other herbaceous species. For the first time, a *TFL1* gene promoter was isolated from the perennial tree crop 'Washington' navel orange (*Citrus sinensis* L. Osbeck). A plant expression vector was constructed containing 1544 bp of the *TFL1* promoter fused upstream of the reporter gene β -glucuronidase (*GUS*) for functional analyses of the spatial and developmental expression of *CsTFL1*. In transgenic tomato plants (*Lycopersicon esculentum*), *GUS* was highly expressed in the four floral organs, green and ripening fruit, and in immature seeds, the latter of which was not observed in *C. sinensis*. *GUS* expression was also detected in stems, particularly in the vascular tissue, but not in young leaves. These results suggest that the *Citrus TFL1* gene displayed a tissue-specific and developmentally regulated pattern of expression.

P-3034

Efficient Regeneration and Transformation System in Some Rice Genotypes Grown Under Egyptian Environments. SAWSAN S. YOUSSEF¹, Reda E. A. Moghaieb¹, S. Ready² and Ahmed M. El-Sharkawy¹. ¹Department of Genetics, Faculty of Agriculture, Cairo University, EGYPT and ²ICGEB, INDIA. Email: gecjica@link.net

The aim of this work is to produce transgenic Egyptian rice plantlets expressing the *bt* gene which renders plant insect tolerance. The pBI-121 plasmid harboring the *bt* gene under the control of 35-S promoter and NOS terminator was used. The embryogenic rice callus explants were transformed with the recombinant plasmid using the gene gun as a mediated gene delivery system. During the selection culture, sub-culturing the explants with a change of fresh medium containing 50 mg/l kanamycin greatly reduced the number of escapes. The nodular structures developed into shoot buds when the embryogenic calli were sub-cultured in the medium supplemented with BA within four weeks. The somatic embryos directly emerged from the body of the explants or indirectly germinated from the embryonic callus. Screening of the putative transgenic plants was done using a kanamycin containing medium. The stable integration of the *bt* gene was confirmed using PCR analysis. The obtained transgenic plantlets were transferred to the greenhouse in order to collect the transgenic seeds.

SILENT ABSTRACTS

P-3020

Plant Regeneration and Shoot Organogenesis from Somatic Cotyledons in Cassava (*Manihot esculenta* Crantz). N. J. TAYLOR¹, S. Sahab¹, M. Fitter¹, K. Jorgenson², and C. M. Fauquet¹. ¹Donald Danforth Plant Science Center, St. Louis, MO 63132 and ²Plant Biochemistry

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In the present study plant regeneration efficiencies of the six African cassava cultivars Tereka, Ebwanatereka, Aladu, Serere, 60444 and Bukalasa, and one Columbian cultivar MCol22 was evaluated. Primary somatic embryos were induced from immature leaf lobes cultured on Murashige and Skoog (MS) basal medium supplemented with 4% w/v sucrose and 12 mg/l picloram. Secondary somatic embryos were induced from cotyledonary-stage primary embryos by culturing on MS medium supplemented with 6 mg/l 2, 4-D in dark. Maturation of green cotyledons was obtained from secondary embryos cultured in light on MS medium supplemented with 0.1 mg/l BAP and used for regeneration studies. Green cotyledons were cut to pieces (2-3 mm²) and transferred to shoot induction medium consisting of MS supplemented with 1.0mg/l BAP and 0.5mg/l IBA. After 20 days of induction in light the status of shoot organogenesis and regeneration efficiency was recorded. The Kenyan cultivar Tereka exhibited the maximum degree of primordia formation, followed by Ebwanatereka, MCol22, Aladu, Bukalasa, Serere and 60444. Explants that formed primordia were subcultured to shoot regeneration medium consisting hormone free MS medium. Tereka exhibited the maximum shoot regeneration frequency with 56% of the cotyledon explants regenerating a shoot, followed by Ebwanatereka at 33%, Aladu 10%, MCol22 9%, 60444 8%, Bukalasa 7% and Serere at 5%. Regenerants from all cultivars were successfully transferred to and established in the soil. Our goal is now to adapt this regeneration system for the recovery of transgenic plants from the East African cassava cultivars described here.

P-3035

A Liquid Phase System Improves Elongation and Rooting of *In Vitro* Cultured *Helianthemum marminorensis*. Importance of Sucrose Concentration*. J. L. CASAS, F. Serrano, and M. Cano. Instituto Iberoamericano de la Biodiversidad (CIBIO). University of Alicante. Crta. San Vicente del Raspeig s/n. E-03690 San Vicente del Raspeig (Alicante), SPAIN. Email: jl.casas@ua.es

A liquid phase system was tested in order to improve the elongation and rooting stages of *in vitro* cultured *Helianthemum marminorensis*, an endemic of the SE of Spain. Shoot tips including three nodes were cultured on Woody Plant Medium with 2% (w/v) sucrose and 0.6% (w/v) Plant agar (Duchefa). After three days 2 ml of liquid medium consisting in half-strength Knop solution containing 3 g l⁻¹ activated charcoal and different (20, 35 and 50 g l⁻¹) sucrose concentrations were added on top of the culture medium. The pH of the liquid medium was adjusted to 5.0 prior to autoclaving. After five weeks, elongation rate and number of nodes per explant were both significantly improved. The improvement was seen to be inversely correlated with the sucrose concentration in the liquid phase decreased in the liquid medium. The best result was thus obtained with 20 g l⁻¹ sucrose (w/v), showing a 4.3-fold increase in length when compared to control (without liquid medium addition). In all cases the percentage of rooted explants was 100%, but the presence of sucrose at 35 g l⁻¹ resulted in the highest number of roots produced per explant.

P-3036

An Efficient *In Vitro* Protocol for Important and Highly Valuable Medicinal Plant *Rauwolfia Serpentina*: An Endangered Medicinal Plant of India. SUDHIR KUMAR SHARMA¹, Rajat Singh², and I. D. Arya¹.

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The natural resources how so ever large are bound to diminish. Many of the important useful species are on the verge of extinction due to over exploitation and habitat destruction. There is therefore, need for intensive agricultural studies leading to genetic improvement and cultivation of these valuable, threatened plant species. Reserpine, an alkaloid of the plant *Rauwolfia*, has gained an established place in the treatment of hypertension and also as a tranquilizing agent in states of tension and anxiety. In its crude form, *Rauwolfia* has been known in Indian materia medica for many centuries. The root and leaves are still sold by quacks in the markets and by medicine-men at village fairs in India. Recently it has attained prominence as a remedy for insomnia. Reserpine act by causing peripheral catecholamine depletion induces a lasting drop in blood pressure and heart rate. The central neuro transmitter depletion would explain its sedative and neuroleptic activity. *Rauwolfia serpentina* Benth known as Sarpagandha in Sanskrit due to its efficacy in snake-bite belongs to family apocyanaceae. The deserved popularity of *Rauwolfia* and its alkaloids has raised questions as to the quality and availability of the roots in future. The plant has poor seed viability and low germination percentage that may be largely ascribed to the presence of cinnamic acid derivatives in the seeds. Moreover, the higher yield of reserpine comes from four year old plants, which are naturally destroyed in harvesting the root. Thus a need arises to generate an efficient protocol for cloning of *Rauwolfia serpentina* in order to generate propagules to replenish depleting forests and meeting the demand of commercial cultivation. In the present study nodal segments with axillary buds were surfaced sterilized with 0.1% HgCl₂ solution for 4-6 minutes, inoculated on different concentration of cytokinin (BAP/Kn). Optimum shoot induction from explants was achieved on different concentration of BAP (1.5-2.0 mg/l). *In vitro* cultures were established on different concentration of BAP (0.5-2.0 mg/l). In 3-4 weeks these shoots were further multiplied and excised, dissected out from the basal portion into shoot clusters of 2-5 shoots and were again subcultured on semisolid MS medium supplemented with 0.5-3.0mg/l BAP. Maximum no. of *in vitro* shoots were rooted on ½ MS supplemented with IBA (0.1-1.0 mg/l) + NAA (0.1-0.5 mg/l). These *in vitro* rooted plantlets were further transferred for hardening and acclimatization in vermiculite/soilrite. These plantlets were supplied with half strength MS medium without organics twice a week and later they were transferred to mist chamber at relative humidity of 80-90% and temperature 30 ± 2°C. Plants were shifted to polybags containing sand: soil: FYM in 1:1:1 proportion and placed in the mist chamber for four to five weeks. After mist chamber stage the plants became hardened and were shifted to open shade house conditions for acclimatization to outer environmental conditions. Tissue culture methods would be a valuable alternative for rapid propagation and conservation of this valuable, threatened plant species, there were previous attempts for the propagation of *R. serpentina* through tissue culture, but considerable research is still required to make it commercially feasible.

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