2005 IN VITRO BIOLOGY MEETING

2005 Meeting of the Society for In Vitro Biology
June 5 -7, Baltimore, MD

LATE SUBMISSION ABSTRACTS

The following abstracts were unable to be included in the Abstract Issue:

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Marian Dorcas Quain, *Tuskegee University, P. Berjak, E. Acheampong, C. Bonsi, V. Brown G. He, and M. Egnin*

SECONDARY METABOLITES - Monday, June 6, 2005, 2:30 pm

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STRESS TOLERANCE - Monday, June 6, 2005, 2:30 pm

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Vladimir Sidorov, *Monsanto Company, L. Gilbertson, P. Addae, and D. Duncan*

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VT-3006 Estrogenic Activity in Tissue Culture Plates Is Not Due To Leaching of Bisphenol A
Laura Davis, *Ursinus College, C. Biswanger, and R. Roberts*

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Salih S. Kocer, *SUNY Stony Brook, E. J. Roemer, and S. R. Simon*

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VT-3011 Adipocytic Differentiation of Stromal Arterial Preadipocytes Induced In Vitro by Rosiglitazone
Miguel Reyes, *Faculty of Medicine U. J. E. D., and B. Lazalde*

INTERACTIVE POSTER SESSIONS

REGENERATION AND MICROPROPAGATION
P-3000
Developing *Dioscorea* Species and *Solenostemon Rotundifolius* Complementary Protocols for Germplasm Conservation and Stable Genetic Integrity. M. D. QUAIN[1,2,3], P. Berjak[2], E. Acheampong[1], C. Bonsi[3], V. Brown[3], G. He[3], and M. Egnin[3]. [1]Tissue Culture Laboratory, Department of Botany, University of Ghana, Legon, Accra GHANA; [2]School of Life and Environmental Sciences, University of Natal, Durban, 4041, SOUTH AFRICA; and [3]Center for Plant Biotechnology Research Tuskegee University, Tuskegee, AL 36088. Email: marianquain@hotmail.com, megnin@tuskegee.edu

Dioscorea sp and *Solenostemon Rotundifolius* are endangered tropical staple crops, due to problems associated with the conventional germplasm conservation and propagation methods. The in vitro slow growth conservation method so far employed have served only short to medium term purposes, therefore, efforts were made to complement the technique by developing cryopreservation protocol. Excised shoot tips and axillary buds of plants under study were subjected to various cryopreservation protocols. Silica gel dehydration-based protocol did not yield survival following cooling. *Dioscorea* sp subjected to plant vitrification solution II (PVS2) yielded 40% survival when tested with tetrazolium salts, 20% plantlet regeneration following slow freezing to -70º C and 10% callus development following freezing in liquid nitrogen at -196° C. *Solenostemon Rotundifolius* yielded no survival on treatment with PVS2 resulting in no survival on cooling. However, survival was obtained following treatment with half strength PVS2, and vitrification solution supplemented with lower concentrations of glycerol and sucrose. Developing somatic embryoids offer better source of explants for long-term cryo-storage. Parameters including carbon source, vitamins, growth regulators, luminance, photoperiod and temperature are being optimized to increase the storage period of in vitro cultures beyond 24 months. Genetic variability of long-term cultures will be assessed using molecular marker techniques such as AFLP. Work supported by UNU/INRA, AgSSIP-World Bank and Tuskegee University.

SECONDARY METABOLITES
P-3001
The Effect of Irradiance Environment on Potency of *Vaccinium angustifolium* (Wild Blueberry) Suspension Culture Extracts Against L1210 Leukemia Cells. TRISTAN F. BURNS KRAFT, Mary Grace, and Mary Ann Lila. Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, 1201 S. Dorner Dr., Urbana, IL 61801. Email: tkraft@uiuc.edu

Vaccinium angustifolium Aiton (wild blueberry) fruits have been intensively researched for potential health benefits and are rich sources of antioxidants. Extracts of wild blueberry fruits have demonstrated the ability to inhibit multiple stages of carcinogenesis. Plant cell culture is an alternative method for producing wild blueberry phytochemicals without many of the associated interfering compounds in fruits, in a more controlled environment that can be manipulated to influence the types and amounts of active compounds. Wild blueberry stock suspension cultures were grown on a 7 day cycle at 25° C on a Gyrotary shaker set at 150 rpm in a solution culture medium. Treatments were set up for 10 days in one of three environments: darkness, transferred from darkness to an irradiated environment (100 micromol m^{-2} s^{-1}) on day 0, and continual growth in an irradiated environment. Cultures were harvested and extracted using 70% aqueous acetone (crude extract). The resulting extract was fractionated using liquid vacuum chromatography on a Toyopearl support, which resulted in five fractions (TP1-TP5). These fractions and the crude extract were tested for ability to inhibit growth of murine leukemia L1210 cells. TP5 was the only fraction with significant activity. TP5 from the dark to light treatment was twice as active as TP5 from cultures in continuous light; TP5 from cultures grown in darkness was not significantly active. Preliminary thin-layer chromatography analysis of TP5 shows that cultures from the dark to light treatment contained higher levels of proanthocyanidins than the other treatments, which indicates that the transfer to an irradiated environment provokes the production of secondary bioactive compounds.

STRESS TOLERANCE
P-3002
Expression of CBF3 Under the Stress Inducible Promoter Rd29A Using Split-seed Explant to Enhance Drought and Cold Tolerance in Maize. DIAA AL-ABED, Parani Madasamy, Talla Reddy, Stephen Goldman, and Sairam Rudrabhatla. Plant Science Research Center, University of Toledo, Toledo, OH 43606. Email: dalabed@utnet.utoledo.edu

Efficient, genotype-independent tissue culture systems are the ideal targets for genetic engineering efforts in any crop species. We demonstrate here an efficient regeneration system based on a novel explant, split-seed. By splitting the seed longitudinally, three different sources of undifferentiated cells namely: Scutellum, Coleoptilar ring and Shoot apical meristems, can be simultaneously targeted to enhance the regeneration and/or increase the ability of DNA transfer. Split-seed explants were used to initiate callus on induction medium supplemented with different concentrations of 2, 4-dichlorophenoxyacetic acid (2, 4-D), or cultured on multiple shoot induction medium supplemented with various concentrations of 6-benzylaminopurine (BAP) and 6-furfurylaminopurine (Kinetin). Callus induction frequency exceeded 92% and adventitious shoots formation frequency was 76% with a range between 2-26 shoots per explant within a period of 2-3 weeks. We further wanted to explore split-seed explant for the genetic transformation of maize. The C-repeat/dehydration-responsive element binding (CBF3/DREB1A) transcription factor under the stress-inducible promoter Rd29A was transformed into split-seed explants via biolistics transformation. Previous reports have shown that the expression of CBF transcriptional factors family enhances drought and cold tolerance in various plant species (A. Pellegrineschi et al, 2004, Glimour, S. J. et al., 2000, Kasuga, M. et al., 1999, Sang-Choon Lee et al., 2004, Stockinger, E. J. et al., 1997 and Yamaguchi-Shiozaki, K. et al., 1994). Since moisture stress is one of the major factors affecting maize crop productivity, our main objectives are to incorporate the split-seed regeneration system with genetic transformation to study the effects of CBF3 expression in maize and to enhance drought and cold tolerance in maize. We identified eighteen putative CBF3 transgenic plants by PCR and were further confirmed by southern blot analysis. For the first time, we report a robust and efficient regeneration protocol coupled with a high frequency transformation system in maize using split-seed explant.

TRANSFORMATION
P-3003
Agrobacterium-mediated Transformation of Seedling-derived Maize Callus. V. SIDOROV, L. Gilbertson, P. Addae, and D. Duncan. Monsanto Company, 700 Chesterfield Pkwy. N., St. Louis, MO 63017. Email: vladimir.a.sidorov@monsanto.com

Efficient production of seedling-derived Type I callus was demonstrated for several corn genotypes including commercial inbred lines. Seeds were germinated on MS-based medium containing 10 mg l^{-1} picloram (Picl) and 3 mg l^{-1} 6-benzylaminopurine (BAP), which induced the development of axillary buds in the area of coleoptilar node. Nodal sections of 7 to 10-d old seedlings were isolated, split longitudinally and placed on callus induction medium supplemented with 2.2 mg l^{-1} Picl and 0.5 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). For lines L4 and L9 the frequency of embryogenic callus induction was 38-42% based on calli per split nodal section. Frequency of callus induction from split nodal sections of seeds germinated on media without growth regulators was 0 to 3%. Seedling-derived callus of 5 genotypes was used for *Agrobacterium*-mediated transformation. Two constructs containing the green fluorescence protein (GFP) gene and genes for either neomycin phosphotransferase II (NPTII) or glyphosate (Gly) selection were used in transformation experiments. Transformation frequency varied from 2 to 15% and about 60% of the R_0 plants had 1-2 copies of transgenes.

EDUCATION POSTER ABSTRACTS

E-3000
Introductory Undergraduate Cell Culture Laboratory Training. D. JAYME, D. Heaton, S. Gold, and G. Frederick, Department of Biochemistry, Brigham Young University–Hawaii, 55-220 Kulanui St., Box 1967, Laie, HI 96762. Email: jaymed@byuh.edu

The undergraduate biochemistry program at BYU-Hawaii traditionally provided a strong foundation in prokaryotic biotechnology applications. However, there was no student exposure to basic eukaryotic cell culture techniques. Given abundant career opportunities for graduates with modest expertise in these techniques, we inaugurated such laboratory training. We rapidly learned that most standard introductory cell culture training programs, both commercial and academic, focused upon applications of cultured cells, rather than basic understanding of fundamental techniques and optimization of nutritional elements of the exogenous fluids. We tested a four-module curriculum with a pilot class of ten biochemistry majors. Fundamental knowledge was communicated through focused lectures, supplemented by reference texts and electronic media. Demonstrations and electronic protocols guided students through laboratory procedures. The primary module encompassed basic issues of safety, aseptic technique, nutrient media preparation, and cell culture laboratory instrumentation and design. The following two modules emphasized fundamental laboratory techniques for cultivation of suspension and adherent cells, respectively. For the final module, the laboratory group was divided into two teams. Each team was supplied a murine hybridoma or primate cell line and challenged to design experiments utilizing previous techniques to optimize yields of a target monoclonal antibody or model virus. Group results were presented electronically and in poster format. This paper presents results from this pilot study and invites feedback from undergraduate educators regarding their experiences, successful or otherwise.

E-3001
Practical Method for Teaching Plant Tissue Culture in Class with Limited Facilities in Developing Country. G. Y. WIJAYA. Faculty of Biotechnology, Universitas Katolik Indonesia Atma jaya, Jakarta 12930, INDONESIA. Email: grace.wijaya@atmajaya.ac.id

In Indonesian National "Competence Based Curriculum 2004" for high School education, topics on "Plant Biotechnology" and "Plant Growth and development" with practicals in "Observing plant growth and development" are included in Biology Subject. The delivery of these topics are still very limited in one-way conventional teaching style. The efforts for delivering related practicals faced difficulties with the large number of students in a class (around 40-50 students) which impacts on the facilities, time, and budget required. The suitable practical methods should be simple, required materials and equipments that are easy to obtained and inexpensive; and possible to be performed by large number of students at once. Plant tissue culture methods that published by Stiff (2005) and Storr (1985) with some modifications were put on trial. As a start, the trial was performed to 40 university students that have never been exposed to such technique before. The result from this first trial showed that the contamination was quite high (above 60%). On the second trial, further modifications were introduced in the type of explant, the way of cutting and sterilized the explant, the type of flask used, and the used of flame or sterile plastic cabinet. It was concluded that the most suitable method was performed by using explant that possible to be cut into required planting size before exposing to bleach; by using flask with small mouth and plastic cap; and by using flame. The used of flame was still very difficult to eliminate without using proper laminar air flow cabinet due to high temperature and humidity of standard classroom used, that do not have any air conditioning facility. The use of media ingredients and equipments that could eliminate the use of flame will be put on the next trial involving high school students.

PLANT POSTER ABSTRACTS

BIOTECHNOLOGY
P-3004
Development of Transgenic Castor Plants with Reduced Levels of Ricin Toxin and Hyper-allergenic 2S Albumin. Y. J. AHN, G. Q. Chen, and T. A. McKeon. U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710. Email: yjahn@pw.usda.gov

Castor plants are the only commercial source of the unique hydroxy fatty acid, ricinoleic acid, which is essential for producing high-quality lubricants, paints, plastics, and coatings. However, castor cultivation poses serious health concerns for the growers due to the presence of ricin toxin and hyper-allergenic 2S albumin in its beans. Genetic engineering appears to be an effective approach to develop safe castor plants with reduced levels of these hazardous proteins. However, efficient transformation techniques for castor plants have not been reported yet. In this study, we have examined regeneration and

genetic transformation of castor plants. Multiple shoot induction from embryo axes has been optimized. Various conditions for Agrobacterium-mediated transformation have been tested to obtain maximal transient and stable GUS expression in infected explants. Establishment of a reliable transformation protocol will allow us not only to obtain safe castor plants but also to perform a broad range of transgenic studies with this important oil crop, castor.

P-3005

A Transcriptional Profiling System to Study Tobamovirus Transfection in *Nicotiana benthamiana*. J. L. BUSTO, P. H. Kaufusi, B. Camara, and M. H. Kumagai. [1]College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, 96822 and [2]Institut de Biologie Moleculaire des Plantes du Centre National de la Recherche Scientifique 67084 Strasbourg, FRANCE. Email: jbusto@hawaii.edu

We are using *Nicotiana benthamiana* as a model plant to study the utility of plant viral vector technology for metabolic engineering and functional genomic applications. The traditional approach to redirect plant pathways has involved the protracted development of transgenic plants. We report here a powerful alternative strategy of combining viral vector transfection with transcriptional profiling technologies to rapidly filter and assess gene expression changes in plant pathways. Inoculation of healthy plants with tobamoviral vectors causes an epigenetic expression of heterologous genes that impact host gene expression. To determine transcriptional changes caused by overexpression or cytoplasmic inhibition of gene expression, potato cDNA microarrays developed by The Institute for Genomic Research (TIGR), and GeneSpring analysis software (Agilent Technologies) were employed. To illustrate, transfection with TTU51 CTP CrtB RZ carrying a phytoene synthase cDNA derived from Erwinia herbicola significantly alters the expression of genes encoding enzymes in the leaf carotenogenesis pathway. There is an unexpected accumulation of endogenous phytoene synthase, validated using quantitative real-time PCR, as well as an upregulation of transcripts both upstream and downstream of the targeted enzyme. In addition, HPLC data shows a 3-fold increase of beta-carotene and an accumulation of colorless phytoene and phytofluene in transfected leaves. Phytoene, which accumulates using this and other viral vector constructs carrying carotenogenic enzymes, does not appear to have a direct role in the regulation of this pathway. Examples of the impact of tobamoviral transfection on other pathways, including plant secretion, will be presented.

P-3006

BCTV Replicon-assisted Expression of Recombinant Proteins in *Agrobacterium*-inoculated Leaf-disks of Tobacco and Tomato Plants. I. S. CHUNG, K. I. KIM, and Y. J. Kim. Department of Genetic Engineering and Plant Metabolism Research Center, Kyung Hee University, Suwon, KOREA 449-701. Email: ischung@khu.ac.kr

Beet curly top virus (BCTV) belongs to geminivirus subgroup II. This virus infects a wide range of dicotyledonous plant hosts and has a monopartite genome that resembles in its organization the A genome components of bipartite subgroup III geminiviruses. In this study, we examined the use of BCTV elements to enhance the expression of recombinant GFP in *Agrobacterium*-inoculated leaf-disks of tobacco and tomato plants. Southern hybridization analysis showed that unit-length DNAs of replicated BCTV could be detected 3 and 6 days after the cultivation of Agrobacterium-inoculated leaf-disks of tobacco and tomato plants. Recombinant GFP was expressed with a molecular size of ~29 kDa in Agrobacterium-inoculated leaf-disks using a BCTV replicon-assisted expression vector system. The expression level of recombinant GFP using BCTV-replicon assisted system was much higher than that using the control vector system without BCTV replicon. Our findings show that BCTV replicon-assisted expression could be potentially useful in the production of recombinant proteins in a plant cell system. This work was supported by a grant from the Rural Development Administration through Bio-green 21 project.

P-3007

Functional Expression and Characterization of Sterol Glucosyltransferase cDNA from *Arabidopsis thaliana*. I. S. CHUNG, H. Y. Chung, N. I. Baek, S. K. Kim, and S. H. Choe. Department of Genetic Engineering and Plant Metabolism Research Center, Kyung Hee University, Suwon 449-701, KOREA. Email: ischung@khu.ac.kr

Sterol glycosides are characteristic lipids of plant membranes. The biosynthesis of these lipids can be catalyzed by sterol glucosyltransferases (SGT). We cloned the SGT cDNA from total RNAs of Arabidopsis thaliana using RT-PCR and investigated its functional expression in DES systems. The expression of recombinant SGT at 5 days after induction using 0.5 mM $CuSO_4$ was confirmed by Western blot analysis. Recombinant SGT was secreted into the medium with a molecular weight of 65 kDa. Recombinant SGT was also rapidly purified to near homogeneity by a simple one-step Ni^{2+} affinity purification procedure. Protein purity was analyzed by SDS-PAGE and silver staining. To measure the purified SGT activity, we also performed in vitro assays with UDP-(U-^{14}C) glucose (donor) and various substrates (acceptors). Recombinant SGT glucosylated only β-sitosterol and stigmasterol. We are currently analyzing transgenic *A. thaliana* plants overexpressing the SGT cDNA. In this presentation we will also describe the function of this SGT cDNA in transgenic *A. thaliana* plants. This work was supported by grants from the Rural Development Administration through Bio-green 21 project and from the Korea Science and Engineering Foundation through the Plant Metabolism Research Center, Kyung Hee University.

P-3008

Genetic Transformation of an Elite Georgia Cotton Line. K. D. DA, P. Ozias-Akins[1], and P. W. Chee. Department of Crop and Soil Science, University of Georgia, Tifton Campus, Tifton, GA 31794 and [1]Department of Horticulture, University of Georgia, Tifton Campus, Tifton, GA 31794. Email: pwchee@arches.uga.edu

Georgia is now the second largest cotton producing state in U. S. and cotton has become the number one cash crop in the state of Georgia. Market demand for improved quality cotton cultivars impels the development of a more efficient protocol for the genetic transformation of commercial cultivars and elite breeding lines. Recent reports in cotton transformation indicated that the green fluorescent protein (GFP) gene is a more efficient visual selection marker compared to chemical selection marker genes. Previous work in our lab have shown that some of the elite Georgia breeding lines are capable of producing embryogenic callus that underwent somatic embryogenesis. Here we report the genetic transformation of an elite Georgia cotton line, GA98033, with the GFP gene via particle bombardment. Our results showed that the bombardment transformation of embryogenic callus and

GFP visual selection method could produce transgenic plants from both high and low regeneration potential cotton genotypes. The transgenic somatic embryos could regenerate directly from single transformed cell or indirectly from transformed callus. The transformation frequency is about 3/bombardment, with the time involved in transgenic somatic embryo formation is 15 and 37 days for transformed single cell and callus, respectively.

P-3009

The Effect of Sugar Ratio on Paclitaxel Production in Suspension Cell Culture of *Taxus chinensis*. J. A. KIM, C. H. Kim, J. Y. Song, and H. J. Choi. Samyang Genex Food & Bio Research Center, Daejeon, 305-717, SOUTH KOREA. Email: jinahne@genex. co.kr

Paclitaxel production pattern of *Taxus chinensis* suspension cell culture on the medium supplemented with sucrose was a biphasic pattern with several days of staggered phase. To improve paclitaxel production by removing staggered phase, *T. chinensis* cells was cultured on the media supplemented with glucose, fructose or various ratio of glucose and fructose with a constant total carbon level. In case of 1:2 (glucose:fructose) ratio, the stagnation of paclitaxel production was not observed at the point of utilized sugar changed, and the highest paclitaxel production was achieved. Base on these results, it was suggested that paclitaxel metabolism shift in cultured cells would be related to change of utilized sugar. These results could give many benefits to commercial production of paclitaxel by not only increasing productivity but also shortening production period.

P-3010

Enhancing the Nutritional Value of Soybean (*Glycine max*) by Genetic Engineering. ZHIWU LI[1], Juliane S. Essig[1], Melissa A. Schapaugh[1], S. Muthukrishnan[2], Bryan E. Hainline[3], Harold N. Trick[1]. [1]Plant Pathology Department and [2]Biochemistry Department, Kansas State University, Manhattan, KS 66506; and [3]Indiana University School of Medicine, Indianapolis, IN 46202. Email: zwli@ksu.edu

Expression of novel proteins in soybean seeds has the potential for overall enhancement of the nutritional quality of the seeds as well as providing specific benefits to certain medical conditions. Here we report two projects addressing these goals. We will discuss the results of introducing the native gamma-zein gene in soybean under control of the beta-conglycinin promoter. This protein, rich in sulfur-containing amino acids accumulated in abundance in transgenic seeds. The percentage of alcohol extractable proteins in total seed proteins ranged between 2.54% and 6.49% in seeds from the transgenic lines expressing γ-zein gene, whereas the corresponding value in seeds from a non-transformed control line was only 0.35%. Transgenic seeds from transgenic progenies strongly expressed the γ-zein protein (up to 819 ng per mg of dry seed flour). Additionally the gamma-zein protein was modified to lack phenylalanine. The goal of this project is to express a PHE-free protein in soybean seeds for Phenylketonuria (PKU) patients: PKU is a serious recessively inherited genetic disorder in which the human body cannot break down the amino acid phenylalanine (PHE). We have constructed a synthetic version of the 27kD γ zein gene by codon optimizing the sequence for soybean expression and by replacing PHE codons with those for Tyrosine. We will report are transformation, recovery, and molecular analyses of this gene. We will also report our efforts to biochemically isolate this protein and quantify the amount of protein being produced.

P-3011

Genetic Authentication of Peppermint Varieties with Microsatellite DNA Fingerprinting. JIE LIU and Tony Chen[1]. A. M. TODD Company, 4091 West 11th Avenue, Eugene, OR 97402 and [1]Department of Horticulture, Oregon State University, Corvallis, OR 97331. Email: jliu@amtodd.com

Varietal identification is essential in plant improvement and associated research. Since environment conditions alter the growth forms and scents of mints, their identification and proprietary protection based only on morphology and oil profile will be difficult to establish. Microsatellite DNA analysis is a state-of-art technology for reliable plant genotyping. We examined total 48 mint DNA samples from different peppermint varieties and populations with 20 microsatellite DNA markers. We found that these markers are very abundant and highly polymorphic in Mentha species. Genetic uniqueness for different mint varieties and their relationships with each other have been described. The ability of microsatellite DNA to reveal high allelic diversity is particular useful in distinguishing between closely related varieties. These markers can also be used in product quality assurance, marker-assisted selection and proprietary protection.

P-3012

Cloning gl4, gl5, gl6, gl14, gl19 Using Map-based Positioning. D. NAMOKOYI[1], Fan Li[2], and Patrick Schanble[2]. [1]Center for Biotechnology, Fort Valley State University, Fort Valley, GA 31030 and [2]Iowa State University, Center for Plant Genomics, Ames, IA 50011. Email: dhirs0@fvsu.edu

Most plants are coated with a complex mixture of lipids referred to as cuticular waxes that serve a variety of roles in plant growth and development including protection from desiccation, ultra-violet radiation, and pathogens. These lipids are composed of very long fatty acids along with their derivatives such as alkanes, ketones, esters, aldehydes and alcohols. The accumulation of cuticular waxes is most visible during the juvenile phase of the maize plants. These phenotypes also present an accumulation of cuticular waxes that are identifiable by the absence of a waxy bloom that results into a shinny, glossy (gl) phenotype. A total of 17 gl has been identified in maize that play a role in the quantities and composition of cuticular waxes. The purpose of this project is to use map-based cloning methods and try cloning gl4, gl5, gl6, gl14, and gl19. Map-based cloning is used to identify the genetic basis of a mutant phenotype but looking for linkage to markers whose physical location in the genome is known. This is help show the relationship between cuticular waxes and VLCFAs and their roles in seed oil levels. Several genes, gl4, gl5, and gl6 showed polymorphism. gl14, and gl19 did not show any polymorphism with markers tested.

P-3013

FLP-mediated Site-specific DNA Recombination in Maize. PEIYU ZENG[1], Colin Lickwar[1], Joel M. Chandlee[1], Albert P. Kausch[1], Jeffrey R. Seemann[1], and Hong Luo[1,2]. [1]Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02881 and [2]HybriGene Inc., 530 Liberty Lane, West Kingston, RI 02892. Email: pzeng@mail.uri.edu

We have evaluated the feasibility of using FLP/FRT site-specific recombination system in maize for genome engineering. The objective of our study was to produce transgenic maize lines stably expressing the yeast FLP recombinase and evaluate in vivo efficacy of FLP-mediated site-specific DNA recombination. We hypothesized that transgenic corn plants containing a recombination-reporter construct, in which the corn ubiquitin promoter and the reporter gusA coding region is separated by the hygromycin marker gene flanked by directly oriented FRT sites will not show GUS activity. When crossed with plants containing stably expressed FLP recombinase, FLP should excise the blocking fragment (hyg gene) thus bringing together the ubiquitin promoter and the downstream gusA gene, giving rise to GUS expression in the hybrid plant. Using Agrobacterium-mediated transformation, we obtained transgenic plants with the FLP-containing construct or the FRT-containing recombination-reporter construct. Transient assay of GUS expression by DNA bombardment demonstrated the function of FLP recombinase in the cells of corn. The next step would be to test the in planta efficacy of FLP mediated recombination. The results obtained will allow for evaluation of the feasibility of using the FLP/FRT system as a tool for genome engineering in maize.

DISEASE RESISTANCE

P-3014

Role of Transcriptional Regulation of *Arabidopsis* FBL Genes in Plant Defense. W. ABOU ALAIWI, S. Zhang, R. V. Sairam, and S. Goldman. Plant Science Research Center, The University of Toledo, 2801 W. Bancroft MS604, Toledo, OH 43606. Email: waboual@utnet.utoledo.edu

FBX proteins contain a structurally conserved F-box motif involved in protein-protein interaction. In addition, FBX proteins often contain one or more protein-protein interaction motifs such as leucine-rich repeats (LLRs), WD-40 repeats and kelch repeats. There are about 700 predicted FBX proteins in Arabidopsis, some of which have been shown to be involved in flower development, disease resistance, sugar metabolism, hormonal signal transduction, cell cycle control, leaf senescence and shoot lateral branching, photomorphogenesis and circadian rhythm. Many of the known disease resistance genes (R genes) encode proteins with LLRs, including TIR-NBS-LRR, CC-NBS-LRR, and few others. A unique group of proteins called FBL proteins contain both an F-box and numerous LRRs motifs. In *Arabidopsis*, about 25 FBL genes were found based on sequence analysis. Among the FBL genes, FBL1 (TIR1), FBL2 (or COI1), and FBL6 (or EBF1) have been shown to play a role in auxin response, jasmonic acid response, and ethylene response, respectively, while FBL5(Max2/ORE9) may regulate shoot lateral branching and leaf senescence. However the rest of the FBL genes are yet known for any functions. We are interested in uncovering the role of transcriptional regulation of these *Arabidopsis* FBL genes in plant defense. Fifteen FBL genes, in addition to three expression markers such as COI1, PDF1.2a, and PR-1, were isolated from *Arabidopsis* seedlings by RT-PCR with EST sequence-specific primers and verified by sequencing. We are analyzing the transcriptional expression profiles of each individual gene, by northern hybridization, in wild-type Arabidopsis plants under abiotic and biotic stress in an attempt to unravel any involvement of these genes in the SA-, JA-, ET-mediated signaling pathways. Eight Arabidopsis mutants whose defense pathways have been changed due to a single gene mutation within its genome will be used to further study the expression of the selected FBL genes. The significance of this work will be discussed in details.

EMBRYOGENESIS/REGENERATION/ MICROPROPAGATION

P-3015

In *Vitro* Propagation and Chemical Characterization of *Hypericum polyanthemum* Klotzsch ex Reichardt. A. P. M. BERNARDI[1,2], D. I. Luz[1], N. Maurmann[1], G. von Poser[2], and S. Rech[1]. [1]Laboratório de Biotecnologia Vegetal and [2]Laboratório de Farmacognosia, Programa de Pós Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, 90610-000, Porto Alegre, BRAZIL. Email: paula@farmacia.ufrgs.br

The flora of Southern Brazil is rich in medicinal plants and most of the species are collected without proper replenishment. From the genus *Hypericum* approximately 20 species are found. *Hypericum polyanthemum* Klotzsch ex Reichardt showed a remarkable analgesic activity: its cyclohexane extract displayed antinociceptive effects, which seem to be mediated by the opioid system. From the apolar extract of this plant three main compounds,6-isobutyryl-5,7-dimethoxy-2,2-dimethyl-benzopyran (HP_1), 7-hydroxy-6-isobutyryl-5-methoxy-2,2-dimethyl-benzopyran (HP_2) and 5-hydroxy-6-isobutyryl-7-methoxy-2,2-dimethyl-benzopyran (HP_3) were isolated and evaluated for the monoamine oxidase MAO-A and MAO-B inhibitory activities, being HP_3 significantly active. Due to the relevant biological properties of *H. polyanthemum* and as the species is critically endangered and included in the Southern Brazilian list of extinction plants we chose to standardize a protocol for in vitro propagation of the species and to analyse the benzopyrans accumulated in the propagated plantlets. The plant was micropropagated using shoot tips explants on MS medium supplemented with different concentrations of BAP. Shoot proliferation was enhanced and rooting was achieved with 0,4 mg/l BAP alone or combined with 0,2 mg/l NAA and regeneration was obtained on growth regulator-free medium. Regenerated plantlets were transplanted to a potting mixture of soil and vermiculite 1:1 under greenhouse conditions and survived acclimatization producing healthy plants. The lipophylic phenolic compounds were evaluated in the aerial parts of the micropropagated plantlets and compared with the field-grown plants. Plantlets displayed the same benzopyrans HP_1, HP_2 and HP_3 found in the field-grown plants.

P-3016

Isolated Microspore Culture of Canadian 6x Triticale Cultivars. F. EUDES and E. Amundsen. Agriculture and Agri-Food Canada, Lethbridge Research Centre, P.O. Box 3000, Lethbridge, Alberta, CANADA T1J 4B1. Email: eudesf@ agr.gc.ca

Isolated microspore culture was conducted on nine Canadian triticale cultivars (X Triticosecale Wittmack) using two induction media developed for wheat, with or without 100 g l-1 Ficoll. Significant interactions were observed for the number of embryos and calluses induced, green and albino plantlets regenerated and fertility of green plants. Ficoll was beneficial in both media to increase numbers of embryos and green plants for all cultivars. Overall, medium NPB99 supplemented with ficoll provided the most suitable condition for most cultivars. AC Alta performed slightly better on CHB3 supplemented with Ficoll. Only cv. Wapiti was not amenable to androgenesis. The cultivars AC Certa, AC Copia, AC Alta, Sandro, Ultima, Frank, Pronghorn and Banjo produced respectively 10, 9, 6, 5, 4, 3, 3 and 1 green plants per Petri dish (35,000 microspores), on their optimum

treatment. Twenty two percent of total lines produced were fertile, and considered doubled haploids. The application of isolated microspore culture to triticale, opens new possibilities in breeding triticale, for the utilization of in vitro selection and genetic engineering.

P-3017
Comparative In Vitro and Early Nursery Performance of Adventitious Shoots from Cryopreserved Cotyledons and Axillary Shoots from Epicotyls of the Same Zygotic Embryo of Control-pollinated *Pinus radiata*. CATHY L. HARGREAVES[1], Lynette J. Grace[1], Susan A. van der Maas[1], Mike I. Menzies[1], Satish Kumar[1], D. Grant Holden[1], Martin N. Foggo[2], Charlie B. Low[1], and Mike J. Dibley[1]. [1]ensis, and [2]67 Tihi Road, Springfield, Rotorua, NEW ZEALAND. Email: cathy.hargreaves@forestresearch.co.nz

The results presented in this talk compare production and performance of adventitious shoots from cryopreserved cotyledons, with axillary shoots formed from epicotyls of the same zygotic embryo of radiata pine (*Pinus radiata* D. Don). Genotypes from 10 control-pollinated families of radiata pine were compared in two treatments for shoot initiation, in vitro growth, rooting and early nursery performance. Plant growth in the nursery bed was assessed by height measurements taken 2 and 7 months after lining out. Following 8 months in the nursery bed, physiological age of all genotypes was assessed prior to field planting. Genotype capture was higher from the cryopreserved cotyledons than from the epicotyls. Early shoot multiplication from both treatments was good although, after four transfer cycles, the epicotyl cultures showed improved elongation and subsequent higher rates of multiplication. Following 6 months of in vitro growth, a sample of shoots from both treatments (9 families, 5-9 genotypes per family) was given an auxin pulse. Shoots from the adventitious origin were slower to root than the epicotyl-derived shoots, although overall rates of rooting were satisfactory (77% and 84% respectively). At lining-out, the plants of adventitious origin were shorter and this was still apparent at lifting for field planting. An assessment of relative physiological age indicated that all plants of adventitious origin showed some increased maturation (6-18 months more than actual chronological ages). With radiata pine, some increase in physiological age may improve form and hardiness in planting stock and may therefore not be disadvantageous.

P-3018
The Effect of Plant Growth Regulators on Pineapple Micropropagation. Kwame K. Williams and THOMAS W. ZIMMERMAN. University of the Virgin Islands Biotechnology & Agroforestry Program, RR1 Box 10,000, Kingshill, VI 00850. Email: tzimmer@uvi.edu

Pineapple is from the tropical American region and has been distributed and grown throughout the world's tropical area. This study was conducted to compare the micropropagation of different pineapple varieties found in the Caribbean, 'Sugar Loaf', 'Black Antigua', 'Jamaican Sugar' and 'Bermuda' with the Cayenne lines grown in other parts of the world. Eleven pineapples varieties were obtained from the USDA Tropical Germplasm Repository. Cuttings of each variety were grown on Murashige and Skoog medium with either Benzyladenine (BA) or BA and Kinetin (Kin) at a concentration of 10 uM. After 90 days the proliferating clumps of shoots were separated and grown on MS medium without plant growth regulators for 60 days. The BA & Kin medium resulted in significantly more plants than BA alone for four varieties. BA alone resulted in a significant increase over BA and Kin for three varieties. There was no difference in plant production between the tissue culture media for four pineapple varieties. Pineapple varieties vary in their response to plant growth regulators for in vitro plant production.

GENE TRANSFER TO PLANTS
P-3019
Rapid Evaluation of Soybean Promoters Using GFP and an Automated Image Collection and Analysis System. JOHN J. FINER[1], Joseph M. Chiera[1], Robert A. Bouchard[1], Yu-Tseh Chi[2], and Peter P. Ling[2]. [1]Department of Horticulture and Crop Science and [2]Department of Food, Agricultural and Biological Engineering, OARDC/The Ohio State University, 1680 Madison Ave., Wooster, OH 44691. Email: finer.1@osu.edu

Two forms of two different soybean promoters were isolated, fused to the GFP coding region and introduced into cotyledonary tissue of lima bean for rapid evaluation of promoter activity. A soybean (*Glycine max*) ubiquitin promoter was amplified from genomic DNA as either a full length fragment (Gmubi) or a truncated form, without putative intronic sequences (Gmupri). A HSP90-like promoter was isolated using the GenomeWalkerT System with the 5' sequence of an early embryo-specific EST, identified from microarray data. A long form (HSP90Stu) and a short form (HSP90Dra) were isolated from the two relevant libraries and evaluated. These 4 promoters were compared to a 35S promoter for timing and intensity of expression. Cotyledons from freshly imbibed lima bean (*Phaseolus lunatus*) cotyledons were excised and bombarded with one of the 5 different promoter constructions. GFP expression was recorded and tracked every hour for 100 hours using an automated image collection system, that consisted of a dissecting microscope, a digital camera and a 2 dimensional robotics platform, all under computer control. Following image collection, GFP expression was quantified as intensity per focus, which was relatively consistent, regardless of foci counts. The promoter which consistently yielded the highest transient GFP expression levels in lima bean cotyledonary tissue was Gmubi. The pre-intronic version of this promoter gave rise to lower expression levels, suggesting a role for the intron in enhancing transgene expression. The truncated version of the HSP90-like promoter gave altered expression compared to the longer version, indicating the loss of regulatory sequences. The level and timing of peak GFP expression, and the general shape of curves, showing GFP expression over time, provide some early clues to the characteristics of different isolated promoters.

P-3020
Towards Marker-free Site-specific Gene Integration in Plants. VIBHA SRIVASTAVA. University of Arkansas, Crop, Soil & Environmental Sciences, 115 Plant Science Bldg., Fayetteville, AR 72701. Email: vibhas@uark.edu

Precise transgene locus can be efficiently obtained by Cre-*lox* mediated site-specific gene integration. Transgenic plants containing site-specific integration locus display much lower expression variability between independent lines, and express transgene consistently over successive generations. However, site-specific integration locus contains selectable marker genes and *cre* gene, which are unnecessary and undesirable in the transgenic plants. Using two site-specific recombination systems, a strategy has been proposed to convert this site-specific integration locus into `clean` locus. The strategy utilizes first recombination system, such as Cre-*lox,* to generate site-specific integration locus,

and a second recombination system, such as FLP-*FRT*, to excise the undesirable genes. It is necessary that the second recombination system is expressed only after the site-specific integration locus is recovered. One approach is to introduce *FLP* gene using sexual crosses and score for recombination in the progenies. Another way is to utilize a inducible *FLP* gene, which can be turned on after the recovery of site-specific integration locus. Since FLP-mediated excision is a non-selectable event, it should occur at high efficiency. We have chosen soybean heat shock promoter (HSP17.5E) to express *FLP* gene in rice and compared its efficiency with HSP17.5E promoter driven *cre* gene. This study is aimed at assessing the utility of FLP-*FRT* and Cre-*lox* systems for generating clean (marker-free) site-specific integration locus.

GENOMES/GENOMICS/BIOINFORMATICS

P-3021

Control of Arginine Decarboxylase Induction in *Arabidopsis thaliana* Grown In Vitro. R. P. FEIRER and B. St. John. Biology Department, St. Norbert College, De Pere, WI 54115. Email: russ.feirer@snc.edu

Polyamines and the enzymes responsible for their biosynthesis exhibit elevations during a number of developmental and physiological processes. In Arabidopsis, arginine decarboxylase (ADC) is the enzyme responsible for biosynthesis of putrescine, which is then converted to the polyamines spermidine and spermine. Elevations of this enzyme are observed during osmotic stress and, not unexpectedly, upon treatment with abscisic acid (ABA), a plant growth regulator involved in a plant's response to water loss. Both exogenously applied ABA and osmotic stress led to increased ADC activity in *Arabidopsis thaliana* grown *in vitro*. Osmotic stress caused elevations of ADC activity in ABA-deficient and ABA-insensitive Arabidopsis mutants, which suggests that more than one signal transduction pathway may control the expression of this enzyme. Lanthanum chloride, a calcium ion channel blocker, was found to reduce the elevation of ADC due to both ABA and osmotic stress. Treatment with hydrogen peroxide, a component in the pathway mediating the plant`s response to ABA, was found to induce ADC activity in the *in vitro* grown *Arabidopsis*. These findings suggest that the induction of ADC can occur via ABA-dependent and ABA-independent pathways.

P-3022

Expression Profiling of Differentially Expressed Genes Possibly Related to Sweetpotato Storage Root Development. H. GAO, M. Egnin, G. He, F. Woullard, and D. Mortley. NASA Center, Tuskegee University, Tuskegee, AL 36088. Email: gaohuiemail@yahoo.com, megnin@tuskegee.edu

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 development of storage roots is one of the most important processes determining yield especially in sweetpotato. Most published works on storage root development have focused on anatomical and physiological characteristic, and hormonal conditions. However, the molecular mechanism of storage root initiation and development in sweetpotato is poorly understood and studied. Knowledge regarding the genetic and molecular nature of the induction process of storage root will tremendously help improve on sweetpotato harvest index. In this study, cDNA-AFLP techniques were employed to investigate temporal expression storage roots development in sweetpotato. Two hydroponically grown varieties using Nutrient Film Technology (NFT) were evaluated. TU-82-155, an early maturing (90 days post planting, DPP) with orange flesh and tinge red skin, and PI 318846-3, a late maturing (135 DPP) with white flesh and off yellow skin, were compared for differential genes expression during storage root development at different time points (14, 21, 28, 35 and 45 DPP). Total RNA was isolated from developing storage roots from five sequential harvests of each variety. cDNA generated from total RNA was subjected to cDNA-AFLP techniques to gain molecular insights and identify transcripts differentially expressed during early stages of sweetpotato storage root development. Eleven primer pairs have been identified that had equal potential for producing the same number of Transcript Derived Fragments (TDFs) in developing storage root specific to initiation, color change, and enlargement stages. The resulting TDF revealed a pattern of up and down regulated gene expression and confirmed the maturation stages of both varieties. Research supported by NASA and USDA/CSREES.

P-3023

Novel Sample Preparation Tool Quickly and Efficiently Prepares Cell Lysates to Facilitate Genomic and Proteomic Research. STEVEN KIRSHER, Robert Dorion, and Samuel Chu. Cartagen Molecular Systems Inc., Seattle, WA 98104. Email: skirsher@cartagen.com

The BioMasher Sample Preparation Device was developed by Nippi Inc. (Tokyo, Japan) to prepare bovine brain cell lysates prior to testing for BSE (Bovine Spongiform Encephalitis). We have found that the BioMasher is a versatile tool well suited for plant genomic and proteomic research. Several plant species were evaluated by PCR using samples prepared with the BioMasher. We compared direct PCR of cell lysates to DNA isolated using standard genomic DNA extraction methods. In addition, we evaluated the BioMasher as an alternative to grinding plant samples in liquid nitrogen prior to genomic DNA extraction.

P-3024

Isolation and Cloning of 'Early Genes' of Anther Development by Suppression Subtractive Hybridization and Their Expression Studies During the Development of Male Gametophyte. RAVINDRA KUMAR[1], Semarjit Shary[2], and Sipra Guha-Mukherjee[2]. [1]Department of Botany, University of Delhi, Delhi-110007, INDIA and [2]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, INDIA. Email: sipra75@rediffmail.com, ravijnu78@yahoo.com

Anther culture is being increasingly used in crop improvement both for production of haploids and also for inducing genetic variations. Present study was undertaken to identify and characterize the genes specifically expressed during anther development. The technique of Suppression Subtractive Hybridization was employed to prepare subtractive cDNA library between the early and late phase of anther development to identify the 'early' genes responsible for development, differentiation, of anthers and for androgenesis. For this the developing anthers of *Nicotiana tabacum* was categorised into 7 stages, stage 1-3 corresponds to the "early" genes, which are differentiation related, specific for anther development and may have role in pollen embryogenesis. Stage 5-7 corresponds to the "late" genes, which are activated during the maturation of anther and are also involved in the pollen tube formation and the fertilization related events. After SSH the isolated cDNAs were

cloned in to pGEMT-Easy vector and transformed in DH5-α cells and positive clones were checked for the blue-white selection. A total of 719 clones were obtained, out of which 299 clones were selected for sequencing after screening through blue-white selection, Colony PCR and Dot/Slot blot. A total of 176 clones were sequenced, analysed computationally using BLASTX and BLASTN program and their expression pattern was analysed during different stages of anther development. Sequence analysis of these clones have revealed 38% redundancy among clones and a total of 114 unique clones were obtained. Among these clones 37% genes were found to be novel and unclassified (14% and 23%, respectively). Rest were comprised of structural, photosynthetic, cell division and growth, metabolism, protein synthesis and processing, defense, biosynthetic, transport, inhibitor and signaling genes. The novel and unclassified genes may hold the keys to understand not only development of male gametophyte but also how pollen embryogenesis is initiated which does not take place in stage 5-7 of *Nicotiana* anthers.

MONOCOT TRANSFORMATION

P-3025

A Gene Tagging System for *Hordeum vulgare.* FRANÇOIS EUDES[1], André Laroche[1], Michele Frick[1], Jennifer Geddes[1] and Laurian Robert[2]. [1]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta T1J 4B1, CANADA and [2]Eastern Cereal and Oilseed Research Centre, Ottawa, Ontario K1A 0C6, CANADA. Email: eudesf@em.agr.ca

An efficient system for production of knockout plants for functional analysis and gene tagging in cereals would be a significant complement to current cereal genome analyses. Tos17, a rice retrotransposon activated by tissue culture, has been successfully used for generation of knockout plants in rice. More recently, it was reported that Tos17 insertions were predominately located within coding sequences and thus provide a unique system to develop knockout plants at a relatively high frequency. Retrotransposons have the advantage over other transposon systems of yielding stable mutations and low copy numbers which facilitate the identification of genes. Using a novel approach that enables the cultivar-independent regeneration of fertile monocots, transgenic barley T_0 was efficiently obtained, and confirmed by southern blot. Results suggest that Tos17 is a very efficient system to generate knockout plants in cereals. Under vegetative growth, the integrated rice retrotransposon Tos17 is inactive based on the absence of retrotransposase activity in barley leaves and stems. Under tissue culture conditions, the retrotransposase activity was stimulated and readily detected via a reverse transcription-PCR assay. A modification in the number of Tos17 copies in barley genomic DNA (from callus tissues) was detected. We also regenerated fertile plants C_0, from 4-5 months callus culture, with novel Tos17 insertion sites. Using real time PCR, we report an increased copy number in C_0 barley genome, and new phenotype in offspring C_1. We will discuss the advantages of this new system for development of knockout plants in cereals and possible impact for genomic studies in barley.

PLANT TISSUE CULTURE

P-3026

In Vitro Micropropagation of Bigtooth Maple. CLARE BOWEN-O'CONNOR, John Hubstenberger, Dawn Van Leeuwen, and Rolston St. Hilaire. Dept. of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003. Email: clareb@nmsu.edu

Bigtooth maple (*Acer grandidentatum Nutt.*) was micropropagated using axillary buds from juvenile shoots of two-year old greenhouse-grown seedlings. Seedlings represented 65 seed sources from locations within New Mexico, Texas and Utah. Shoots were proliferated from nodal segments and were maintained over two years by sequential subculture on Driver-Kuniyuki Walnut (DKW) media containing 10 micromoles of zeatin. Proliferated shoots (microshoots) were then placed in Phytatrays II™ containing DKW media with no plant growth regulator (DKW0) to reduce the high cytokinin levels used for shoot proliferation. Microshoots were then induced to form roots for 15 days by placing them on DKW media containing IAA (indole acetic acid) at 0.01, 1, 2.5, 5, 10, 15 or 20 micromoles. Rooting frequency, the number of leaves and callus area were recorded every 30 days for 60 days. With 10 micromoles IAA in DKW, 29% of the shoots formed roots. However, as much as 71% of shoots for one of the three Guadalupe Mountain, Texas sources rooted without auxin treatment after 30 days. Indole acetic acid inhibited shoot proliferation. Average leaf size was 307 mm^2. Indole acetic acid induced rooting in microshoots, but limited shoot proliferation of bigtooth maple after 15 days of root induction. Plantlets were successfully reestablished in a peatmoss:perlite (1:1 v/v) mixture after root induction.

P-3027

Short-term Storage of In Vitro Vitis Shoots. DAVE ELLIS, Trevor Roberts, Marie Turner, and Leigh Towill. National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, CO 80521. Email: elvis@ars.usda.gov

To our knowledge there is no robust, genotype-independent method to preserve valuable Vitis germplasm other than maintenance in the field. Such maintenance is labor and land intensive as well as subject to loss from disease, pests and abiotic stresses. The USDA-ARS National Plant Germplasm System (NPGS) currently has over 3200 vegetatively-propagated Vitis accessions maintained in the field, often by as few as two replicate plants. The National Center for Genetic Resources Preservation (NCGRP) focuses on research aimed at the long-term storage and preservation of the germplasm in the NPGS. While cryopreservation of important agricultural vegetatively-propagated material, is the goal, such methods do not exist at present for Vitis. The present study therefore is investigating the preservation of this valuable Vitis germplasm as in vitro shoot cultures at 5° C, 10° C and 15° C for 1, 3, and 6 months in an effort to reduce the labor involved in maintenance of in vitro cultures. As might be expected, the response was genotype-specific but, generally, recovery was faster at 15° C than at 5° C and survival after cold exposure was time dependent. With most genotypes, initial recovery was good but at longer exposure times to the cooler temperatures, senescence began in many genotypes 6 weeks after removal from the cold.

P-3028

Cryopreservation of 12 *Allium sativum* (Garlic) Accessions: A Comparison of Plant Vitrification Solutions (PVS2) and PVS3. DAVE ELLIS[1], Dianne Skogerboe[1], Christina Andre[1], Barbara Hellier[2], and Gayle Volk[1]. [1]National Center for Genetic Resources Preservation, USDA-ARS, Fort Collins, CO 80521 and [2]Western Regional Plant Introduction Station, Pullman, WA 99164. Email: elvis@ars.usda.gov

The National Plant Germplasm System (NPGS) maintains more than 200 *Allium sativum* (garlic) accessions at the Western Regional Plant Introduction Station in Pullman, WA. Many accessions must be grown out in the field annually since garlic plants do not produce seeds and bulbs are difficult to store for extended lengths of time. Shoot tips excised from garlic cloves can be successfully cryopreserved using either Plant Vitrification Solution 2 (PVS2; 15% DMSO, 15% ethylene glycol, 30% glycerol, 0.4 M sucrose) or Plant Vitrification Solution 3 (PVS3; 50% sucrose, 50% glycerol). We compared regrowth of shoot tips representing diverse garlic germplasm after exposure to either PVS2 or PVS3 during the cryopreservation procedure. At the National Center for Genetic Resources Preservation, we consider accessions successfully preserved if a minimum of 40% of explants exhibit regrowth after liquid nitrogen exposure and at least 60 viable shoot tips in remain in long-term storage. Eleven of twelve diverse garlic accessions were successfully cryopreserved using either PVS2 or PVS3 as cryoprotectants. Seven genotypes had higher regrowth levels after exposure to PVS2, three genotypes had higher regrowth levels after exposure to PVS3, and one genotype performed well using either vitrification solution. This project is part of an ongoing project aimed at cryopreserving accessions of the NPGS garlic collection.

P-3029

Establishment of a Simple and Highly Efficient Protocol for Somatic Embryogenesis and Plant Regeneration from Proembryonic Mass Suspension Culture in Seedless Grape 'Autumn Royal' (*Vitis vinifera* L.). Y. JITTAYASOTHORN[1, 3], J. Lu[1], X. Xu[1], P. Thipyapong[2], and N. Boonkerd[3]. [1]Center for Viticulture and Small Fruit Research, CESTA, Florida A&M University, Tallahassee, FL 32317; [2]School of Crop Production Technology; and [3]School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, THAILAND, 30000. Email: yingyos_j@hotmail.com

Successful grape biotechnologies, such as gene transformation and In Vitro selection, require a high productivity of synchronized somatic embryos and efficient regeneration system. Although somatic embryos could be multiplied by subculturing on solid medium, yields are generally low. In addition, quality of somatic embryos cultured on solid medium varied in each subcultivation, which is a major factor influencing regeneration frequency. A simple and highly efficient suspension culture and plant regeneration procedure was developed for 'Autumn Royal' seedless (*V. vinifera* L.) in our laboratory. Proembryonic masses (PEMs) suspension culture of 'Autumn Royal' seedless was established from primary somatic embryos at the second subculture in MGGN medium. The PEMs appeared in small clusters (SPEMs, <1 mm) and large clusters (LPEMs, ≥1 mm). The PEMs, yellowish in color, grew rapidly in the liquid medium. Browning was neither observed in the liquid medium nor the PEMs. More than 1,000 mature somatic embryos could be produced from approximately 50 mg of SPEMs on solid MSA medium within 14-16 weeks. The somatic embryos were easily germinated (100%) in MSA medium and up to ninety-five percent of them were converted into normal plantlets.

P-3030

Development of an Automated Petri Plate Pourer. J. G. LAYTON[1], J. Koerber[1], J. Hellebusch[1], N. Malterer[2], S. Dulle[1], and S. Norris[1]. [1]Monsanto Company, Chesterfield, MO 63017 and [2]Scinomix Corp., Earth City, MO 63045. Email: jeanne.g.layton@Monsanto.com

An automatic Petri plate pourer has been designed by Scinomix Corporation in collaboration with the Monsanto Automation Group and Media Preparation Team to fill plates in order to reduce ergonomic strain associated with this task. Repetitive hand, back, and wrist motions required to dispense semi-solid medium into Petri plates can be a source of ergonomic injury. The equipment has successfully pumped media into Petri plates at a fill rate of 6-7 seconds per plate with no splashing or bubbles observed. Ergonomic, operational safety, and sterility issues were considered in overall design.

P-3031

Indirect Organogenesis in *Agave tequilana*. KARLA K. VALENZUELA-SÁNCHEZ, Raul E. Juárez-Hernández, Andrés Cruz-Hernández, Víctor Olalde-Portugal, María Elena Valverde, and Octavio Paredes-López. Depto. de Biotecnología y Bioquímica, Unidad Irapuato, Centro de Investigación y de Estudios Avanzados del IPN, Apdo. Postal 629, 36500 Irapuato, Gto., MEXICO. Email: kvalenzu@ira.cinvestav.mx, oparedes@ira.cinvestav.mx

Agave as other plants like maize, beans and amaranth have been very important crops in Mexico since prehispanic times, also this country is considered origin and biodiversity center for the Agavaceae family. *Agave tequilana* Weber cv. Azul is the most widely cultivated species; it is the source of carbohydrates and additional minor components for the production of the alcoholic beverage called "tequila", a Mexican culture distinctive with high international demand. *A. tequilana* reproduction may be sexual (seeds) or asexual (from rhizomes or bulbils), although, in field the propagation has been carried out from rhizomes, as a consequence the genetic diversity is limited which generates plants vulnerable to the occurrence of new and more aggressive pathogens which could result in severe diseases over large geographic areas. Conventional plant breeding techniques might be an alternative to solve these problems, nevertheless their application is complicated in this type of crops because the long juvenile period (6 to 10 years for *A. tequilana*). Therefore, genetic engineering tools may help to carry out the introduction of genetic variability to the crop providing the opportunity to study the regulation of genetic expression. Under this concern, by testing different tissues as explant source for callus formation, maintenance and regeneration capacity under the effect of different auxin (2,4-D and NAA) and cytokinin (BAP) concentrations, we have developed an indirect organogenesis system in *A. tequilana* for its further use in gene transfer protocols.

P-3032

Adventitious Shoot Regeneration from Leaf Cultures of Choke Cherry (*Prunus virginiana* L.). C. SRINIVASAN and R. Scorza. USDA-ARS Appalachian Fruit Research Station, 2217 Wilshire Road, Kearneysville, WV 25430. Email: srinivas@afrs.ars.usda.gov

Prunus species are generally recalcitrant to regenerate adventitious shoots from vegetative organs such as leaves. Although there are few reports of regenerating transgenic Prunus plants, most of the reports cannot be routinely used for Prunus transformation primarily due to very low regenerative competence. In order to develop an efficient regeneration for Prunus, we are evaluating regenerative competence of explants from wide range of Prunus species. Choke cherry (*Prunus virginiana* L.) is one of the popular Prunus species used for wild life conservation and wild life habitat in North America. Shoot multiplication cultures were established in vitro from four genotypes of *P. virginiana* by culturing in agar solidified Murashiege and Skoog (MS) medium containing 10 µM benzyl adenine (BA). To induce adventitious shoot regeneration, young leaves (1-6 mm long) excised from actively growing shoot cultures were cultured on different plant culture media supplemented with 5-20 µM BA or 1-5 µM thidiazuron (TDZ). Leaves cultured under light did not regenerate any shoots. Culture of 1-4 mm long leaves in dark for three weeks on Woody plant medium containing 5 µM BA and transfer to Schenk and Hildebrandt (SH) medium containing 10 µM BA induced 49 percent of the leaves to regenerate adventitious shoots. *P. virginiana* cv 'Schubert' produced more adventitious shoots than three other genotypes tested. Browning of leaf cultures appeared to reduce shoot regeneration. Methods to reduce browning and improving shoot regeneration from leaves by using polyvinyl pyrolidine, polyamines, and cysteine will be discussed.

P-3033
Towards Using Endosperm Tissue as a Transient Transformation Model System for Seed Traits in Soybean. M. A. SCHMIDT and E. M. Herman. USDA/ARS, Donald Danforth Plant Science Center, St.Louis MO 63132. Email: mschmidt@danforthcenter.org

Soybean (*Glycine max* L.) is one of the world's most important crops due to the high content of both oil and protein in its seed. For this reason, the general improvement of soybean through biotechnology has largely focused on seed-specific traits. A significant time delay is incurred with the genetic engineering of seed traits into soybean. Tissue culture/ transformation typically take approximately nine months and that is followed by four months for the resultant plant to mature and set seed. Hence, about one year will pass before the initiation of a soybean seed-specific engineering project and the analysis of a desired phenotype. Considering the substantial time investment, efforts to increase the likelihood of obtaining the desired seed phenotype are being made by designing a system in which constructs, genes, promoter expression and cellular localization can quickly be assessed before embarking on a stable soybean transformation project. We are currently using aseptically isolated endosperm tissue from mature soybean cotyledons as a model system to test seed expression of constructs and to visualize localization of introduced proteins within cells. Endosperm tissue was chosen because it is easy to obtain and through protein profiling and electron microscopy analysis it appears to be quite similar to a mature seed. It has also proven to be ideal for immuno-labeling and subsequent microscopy visualization as it is composed of a single cell layer. We will report on the protein and structural similarities between endosperm and seed and show examples of transgene expression in the endosperm. For instance, the endoplasmic reticulum (ER) localization of KDEL constructs and the compartmentalization of various transgenes within protein bodies will be shown. This work demonstrates that the endosperm tissue could successfully be used to transiently transform constructs as a means to assess if the desired phenotype will result before engineering those constructs into soybean.

PLANT TRANSFORMATION
P-3034
Improved Production of Phytosterol from Transgenic Calli of *Chrysanthemum coronarium* L. I. S. CHUNG, S. Y. Lee, H. C. Kim, and N. I. Baek. Plant Metabolism Research Center, Kyung Hee University, Suwon, KOREA 449-701. Email: ischung@khu.ac.kr

We describe the optimal production of phytosterol from normal and transgenic calli of *Chrysanthemum coronarium* L. Normal calli were induced on Murashige and Skoog medium containing 2, 4-D from *C. coronarium* L. A suspension culture was established and the effects of the seeding density and nitrogen, phosphate and carbon sources on phytosterol production from the suspension culture were studied. Conditions for air-lift bioreactor culture were optimized for phytosterol production. The SMT gene encoding the key enzyme for phytosterol synthesis was cloned and transformed into the calli for overexpression of this gene using Agrobacterium-mediated transformation. We are currently investigating the integration and the expression of the SMT gene, and the level of phytosterol formation in transgenic calli. The potential of transgenic calli of *C. coronarium* L. for phytosterol production will also be examined. This work was supported by a grant from the Rural Development Administration through Bio-green 21 Project.

SILENT ABSTRACTS
P-3035
In Vitro Regeneration of Cowpea (*Vigna unguiculata* L. Walp.) Plants Through Organogenesis. M. MANOHARAN[1], L. S. Dahleen[2], S. O. Okiror[1], and J. Garner[1]. [1]Department of Agriculture, University of Arkansas at Pine Bluff, AR 71601 and [2]USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105. Email: manoharan_m@uapb.edu

Cowpea, *Vigna unguiculata* L. Walp., used as a grain crop, animal fodder or as a vegetable, is widely grown in tropical and subtropical regions including the southern United States. The protein in cowpea seed is rich in the amino acids lysine and tryptophan, compared to cereal grains and therefore cowpea is valued as a nutritional supplement to cereals. In Arkansas and southern Mississippi delta, cowpea is grown by small acreage farmers for fresh produce and large acreage farmers for dried bean. Cowpea production has declined sharply in recent years due to insects such as cowpea weevil and pod borer. Currently, there are no cultivars that resist insect damage. Efforts to incorporate insect resistant genes through genetic transformation into cowpea did not succeed, due to lack of an efficient regeneration system. We are screening four different genotypes (Early Scarlet, Coronet, Quick Pick and 87-435-68) to establish an efficient regeneration system in cowpea. Embryonal axes were cultured on MS medium with different concentrations of various growth hormones. Among the four cultivars tested, Early Scarlet responded well to MS medium with 2.0 mg/L BAP and 1.0 mg/L zeatin. After 6-8 weeks, callus with shoots were transferred to MS medium with 1.0 mg/L IBA for shoot elongation and rooting. After four weeks, fully established plants were transferred to peat pellets first and then to the greenhouse. Further improvements of the regeneration protocol are in progress.

P-3036
Use of Bioreactors on Growth of *Pinus nigra* Somatic Embryo Culture. W. T. P. S. K. SENARATH. Department of Botany, University of Sri Jayewardenepura, Nugegoda, SRI LANKA. Email: senerath@yahoo.co.uk

Selecting elite trees from mature plantations for breeding programs is recommended since seeds collected from wild are open to genetic variation. Clonal propagation through in vitro techniques is one of the most appropriate tools to be used. This could be achieved easily through somatic embryogenesis, which should lead to the production of synthetic seeds of identical clones. Embryogenic cell suspensions were maintained on MS containing modified CD medium supplemented with BAP and 2,4-D. Subculture were made in every 4 days. Embryo development was monitored and assessed microscopically. To assess the embryo developed embryos were stained with acetocarmine (2:1 v/v). The scale-up of *pinus nigra* somatic embryogenesis from shake flasks to 5 liter bioreactors was investigated. Proliferation of embryogenic suspensions in air-lift and bubble bioreactors resulted in improved growth, in comparison to shake flasks. There was no observed lag phase and growth followed a linear phase for four weeks. Although no stationary phase was observed viability declined after three weeks. Growth of the embryogenic suspensions in air-lift and bubble bioreactors resulted in somatic embryo aggregation and embryos were present in clusters. Mechanically agitated bioreactors appeared less suitable for somatic embryo proliferation than gas-liquid bioreactors and shake flasks. In a stirred tank and a hanging stirred bar bioreactor the linear growth phase was followed by a stationary phase and the yield of somatic embryos was reduced. Fractured somatic embryos and cell debris were recorded in mechanically agitated bioreactors, particularly in the hanging stirred bar bioreactors, where few intact embryos remained. Carbohydrate analysis of shake flask cultures revealed that sucrose was rapidly hydrolyzed into glucose and fructose. Both glucose and fructose were removed from the medium and fructose was utilized preferentially over glucose. This pattern of carbohydrate utilization was repeated under all bioreactor conditions except the bubble bioreactor culture, where glucose was utilized preferentially. Somatic embryos from shake flasks and bioreactors were matured on solid medium. The number of stage-3 embryos produced was significantly higher from gas-liquid bioreactors than mechanically agitated bioreactors.

P-3037
Optimization of a Micropropagation Protocol for Douglas-fir. ABDOULAYE TRAORE, Chien-Chih, and John Carlson. School of Forest Resources, 419 Life Sciences Building, University Park, PA 16802. Email: axt14@psu.edu

Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) is an important plant species for the timber, landscape and Christmas tree industries of North America. Christmas tree farms are established using open pollinated seeds and because of that, the trees are genetically and phenotypically diverse. Clonal propagation using micropropagation can be used to multiply and make true-to-type copies of these excellent trees. To obtain efficient in vitro conditions for Douglas-fir micropropagation, we are optimizing each step in the process using trees selected by breeders as improved Christmas tree genotypes. First we tested various surface sterilization treatments based on the use of bleach versus ethanol flaming. Next, using the best surface sterilization treatment, we tested various concentrations of BA on culture establishment and development. The bleach treatments resulted in the highest percentage of healthy bud cultures (over 90% for winter buds). Successful sterilization was also achieved by flaming, but bud injury was observed. In addition, sterilization by either approach required subsequent bud dissection to remove the outer scales; otherwise most buds were lost to contamination. After successful sterilization and culture initiation, bud expansion was the highest (50%-98%) in the presence of low concentrations of BA, while higher concentrations of BA reduced bud expansion (0-60%), but promoted bud multiplication. At moderate levels of BA, most of the shoot multiplications observed were by axillary bud growth. However, at the highest BA concentrations, shoot multiplication occurred by organogenesis at the base of needles and increased with increasing BA concentration. Shoots produced by both axillary bud growth and shoot organogenesis from needle tissue continued to grow when transferred to hormone free media and formed extended shoots with multiple branches.

VERTEBRATE/TOXICOLOGY POSTER ABSTRACTS

CELLULAR MODELS
VT-3000
Development of an In Vitro Model Representative of Lymphoid Organ of Chicken Embryo. N. B. ALITHEEN, A. M. Ali, and P. McCullagh. Dept. of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, MALAYSIA. Email: noorjahan@putra.upm.edu.my

In vitro systems enable experimental manipulation and can provide an avenue to understand developmental process and also to study the host responses to pathogens. The B cell development in the bursa of Fabricius of birds, ileal Peyer's patch of sheep and the gut-associated lymphoid organ of rabbits are all dependent on participation and maintenance of these microenvironments by an epithelial component. A three dimensional in vitro system was developed to simulate the organogenesis of lymphoid organ in chicken embryo. The system was developed by applying similar technique that was used to generate the Peyer's patches in fetal lamb. Chicken embryos of gestational ages 15-18 days were used in this experiment. The spleen was removed and a single cell suspension was made. Epithelial cells from proventriculus were obtained through enzymatic disaggregation. Epithelial cells and spleen lymphocytes were mixed at the ratio 2:1 and pelleted by centrifugation. After 48 hours in culture, interaction between epithelial component and lymphocytes took place to form a mini-organ. An outer layer consists of well-defined epithelial cells was observed in the mini-organ. H&E sections of the mini-organ clearly reveal the overlying epithelium and lymphocytes clusters in the inner part. The results obtained showed that interaction between epithelial cells and spleen lymphocytes of chicken embryo occurs to generate the three dimensional architecture. This model is expected to facilitate studies of host-pathogen interaction. Thus, the establishment of the in vitro system could be used to study the mechanism of immune responses upon infection.

VT-3001

Measures of Production of Oxidant Species In Vitro and in Cells: Assessment for Evaluation of Possible Health Risks. F. DACCUEIL, A. Fijman, E. J. Roemer, and S. R. Simon. Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11794-8691. Email: DacFarah03@yahoo.fr, fdaccuei@ic.sunysb.edu

Phagocytes, such as neutrophils and macrophages undergo a so-called "oxidative burst" as a component of the host response to foreign substances. When these cells encounter such materials, they transmit signals originating from the plasma membrane, which result in the activation of pathways, which produce different reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. While ROS can destroy microbes, they can also injure the tissues where they are produced; especially in the lung. We have employed several assays to measure the generation and the potential inhibition of different ROS selectively. The assays used two different fluorescent probes: aminophenylfluorescein (APF), which is highly specific for hydroxyl radicals and hypochlorous acid, and dichlorodihydrofluorescein (DCFH), which is a more nonspecific ROS probe. APF was employed as a probe of hydroxyl radicals formed in vitro from the Fenton reaction. Hydrogen peroxide and ferrous perchlorate were used in this reaction to generate hydroxyl radicals in the absence of cells. Increasing concentrations of ferrous iron in the presence of a constant concentration of hydrogen peroxide produced proportionately greater relative fluorescence intensity from APF. To inhibit the formation of the fluorescent product, we employed extracts of a plant, feverfew, which has been claimed to have beneficial antioxidant activity. Extracts of this natural product were found to diminish the signal generated by a mixture of ferrous iron and hydrogen peroxide, also in a dose dependent manner. Assays on hydroxyl radical formation by viable phagocytes are still in progress. We have successfully employed a human macrophage cell line as the source of ROS which can be detected by DCFH. The cells were pretreated with LPS to enhance their phagocytic activity. As phagocytic challenges, we employed small particulate suspensions of minerals, including pyrite and quartz. These assays have demonstrated dose- and agent-specific increases in fluorescence from the oxidation of DCFH to its fluorescent product, DCF which can be attributed specifically to uptake of the particulates by the activated macrophage-like cells. We hope to correlate the magnitude of the ROS levels generated by these cells and detected with DCFH or with other probes to additional measures of the response of phagocytes to foreign particulates in order to assess the health risks of exposure to such materials in vivo and to evaluate the potential therapeutic applications of natural antioxidants. (Supported in part by Integrated Botanical Technologies.)

VT-3002

Effects of Mineral Metal Sulfides and Metal Oxides on Human Inflammatory Cells. D. M. HABIEL[1], K. Patel[1], S. Abbate[1], M. Schoonen[2], S. R. Simon[1], and E. J. Roemer[1]. [1]Departments of Pathology and Biochemistry, [2]Department of Geosciences, SUNY Stony Brook, Stony Brook, NY 11794-8691. Email: mhabiel@ic.sunysb.edu

When humans are exposed to mineral dusts in the environment, their health is often adversely affected. Alveolar macrophages that are exposed to fine-grained minerals will phagocytose the particles. This process is controlled by cytokines. Prolonged exposure to mineral particles may lead to generalized increase in cytokine production. Increased production of cytokines can lead to a chronic, systemic inflammatory response, resulting in organ injury and eventually death. We have employed MonoMac 6 cells as an in vitro model of airway macrophages to examine aspects of the human inflammatory immune response to minerals ingested in the airways. MonoMac 6 (MM6) is a human monocytic cell line established in 1985 from the peripheral blood of a 64-year-old man with acute monocytic leukemia. Cells of this line retain many of the characteristics of normal human monocytes and can be induced to differentiate by a number of treatments. To induce phagocytosis, we pre-incubated MM6 cells with low concentrations (10 ng/ml) of lipopolysaccharide (LPS), a major constituent of the cell wall of gram negative bacteria. The cells were then incubated for 24 hours with varying concentrations of Pyrite (FeS_2), Nickel Disulfide (Ni-pyrite, NiS_2), Quartz or Titanium Dioxide. Cytotoxicity is measured with MTS, a tetrazolium salt based assay, and IL-8 release is evaluated using a commercially available ELISA kit. The MM6 cells release IL-8 in response to incubation with pyrite in a dose dependant fashion. Quartz and titanium oxide do not induce a similar response. Ni-pyrite was the only mineral to show significant cell toxicity at the doses used and also triggered the release of the highest levels of IL-8. When differentiated cells and pyrite were incubated on opposite sides of a porous membrane so that the cells and the test materials were not in direct physical contact, no response was elicited. Phagocytosis of inert beads did not induce an IL-8 response. The data suggest that phagocytosis and/or direct contact between minerals and MM6 cells is necessary to induce an increased release of IL-8. (Supported in part by DOE-Basic Energy Science grant #DEFG0296ER14633 and the Center of Environmental Molecular Science at SUNYSB.)

VT-3003

Long-term Propagation of Pluripotent Mouse ES Cells in a Serum-free Medium Without Feeder Cells. M. Furue[1], T. Okamoto[2], Y. Hayashi[3], H. Okochi[4], M. Fujimoto[4], Y. Myoishi[2], T. Abe[5], K. Ohnuma[3], G. H. Sato[6], M. Asashima[3,5], and J. D. SATO[7]. [1]Kanagawa Dental College, Yokosuka 238-8580, JAPAN; [2]Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima 734-8553, JAPAN; [3]Graduate School of Arts and Sciences, Tokyo University, Tokyo 153-8902, JAPAN; [4]International Medical Center of Japan, Tokyo 162-8655, JAPAN; [5]Graduate School of Science, Tokyo University, Bunkyo-ku, Tokyo 113-0033, JAPAN; [6]Ministry of Fisheries, Massawa, ERITREA; and [7]Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672. Email: dsato@mdibl.org

We developed a serum-free medium (ESF7) that allowed the long-term propagation of pluripotent ES-D3 mouse embryonic stem cells in the absence of feeder cells. Cells maintained in this medium for over two years retained expression of the transcription factor Oct-3/4, the ES cell marker SSEA-1, and alkaline phosphatase. Leukemia inhibitory factor (LIF), a component of ESF7 medium, elicited increased expression of nanog and Rex-1 and decreased expression of FGF-5. These effects were concentration-dependent. In the absence of serum LIF exhibited a novel mitogenic activity for ES-D3 cells, which was completely obscured in serum-supplemented medium, while LIF withdrawl resulted in apoptosis. ES-D3 cells propagated in ESF7 medium remained pluripotent as they could be induced to differentiate in the absence of LIF by serum, BMP4 or FGF-2. BMP4 promoted differentiation into simple epithelial cells while FGF-2 promoted differentiation into neuronal and glial-like cells.

Serum induced the expression of genes characteristic of derivatives of all three embryonic germ layers: the primitive ectodermal marker FGF-5; the mesodermal marker brachyury; and the endodermal marker GATA4. As this serum-free culture system supports pluripotent ES cell propagation, it should aid in elucidating signaling pathways that regulate ES cell differentiation.

CELLULAR AND MOLECULAR BIOLOGY

VT-3004

Metabolic Profiling of and Assessing Polymorphisms in Leptin and Obesity Genes in African American. V. BROWN[1,2], M. Egnin[1], E. Powell[2], R. D. Pace[1], E. Kebede[1], and M. Gore[3]. [1]College of Agricultural, Environmental & Natural Sciences; [2]College of Veterinary Medicine & Allied Health Sciences, Tuskegee University, Tuskegee, AL 36088; and [3]Advanced Physicians Weight Management, Montgomery, AL 36117. Email: vbrown@tuskegee.edu, megnin@tuskegee.edu

Obesity, a multifactorial disease arising from complex interactions between genetic predisposition, nutritional and environmental factors, increases the risk for cardiovascular and metabolic diseases and conditions such as diabetes, hypertension and dyslipidemia. Our goal is to apply enabling tools to understand obesity and improve treatments to reducing obesity and related disorders. The project aims to metabolically profile serum leptin levels in a cohort of healthy rural African Americans, determine its relation to the commonly used anthropometric measures of obesity and to patterns of fat distribution in the body, and to assess polymorphisms caused by genetic abnormality in leptin receptors and genes to determine the mechanisms associated with the development of obesity. The associations between obesity (or BMI >25) and chronic disease disproportionately affect ethnic and socio-economic groups such as African Americans. The prevalence worsens with age and is different between genders. The greater genetic predisposition to metabolic disorders in the African American community has contributed to health disparity issues. As a result, the U.S. Department of Health and Human Services has given these chronic diseases priority for research in minority populations. Leptin, an adipocyte-derived hormone encoded by the obese gene, is released in the bloodstream to modulate energy expenditure. Leptin levels are increased and correlate with individual BMI in overweight and obese individuals. Recent molecular genetics studies of leptin in mice, pig, and human have identified several polymorphisms linked to obesity (Boston, 2001; Yoshio, 2002; Wei et. al., 2004)). Molecular data to support comparable function of leptin in African Americans is limited. Since adipocytes express leptin, it is reasonable to study whether leptin interferes with endocrine alterations observed in obesity, or whether leptin can be manipulated for weight reduction in African Americans. Research supported by NIH-NCMHD, USDA and Tuskegee University.

VT-3005

A Strategy for Determining Potential of Minerals to Induce Lung Disease Based on ROS Generation and Reactivity Toward RNA. COREY A. COHN and Martin A. Schoonen. Department of Geosciences and Center for Environmental Molecular Science, Stony Brook University, Stony Brook, NY 11794-2100. Email: ccohn@ic.sunysb.edu

Inhalation of mineral dusts can cause oxidative stress and lung disease. Occupationally inhaled particles include a range of minerals with varying physico-chemical characteristics. The mechanisms through which mineral particles may cause lung disease are unclear, which makes it difficult to predict a pathological response for a given mineral. However, it is generally accepted that generation of Reactive Oxygen Species (ROS) and oxidation of biomolecules are important indicators of potential toxicity. As examples, quartz and asbestos have been shown to generate ROS, degrade nucleic acids and cause lung disease. Some other minerals, such as metal sulfides, show evidence for oxidative reactivity, but the mechanisms and their possible contribution to lung disease remain unknown. Here, we present a strategy for screening mineral dusts for mineral-generated ROS and reactivity toward RNA as a precursor to in vitro and possibly in vivo studies. In step one, yeast RNA is added to two mineral/aqueous slurries, one with and one without the iron chelator EDTA. RNA is quantified using a fluorescent dye. RNA is rapidly degraded if the mineral produces ·OH; EDTA lends insight into the role of iron. In step two, the reactivity of the mineral slurry toward adenine is examined. Adenine, monitored by UV-Vis absorbance, is more slowly decomposed compared to RNA. However, by adding the radical scavenger ethanol and the H_2O_2-decomposing enzyme catalase to duplicate adenine mineral slurries, it is possible to evaluate if the mineral generates ·OH and H_2O_2. If H_2O_2 is generated, it can be quantified using Leuco Crystal Violet (LCV), which oxidizes in the presence of peroxide and Horseradish Peroxidase (HRP). In iron-containing solutions, iron chelation is necessary to preserve H_2O_2. ·OH can be identified by EPR spin trap techniques. This multi-step strategy may prove useful for determining potential toxicity for a range of mineral particles.

VT-3006

Estrogenic Activity in Tissue Culture Plates Is Not Due to Leaching of Bisphenol A. L. DAVIS, C. Biswanger, and R. Roberts. Department of Biology and Biochemistry & Molecular Biology Program, Ursinus College, Collegeville PA 19426. Email: rroberts@ursinus.edu

Bisphenol A (BPA) is an environmental endocrine disrupting chemical that binds to estrogen receptors. Endocrine disruptors such as BPA are believed to be responsible for increased puberty and impairment of reproductive organs in mice, and have been implicated in human breast cancer proliferation *in vitro*. BPA is produced by the acid-catalyzed reaction of acetone and phenol and is used in the manufacture of polycarbonate, epoxy, and plyester-styrene resins. Tissue culture plates have been shown to contain impurities with estrogenic properties using an estrogen-response element-linked luciferase reporter assay (Ishikawa 2001). To determine more specifically the nature of the estrogenic compounds in plastic lab ware, we analyzed the level of BPA leached from plates obtained from six different manufacturers. Plates spiked with 50 nM BPA in 10 mL ddH_2O or complete phenol-red free RPMI were incubated at 37° C. Aliquots were removed at days 0, 1, and 7, derivatized with pentaflurobenzyl bromide (PFBBR) and analyzed by gas chromatography mass spectroscopy. When compared to a standard BPA concentration curve (R^2=0.978) none of the plate showed an increase or decrease in BPA concentration. Finally, different murine spleen suspensions in complete phenol-red free RPMI are currently being tested to see if the BPA concentration changes over an 18 hour period in the presence of primary tissue.

VT-3007
Estrogen Receptor Alpha Is Upregulated by In Vitro Estradiol Exposure. R. LENHART, Z. ICHTER, B. Scipioni, H. Mellert, M. Heayn, D. Getnet, and R. Roberts. Department of Biology and Biochemistry & Molecular Biology Program, Ursinus College, Collegeville, PA 19426. Email: rroberts@ursinus.edu

The connections between the immune system and the endocrine system are not fully understood, yet the progression of some autoimmune diseases, such as Systemic Lupus Erythematosis, is known to be affected by hormones. The classical mechanism by which estrogens affect cells is through estrogen receptors (ER). Estradiol (E2) binds to the ER and the complex migrates to the nucleus. There it can directly or indirectly affect transcription at an estrogen response element (ERE). Using the program Dragon ERE Finder, we found four putative ERE associated with the murine ER-alpha gene, indicating a possible feedback mechanism in ER regulation by E2. We have demonstrated that ER-alpha in splenocytes isolated from a murine model for Systemic Lupus Erythematosis (NZBWF1) are upregulated upon an 18 hour exposure to estradiol. Statistical analysis of trypan-blue exclusion assays indicate that the upregulation is due to the exposure itself and not to any cell death that is occurring during the 18 hour incubation period. Thus, a feedback loop exists for regulation of ER by E2.

INFECTIOUS DISEASES/CELLULAR PATHOLOGY
VT-3008
Pyrite Induced Inflammatory Response in Human Derived Immortalized Monocytes. A. FIJMAN[1], F. Daccueil[2], M. Schoonen[3], E. J. Roemer[2], and S. R. Simon[2]. [1]Pulmonary Medicine, [2]Departments of Pathology and Biochemistry, [3]Department of Geosciences, SUNY Stony Brook, Stony Brook, NY 11794. Email: longislandmd@hotmail.com

Pyrite is a widely distributed and naturally occurring iron sulfide that exists in many locals allowing for its potential interaction with the human airway. Other ferrous compounds, such as those found in particulate air pollutants, have been implicated as having potentially deleterious effects on human health. The aim of this study is to define the inflammatory potential of pyrite particles by measuring production of reactive oxygen species (ROS). Both unstimulated MonoMac-6 (MM6) cells, E. coli endotoxin-pretreated MM6 cells in buffer and acellular buffer solution controls were incubated in 96 well plates with dichlorodihydrofluorescein (DCFH), a non-specific fluorogenic probe for reactive oxygen species, in the absence or the presence of escalating measured amounts of pyrite particles. Plates were then scanned for fluorescence from the oxidized product (DCF) at 2 hours and again at 20 hrs. On separate 96 well plates, cells were similarly incubated with the tetrazolium salt MTS to measure viability and metabolic activity and were scanned at the same intervals for absorbance from the resulting formazan. In the presence of doses of pyrite ranging from 100 to 800 ug/ml, MM6 cells generated significantly greater DCF fluorescence than acellular or particle-free cell controls, denoting an inflammatory response. It was further demonstrated that cell-mediated conversion of DCFH to DCF was greater at increasing doses of pyrite. Furthermore, at the higher pyrite doses, conversion of MTS to its formazan declined significantly, as reflected in lower absorbance, indicating toxicity to the MM6 cells. Toxicity, as reflected in conversion of MTS to formazan, was diminished in endotoxin-pretreated cells when compared to naïve cells exposed to pyrite. This first characterization of the capacity of pyrite to trigger an inflammatory response and induce cytotoxicity in a mononuclar phagocyte cell line supports a potential role for pyrite as a pathogenic agent among mineral particulates. (supported in part by DOE-Basic Energy Science grant #DEFG0296ER14633 and the Center of Environmental Molecular Science at SUNYSB).

VT-3009
Metalloproteinase Inhibitors, Non-antimicrobial Chemically Modified Tetracyclines and Ilomastat, Protect Human Peripheral Blood Monocytes from Anthrax Lethal Factor-mediated Cytotoxicity and Block Lethal Toxin-induced Suppression of Innate Immunity. S. S. KOCER[1], E. J. Roemer[1,2], and S. R. Simon[1,2]. [1]Department of Biochemistry and [2]Pathology, SUNY Stony Brook, Stony Brook, NY 11794-8691. Email: sskocer@hotmail.com

After the recent cases of anthrax in the United States, anthrax has become a worldwide bioterrorism concern. Anthrax lethal toxin (LeTx), a major virulence factor produced by Bacillus anthracis, has been demonstrated to proteolytically inactivate members of the mitogen activated protein kinase kinase (MAPKK or MEK) family with consequent suppression of the innate immune response in human macrophages and massive cytotoxity to murine macrophages. If diagnosis of B. anthracis infection is not made early in the course of the disease in humans, progression invariably occurs with attendant high morbidity and mortality, even if aggressive antibiotic therapy is instituted. A safe, easily administered agent to neutralize the effects of LeTx would provide a valuable defense against the effects of anthrax in populations exposed to B. anthracis spores. Previously we have shown that CMT-300, CMT-308 and Ilomastat, agents initially characterized as matrix metalloproteinase inhibitors which are in early stages of development as pharmaceuticals, effectively inhibit the zinc metalloproteinase activity of Lethal Factor (LF), the proteolytic subunit of LeTx, and inhibit LF-mediated cleavage of a synthetic peptide substrate based on the N-terminal consensus sequence of MEKs. This study shows that these same inhibitors effectively block cleavage of intracellular MEKs in viable monocytes and dendritic cells, diminish LeTx-induced cytotoxicity and prevent LeTx-triggered suppression of inflammatory cytokine release in human monocytes. CMT-300 and -308 have high oral bioavailability, low incidence of adverse side effects in human subjects, and can enter viable cells in a so-called post-exposure prophylactic mode to inhibit LF which has already been internalized. These results offer promise for a strategy to combat effects of Bacillus anthracis LeTx, based on pleiotropic actions of CMTs and Ilomastat. (CMT-300 and CMT-308 were generous gifts of CollaGenix Pharmaceuticals Inc; supported by NIH (NIAID) R21-AI53524.)

SILENT ABSTRACTS
VT-3010
An Algorithm to Select Highly Effective Target-specific siRNA. T. ALSHEDDI and A. Baranova. George Mason University, Fairfax, VA 22030. Email: talshedd@gmu.edu, abaranov@gmu.edu

Designing mammalian siRNAs with maximized specificity is one of the hottest topics in modern biology. We have developed a new algorithm that allows one to map all unique short-string sequences ("the targets") with lengths of N = 9-15 nt within existing large sets of sequences, e.g. a set of all known transcripts in certain organism. The algorithm assures the uniqueness of the

"target" in sequence set of study. The "target" is considered unique if present only in one of the sequences from study set; otherwise all the identical short-string sequences including primary target will be masked. The main purpose of this algorithm is to generate 21 bp siRNA sequences characterized by minimal off-target hybridization. The algorithm steps are as follow: **Generate all possible sequences with length 'N':** A model mouse transcriptome was transformed into set of overlapping sequence fragments of length "**N**" where first nucleotide of the first fragment corresponds to Position 1 in Gene 1. **Standardize targets and remove redundancy:** The targets with one or more letters different from than A, G, C or T were artificially transformed into the permutated equivalent AGCT-containing sequences. All duplicated targets were removed. **Locate targets within candidate siRNA antisense:** Unique targets were concatenated to their suffix. In some cases resulting 21 nt sequences represent chimeric target identical to target(s) already removed in the previous step. To avoid chimerism, only targets followed by (21-N) downstream targets were preserved in the output file. We searched for unique target 9 to 15 nt in size in mouse transcriptome. No unique targets of length 9 and 10 and only five 11-nt targets were found. 12-nt targets cover 28% of the mouse transcriptome. 97%, 99% and 100% of the mouse transcriptome have been covered with N=13, 14 and 15 respectively. We are currently in the process of creating a public database with siRNA suitable gene regions in mammalian genomes. siRNAs with the unique kernel positions will provide quality of gene suppression by minimization of off-target effects and will simplify routine siRNA studies.

VT-3011

Adipocytic Differentiation of Stromal Arterial Preadipocytes Induced In Vitro by Rosiglitazone. M. REYES and B. Lazalde. Faculty of Medicine U.J.E.D., Food & Nutrition Research Center, Durango, Dgo. MEXICO 34000. Email: mareyes@ linux.ujed.mx

Thiazolidinediones are insulin sensitizing agents which act through activation of PPAR-γ and are prescribed for treating diabetes mellitus type 2; increasing of adiposity is a collateral effect. The aim of this work was to evaluate the in vitro effect of the thiazolidinedione rosiglitazone (Avandia™) on adipocytic differentiation of stromal preadipocytes contained in arterial walls. Mixed primary cultures were derived from rat aortic rings incubated in Dulbecco's MEM supplemented with 10% fetal bovine serum. After ten days the rings were removed and the medium was replaced by Dulbecco's MEM with 0.2% albumin and rosiglitazone at 1, 10, 100 or 1000 nM during three days; presence of lipid droplets was evaluated by light microscopy after staining with oil red O. Experiments were done by triplicate. Adipocytic differentiation in cultures incubated with rosiglitazone at 1 and 10 nM was minimal when compared to controls without the drug, with only few cell showing scant fine oil droplets. On the contrary, rosiglitazone at 100 nM exerted a clear adipogenic effect with a great number of cells filled with fine and medium size lipid droplets; this effect was extreme at 1000 nM at which many cells showed lipid macrodroplets many of which detached from cells and floated on the culture medium. These results showed that primary cultures derived from aortic rings are a good model for studying adipocytic differentiation. The effect of rosiglitazone observed in vitro in this work suggests that thiazolidinediones in vivo could have undesirable lipostatic effects in sites such as arterial walls; currently there are not reports about studies in human beings to this respect, therefore, further studies in this issue are warranted.

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