2009 IN VITRO BIOLOGY MEETING

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ANIMAL POSTER ABSTRACTS

CANCER BIOLOGY

A-3000

Variations of the Extracellular Matrix Alters the Organization of Breast Tissue in Three-Dimensional Cultures. M. SWAMYDAS, J. M. Hudak, and D. Dreau. University of North Carolina - Charlotte, Charlotte, NC. Email: mswamyda@uncc.edu

Epithelial-stromal interactions are key regulating factors in the development of mammary gland. In addition to its role in normal development, extra cellular matrix (ECM) also modulates breast cancer. Matrix stiffness influences tissue growth and morphogenesis by modulating cell contractility, cell migration and maturation. However, the precise role of the chemical composition and density of the ECM on the development of breast cancer has yet to be fully elucidated. To determine the physical and chemical effects of the ECM composition and density on migration and organization of breast structures under normal and cancerous conditions, 3D cultures using mammary epithelial cells (NMuMG) in combination with pre-adipocytes (D1) were grown in various ECM. Following a 10-day incubation, the number and area of structures were quantified. When co-cultured with D1 pre-adipocytes, D1 cells surrounded NMuMG epithelial cells and formed acinar structures with lumen formation. Both the number and area of acinar structures in cultures grown in Matrigel® and collagen in combination with agarose were higher than those observed in cultures grown in either agarose, Matrigel® or collagen alone ($p<0.05$). In 3D conditions, both 4T1 and NMuMG cells migrated toward conditioned media derived from stromal MOVAS and D1 cells. These results further underline the key role of the microenvironment in the development of breast tissue and migration associated with breast cancer. (This work was supported by grants from the Department of Defense Era of Hope Program and the National Science Foundation.)

A-3001

Expression of B7-H4 in DCIS and Association with Progression to Invasive Breast Cancer. A. GOLDBERG, S. Burke, and K. R. Shroyer. Department of Pathology, Stony Brook University Medical Center, Stony Brook, NY. Email: abgoldberg8614@gmail.com

Despite advances in the early diagnosis and treatment of breast cancer, over 40,000 women die of breast cancer in the United States each year. B7-H4 (DD-O110) is a relatively recently described breast cancer biomarker and is a member of the B7 family that is involved in the regulation of antigen-specific immune responses, including T cell activation. This study is being conducted to further investigate the expression of B7-H4 in normal breast tissue, ductal carcinoma *in situ* (DCIS), and in invasive breast carcinoma. In previous immunohistochemical studies, B7-H4 was found to be universally over-expressed in both ductal and lobular breast cancers, suggesting that B7-H4 is related to malignant transformation and could serve as a prognostic marker or therapeutic target for combating breast carcinomas. By contrast, lower levels of expression have been observed in benign ductal cells. Cancer biomarker specificity may reflect differential expression of specific biomarker epitopes that are associated with malignant transformation. The goal of the current study is to determine if there is differential expression of B7-H4 epitopes in the sequential transition from normal ductal epithelium to DCIS and to invasive carcinoma. Additionally, this study will determine whether B7-H4 staining pattern or intensity in DCIS cases can be used to evaluate prognosis, that is, predict the likelihood of progression to invasive ductal carcinoma. This will be determined by the immunohistochemical analysis of specific B7-H4 epitopes in a series of formalin-fixed tissue blocks from the archival collections of the Department of Pathology at Stony Brook University Medical Center. Differentially expressed B7-H4 epitopes will be further evaluated to determine if they are predictive of progression from DCIS to invasive carcinoma. These data will determine if B7-H4 is a prognostic marker of disease progression from DCIS to invasive ductal carcinoma and subsequent in vitro studies will be indicated to determine the potential role of B7-H4 as a novel therapeutic target for pre-invasive and invasive breast cancer.

A-3002

P53 Gene Expression During Osteoblastic Differentiation of C2C12 Cells. K. DAVIS, E. Hays, and N. Chandar. Midwestern University, Biochemistry Department, 555 31st St., Downers Grove, IL 60515. Email: Kendra.Davis@mwumail.midwestern.edu

C2C12 cells, a bipotential line, require Bone Morphogenetic Protein-2 in order to differentiate and express osteoblast specific genes. Our lab has previously shown an important role for p53 in bone differentiation. p53 mutations result in increased cell growth and impaired osteoblast differentiation. C2C12 cells were exposed to differentiating promoting (DP) media containing ascorbic acid and β-glycerophosphate, which is commonly used with preosteoblastic cells. DP media promotes the production of matrix proteins and eventual mineralization in culture. The differentiation properties of C2C12 were compared with MC3T3, a determined preosteoblastic cell line. Alkaline phosphatase, osteocalcin, (bone specific genes) and p53 expression were measured and found to be induced by DP treatment in C2C12 cells. In separate experiments, we attempted to knockdown p53 in C2C12 cells using shRNA technology. Reduction in p53 dosage produced a drastic change in morphology of C2C12 cells. We were able to observe multinucleated myoblasts in culture along with cells that resembled osteoblasts. Our results suggest that expression of p53 is associated with changes in bone specific gene expression of C2C12 cells. Downregulation of p53 also seemed to affect differentiation behavior of these cells, thus showing the important role of p53 in the differentiation process.

CELLULAR IMMUNOLOGY

A-3003

Modulation of Inflammatory Mediators in Corneal Tissue Models in Response to Sulfur Mustard Exposure. DENISE M. MILHORN, Angie Adkins, Adam Swartz, and Marian Nelson. Cellular and Molecular Biology Branch, Research Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. Email: denise.milhorn@us.army.mil

Acute corneal injury resulting from ocular sulfur mustard agent (SM) exposure resembles an inflammatory response involving the infiltration of lymphocytes and the production of inflammatory mediators. Chronic SM corneal injury can become a serious clinical problem progressing to permanent eye damage and even blindness. The purpose of the current study was to investigate the activation of mitogen activated protein kinases (MAPKs) and subsequent inflammatory cytokine/chemokine production in response to SM ocular exposure in vitro. Corneal epithelial cells and corneal tissue models were exposed to increasing amounts of SM (50-300 μM) over a 24 hour time period. Supernatants were collected at 1, 4, 8, 16 and 24 hrs post-exposure and analyzed for cytokine/chemokine production by electrochemiluminescence (Meso Scale Discovery). Additionally, tissue/cell lysates were collected at 15, 30, 60 and 90 minutes post-exposure and analyzed by western blot for MAPK phosphorylation/activation. SM exposure resulted in the phosphorylation/activation of p38 and c-jun N-terminal kinase MAPK, (but not extracellular signal-regulated kinases 1 and 2) at 15 minutes post-exposure, increasing over the time course examined. Additionally there was an up-regulation of interleukin (IL)-1β, tumor necrosis factor (TNF) α, IL-6, and IL-8 at 8, 16 and 24 hrs post exposure. Together, these data suggest that ocular SM exposure activates inflammatory mediators, which may contribute to acute as well as chronic injury seen in human victims of ocular sulfur mustard exposure. Identifying the direct involvement of specific signaling pathways in SM induced corneal inflammation may identify critical points of therapeutic intervention for drug development.

DIFFERENTIATED CELLS

A-3004

Development and Characterization of Embryonic Stem Cell-derived Neurons as a Botulinum Inhibitor Drug Discovery Platform. P. M. MCNUTT, M. T. Mesngon. United States Army Medical Research Institute of Chemical Defense, APG-EA, MD 21010. Email: patrick.mcnutt@us.army.mil

Weaponized BoNT presents a significant civilian and force health threat, and the neurotoxin has been labeled a CDC Category A agent. There is no effective pharmacological prophylaxis or post-exposure therapy for BoNT intoxication. In part, the failure to develop a viable therapeutic can be attributed to the lack of a physiologically relevant neuron tissue culture system compatible with drug discovery methodologies. We present evidence that neurons differentiated from murine embryonic stem cells (mESCs) are a viable drug discovery platform. mESCs are differentiated into homogenous (>95%) neuron cultures (ESNs) over eight days using a 4/4 differentiation strategy. ESNs express botulinum substrate proteins SNAP25, VAMP2 and syntaxin, exhibit action potentials and undergo trans-synaptic signaling. Exposure of ESNs to BoNT/A and /B results in: a) proteolysis of SNAP25 and VAMP2, respectively; b) inhibition of neurotransmitter release as measured by HPLC; and c) disruption of network connectivity as characterized with multielectrode arrays. We also assess the use of genetically encoded fluorescent botulinum reporters in transgenic ESNs to quantify BoNT activity via Forster resonance energy transfer. We have developed three complementary assays to characterize candidate BoNT inhibitors in real-time, nondestructive screening assays, and assessed their efficacy using known BoNT inhibitors. It is anticipated that this model system will have widespread applicability as a cell-based screening system for candidate BoNT therapeutics, as well as for other neuron-specific diseases.

IN VITRO TOOLS TECHNIQUIES AND OPTIMIZATION

A-3005

Optimization of Culture & Measurement Conditions for Improved Stability & Sensitivity of Cell Physiology Monitoring Systems for Toxicology Applications. Evamaria Stuetz[1], Rebekka Kubisch[1], Sabine Drechsler[2], RALF EHRET[2], and Maximilian Fleischer[1]. [1]Siemens AG, Corporate Technology, CT PS 6, Otto-Hahn-Ring 6, 81739 Munich, GERMANY and [2]Bionas GmbH, Friedrich-Barnewitz-Strasse 3, 18119 Rostock, GERMANY. Email: ralf.ehret@bionas.de

Long term and on-line monitoring of water quality with cell-based systems requires at least two aspects to be addressed: a) the measurement system including the cellular system has to be stable and to deliver stable data over time and b) the sensitivity of the cell-based system has to be adequate for the proposed application. In this study the Bionas® 2500 *analyzing system* detects physiological parameters of living cells (HepG2, V79) in a label-free and non-invasive assay. Cells are placed on a sensor chip and supplied with medium in a perfusion system. The multiparametric sensor chip continuously measures a) the oxygen consumption (respiration), b) the extracellular acidification (glycolysis) and c) the cell impedance. Whereas the oxygen consumption and the acidification determine the acute rates of the cellular energy metabolism (bioenergetics) the cell impedance detects alterations in the cellular adhesion/confluence and morphology. A perfusion system supplies the cells with nutrients. Moreover, it guarantees highly defined cell environmental conditions throughout the whole experiment. The composition of the running medium (the media used in the system for the measurement) has shown to be very important concerning signal stability during the experiment and sensitivity to toxicants. To examine adaptation effects the culture medium (before the cells are seeded on the chips) was also modified. Although the composition of the medium was only changed in "simple" parameters (basal media DMEM, MEM, DMEM/HAM's, glucose, glutamine, galactose – no special additives), the effects on stability and sensitivity were significant. Summarized, simple changes in the culture and measurement medium may affect the outcome of in vitro experiments – not only for toxicological applications.

A-3006

Automating the Identification and Analysis of the Longest Telomeres: a General Signature of Adult Stem Cell Compartments. A. CHOLEWINSKI[1], J. Zimmermann[2], I. Flores[3], A. Canela[3], E. Vera[3], A. Tejera[3], G. Cotsarelis[4], and M. A. Blasco[3]. [1]Definiens Inc., 55 Madison Avenue, Suite 400, Morristown, NJ 07960; [2]Definiens AG, Trappentreustrasse. 1, 80339 Münichen, GERMANY; [3]Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Madrid E-28029, SPAIN; and [4]University of Pennsylvania School of Medicine, M8 Stellar-Chance Laboratories, Philadelphia, PA 19104. Email: acholewinski@definiens.com

Identification of adult stem cells and their location (niches) is of great relevance for regenerative medicine. However, stem cell niches are still poorly defined in most adult tissues. We have previously shown that the longest telomeres are a general feature of adult stem cell compartments. Using confocal telomere quantitative fluorescence in situ hybridization (telomapping), we have found gradients of telomere length within tissues, with the longest telomeres mapping to the known stem cell compartments. Applying the Definiens Cognition Network Technology® to previously generated data we have been able to automate this identification of the various cellular compartments within the mouse small intestine.

A-3007

Context-based Analysis of Multidimensional Experimental and Simulated Image Data. A. CHOLEWINSKI[1], O. Feehan[2], M. Athelogou[2], G. Schmidt[2], and G. Binnig[2]. [1]Definiens Inc., 55 Madison Avenue, Suite 400, Morristown, NJ 07960 and [2]Definiens AG, Trappentreustrasse. 1, 80339 Münichen, GERMANY. Email: acholewinski@definiens.com

Multidimensional image data arises frequently as an output of experimental processes in contemporary biology. Extracting knowledge from these images through image analysis can lead to the discovery of new information either directly or by providing parameters for further experiments or simulations. The presented work focuses on the analysis of experimental and simulation results involving the process of endocytosis in hepatocytes.

PLANT POSTER ABSTRACTS

IN VITRO BIOLOGY: CRYOPRESERVATION/LONG TERM STORAGE

P-3000

Indirect Somatic Embryogenesis in *Coffea arabica* L. Elite Clones. J. C. Rezende[1], C. H. S. CARVALHO[2], A. C. R. Santos[3], J. B. Teixeira[4], J. B. Pasqual[5]. [1]Epamig, Minas Gerais, BRAZIL; [2]Embrapa Coffee, Alameda do Café, 1000, Varginha, BRAZIL, 37026-400; [3]Procafé Foundation; [4]Embrapa Genetic Resources and Biotechnology; and [5]Federal University of Lavras, BRAZIL. Email: carlos.carvalho@embrapa.br

Indirect somatic embryogenesis is the method of choice for mass propagation of heterozygous coffee plants and several protocols for callus induction have been reported for both arabica and robusta coffee. Most protocols use a two step callus induction. Initially the explants are placed in a primary medium for about one month and then transferred to a secondary medium. Kinetin or the combination of auxin and kinetin in one medium, or a two step callus induction system using different combinations of auxin/kinetin have been used. This work was conducted to assess the potential of embryogenic callus production of 10 *Coffea arabica* elite clones with leaf rust and leaf miner resistance, and also to test several combinations of 2,4-D (2.5 and 20.0 uM) and 2-iP (2.5 and 20.0 uM) for callus induction. Embryogenic callus

induction was highly genotype-dependent and a large variation among clones selected in F_4 families was observed. Callus induction was also dependent of the time the explants were collected and of the 2,4-D/2-iP combination.

P-3001
Overcoming *In Vitro* Rooting Deficiency in *Corymbia maculata* Microshoots. B. STEINITZ and Y. Tabib. Department of Vegetable Research, Institute of Plant Sciences, ARO, The Volcani Center, Bet Dagan 50250, ISRAEL. Email: steinitz@volcani.agri.gov.il

Corymbia maculata (syn. *Eucalyptus maculata*) is considered recalcitrant in terms of vegetative propagation mainly due to very poor rooting ability of cuttings, which hampers vegetative multiplication of elite trees. In a preliminary work we found that absence of rooting is characteristic also for microshoots prepared from *C. maculata* seedlings germinated and raised in vitro, indicating thereby that rooting failure is not limited to explants derived from mature trees. We attempted overcoming lack of adventitious root regeneration in in vitro shoots. Culture initiation was from a seedling. Multiplication of shoots was accomplished by single stem node explants grown on a shoot induction medium including BA and NAA. For adventitious root induction experiments, shoot tips were harvested and planted onto a root induction medium (RIM). No rooting occurred in shoots planted onto RIM with IAA, IBA, or NAA when growth regulators were administered at different concentrations or different treatment durations. The hurdle in rooting was eventually overcome by adding silver ions (Ag^+) to RIM with IBA. Details of the conditions allowing abundant rooting in above 80% of microshoots will be presented. Since Ag^+ is well known to be a potent ethylene action inhibitor, our results suggest that ethylene generated by cultures plays a central role in preventing adventitious root induction in *C. maculata*. Moreover, our study hints for the first time at the possible involvement of ethylene as a hormone controlling adventitious root formation in *Eucalyptus* species.

IN VITRO BIOLOGY: ANALYSIS OR PRODUCTION OF PLANT METABOLITES
P-3002
Developmental Profile of Storage Reserve Accumulation in Soybean Somatic Embryos. S. A. SPARACE, Y. He, K. R. Clark, and T. E. Young. Biological Sciences Dept., Clemson University, 132 Long Hall, Clemson SC 29634. Email: smsprc@clemson.edu

The growth and development of somatic embryos of soybean (*Glycine max* L., cv. 'Jack') in liquid culture over the course of 8 weeks have been documented in relation to the accumulation of the main storage components (protein, lipid, starch and soluble carbohydrate). Under routine growth conditions, explants of embryonic tissues show almost typical sigmoidal grow kinetics. After 3 weeks in culture, embryos slowly accumulated 0.3 gfw per culture flask. At 4 weeks, embryo growth increased sharply and continued until 8 weeks where growth terminated at 4.1 gfw per flask. Over the 8-week course of development, protein content increased from an initial 10% fw to 31% fw at 2 weeks and then gradually decreased to about 15% fw at weeks 7 and 8. Starch content increased exponentially from an initial 0.25% fw to a maximum of 2.3 % at weeks 4 and 5 and then decreased to 0.1% at weeks 7 and 8. The % fw of soluble sugar was initially 3.3%, which decreased and then increased to a maximum of 3.7% at 3 weeks, and then gradually decreased to about 1.5 % at 8 weeks. Lipid content was initially 0.8% fw, but steadily decreased to 0.3% at 3 weeks and then increase sharply to a maximum of about 1 % at 5 weeks, and finally decreased to 0.5% by the 7^{th} and 8^{th} weeks in culture. These observations indicate that the pattern of accumulation of storage reserves in somatic embryos is similar (but not identical) to that of zygotic embryos. This work was supported by Grant no. 8233 from the United Soybean Board to S.A. Sparace.

IN VITRO BIOLOGY: CROP IMPROVEMENT THROUGH IN VITRO TECHNIQUES
P-3003
Tetraploid Black Locust, a Promising Tree Resource for Biomass Energy and Forage. YUN LI, Guojun Zhang, and Jinzhong Jiang. Key Lab. of Genetics and Breeding of Forest Tree and Ornamental Plant of MOE, College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing 100083, P. R. CHINA. Email: yunli63@163.com

Tetraploid black locusts (tetraploid *Robinia pseudoacacia*) has been genetically improved in China since 1997. The improved trees are fast growing, with high drought tolerance, large biomass production. Stems or branches can be efficiently converted to energy by combustion. Leaves and thin supple twigs are used as forage with a high protein content. Our study had five objectives: 1. The rapid propagating of cuttings by tissue culture and in vitro propagation. The propagating rate was improved more than 10 times, the transplant survival rate was improved more than 96%. The cost of each seedling was reduced to 2 US cents. 2. The selection of improved genotypes for bio-energy and forage production. Superior clones were selected. Some superior clones are special for bio-energy and others are special for forage. 3. The composition and nutritional quality of leaves and thin supple twigs were evaluated. And dairy cow Ruminal in situ digestibility was analyzed in dairy cows. The results prove that tetraploid black locust is a promising tree variety for forage, The CP is 23.56%, DM is 25.36%, NDF is 26.27%, ADF is 15.48%, EE is 3.43%, Ash is 5.618, Ca is 0.62%, and P is 0.33% in the leaves. Tetraploid black locust is similar to Leymuschinensis (it is one of forage plants) of Ruminal in situ digestibility of DM, NDF, ADF, CP, EE and OM. It has a superior flavor, balanced nutritional content, is highly digestible and increases weight gain in cows and goats. We also developed processing protocols and facilities. 4. The energy conserved in stems and branches was evaluated. The combustion value is more than 4982.3 calories/gram from branches and leaves of one-year-old ratoons. Processing facilities for the combustion of biomass were set up. 5. Cultivation and management practices were optimized. Under optimized management survival rate reaches more than 91%. Annual dry biomass production for combustion is 25 000 kg/hectare or wet biomass production for forage is 75 000 kg/hectare. *Note: dry material (DM), organic material (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF).*

IN VITRO BIOLOGY: REFINED TOOLS FOR GENETIC TRANSFORMATION
P-3004
Evaluation of the D-amino Acid Selectable Marker Gene, dsdA for Use in Maize Transformation. Fang-Ming Lai, Laura Privalle, Kangfeng Mei, Durba Ghoshal, Yuwei Shen, Jeff Klucinec, Klaus Daeschner[1], Luke Mankin, and TODD JONES. BASF Plant Sciences LLC, 26 Davis Drive, Research Triangle Park, NC 27709 and [1]BASF Plant Science Company, GmbH, Agricultural Center, 67117, Limburgerhof, GERMANY. Email: todd.jones@basf.com

In this study, we evaluated the D-serine ammonia lyase (*dsdA*) gene from *E. coli* as a selectable marker for maize transformation. D-serine ammonia lyase is a substrate-specific enzyme that metabolizes D-serine into pyruvate, ammonia and water. D-serine inhibits germination of isolated immature maize embryos and growth of embryogenic callus from wild-type plants in a concentration range between 2 – 15 mM. Transgenic plants were recovered under the preferred selection conditions with *dsdA* as the selection marker at efficiencies comparable to using an *ahas* selection marker gene as a control. Immature embryos infected with an *Agrobacterium* strain containing an *ahas* gene construct without *dsdA* did not yield any transgenic events on the selection medium with 10 mM D-serine, indicating D-serine provided tight selection without escapes. Molecular analysis confirmed the integration of the *dsdA* gene into the genome of the transgenic plants. No adverse

phenotypes were observed in the greenhouse and expression of the *dsdA* marker had no affect on agronomic characteristics or grain yield in a multilocation field trial. Seed composition analysis results showed that there were no significant differences in contents of seed protein, starch, fatty acids, fiber, phytic acid, and free amino acids between transgenic and non-transgenic control plants. These data indicate that the *dsdA* gene is properly expressed in maize and the DSD protein functions appropriately to metabolize D-serine during in vitro selection. The *dsdA* gene in combination with toxic levels of D-serine represents a new and effective selectable marker system for maize transformation.

P-3005

Marker-Free Transformation Systems with Intellectual Property Freedom-to-Operate (FTO) for the Development of Genetically-enhanced Plants. H. T. T. LE[1,2]*, R. E. Figueroa-Balderas[1], S. W. Bird[1], C. L. Chi-Ham[1], and A. B. Bennett[1]. [1]PIPRA, University of California, One Shields Avenue, PRB Mail Stop 5, Davis, CA 95616-8631 and [2]Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Hanoi, VIETNAM. *Vietnam Education Foundation Visiting Scholar. Email: hthle@ucdavis.edu, abbennett@ucdavis.edu

Plant genetic engineering has positively impacted global production of major food crops. However, as with most *Agrobacterium*-based transformation systems, the published protocols incorporate a large number of patented technologies. A fundamental hurdle in developing commercial genetically engineered (GE) crops is the inherent cost of obtaining intellectual property (IP) with freedom-to-operate (FTO) for plant transformation enabling technologies. Consequently, access to enabling technologies is critical to support world-wide innovation in agricultural biotechnology. In addition, it is increasingly desirable to produce GE crops that do not carry selectable markers. PIPRA is developing several plant transformation technology platforms useful to produce GE crops for research, commercial, and humanitarian uses. A transposon-based system enables removing DNA that is not necessary after transformation. Thus, it is possible to produce GE crops that only contain the trait gene and even all plant-based DNA components. Similarly, a site-specific recombinase-mediated excision system removes marker genes and unwanted DNA post-transformation, retaining only the trait genes. A third system involves co-transformation using separate plant binary vectors containing either selectable markers or gene of interest cassettes in two *Agrobacterium* strains. Co-transformation with separate plasmids results in a percentage of unlinked gene insertions which can be segregated in subsequent generations. We are optimizing the performance of individual components and validating these distinct systems in both model and crop plants. These plant transformation technology platforms address major IP hurdles, offer maximum global FTO, and address regulatory and consumer issues as they include technologies to generate marker-free and, if desired, cisgenic plants, engineered with DNA from plant origin. PIPRA is implementing a licensing model to make the technologies in these platforms available. These marker-free transformation platforms will be useful to generate new GE crops.

P-3006

Development of a High Throughput Genetic Transformation Platform for the Production of Disease Resistant and Nutritionally Enhanced Cassava (*Manihot esculenta*). N. J. TAYLOR, T. Moll, C. Corbin, and C. M. Fauquet. International Laboratory for Tropical Agricultural Biotechnology, Donald Danforth Plant Science Center, 975, N. Warson Rd, St Louis, MO, 63132. Email: ntaylor@danforthcenter.org

Cassava is, after rice and maize, the most important source of dietary calories in the tropics. As a vegetatively propagated, heterozygous plant, conventional breeding is limited in its ability to stack beneficial traits within germplasm preferred by resource poor farmers or industrial scale producers. Transgenic technologies therefore offer significant potential for the improvement of the crop. To this end, we have developed a genetic transformation platform that raises transgenic capacities in cassava close to those available for the other major staple food crops. This high throughput system is being exploited for the production and molecular analysis of transgenic cassava within projects aimed at increased virus resistance and enhanced nutritional content of the storage roots. Based on Agrobacterium-mediated transformation of embryogenic callus from the Nigerian variety 60444, continued optimization of the selection and regeneration stages has reduced the time from gene insertion to transgenic plant regeneration to less than five months, and in 2008 facilitated the production of more than 1700 independent transgenic events at the Danforth Plant Science Center. Low cost, DNA dot blot analysis has been mated to plant production to enable determination of transgene copy number on up to 150 in vitro transgenic plantlets per week, thereby allowing rapid selection of low copy transgenic events for subsequent expression studies. The above system has proved robust, delivering 100s of transgenic plants for greenhouse testing and field trials, and has been shown capable of generating plants with up top four stacked transgenes for nutritional enhancement without loss of transformation efficiency. Information will be presented describing the transformation platform, molecular analysis of the resulting plants and progress in adapting the system to handle farmer preferred cassava cultivars from East and West Africa.

P-3007

Studies on Pure Mlb® Technology and Its Impact on Vector Backbone Integration in Transgenic Cassava. SAREENA SAHAB, Brent Trauterman, Jitender Yadav, Nigel Taylor, and Claude Fauquet. ILTAB, Donald Danforth Plant Science Center, St. Louis, MO. Email: ssahab@danforthcenter.org

Imperfect T-DNA processing is common during Agrobacterium-mediated transformation, resulting in integration of binary vector backbone (VBB) sequences into the plant genome. Regulatory restrictions prevent such transgenic plants being developed for commercial deployment. Up to 75% of transgenic cassava plants regenerated in our laboratory by co-culture of friable embryogenic callus with *A. tumefaciens* were found to contain vector backbone sequences. A typical population of such plants consisted of ~25% free of VBB sequences, 15-20% showing integration of the whole vector backbone (containing both left and right borders) 30-40% with beyond the LB only and 20-25% beyond the RB only. To reduce the frequency of VVB integration, multiple left border (PureMlb®) technology was employed and the binary vector pCambia2300 modified to carry up to three, stacked left border repeats. Transgenic BY2 cells, tobacco and cassava plants were transformed with *A. tumefaciens* strain LBA4404 carrying PureMlb® and regenerants screened by PCR for presence of VBB sequences. In all three plant systems use of PureMlb® technology reduced VBB integration past the LB close to 20% but was ineffective for suppressing beyond RB integration, indicating both the effectiveness and limitations of this technology. PureMlb® has now been adopted as a standard component of cassava transformation technologies within our laboratory.

IN VITRO BIOLOGY: EMBRYOGENESIS/MICROPROPAGATION/REGENERATION

P-3025

Effect of Auxins on the Induction of Somatic Embryogenesis from Leaf Explants of Four Rose (*Rosa hybrida* L.) Cultivars. S. KRASNYANSKI, T. Nguyen, and G. Allen. North Carolina State University, Dept. of Horticulture, Campus Box 7550, Partners II Bldg., Room 1208, Raleigh, NC 27606. Email: sfkrasny@ncsu.edu

Roses are one of the most valuable cut flowers because of particular traditions around the World and their great appeal to consumers. They are in constant demand in Europe, US and South America. However, roses are highly susceptible to various pathogens. Depending on the genotype the vase life of cut flowers can be very sensitive to poor

postharvest handling. Genetic manipulations via transformation could play an important role in improving disease resistance and cut flowers' qualities. To successfully employ genetic transformation as a tool to improve quality of roses, it is essential to have an efficient plant regeneration system. Plant regeneration via embryogenesis is preferable to organogenesis due to the higher possibilities of chimera plants production through organogenesis. In this study we investigated the effect of different concentrations of four auxins (NAA, dicamba, 2,4-D and picloram) on the induction of somatic embryogenesis through initiation of embryogenic callus and somatic embryos directly from leaf explants of four elite rose cultivars, which included Charlotte, Classy, Freedom and Forever Young. Leaf explants from in vitro maintained stock plants were used in our experiments, and the auxin concentrations tested varied from 1 to 10 mg/L. Explants subjected to various auxins were first cultured for 30 days in the dark and then subcultured on three different MS media supplemented with 1.0 mg/L TDZ + 0.1 mg/L GA_3, 1.0 mg/L BA + 0.1 mg/L GA_3, and with no growth regulators as a control. The first somatic embryos were observed after 25 to 30 days of culture. The best induction of somatic embryogenesis was obtained on the MS media supplemented with 5 mg/L 2, 4-D for Classy, and 2.5 mg/L for Charlotte followed by subculture on the medium supplemented with 1.0 mg/L TDZ + 0.1 mg/L GA_3. No somatic embryogenesis could be induced with any of the combination of growth regulators in Forever Young or Freedom. The efficiency of induction of somatic embryogenesis through initiation of friable embryogenic callus and direct development of somatic embryos from leaf tissue was 25% for Classy and 18.6% for Charlotte. Both embryogenic callus and somatic embryo cultures have been maintained for over a year and currently are being successfully used in transformation experiments.

P-3026

Large-scale Plantlet Conversion from Cotyledonary Somatic Embryos of *Kalopanax septemlobus* Tree Using Bioreactor Culture. SUN JA KIM[1], Yaser Hassan Dewir[2], and Heung Kyu Moon[1]. [1]Division of Biotechnology, Korea Forest Research Institute, Omokdong 44-3, Suwon 441-350, SOUTH KOREA and [2]Department of Horticulture, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, EGYPT. Email: hkmoon@forest.go.kr

A suitable bioreactor system for a large-scale embryo-to-plantlet conversion of *Kalopanax septemlobus* was established. In temporary immersion with net (TIN) bioreactor, 85% of embryos successfully produced plantlets whereas only 29.3% conversion rate was obtained in continuous immersion with net (CIN) bioreactor. Embryos cultured in TIN bioreactor produced more vigorous plantlets in terms of fresh weight, height, root length, number of roots and number of leaves. In CIN bioreactor, *Kalopanax* plantlets showed high MDA content and increased activities of ROS-processing enzymes, such as APX and GR, indicating the occurrence of oxidative stress during culture. However, both SOD and CAT showed similar activities in plantlets grown in different bioreactors. Plantlets grown in CIN bioreactor exhibited low survival rate (75.8%) compared to those grown in TIN bioreactor (100%). MDA content decreased with progression of acclimatization, indicating a decrease in oxidative stress. However, MDA level in CIN derived plantlets was higher than that in TIN derived plantlets. In TIN derived plantlets, both SOD and GR activities increased after 1 wk and thereafter they decreased. CAT activity decreased while APX activity started to increase after 1 wk of acclimatization. The results indicate that *Kalopanax* plantlets are able to overcome oxidative stress mainly through SOD activity. However, the levels of antioxidant enzyme activities were higher in CIN derived plantlets than in TIN derived plantlets. *Kalopanax* plantlets obtained from TIN bioreactor performed better during the acclimatization phase and showed higher survival rate than those obtained on CIN bioreactor or conventional culture systems.

GENETIC TRANSFORMATION FOR CROP IMPROVEMENT: BIOTIC STRESS TOLERANCE

P-3008

Fungal Pathogen Protection in Sugarcane by the Expression of a Plant Defensin Peptide. NAGLAA A. ABALLAH[1,2] and Kh. Radwan[2]. [1]Department of Genetics, Faculty of Agriculture, Cairo University, EGYPT and [2]Agricultural Genetic Engineering Research Institute (AGERI), ARC, EGYPT. Email: naglaa_a@hotmail.com

Sugarcane is considered as the main source for sugar production in Egypt with a total production area of 255,000 feddans planted in 2006. However, sugarcane cultivation is threatened by different pests, such as insects, fungi and viruses. Pokkah boeing is a challenging disease that affect sugarcane production in many different areas around the world. Plant defensins are a family of antifungal proteins that have a remarkable structural conservation and rich diversity of variants. The constitutive expression of these proteins in transgenic crops affords strong protection from fungal attack. To reach our goal, regeneration and transformation system for two Egyptian sugarcane cultivars Ph and C9 were established through somatic embryogenesis. The effect of 2,4-D, NAA, IAA and kinetin on the regeneration of sugarcane was studied. Microprojectile-mediated transformation was used to transform the Egyptian sugarcane cultivar C9 with the MsDef 4 gene cloned into a plant expression vector containing hygromycin resistance gene as a plant selectable marker. The putative transformed plants were cultivated on a selection media containing hygomycin. Nevertheless, the transformed plants with fungal resistant gene were evaluated with PCR analysis as well as the Southern and western techniques to confirm the presence and expression of the transgenes. Further studies will evaluate the resistance of transgenic lines to fungal infection.

P-3009

Effect of Antimicrobial Synthetic Peptide D4E1 on Infestation of Cotton Seed Germination Rates in the Presence of Cotton Seedling Disease. LAKISHA ODOM, C. Bonsi, R. Ankumah, J. Jaynes, M. Egnin, L. Ogden, and D. Mortley. Department of Agriculture and Environmental Science. Tuskegee University, AL, 36088. Email: LO0026162@tuskegee.edu

Cotton seedling disease is a fungal disease complex comprised of several fungal pathogens. In Alabama, those pathogens are *Rhizoctonia Solani* and *Pythium* belonging to the fungal classes *Basidomycetes*, and *Oomycetes*, respectively. Cotton Seedling Disease, which results in loss of cotton production revenues totaling over 10 million dollars per year, in Alabama alone, has no known disease resistant cultivars. Synthetic antimicrobial peptides have been used in previous research in order to confer phytopathogen control. In an effort to confer resistance through genetic modification, a synthetic antimicrobial peptide *D4E*1, which has been shown in vitro and *in-planta* to have broad spectrum antimicrobial action against many fungal orders, has been transformed into cotton seeds to examine the efficacy of this peptide on the control of Cotton Seedling Disease Complex in transformed cotton plants in a field setting. Two 150 x150 ft test plots were arranged in a completely randomized design and were assigned either one of 3 lines of cotton seed transformed with *D4E1* (designated 357, 358, and 373) or a control line containing GUS marker gene. In test plot 1 there was a significant difference ($P \leq 0.05$) between line 358 and the control and line 373 indicating that those lines had overall healthier cotton seedlings than the control. In field plot 2 there was no significant difference between lines and the control. When comparing mean cotton seedling germination scores, in field plot 1, there was a significant difference ($P \leq 0.05$) between each of the lines and the control and in test field 2 there was no difference between the lines and control. *Work Supported by USDA and Tuskegee University GWCAES.*

P-3010

Barley Yellow Dwarf Virus Subgenomic RNA Knockout Mutants Replicate and Cause Disease in Oat Plants. J. R. JACKSON[1,2], S. Liu[2], and W. A. Miller[2]. [1] Plant Biotech & Genomics Lab. Tuskegee University, Tuskegee AL 36088 and [2]Interdepartmental Genetics Department of Plant Pathology, Iowa State University, Ames, IA 50011. Email: jacksonj@tuskegee.edu

Barley Yellow Dwarf Virus produces three subgenomic RNAs (sgRNAs) during its infection cycle. sgRNA1 serves as the messenger for translation of the coat protein, the coat protein C-terminal extension (read-through domain), and a protein required for systemic infection in plants (P4). In contrast, sgRNA2 and sgRNA3 are not believed to be mRNAs. Instead, sgRNA2 appears to function as a ribo-regulator of viral translation in virus-infected protoplasts. SgRNA3 does not encode an ORF and its role is unknown. To understand the roles of sgRNA2 and sgRNA3 in BYDV infections, infectivity of viral RNA containing knockout mutants of these RNAs were tested in whole plant infections. We report that these sgRNA knockout mutants elicit infection in oats and induce similar symptoms and disease onset as the wild-type virus. An ELISA revealed that coat protein levels in the sgRNA2 and double knockout mutants were significantly higher than those induced by the control virus. There was no significant difference between the levels of coat protein in the control and sgRNA3 knockout mutant. These results reveal an unexpected modest inhibitory effect of sgRNA2 on virion accumulation, and a lack of effect of sgRNA3 on virus accumulation and disease symptoms. *Work supported by the NIH Ruth L. Kirschstein NRSA Minority Fellowship grant no: 1F31AI056673-01.*

GENETIC TRANSFORMATION FOR CROP IMPROVEMENT: ALTERING PLANT DEVELOPMENT

P-3011

Generation of New Petunia Cultivars Using Transcription Factors – The Black Leaved Petunia. KEVIN DAVIES[1], Nick Albert[1, 2], Simon Deroles[1], Murray Boase[1], David Lewis[1], Huaibi Zhang[1], Kathy Schwinn[1], and Paula Jameson[2]. [1]New Zealand Institute for Plant & Food Research Limited, Private Bag 11-600, Palmerston North, NEW ZEALAND and [2]School of Biological Sciences, University of Canterbury, Private Bag 4-800 Christchurch, NEW ZEALAND. Email: daviesk@crop.cri.nz

Anthocyanin biosynthesis is one of the best-characterised metabolic pathways in plants. In addition to the biosynthetic genes being known, much is understood of the genetic regulation of the pathway through the action of different families of transcription factors (TFs) and regulatory proteins, which include those of the MYB, bHLH and WD40 type. We have studied the effect of the expression of selected TFs on the generation of colour in petunia with the aims of understanding anthocyanin regulation in petunia and developing new cultivars with dark foliage. The *Lc* (maize bHLH) petunia model, which displays enhanced, light-induced vegetative pigmentation, was used to investigate how high-light affects anthocyanin biosynthesis. *Lc* petunia plants displayed intense purple anthocyanin pigmentation throughout leaves and stems when grown under high-light conditions, either in the field or in growth cabinets, yet remain acyanic when grown under shade conditions. The additional pigmentation in *Lc* petunia leaves appears to screen underlying photosynthetic tissues, increasing light saturation and light compensation points without reducing the maximal photosynthetic assimilation rate. Experiments with other TF types suggest the high-light induced anthocyanin pigmentation is regulated by endogenous MYB transcription factors, and that the appropriate TF genes can deliver cultivars with light-independent foliage pigmentation.

P-3012

Enzyme Production in Plants. S. CHAIWONGSAR, T. Ziegelhoffer, J. R. Raasch, and S. A. Phillips. University of Wisconsin-Madison Biotech Center, 425 Henry Mall, Madison, WI. Email: schaiwongsar@wisc.edu

The purpose of this project is to explore the utility of transgenic plants as vehicles for the production of lignocellulose-degrading enzymes. The two major objectives of this project are: 1) to take advantage of plant agricultural productivity to both decrease the unit cost and increase the production capacity for these enzymes and 2) to determine if the expression of these enzymes in plants can improve biomass digestibility and effectively lower process costs for ethanol production from biomass. To achieve these goals, we employed Gateway Vector, a medium-high throughput system, to express various lignocellulose-degrading enzymes including E1 and E2 endoglucanases (endocellulases) in *Arabidopsis* plants. We demonstrated that the enzymes could be highly expressed in transgenic plants with the minimal effect to the plant growth and development. The collapsed xylem in transgenic plants suggested the alteration in cell wall composition. The digestibility assay will be further investigated for those transgenic plants.

P-3013

Characterization of Local Sweetpotato Cultivars as Bio-fuel Crop. B. J. MIN[1], M. Egnin[1], C. Bonsi[1], D. Mortley[1], S. Traore[1], and M. Gao[2]. [1]Plant Biotech & Genomics Research Lab, George Washington Carver Agricultural Experiment Station, Tuskegee University, AL 36088 and [2]Biotech Center, Alcorn State University, MS 39056. Email: byungjin.min@tuskegee.edu, megnin@tuskegee.edu

The sweetpotato (*Impomoea batatas L., Lam*), which is cultivated in tropical and subtropical regions, has been considered as a useful source of food, animal feed and industrial material. With environmental contamination has soared due to petroleum-based fuel and crude oil price continue to rise, Bio-ethanol is considered as one of the alternative to conventional gas. Based on biomass from crops such as maize, sugar cane, potato starch, fuels containing bio-ethanol could potentially reduce petroleum-based fuel consumption and lower price. Reports have shown that sweetpotato carbohydrate yields are greater than that of corn with great potential for bio-ethanol production. For formulating food products and bio-fuel, a total of 14 sweetpotato cultivars grown at Tuskegee University GWCAES field were evaluated for their starch yield, physical properties and enzyme activities. The color range of freeze-dried sweetpotato meal was white to yellow. White flesh showed higher L* value while yellow flesh showed higher b* value. For the starch content, freshly harvested sweetpotatos were washed, peeled, chopped, blended and filtered. The starches extracted were dried in a vacuum chamber at 20°C then the yield was calculated on a wet basis. Starch yields of all cultivars ranged between 15 and 30%. Three cultivars DM01-158-097 (28.4%), TIB4 (29.2%) and UK-RED-WHITE (28.8%) had high levels of starch. Amylose levels ranged between 18.0 ~ 19.4, while that of amylopectin ranged between 80.6 ~ 82.0. No significant difference between amylose and amylopectin ratio in all cultivars tested was observed. Alpha amylase is being determined by fluorometer to evaluate its activity in all cultivars. Results on freeze-dried sweetpotato subjected to ELISA to determine the levels of alpha and beta amylase will be presented. *Work supported by Tuskegee University, USDA/CSREES, and Alcorn State University.*

P-3014

Expression of Synthetic Tumor Reducing Peptide Genes in Sweetpotato as Therapeutic Drugs Against Cancer. S. TRAORE, M. Egnin, F. Sanders, S. Samuels, T. Radwan, B. J. Min, and J. Jackson. Plant Biotech & Genomics Lab, Tuskegee University, Tuskegee, AL 36088. Email: sytraore@gmail.com, megnin@tuskegee.edu

The emergence of molecular farming in transgenic technologies offers the possibility of manipulating existing genes or designing *de novo* peptide genes using a more rapid and targeting approach. Though

successful, traditional methods for drugs and vaccine production are hampered by lack of post-translational modifications, high costs of production, and severe side effects. Consequently, there is a need to explore new approaches for drug discovery and production that could reduce or eliminate problems associated with conventional methods. Attempts are being made to express many proteins of therapeutic use at high levels in plants and to use them as bio-reactors in the modern era against diseases such as HIV, diabetes, hypertension, obesity, hepatitis B and C and cancer which kills over 7 millions people annually worldwide. As such, synthetic tumor reducing peptides able to reduce significantly the volume of cancer tumors have been developed at Tuskegee University. Plant-optimized synthetic genes encoding these novel peptides have been *de novo* designed, constructed and cloned for use in sweetpotato transformation. The genes composed of two sequences of exons separated by an intron sequence were driven by CaMV 35S promoter at the 5' end and Nos terminator at 3' end and cloned into binary plasmids *pCGN1557 and pCGN1558* at the right border along with a *nptI*I gene at the left border of the T-DNA and mobilized in *E. coli* DH5α. Resulting positive recombinant plasmids were confirmed through restriction digest. Confirmed recombinant plasmids were mobilized in disarmed *Agrobacterium tumefaciens* strains EHA101. Positive strains carrying the recombinant plasmids were utilized in sweetpotato transformation. Fifteen putative transgenic plantlets were recovered on selection medium and subjected to Southern blot to confirm the integration of the synthetic genes. Results on gene expression at the transcriptional and translational levels by RT-PCR and Western blot analyses will be presented. *Work Supported by NIH-EXPORT, USDA and Tuskegee University GWCAES.*

P-3015

Development of Transgenic Sweetpotato [*lpomoea batatas (L. lam*)] Expressing *jc41N* and *jc41ND* Genes as Plant-based Vaccines Against HIV. S. SAMUELS, M. Egnin, J. Jaynes, S. Traore, B. Min, J. Jackson. Department of Agriculture & Environmental Sciences. Tuskegee University, Tuskegee, AL 36088. Email: ssamuels1822@yahoo.com megnin@tuskegee.edu

Recombinant proteins are now being used in the medical field as a method of treatment for many diseases such as cancer and HIV. Plants have been found to be an inexpensive and efficient means of production and expression of these therapeutic proteins. Based on these facts and the principles of plant transformation technology, synthetic antiviral peptides that are capable of inhibiting the progression of HIV have been developed at Tuskegee University by Dr. Jesse Jaynes. These synthetic peptides, jc41N and jc41ND, have lytic properties and are extremely toxic to cells; however, jc41ND contains an additional amino acid sequences that renders it inactive and non-toxic to cells when expressed. In order to express these peptides *in planta*, *de-novo* synthetic gene constructs were designed with an intron sequence to facilitate cloning in bacteria and accumulation in plants without penalty or lethality. The objective of this research is to clone and express these peptides in the sweetpotato cultivar, PI318846-3 (D-3) as an alternative production and delivery system for HIV treatment. A CaMV 35S promoter sequence was fused to each construct and the resulting recombinant plasmid are used to transform sweetpotato via *Agrobacterium tumefaciens*-mediated system. Putative transgenics will be tested to screen for the presence of each peptide genes in sweetpotato. Confirmation of stable integration and expression these peptide genes into the D-3 genome will be done via Southern and northern analyses. *Work Supported by NIH-EXPORT, USDA, Tuskegee University GWCAES and UC-Davis CREATE Igert.*

P-3016

Genetic Engineering of Phenylpropanoid Pathway of Cowpea (*Vigna unguiculata* (L.) Walp.) to Enhance Its Isoflavone Level. NAVNEET KAUR and John Brad Murphy. University of Arkansas, Dept. of Horticulture, 316 Plant Science Building, Fayetteville, AR. Email: nkaur@uark.edu

Isoflavones are phenylpropanoids, that are found mainly in legumes particularly soybean. In plants, they help in establishing a relationship with the nitrogen-fixing bacteria *Rhizobium*, act as chemoattractants, antifeedants, and anti-microbials that provide disease resistance in plants. When consumed in the human diet, they can reduce menopausal symptoms, cardiovascular disease symptoms, aid in the prevention of prostate and breast cancer, and also reduce osteoporosis. Cowpea is a highly nutritious crop, being rich in proteins and carbohydrates, but the isoflavone content is negligible. So, it would be beneficial for millions of people in developing countries such as Africa and Asia where cowpea is consumed as a staple crop, if the isoflavone content could be increased. The first committed step in the production of isoflavones is performed by a cytochrome P450-like enzyme, isoflavone synthase, which catalyzes a 2, 3 aryl migration of flavanones to isoflavones. We have cloned and characterized two isoflavone synthase genes (*Vu*IFS1 & *Vu*IFS2), with their respective promoters, from cowpea (*Vigna unguiculata* (L.) Walp.). We found that there are three poly (A) signals in the 3'UTR of *Vu*IFS1 mRNA, potentially resulting in three transcripts of different lengths. RT-PCR analysis indicated that both were expressed in stem and leaf tissues only. Both gene products were able to produce daidzein and genistein from liquiritigenin and naringenin, respectively, in a yeast in vivo experiment. After introducing soybean IFS1 with its native promoter, daidzein and genistein levels increased in transgenic cowpea calli. Conjugated forms of isoflavones such as daidzin, malonyl-glucosyl daidzin, genistin, malonyl-glucosyl genistin, glycitein (O-methylated form of liquiritigenin) were also formed, which indicates that cowpea endogenous glucosyltrasferase enzymes were able to recognize newly formed isoflavones. These results suggest that, once transgenic plants are obtained, cowpea with enhanced isoflavone levels may be produced.

P-3017

Physiological Characterization of Co-expressing of a *CAX1* Calcium Transporter and a *CRT* Calcium-binding Protein in Plant. QINGYU WU, W. Lim, and S. H. Park. Department of Horticulture, Forestry & Recreation Resources, 2021 Throckmorton Plant Science, Kansas State University, Manhattan, KS 66506. Email: qingyuwu@ksu.edu, shpark@ksu.edu

Dietary calcium (Ca) deficiencies constitute a grave and increasing global concern by the incidence of osteoporosis, a major disease worldwide. Increasing nutritionally available Ca in vegetables and fruits can potentially make them an important source of dietary Ca. One potential model to alter the Ca content in plants is to directly engineer high expression of plant endomembrane Ca transporters in the edible portion of the plant. Currently our data suggest that ectopic expression of Ca transporters (CAX) may control Ca to more desirable levels in tomato, potato, lettuce and carrot plants. Another potential model would be to manipulate Ca-binding proteins in the endoplasmic reticulum to store more Ca. Recent studies in plants also suggest that Ca-binding proteins (CRT) play a key role in the regulation of Ca^{2+} status of the plant ER and that the ER, in addition to the vacuole, is an important Ca store in plant cells. This suggests that by co-expression of the various *CAX* genes in combination with CRT, we may be able to further increase Ca in fruits and vegetables. The objective of this research was to physiologically characterize and understand the effects of modulating the expression of *Arabidopsis* Ca transporters individually and in combination with CRT in tomato. The co-expression of *CRT* and s*CAX1* in tomato demonstrate more Ca level than controls and does not appear to alter plant growth and development. Furthermore, increased Ca levels in co-expressing fruits do not appear to alter fruit morphology or yield.

GENOMICS: GENE MINING/GENE EXPRESSION PROFILING

P-3018

Quantitative and Comparative Gene Expression Profiling of Differentially Expressed Genes in Sweetpotato Storage Root Development. M. Egnin, F. SANDERS, H. Gao, G. He, S. Traore, D.

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Real Time Reverse Transcriptase quantitative PCR is now the method of choice to quantitatively profile gene expression, especially in developmental biology. Sweetpotato (*Ipomoea batatas* L.) is one of the most important root crops widely grown throughout the tropics and warm temperate regions of the world. In tuber and root crops, the initiation and the development of storage roots is one of the most important processes determining yield. Anatomical and physiological characteristics, and hormonal conditions relating to the development of storage root crops have been well studied and described. Recent studies have focused on specific developmental time points to elucidate the molecular mechanisms controlling storage root initiation in sweetpotato, which is still poorly understood. Knowledge regarding the molecular nature of storage root formation processes at different time points early on in development will provide molecular insights that could contribute greatly to our understanding of sweetpotato harvest index. In this study, quantitative transcript profiling techniques were employed to investigate changes in gene expression levels during sweetpotato storage root development. Cultivars NCC-58, TU82-155 and J6/66 were selected based on the difference in their storage root thickening potential when grown in nutrient film (NFT) and invitro techniques. RT-PCR was performed on total RNA utilizing Amersham NotI-Oligo-dT-bifunctional primer to generate adequate quantities of cDNA for AFLP identification of transcripts differentially (TDF) expressed during early stages of sweetpotato root development. Selected TDFs, possibly related to storage root development, were sequenced for generation of specific primers, and Blasted. Results from quantitative gene expression profile utilizing Real time qPCR will be reported. Research supported by NIH, USDA/CSREES, GWCAES and NASA.

P-3019

Optimizing the Pharmacological Activity of Medicinal Plants Using Natural Product Genomics Technology. B. C. LI[1], N. Monks[2], D. Brown[1], S. Gunjan[1], D. Falcone[3], and J. M. Littleton[1,2]. [1]Kentucky Tobacco Research Development Center, KTRDC-UK, 1401 University Drive, Lexington, KY 40546-0236; [2]Naprogenix, Inc. KTRDC-UK 1401 University Drive Lexington, KY 40546-0236; and [3]University of Massachusetts at Lowell, 1 University Avenue, Lowell, MA 01854. Email: bli2@uky.edu

Medicinal plants are extremely important sources for natural-products drug discovery since they contain pharmacologically valuable secondary metabolites. However, these secondary metabolites are generally extremely low in quantities in the plants, which make it very difficult to produce them at large scale commercially. We are taking a genomic approach to tackle this issue. Specifically, we use activation tagging mutagenesis (ATM) and *Agrobacterium rhizogenes*-mediated hairy root transformation system to the medicinal plants in an effort to produce mutant hairy root that would either yield higher level of the valuable secondary metabolites or produce new novel compounds. ATM, which takes the advantage of the multimerized transcriptional enhancer elements that are placed adjacent to the right border of T-DNAs, has been successfully used for mutant generation and gene discovery for a number of plant species for numerous traits. The hairy root system is ideal for this application since roots have been found to be able to produce most of the compounds an adult plant can. We focus on several medicinal plant species, and have developed an efficient hairy root transformation system for some of them, including *Lobelia cardinalis* and *Catharanthus roseus*. Our current focus is on the production and characterization of the mutant hairy roots generated by the ATM system.

P-3020

Cloning and Functional Analysis of a PR5 Protein from European Plum (*Prunus domestica* L.). A. El-Kereamy [1,2], I. El-Sharkawy [1], A. Taheri [1,] D. Errampalli [3], and S. JAYASANKAR[1]. [1]University of Guelph, Department of Plant Agriculture, 4890 Victoria Ave. N., P.O. Box 7000 Vineland Station, ON L0R 2E0 CANADA; [2]University of Guelph, Department of Cellular and Molecular Biology, Guelph, ON, N1G2W1, CANADA; and [3]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre. 4902 Victoria Av. N., P.O. Box 6000 Vineland Station, ON L0R 2E0, CANADA. Email: jsubrama@uoguelph.ca

European plum (*Prunus domestica*) is an important tree fruit crop, which is affected by a number of fungal diseases, especially during ripening resulting in serious economical loss. Although, different cultivars differ in their resistance to the fungal diseases, little is known about the molecular mechanism underline this varietal variation. A major disease that attacks all the *Prunus* fruits is brown rot caused by the fungus, *Monilinia fructicola*. Pathogenesis related proteins (PR-proteins), especially PR5, have been shown to be involved in disease resistance. In the present study we cloned a full length cDNA and promoter region of a Pathogenesis-related protein (PR-protein) class 5 named *pd*PR5. We studied its expression using four cultivars that show differential responses to brown rot resistance. Our results show that *pd*PR5 is generally induced during the ripening stage in *P. domestica*. Expression of *pd*PR5 is constitutively higher in the fruits of resistant varieties (Stanley and Violette) but increased only after infection in the susceptible varieties (Veeblue and Victory). Transgenic *Arabidopsis* plants over-expressing this *pd*PR5 gene exhibited resistance to the fungal pathogen *Alternaria brassicicola* in the detached leaves assay. Over-expression of PR5 also alters the expression of PAL, CYP79B3 and PAD3 genes, all intermediaries in the phytoalexin pathway, in the transgenic lines suggesting that *Pd*PR5 over expression alters the host defense pathway after fungal infection.

P-3021

The Molecular Characterization of ERF Proteins in Peaches Reveals Antagonistic and Redundant Effects to Disease Resistance. S. SHERIF, G. Paliyath, and S. Jayasankar. University of Guelph, Department of Plant Agriculture, 4890 Victoria Av. N., PO Box 7000 Vineland Station, ON, L0R 2E0 CANADA. Email: ssherif@uoguelph.ca

Ethylene Responsive Factors (ERFs) are a major class of transcription factors in plants. The role of certain ERFs has been studied extensively in *Arabidopsis thaliana* and other model plant species but less is known about the role of these TFs in fruit tress in response to biotic stress. *In silico* analysis and cloning using degenerate primers approach have led us to identify eight members of ERFs in peaches that could be classified into three distinct groups (El-Sharkawy et al. 2008). The gene expression analysis of these proteins by qRT-PCR has showed different expression patterns after the infection by *Xanthomonas pruni* in both resistant and susceptible peach verities. Although some ERF members are increased significantly in the resistant variety, others behave in an opposite way. In order to know under which signal transduction pathway these genes can be controlled, we investigated the effect of salicylic acid (SA), Jasmonic acid (JA) and ethylene (ET) on the expression of these genes. Most ERFs are induced largely by JA and to a lesser extent by ET. SA does not seem to induce these ERFs to any notable levels. The direct interaction among these proteins has been studied by Bimolecular Fluorescence complementation assay (BIFC). The preliminary results showed interactions among members of different subgroups. On the other hand, the functional potency of these proteins in vivo has been confirmed by their ability to induce the expression of the GUS gene with GCC-core sequences in its promoter. The degree of GUS activity with different combinations of ERF-effecter proteins can provide extra evidence on the interactions among these TFs. All together these data indicate that ERF proteins in peach not only regulate the expression of the genes associated with disease

resistance but also they can interact with each others as a putative negative feedback mechanism.

P-3022

Identifying Trithorax and Polycomb Related Genes in Tomato. M. T. SADDER[1,2], Z. Avramova[3], and A. Alsadon[1]. [1]Center of Excellence in Biotechnology Research, King Saud University, P.O. Box 2460, 11451 Riyadh, KINGDOM OF SAUDI ARABIA; [2]Faculty of Agriculture, University of Jordan, Amman, 11942, JORDAN; and [3]University of Nebraska at Lincoln, Biological Sciences, 302 Manter Hall, Lincoln, NE 68588. Email: sadderm@hotmail.com

Genes from the Trithorax- (trxG) and Polycomb-Groups (PcG) encode histone modifying activities. Because of their potential to change chromatin structure and as a consequence, to alter gene expression, these activities belong to the group of the epigenetic regulators. The ability of epigenetic factors to regulate global genome re-structuring and re-programming defines them as the major force driving plant developmental, environmental response, and adaptation mechanisms. Most of our current knowledge about the function of these factors in plants comes from studies of the model system Arabidopsis. We are interested in extending available knowledge to a major crop, tomato, in an attempt to reveal the evolution of the genes encoding epigenetic regulators in a highly-cultivated and selected plant system. By comparative genomics, using the public databases, we shall aim at revealing and analyzing trxG and PcG-related genes in tomato. We use bioinformatics tools (multiple alignment using ClustalW) to retrieve possible candidate sequences based on available data from several plant genomes. Consensus amino acid domains are used *in silico* as probes to fish candidates from Genbanks through tblastn tool. Domain identity is verified by Simple Modular Architecture Research Tool. VectorNTI tools aid in designing dedicated primers. RNA isolated from selected tissues is subjected to reverse transcription-PCR. Sequenced clones will be analyzed for splicing sites using both tblastx tools and NetGene2 World Wide Web Server. Retrieved genes will be analyzed in an evolutionary context by reconstructing phylogenetic trees. An array of tomato breeding lines and genotypes harboring desired traits will be used as entries in the mining procedure. Currently, we have retrieved at least three trithorax-related gene and a partial segment of the most conserved trithorax clone from tomato was cloned. Further analyses will include promoter elements analysis, gene polymorphism analysis using SNP mining, expression profiling and protein analysis.

P-3023

Characterization of Two Distinct 4-Coumarate:CoA Ligase Genes in Loblolly Pine. HAN-YI CHEN, Scott A. Harding, and Chung-Jui Tsai. University of Georgia, Warnell School of Forestry and Natural Resources, Department of Genetics, 120 E Green Street, Athens, GA 30602-2152. Email: chenh@warnell.uga.edu

The enzyme 4-coumarate:CoA ligase (4CL) catalyzes the conversion of hydroxycinnamates to the corresponding high-energy CoA-thioesters. It channels phenylpropanoid carbon into several downstream pathways for the synthesis of lignin and flavonoids that are central to plant development, structure, and defense. In loblolly pine (*Pinus taeda*), *Pta4CL1/2* are highly expressed in xylem and involved in lignin biosynthesis. Recombinant Pta4CL1 protein exhibits high activity toward three substrates, 4-coumarate, caffeate, and ferulate. We have identified a new gene, *Pta4CL3*, which is phylogenetically distinct from *Pta4CL1/2*. Pta4CL3 is clustered in a clade mainly composed of angiosperm 4CLs associated with the biosynthesis of non-lignin phenylpropanoids. *Pta4CL3* transcripts are more abundant in root tips and immature needles, but are essentially absent in xylem. Recombinant Pta4CL3 protein exhibits higher activity toward 4-coumarate, followed by caffeate. Under nitrogen deficiency treatment, *Pta4CL1/2* are up-regulated in immature needles, xylem, and elongating roots, while *Pta4CL3* was up-regulated in elongating roots and root tips. Our results suggest that *Pta4CL3* is involved in non-lignin phenylpropanoid biosynthesis, especially in root tissues. *Pta4CL1/2* appear to function in both lignifying and green/soft tissues. Both *Pta4CL1* and *Pta4CL3* have been ectopically expressed in aspen (*Populus tremula* × *Populus alba* clone 717-1B4) for *in planta* characterization of their roles in phenylpropanoid metabolism.

P-3024

Functional Genomics Approach Towards the Improvement of Sugarcane for Biofuel Production. M. B. DAMAJ[1], P. P. Beremand[2], J. L. Jifon[3], I. S. Curtis[1], M. A. Silva[3], V. Ochoa[1], D. Rossi[1,3], D. Odokonyero[1], T. L. Thomas[2], and T. E. Mirkov[1]. [1]Department of Plant Pathology and Microbiology, Texas AgriLife Research, Weslaco, TX 78596; [2]Department of Biology, Texas A&M University, College Station, TX 77843-3258; and [3]Department of Horticulture, Texas AgriLife Research, Weslaco, TX 78596. Email: mbdamaj@ag.tamu.edu

Sugarcane (*Saccharum* spp.), a major contributor to the world sugar industry, is gaining attention as a potential bioenergy crop. As a C_4 plant, it is capable of high biomass production especially in sub-tropical regions. However, the major limiting factor to plant production is the availability of a constant water supply. Thus, tolerance to drought stress is an important trait for manipulation through molecular breeding and genetic improvement. Here, we have investigated the molecular and physiological potential of commercial sugarcane varieties as drought tolerant, following treatment with stress regulators. Salicylic acid (SA) and jasmonic acid (JA) are major signaling molecules involved in the regulation of biotic and abiotic stresses via signaling crosstalk. Using transcriptional profiling, we examined changes in the abundance of transcripts corresponding to 6,024 genes in the Affymetrix sugarcane genechip in response to treatment with SA, JA or methyl jasmonate (MeJA). A large number of genes were co-induced by SA, JA and MeJA. The most significant co-induction pattern was observed for genes belonging to (a) defense genes involved in lignin biosynthesis and phenylpropanoid pathway, and (b) drought-responsive genes. The data revealed a high level of crosstalk between the SA-/jasmonate-induced defense and drought signaling pathways. A focused study was performed to assess changes in relative water content, total chlorophylls and photosytem II chlorophyll *a* flurorescence, in SA-, JA- and MeJA-pre-treated sugarcane following exposure to simulated drought. Pre-treatment of sugarcane with the stress regulators before simulated drought, had a protective effect against chlorophyll degradation and water loss. The mechanisms of coordination between SA-/jasmonate-induced defense and drought-responsive pathways, as well as the protective role of the SA/jasmonates in drought stress, will be useful in genetic improvement of sugarcane to allow this important bioenergy crop to be further cultivated into more arid environments.

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