

2006 IN VITRO BIOLOGY MEETING
2006 Meeting of the Society for In Vitro Biology
June 3 -7, Minneapolis, MN

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ANIMAL POSTER ABSTRACTS

BIOTECHNOLOGY

A-3000 In Vivo-like Mammalian Cell Growth and Differentiation on Synthetic Nanofibrillar Surface. MUHAMMAD LODHI¹, Shannon Larkin¹, Gary Opperman¹ and Beth Jones². ¹SurModics, Inc. 9924 West 74th Street, Eden Prairie, MN 55344 and ²Donaldson Company, P.O. Box 1299, Minneapolis, MN 55440. Email: mlodhi@surmodics.com

Extracellular matrix (ECM) is an intricate network of several proteins and glycoproteins, including collagen, and proteoglycans and provides the architecture and support to tissues in vivo. Some of the ECM proteins, such as collagen, fibronectin and laminin are used for in vitro cell culture to mimic the intrinsic environment but are limited due to several functional and regulatory reasons. Current commercially available synthetic materials fail to produce reliable cell growth and differentiation of cells. We have developed surfaces made with polyamine activated electrospun nanofibers to attach and differentiate PC12, HEK293, BAE and mouse embryonic stem cells ES-D3. Cells attach, proliferate and differentiate as well or better than surfaces coated with proteins or hydrogels. PC12 cells attach better than many natural and synthetic hydrogels as well as surfaces coated with other proteins and reagents. Upon induction with NGF, PC12 cells differentiated into process bearing neurite outgrowth. ES-D3 cells not only proliferate well but also differentiate into neurons, oligodendrocytes and astrocytes when induced with different reagents and identified with O4, MAP-2, NF and GFAP markers. Data also suggest that nanofibrillar surfaces help maintain ES-D3 and PC12 cells in the undifferentiated form for a longer period of time without cytotoxic effects.

A-3001

Cytotoxic Effect of Cyanobacteria Extracts from Baja California Isolates on HCT-116 Cell Line Culture. MA-ENRIQUETA MUÑOZ M¹, Elizabeth Ponce², Hector Sigala¹, Claudia Delgadillo¹. ¹Facultad de Ciencias Química e Ingeniería, Laboratorio de Biotecnología, Universidad Autónoma de Baja California, Tijuana, BC. Av. Tecnológico 14418 Otay Mesa and ²Centro de Investigación Científica y de Educación Superior de Ensenada, BC, MÉXICO. Email: memm@uabc.mx

The programs of bioprospection with cyanobacteriae began in 1970 with reports about their toxicity, but due to the difficulties of it collects and the nonreproducibility of the samples in laboratory, the efforts in this field were truncated and by such reason they have received very little attention. It was not until 1980s that the biopharmaceutical programs for treatments of diseases like the cancer and the AIDS, identified to cyanobacteriae like one of the most prominent groups of microorganisms able to produce new potentially bioactive products. The biotoxines of cyanobacteriae are between poisons more powerful than they are known. The refinement of methodologies using cultures of cellular lines has allowed to make determinations of compound whose values are underestimated or not detected by the common tests. Isolates of

cyanobacteria along the Baja California were cultured and the extracts obtained resuspended in DMSO. These extracts were used in confluent cellular cultures multilayer and monolayer of cellular line HCT-116. The cytotoxicity test was considered by the metabolism 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT), using DMSO, etoposide, antibiotics and media as controls, the cell growth inhibition settled down in 0% and 50%. The colorimetric tests were measured at the end of 72 hours of culture and the results correlated with the visual examination of the cells at the end of the test. Variable patterns of answer were obtained to the different extracts and dilutions. Some of the extracts with low cytotoxicity inactivated the presence of Mycoplasma without damage of the cellular metabolism nor of the proliferation of cellular cultures.

CANCER BIOLOGY

A-3002

Validation of a Non-radioactive Flow Cytometry-based Unscheduled DNA Synthesis (FL-UDS) Assay. C. Kirk¹, M. Iatropolous², G. DEGEORGE¹, and G. Williams². ¹MB Research Labs, In Vitro Toxicology, Spinnerstown, PA and ²New York Medical College, Dept. of Pathology, Valhalla, NY. Email: mbinfo@mbresearch.com

The possible genotoxic potential of new chemicals and drugs drives the growing need for an inexpensive, reliable genotoxicity screening assay. The Unscheduled DNA Synthesis (UDS) assay has been proven to identify and characterize genotoxic chemicals by detecting repair of damaged DNA, via measurement of the incorporation of 3H-Thymidine following the induction of various types of genetic lesions. Since this DNA repair is distinct from de novo DNA synthesis observed in normally dividing cells, it is commonly referred to as "Unscheduled" DNA Synthesis. MB Research has developed and optimized a high-throughput non-radioactive UDS assay using flow cytometry, termed FL-UDS. This assay measures incorporation of fluorescently-labeled or conjugated thymidine analogs, rather than radioactive nucleotides. The FL-UDS assay is run on an automated microplate-driven platform, which is to be more cost-efficient, shortens study time (1 wk vs. 16 wks), can interrogate a much larger number of cells (10,000 vs. 50 - 100) and increases both accuracy and throughput. Another key improvement of FL-UDS over the radioactive UDS is that FL-UDS methodology can resolve three types of genotoxic agents: activation-dependent, activation-independent and those that are detoxified by biotransformation. We have evaluated 15 known genotoxins and non-genotoxins and successfully classified 14 of the 15 test chemicals (Accuracy = 93%). Due to its many improvements over the standard UDS assay in genotoxicity assessments (especially lower cost and faster turnaround), the FL-UDS assay is proving to be of considerable commercial value to the pharmaceutical, biotech, chemical, cosmetic and consumer products industries.

CELLULAR AND MOLECULAR BIOLOGY

A-3003

A Non-animal Phototoxicity Test Using Epidermal Tissue Models and Cytokine Endpoints. L. Pratt, J. Sallit, M. Reeder, B. Bowen, and G. DEGEORGE. MB Research Labs, In Vitro Toxicology, Spinnerstown, PA 18968. Email: mbinfo@mbresearch.com

The phototoxic potential of chemicals, cosmetics, dietary supplements and pharmaceuticals are a growing concern in the consumer products and chemical industry. Animal models of phototoxicity are expensive, slow, subjective, and not amenable to high throughput. Currently in the US, there are no regulatory agency-accepted alternative or in vitro phototoxicity tests. To address the need we have conceived a high-throughput in vitro screening test for phototoxicity designated the Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS). The EPARS test overcomes many of the limitations of the 3T3 NRU Phototoxicity Test; which has been validated in Europe by ECVAM: 1) EPARS is based upon a differentiated tissue model that closely parallels human skin morphology, instead of a fibroblast monolayer; 2) formulations of test articles can be topically applied instead of the often problematic solubilization of formulations into culture media; 3) the tissues are composed of human primary keratinocytes which are more relevant model than a mouse tumor cell line. In EPARS, the test substance is applied topically to the reconstituted human skin models, with and without UV irradiation. Overall, the EPARS test has proven to be an accurate and sensitive test for detecting phototoxic (photo-irritating) substances. Phototoxic effects are determined by comparing the viability of irradiated vs. non-irradiated tissues by MTT uptake. In order to increase the sensitivity and specificity of the test, we have measured the release of cytokines into the culture media via ELISA. PGE₂ release was shown to be an early predictor of the toxic effects demonstrated in the viability assay. Release of IL-1 alpha, IL-1ra, IL-8 & TNF-alpha supported the results of the cell viability. The effects of irradiation +/- chlorpromazine were further characterized by gene expression (cDNA microarray) analysis.

CELLULAR MODELS

A-3004

Developing a Reproducible Positive Control for Evaluation of Reactive Oxygen Species Generated by Monomac 6 Cells. F. DACCUEIL, Y. Fukuda, S. R. Simon, and E. J. Roemer. Departments of Biochemistry and Pathology, Stony Brook University, Stony Brook, N.Y 11794. Email: fdaccuei@ic.sunysb.edu

Human airway epithelial cells and inflammatory cells respond to a challenge from mineral dusts by generating multiple reactive oxygen species (ROS). We evaluated three different probes for detection of cell-generated ROS: aminophenyl-fluorescein (APF), dichlorodihydrofluorescein (DCFH), and 4-((9-acridinecarbonyl)-amino)-2, 2, 6, 6 tetra-methylpiperidine-1-oxyl (Ac-Tempo). APF reacts with hydroxyl radicals to generate the fluorescent product fluorescein; Ac-Tempo reacts specifically with secondary radicals formed from reaction of hydroxyl radicals with phenol and glutathione to yield the fluorescent acridine piperidine derivative; and DCFH is oxidized to the fluorescent dichlorofluorescein by a broader range of ROS. We developed a reproducible positive control for our assays using the potent activating agent phorbol myristate acetate (PMA) at a concentration of 100 ng/ml in combination with *E.coli* lipopolysaccharide (LPS). To enhance the responsiveness of MonoMac6 cells to the secondary stimulus; either various mineral dusts or PMA; they were pretreated with LPS at a concentration of 50 ng/ml for 24 hours prior to stimulation. Emission of the fluorescent products from the ROS

assays was recorded on a Cytofluor 2350 multiwell microplate spectrofluorimeter with excitation at 488 nm and emission at 515 nm for detecting fluorescein derivatives and excitation at 360 nm and emission at 460 nm for detecting acridine. Our data indicate that under these conditions MonoMac 6 cells generate Reactive Oxygen Species that can be measured by DCFH and APF but not by Ac-Tempo. We now have a robust positive control for our protocol employing the human monocyte cell line MonoMac 6 for detection of ROS, generated in response to stimuli. We have used this protocol to show that different mineral dusts can stimulate a human monocyte line to generate ROS which could have the potential to injure tissues if the reactions were to occur in the lungs or airways. Supported by NSF-EAR 0601994 and NSF-IGERT training grant, Minority Access to Research Career (MARC) Fellowship.

GENOMES/GENOMICS/BIOINFORMATICS

A-3005

'Omics' Technologies Enabling the 3Rs in Drug Discovery and Development: Treating Human Disease by Studying Humans. S. DHRUVAKUMAR. People for the Ethical Treatment of Animals (PETA), Norfolk, VA 23510. Email: SadhanaD@peta.org

The first step of drug discovery is "target identification" in which the disease state is studied to identify a molecular target for modulation through chemical intervention (i.e., a drug). Until recently, target identification relied heavily on finding targets by studying animal models of human disease (natural or engineered). However, animal-based targets are only successful to the extent that the relevant biology is replicated in humans – a hit-or-miss proposition due to differences between species and between the animal and human diseases. However, a new paradigm is emerging as genomics, proteomics, and other 'omics' technologies are facilitating the molecular level study of humans. Targets are increasingly found by studying human tissue (e.g., normal vs. diseased or early vs. late stage) for differential gene or protein expression to identify genes that are involved in the disease process. Cellular pathways associated with the human disease can be mapped using such techniques, leading to the identification of high-quality human-relevant disease-specific targets. Another application of 'omics' technologies comes later during drug development, when these technologies can be used to identify early biomarkers of drug efficacy or toxicity. Early stage human drug testing or 'experimental medicine' utilizing such biomarkers is increasingly popular and is replacing some animal preclinical testing. The identification of 'omics' biomarkers is also being done within animal experimentation. While this enables more humane endpoints, the fact remains that attempting to extrapolate from animal-based human drug research is inefficient compared to studying humans directly, and resources should shift more quickly towards human-based 'omics' approaches.

VIROLOGY

A-3006

Rational Vaccine Design: Rendering Black-box Animal Potency Testing Obsolete. S DHRUVAKUMAR. People for the Ethical Treatment of Animals (PETA), Norfolk, VA 23510. Email: SadhanaD@peta.org

Every batch of vaccines is tested for potency and safety, currently consuming approximately 10 million animals per year (an estimated 10% of all animal use in biomedical experimentation globally). Animal potency testing involves a pathogenic challenge, and thus causes much suffering. Animals can be entirely replaced in potency testing by physico-chemical methods of quantifying antigens (the part of the vaccine that stimulates the protective

response) if they are known, but for most currently used vaccines which were developed empirically, the protective antigens are not known. This is problematic because poorly characterized vaccines are difficult to test in ways other than black-box animal studies, but animal potency tests are often unpredictable due to species differences as well as non-biological routes of exposure (e.g., rabies inoculation through intracerebral injection in the NIH test). Antigen quantification systems have been devised for some older vaccines, but resources may be better spent focusing on new vaccines and novel methods of vaccine development. As researchers deduce the sequences and structures of pathogenic proteins and develop a detailed knowledge of their roles, they can purposefully design vaccines with defined components in order to maximize effectiveness and minimize safety concerns. Computational immunology can help predict which epitopes are likely to be the best targets. Fortunately, rational vaccine design by its nature goes hand-in-hand with non-animal testing since designing vaccines to contain defined antigens enables the direct measurement of antigenic content, thus rendering the use of animals in potency testing obsolete.

SILENT ABSTRACT

A-3007

Up-regulation of the TNF- α mRNA Steady-state Level in Sinusoidal Endothelial Liver Cells Exposed to Cold in Presence of EGB-761T. EDUARDO PORTILLO-DELCAMPO, Franciannella Reyes-Vargas, Miguel R. Reyes. Faculty of Medicine UJED. Durango, Dgo., 34000. MEXICO. Email: laloporto@hotmail.com

Cold-induced injury of sinusoidal endothelial liver cells (SEC) mediated by free radicals is an early event that results in loss of up to 8% of transplanted livers. EGB-761T (EGB), an antioxidant flavonoid-rich standardized extract of Ginkgo biloba leaves prevents the cold-induced apoptosis of rat SEC (Hepatology 40 (S1), A499, 2004); however, mechanisms distinct to the antioxidative effect of EGB could be involved because genomic and metabolic effects of EGB have also been described. Our aim was to study in vitro the effect of EGB on the steady-state level of TNF- α mRNA in SEC. Confluent monolayers of a rat SEC line cultured at 37 °C in Dulbecco's MEM with 0.2% albumin were maintained by 24 h at 2 °C in presence of 0.2% EGB; afterward, cells were rewarmed to 37 °C during 1 h; positive controls were cold-exposed cultures without EGB, negative controls were unexposed cultures. TNF- α mRNA was measured by semi-quantitative RT-PCR, experiments were done by quadruplicate. Cold-exposed cultures showed a partially detached monolayer with rounded apoptotic cells; EGB-treated monolayers maintained integrity and looked morphologically similar to negative controls. TNF- α amplicons in negative controls were barely detectable; positive controls and cultures with EGB showed a significant increase in the TNF- α mRNA steady-state level of 3.2- and 3.0-fold respectively. Since TNF- α elicits survival and death signals, its up-regulation appeared to be associated to cold exposure because this up-regulation was similar in both positive controls and EGB-exposed cultures. However the dramatic difference in outcome suggests that TNF- α signalled different pathways. By the potential usefulness of flavonoids in the organ preservation field further studies are warranted.

EDUCATION POSTER ABSTRACT

E-3000

2005 Survey of SIVB Students and Postdocs. P. J. WEATHERS, B. E. O'Neil, and S. J. Pals. Department of Biology and

Biotechnology, Worcester Polytechnic Institute, 100 Institute Rd., Worcester, MA 01609. Email: weathers@wpi.edu

At last year's annual meeting the attending students and postdocs were queried about their desire to have a student subgroup of the SIVB. The initial number of responses was low, so to enlarge the response pool, the survey was also sent out via email to other students in the Society. There were 28 respondents. The survey gathered demographic data along with respondents' opinions about the formation of a student subgroup. Data were analyzed using SAS statistical program.

Key results were:

1. A sincere interest in starting a student subgroup.
2. The respondents also displayed a willingness to serve as leaders of this group.
3. The student respondents indicated that in their opinion, formation of a subgroup might increase membership numbers for the Society.
4. The most important benefits that were desired were: free conference registration, a student-only session at the annual SIVB meeting, resume, job, and internship postings, and scholarship opportunities.
5. Having such benefits would positively influence their decision to later become full paying members of the SIVB.

PLANT POSTER ABSTRACTS

BIOTECHNOLOGY

P-3000

Transgenic Mitigation Technology for Reducing Risks of Transgene Flow. H. AL-AHMAD^{1,2} and J. Gressel¹. ¹Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, ISRAEL and ²Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996-4561. Email: halahmad@utk.edu, Jonathan.Gressel@weizmann.ac.il

Transgenic crops can interbreed with related weeds and crop cultivars. Depending on the transgene, this may increase the fitness of the hybrid offspring. Two mechanisms have been suggested to control transgene flow and establishment: either containing the transgene(s) within the biotech crop, and/or by employing transgenic mitigation (TM) techniques to minimize the environmental effects of the primary transgenic trait (e.g. herbicide resistance, pharmaceutical trait, etc.) should it escape. In TM technology, mitigator genes that lower the competitive ability of transgenic hybrids are linked in tandem to the primary transgene. Such mitigator genes are neutral or positive to the biotech crop, conferring traits such as dwarfism, no secondary dormancy, non-shattering of seedpods, etc. The TM concept was tested in tobacco (*Nicotiana tabacum*), and oilseed rape (*Brassica napus*) that may remain in fields as volunteer weeds and the latter can interbreed with nearby weedy *B. rapa*. The two TM crops were transformed with a tandem construct of *ahas*^R conferring herbicide-resistance in tandem with Δ gai for dwarfism. In both plant systems, the risk of transgene establishment in transgenic volunteer or intraspecific hybrids was effectively reduced at different levels of competition, at the close spacing typical of weed populations, under greenhouse and screen-house conditions. The yield of transgenic monocultures was significantly higher than the corresponding wild type of both crop species due to the increased harvest index conferred by dwarfism. The yield of TM *B. napus* was double the wild type when growing by itself. In contrast, TM hybrids with wild type *B. napus* or with weedy *B. rapa* were unfit to reproduce well when grown interspersed with the non-transgenic cohorts.

Their reproductive fitness based on seed yield relative to the non-transgenic *Brassica sibs* was between zero and 11% depending on planting density, demonstrating the advantage of the TM technology to minimize the risk of transgene establishment and spread, while increasing crop yield.

P-3001

Mass Production of Corosolic Acid by Suspension Culture of *Eriobotrya japonica*. CHANG-HEON KIM, Jin-Ah Kim, Jin Suk Cho, Moon-Suk Kim, Sang-Hyun Pyo, Jai Young Song, and Hojoon Choi. Samyang Genex Food & Bio Research Center, Daejeon, 305-717, KOREA. Email: chkim@genex.co.kr

Corosolic acid is a biologically active triterpenoid, which has been shown to stimulate glucose transport activity, and therefore have potential benefits for regulating blood sugar levels. It is known to be contained in *Lagerstroemia speciosa* (banaba), *Eriobotrya japonica* (loquat), *Rhabdosia japonicus* (Isodonis Herba) and many other plants. Recently, accumulation of corosolic acid in callus cultures of *Eriobotrya japonica* was reported. In this study, suspension cultures of *Eriobotrya japonica* were investigated as a commercial source for corosolic acid. Callus cultures derived from leaves of loquat on various media were used to induce suspension cultures. Cultures grown in LS and MS media showed more rapid growth rate than cultures grown in B5 and mB5 media. However, productions of corosolic acid were significantly higher in B5 and mB5 media than LS and MS media. The effects of initial sugar levels and inoculum size were also investigated. Yields of corosolic acid in cultures grown in higher initial sugar level were 3-5 fold higher than cultures grown in lower initial sugar level. In addition, several types of elicitors were evaluated for improving productivity of corosolic acid in loquat suspension cultures. In combined culture conditions of these results, the maximum corosolic acid production was reached to 2151 mg/L/14days.

P-3002

Environmental Impact of Antibiotic Resistance Transposed from Genetically Modified Organisms to the Soil Bacteria is Insignificant. P. AROKIARAJ¹, D. Antarjo², R. Leela-Vathy¹, and S. Kamaruzaman². ¹Malaysian Rubber Board, Rubber Research Institute of Malaysia, Biotechnology and Strategic Research Unit, 47000 Sungei Buloh, Selangor, MALAYSIA and ²University Putra Malaysia, Department of Plant Protection, 43400 UPM, Serdang, Selangor, MALAYSIA. Email: parokiaraj@lgm.gov.my, kama@agri.upm.edu.my

The Rubber Research Institute of Malaysia routinely employed the neomycin phosphotransferase II (nptII) cDNA as a selection marker for generating transgenic *Hevea brasiliensis* (Rubber) plants. One of the concerns in the planting of genetically modified crops was the theoretical possibility of antibiotic resistance transferred to soil bacteria when vegetative materials of the transgenic plant falls to the ground and decompose. As such, a study was carried out to show the prevalence of antibiotic resistant bacteria in soil areas at 10 sites surrounding the transgenic plots as well as from soil samples at 10 sites collected from the natural habitat of a rubber plantation. From this study, a profile of antibiotic resistant bacteria to one or to multiple antibiotics was generated by plating on antibiotic nutrient media at 28°C and at 37°C. Bacterial isolates were identified by Biolog® identification system based on the carbon source utilized. Preliminary results seemed to confirm that resistance to kanamycin, ampicillin, carbenericillin, tetracyclin and chlroamphenicol is common among soil samples studied in the designated areas. The high prevalence of kanamycin resistant organisms already present in the environment would not cause a significant environmental impact by the presence of the kanamycin

resistant rubber plants as the rate at which such transfer could take place, if at all, was of so small a magnitude. Hence, these results might show that the transposition of antibiotic resistance from genetically modified rubber trees to soil bacteria was of no significance.

P-3003

Employment of Efficient Marker-free Transformation Methods. C. RICHAEAL, T. W. Weeks, J. Ye, and C. M. Rommens. Simplot Plant Sciences, J.R. Simplot Co., Boise, Idaho 83706. Email: Craig.Richael@Simplot.com

As part of a larger goal to design transgenic crops plants with no foreign DNA, three independent marker-free methods were developed to transform plants. In contrast to earlier strategies, these new methods are highly efficient and commercially applicable. The first marker-free method takes advantage of transient expression of the kanamycin resistance nptII gene coupled with selection against its stable integration with the gene of interest. On average, twenty percent of plants derived from this strategy contain the gene of interest while lacking the marker gene. The efficacy of this method has been demonstrated for the Solanaceous crops potato, tobacco and tomato. Results from recent experiments confirm its applicability to a Brassica species as well. The second marker-free method is based on the ability to transform the exposed meristems of germinated seedlings following an extensive vortexing step, and was found to be cultivar and species independent. Alfalfa plants transformed with agronomically important traits are being generated with marker-free efficiencies in the T1 generation of one to five percent. The third marker-free strategy employs an Agrobacterium binary plasmid with the gene of interest within the right and left borders and the isopentenyl transferase gene (ipt) in the vector backbone. Overexpression of the plant hormone biosynthetic gene serves to drive regeneration of normal, marker free shoots and functions as a visual marker for integration of the backbone sequence within the plant genome. The method precludes the use of complicated regeneration media with hormones and selection agents. Successful production of marker and backbone free plants has currently been accomplished for crops including tomato, tobacco and potato with frequencies ranging from three to ten percent. The three transformation strategies explained here are currently being applied to improve the agronomic performance and/or nutritional characteristics of plants using only native DNA.

P-3004

Properties and Characteristics of Recombinant Plant-made Vaccines. L. SCHULENBERG, M. Henry, J. Van Eck, A. Walmsly, D. Kirk, H. Mason, C. Arntzen, M. Fanton, T. Miller, C. Mihaliak, and S. Webb. Dow AgroSciences, LLC, 9330 Zionsville Road, Indianapolis, IN 46268. Email: lschulenberg@dow.com

Plant-based expression systems have long been considered a platform for the production of vaccines and other therapeutic proteins. We have recently demonstrated the successful production of hemagglutinin neuraminidase (HN) glycoprotein from the Newcastle Disease Virus in tobacco suspension cultures. This plant-cell-culture system is a safe, efficient and effective production platform for the expression of biologically active protein antigens. Furthermore, Dow AgroSciences has recently received the first licensure for a plant-made vaccine from the USDA/APHIS - Center of Veterinary Biologics. The poster will detail the technical characteristics of the Concert™ plant-cell production system and the HN antigen.

P-3005

Evaluation of RNAi Technology for Rice Blast (*Magnaporthe grisea*) Resistance. V. SEGOVIA, S. R. Stevens, B. Valent, and H. N. Trick. Department of Plant Pathology, Kansas State University, Manhattan, KS 66502. Email: vsegovia@ksu.edu

The fungal disease rice blast, caused by *Magnaporthe grisea*, is one of the most important diseases of rice (*Oryza sativa*). Although some resistant rice cultivars have been developed through breeding, blast resistance is either partial or limited to a handful of fungal isolates. One possible method of increasing fungal resistance is through the use of RNA interference (RNAi). RNAi and gene silencing have become potentially important tools to control diseases by blocking the production of specific proteins in the plant/pathogen interaction. The availability of genome sequence for both *M. grisea* and rice facilitate the evaluation of this novel approach. To test our hypothesis, three *M. grisea* genes were selected for this study and two delivery methods were evaluated: exogenous application of dsRNA transcripts to fungal cultures and the production of RNAi expressing transgenic rice plants. Primers for the selected genes were based on the *M. grisea* database (<http://www.broad.mit.edu/annotation/fungi/magnaporthe/>) and approximately 300 bp of each gene was selected as our target sequences. For the exogenous application dsRNA molecules were generated using MEGAscript RNAi Kit (Ambion) on Silencer siRNA Cocktail Kit (Ambion). For the exogenous application, pANDA vectors were used to generate siRNA in transgenic rice via *Agrobacterium*-mediated transformation. Both the results of the in vitro assays and analysis of transgenic plants will be discussed.

P-3006

Biotechnological Advances in Medicinal and Nutraceutical Plants. Anand K. Yadav, Nirmal Joshee, Bipul K. Biswas, and ASHISH YADAV. Agricultural Research Station, Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030. Email: yadava@fvsu.edu

The biotechnology research on medicinal and nutraceutical plant species is in high-priority area in the Specialty Plants Biotechnology Laboratory of Agricultural Research Station at Fort Valley State University. Primary objectives underlying potential activities are the introduction of specialty plants germplasm from different sources around the world followed by their evaluation for required/desirable quality characteristics. Furthermore, developing efficient biotechnological protocols for plant regeneration, their mass multiplication and conservation are also focused. Analyzing various plant parts for bioactive properties of selected secondary metabolites and genetically transforming them to enhance/improve their desired quality characters and increase their cold tolerance for wider adaptability. We are working on some important medicinal plant species of great potential, viz., *Scutellaria*, *Bacopa*, *Ocimum*, *Centella*, *Withania*, *Commiphora*, *Azadirachta*, *Curcuma*, *Datura*, and *Basella*. Further, we are also investigating selected nutraceutical plants, viz., guava, peach, phalsa, jamun, moringa, papaya, amla, and bael. We have developed biotechnological protocols for plant regeneration, somatic embryogenesis, synthetic seeds, and the mass multiplication in most of these plants. Additional studies on genetic transformation and in vitro production of secondary metabolites are also underway.

P-3007

Efficient Paclitaxel Production and Release in *Taxus chinensis* Cell Suspension Cultures Adapted to Chitosan. CHANGHE ZHANG^{ab}, P. S. Fevereiro^a and Guangyuan He^b. ^aInstituto de Biologia Experimental e Tecnologica/Instituto de Tecnologia Quimica e Biologica (IBET/ITQB), Apartado 12, P-2781-901

Oeiras, PORTUGAL and ^bSchool of Life Science & Technology, Huazhong University of Science & Technology, Wuhan 430074, CHINA. Email: zhang@itqb.unl.pt

The aim of this work was to characterize the growth and paclitaxel production and release of *Taxus chinensis* cell suspension cultures adapted to chitosan stress by comparing with normal non-adapted cells. Without elicitor treatment, the constitutive paclitaxel yield and extracellular accumulation in the chitosan-adapted cell cultures were 2 and 6-fold those in the non adapted cell cultures, respectively. Upon elicitor treatment, the severe growth suppression observed in the non-adapted cell cultures was significantly alleviated or even eliminated in the chitosan-adapted cell cultures. Elicited by Ag⁺, methyl jasmonate (MJ), fungal elicitor or chitosan, the paclitaxel yield and the extracellular accumulation of the chitosan-adapted cell cultures was 4.6, 3.2, 2 or 2-fold and 2.8, 3.2, 3 or 1.3-fold those of the non-adapted cells, respectively. The efficient paclitaxel productivity and release capability observed in the chitosan-adapted cells couldn't be achieved by chitosan or MJ pretreatment (priming) of the non-adapted cells several days before elicitor treatment. The highest yield at 47.5 mg/l was obtained in the MJ-elicited chitosan-adapted cell cultures with MJ-priming. This yield was 2.2 and 50-fold those of the non-adapted cell cultures under the same treatment and without elicitor treatment, respectively. Further more, the phenylalanine ammonia-lyase (PAL) activity and extracellular protein content of both cell cultures were also evaluated in the presence and absence of elicitor treatment.

EDIBLE VACCINES

P-3008

A Plant-derived Vaccine Candidate for Hepatitis B Virus and Human Papillomavirus. Z. HUANG¹, L. Santi¹, R. C. Rose², C. J. Arntzen¹, and H. S. Mason¹. ¹Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, AZ 85287-5401 and ²School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642. Email: zhong.huang@asu.edu

A region of amino acid 108-120 (LVEETSFIDAGAP) of the human papillomavirus (HPV) L2 minor capsid protein has been found to induce antibodies that cross-neutralized different types of HPV. Here we described the expression of this neutralizing "Kawana" epitope as a N-terminal fusion to hepatitis B surface antigen (designated as "K-HBsAg") in several plant expression systems and its immunogenicity in mice. Using a plant virus-based transient system, the K-HBsAg was produced at levels up to 1.4 µg per mg total soluble protein in *Nicotiana benthamiana* leaves. Transiently expressed K-HBsAg accumulated as full-length product, presented the neutralizing HBsAg 'a' determinant, and assembled virus-like particles (VLPs) that present the L2 epitope as demonstrated by sucrose gradient sedimentation. Partially purified K-HBsAg evoked high-titer anti-HBsAg and moderate-titer anti-HPV L2 serum responses in Balb/c mice when injected systemically. More importantly, IgG in sera from K-HBsAg immunized mice bound to HPV16 L1/L2 VLPs and competed for binding sites on the VLPs with a L2 peptide-immunized rabbit serum, which is known to neutralize authentic HPV virions. Our results demonstrate that the K-HBsAg can stimulate protective HBV- and HPV-specific antibody responses, and is thus a promising vaccine candidate against both HPV and HBV. In addition, we generated transgenic *N. benthamiana* and potato plants expressing the K-HBsAg at levels up to 0.9 µg/mg total soluble leaf protein and will test their oral immunogenicities in mice.

EMBRYOGENESIS/REGENERATION/ MICROPROPAGATION

P-3009

Age-dependent Biological Characteristics of Embryonal Mass of Maritime Pine in Relations to the Embryogenic Potential. K. KLIMASZEWSKA, C. Noceda, G. Pelletier, P. Label, R. Rodriguez, M. A. Lelu-Walter. Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 rue du P.E.P.S., Quebec, CANADA. E-mail: kklimaszewska@cfl.forestry.ca

Somatic embryogenesis (SE) in conifers is at the base of nearly all biotechnology products that potentially might be developed including transgenic trees. SE is a powerful tool for clonal propagation that has opened avenues for deployment of superior clonally replicated planting stock in forest plantations. Although the SE process can be managed for high efficiency there are still several problems that require better understanding of underlying causes. One phenomenon, which occurs after the long-term culture of embryogenic tissue, particularly of pines, is the loss of ability to produce mature somatic embryos and consequently plants. Such unproductive cultures must be replaced, which is both expensive and undesirable. In an attempt to get an insight into the differences between young, primary lines of embryonal mass of maritime pine that produced somatic embryos and the same lines of significantly increased age that stopped producing somatic embryos, we analyzed in both types of material the levels of endogenous hormones and their metabolites, polyamines, global DNA methylation and DNA methylation patterns. DNA methylation plays an important role in regulation of gene expression and may cause epigenetic change leading to suppressed embryogenic potential. To determine if the DNA methylation could be altered, we exposed the cultures to different concentrations of hypomethylating agents such as 5-aza-2'-deoxycytidine, 5-aza-cytidine and zebularine and tested the embryogenic potential of a line after the treatment. For comparison, we induced secondary SE from a few mature somatic embryos of the same lines and carried out the same as above analyzes. Secondary SE has been implicated in restoring/enhancing the embryogenic potential of a given line and therefore theoretically the tissue was expected to be biologically similar to the primary young line. Our results indicated that embryogenic cultures of different ages varied with respect to the polyamines levels and DNA methylation pattern but not with respect to the hormone concentration. Treatment of the cultures with the hypomethylating agents resulted in different methylation patterns and somatic embryo production ability.

GENOMES/GENOMICS/BIOINFORMATICS

P-3010

Potential Dual Role Members of the GRAS Gene Family Play in *Medicago truncatula* Symbiosis and Defense R. J. MORPHEW, M. A. Graham, K. A. T. Silverstein, and K. A. VandenBosch. University of Minnesota, Department of Plant Biology, Saint Paul, MN 55108. Email: morp0005@umn.edu

Members of the GRAS family of transcriptional regulators have diverse functions in many plant species (Pysh et al. 1999). In *Arabidopsis thaliana*, members of the family have been shown to play roles in Gibberellin acid signal transduction, meristem development, and radial patterning in roots. In *Medicago truncatula*, two members of the GRAS family, NSP1 and NSP2, are necessary for the symbiotic relationship between the plant's roots and nitrogen fixing bacteria (rhizobia) (Smit et al. 2005, Kalo et al. 2005). Two GRAS genes have been identified in rice that are upregulated after treatment with an oligosaccharide elicitor, but

possible roles for GRAS proteins as regulators of defense responses has not been explored (Day et al. 2004). We propose that some GRAS family members may play a role both in defense responses as well as in symbiosis with mycorrhizal fungi and/or rhizobia. Here, we will present a strategy to investigate this hypothesis, and summarize what is known about expression of gene family members in *M. truncatula*. The phylogenetic relationship among known members of the GRAS family from various species of plants will be addressed.

IN VITRO TOOLS, TECHNIQUES, AND OPTIMIZATION

P-3011

An In Vitro Culture System to Study the Role of Secondary Metabolites in the Interaction Between AM Fungi and Purified Cell Walls from Host Roots. G. NAGAHASHI. USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA 19038. Email: gnagahashi@errc.ars.usda.gov

Arbuscular mycorrhizal fungi are obligate, mutual symbionts that are able to extract nutrients from the soil and make them available to a host root in exchange for sugar molecules. In an effort to complete the fungal life cycle without a host, aseptic germinated spores from *Gigaspora gigantea* and purified carrot root cell walls (plant extracellular matrix) were used in an *in vitro* culture system to study particular steps of the life cycle. Large contiguous pieces of carrot cell walls, frequently 4-5 cells in length, were isolated with a Parr nitrogen bomb. The epidermal/cortical cell walls were then purified by removing vascular walls via a step sucrose gradient. To increase appressoria formation on the surface of purified walls, the culture plate (containing walls and geminated spores) was physically manipulated to maximize hyphal branching. This system has provided for a new bioassay which allows us to study the formation of penetration hyphae which develop immediately after appressoria formation. The formation of penetration hyphae are most likely induced by secondary metabolites from the host. This stage of fungal development is the 4th out of 7 steps in the life cycle which can be completed without the presence of a living host root. Completion of the life cycle, *in vitro*, would provide a method to generate large quantities of pure, noncontaminated AM fungal spores. These spores could potentially replace or supplant the use of chemical fertilizers.

PLANT TISSUE CULTURE

P-3012

Plant Regeneration and Transformation in Castor (*Ricinus communis* L.), an Important Oil Crop. YEH-JIN AHN, Louisa Vang, and Grace Q. H. Chen. US 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, efficient transformation techniques for castor plants have not been developed. In this study, we examined plant regeneration and genetic transformation of castor plants. Explants from young castor plants produced adventitious shoots (24 shoots per explants). When developed and elongated, 93% of shoots were rooted. Regenerated plantlets were acclimated in the soil successfully. To our knowledge, this is the first study reporting an efficient protocol for adventitious shoot formation from non-meristematic tissues in castor. Using this protocol, various conditions for *Agrobacterium*-mediated and biolistic transformations were tested for the genetic transformation.

Establishment of a reliable transformation protocol will allow us to perform a broad range of transgenic studies in castor.

P-3013

A Biological Selection System for Obtaining Somatic Embryos and Plant Regeneration of Common Bean *Phaseolus vulgaris* L. L. JOSÉ LUIS CABRERA-PONCE, Guadalupe Sánchez-Martínez, Fernando Hernández-Godínez, Claudia Geraldine León-Ramírez, Luis Herrera-Estrella. Depto. Ingeniería Genética, Unidad Irapuato, Campus Guanajuato, Centro de Investigación y de Estudios Avanzados del IPN. Apdo. Postal 629, C.P. 36500 Irapuato, Gto., MÉXICO. Email: jcabrera@ira.cinvestav.mx

Somatic embryogenesis from 15 genotypes of *Phaseolus vulgaris* L. was obtained. A biological selection system was applied to select totipotent tissue from the shoot apex and cotyledonary tissues of mature zygotic embryos. Osmotic treatments were evaluated using equimolar concentrations of mannitol+sorbitol (0.2M each) and 12 % sucrose. All obtained calli were embryogenic, and were developed after 45 days in culture, the rest of the zygotic embryo, composed of non-totipotent cells became necrosed and died. The best results were obtained by culturing zygotic embryos in 12% sucrose for 48 hours in MS medium containing 10 mg/L BAP and 40 mg/L adenine, and then transferred to fresh medium without the osmotic and were incubated under a 16 hours light photoperiod, at 30 μ moles/m².seg of irradiance. Adenine was found to be an important factor to induce and maintain the response. An efficiency of 75% of the explants responded forming somatic embryos. Embryogenic callus were satisfactorily propagated, by transferring them, at one month intervals, to the same medium. Their embryogenic capability was maintained for at least three years, keeping its high quality of embryogenic capability. Plant regeneration was obtained in two steps: 1.- Induction of roots from embryogenic callus by culturing in MS medium with 0.2 mg/L BAP, 0.1 mg/L kinetin for two months, and 2.- Germination of complete plants derived from embryos/roots coming from rooted callus. The frequency of conversion of plants was 25%, one plant can be regenerated from 100 mg of fresh weight of callus. All regenerated plants were successfully transferred and maintained in a greenhouse, all produced seeds. This protocol is being used satisfactorily for genetic transformation by biolistics and *Agrobacterium tumefaciens* methods.

P-3014

In Vitro Production of Metabolism-enhancing Phytochemicals from *Rhodiola heterodonta* and *Rhodiola semenovii*. DIANA M. CHENG, R. B. Rogers, G. Yousef, M. Grace, and M. A. Lila. Department Natural Resources and Environmental Sciences, University of Illinois Urbana-Champaign, 1115 Plant Sciences Lab, 1201 S. Dorner Dr., Urbana, IL 61801. Email: dcheng2@uiuc.edu

Rhodiola heterodonta and *Rhodiola semenovii* are recognized as donors for metabolism-enhancing and endurance-promoting (adaptogenic) extracts by indigenous people of Uzbekistan and Kyrgyzstan. These plants are related to the poplar adaptogenic plant, *Rhodiola rosea* (goldenroot) and are taken in alcoholic tinctures. Phenolic and cyanogenic glycosides along with proanthocyanidins are the compounds purported to provide the medicinal properties. *R. heterodonta* and *R. semenovii* callus cultures were established from shoots of microplants and

maintained on MS media and supplemented with 1.5 mg/L BAP and 0.5 mg/L NAA. Large callus aggregates were established and maintained on liquid media of the same composition with subculture intervals of 2 weeks. The phytochemical profile of in vitro cultures of each species was analyzed by HPLC. Preliminary results show that phenolic and cyanogenic glycosides were not detected in control cultures. Elicitation of *R. heterodonta* on the day of subculture with 250 μ M methyl jasmonate resulted in the detection of salidroside at 18.9 μ g/g (fresh weight of cells) and viridoside at 83.6 μ g/g. When *R. semenovii* callus cultures were similarly elicited, the phenolic glycosides were not elicited, however, lotaustrolin, a cyanogenic glycoside, was detected at 168.6 μ g/g.

P-3015

Improvements In Vitro Rooting of *Asimina tetramera* with Silver Thiosulfate or Activated Charcoal. M. A. JASKOWIAK, V. C. Pence, and S. Charls. Cincinnati Zoo and Botanical Garden, Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati, OH, USA, 45220. Email: mjaskowiak@cincinnati-zoo.org

Asimina tetramera (four-petaled pawpaw) (Annonaceae) is a federally endangered species endemic to Florida. Micro-propagation protocols have been developed for this species as part of the Endangered Plant Propagation Program at CREW, but, like many other woody species, in vitro rooting percentages have been low. Rooting was first achieved in *A. tetramera* when 50 or 100 μ M silver thiosulfate (STS), an inhibitor of ethylene action, was added to WP medium containing 0.5 mg/L IBA (Charls, et al. 2003), suggesting that high levels of ethylene inhibit root initiation in these species. However, only about 25% of shoots developed roots on this medium. Because two other ethylene inhibitors, silver nitrate and aminovinylglycine, that were tested did not increase rooting further, other media and environmental conditions were investigated. Shoot cultures were maintained on MS medium with 3% sucrose, 2.4% agar, and 1 or 2 mg/L BAP. For rooting experiments, healthy cuttings with at least three nodes from four-six week-old cultures were used and transferred to WP medium with MS organics and 1 mg/L IBA. The plants were incubated at 21° or 26°C and at 20 or 40 μ E/m²/sec PAR. There was no difference in rooting observed at the two light levels, but at 21°C, an average of 27% of the shoots produced roots, compared with 7% at 26°C (p<.05). In separate experiments, shoots were transferred to WP medium with 50 mg/L IBA for 7 or 10 days and then transferred to WP medium with 1% charcoal. There was a higher percentage of root initiation after 10 days of culture on IBA (64%) than after 7 days of culture (34%) (p<.05). These results suggest that temperature, auxin level and the time of exposure to auxin, in addition to ethylene levels, are important in stimulating root initiation in micropropagated shoots of *A. tetramera*. Further studies are underway to examine the timing of the auxin stimulation of root initiation in these species.

P-3016

Improvement of Soybean Transformation Using *Agrobacterium rhizogenes*. M. J. KIM, R. Collier, Veena, and C. G. Taylor. Donald Danforth Plant Science Center, Saint Louis, MO 63132. Email: mkim@danforthcenter.org

Soybean (*Glycine max*) is a valuable economic crop, and the current transformation methods have low transformation efficiencies, 3-5% typically. It is of great interest to the soybean research community to find new ways to increase transformation efficiencies of soybean. Recently, our lab has modified a highly virulent strain of *Agrobacterium rhizogenes*, strain K599. Due to its

ability to transform many different legumes *A. rhizogenes* strain K599 has become the predominate strain of choice for making transgenic "hairy roots" in soybean. Using homologous recombination, the T-DNA for *A. rhizogenes* strain K599 was removed creating a disarmed *Agrobacterium*. In transformation test of *Arabidopsis* the newly disarmed *A. rhizogenes* improved transformation efficiencies by 2.5x fold as compared to the widely used *A. tumefaciens* strain C58C1. This newly disarmed *A. rhizogenes* is now being tested in several different model transformation systems, including soybean. Transformation efficiency data will be presented on using the disarmed *A. rhizogenes* in a standard *Agrobacterium*-cotyledonary node transformation system.

P-3017

Production of Secondary Metabolites and Recombinant Proteins in Hairy Roots Cultured in the Liquid Lab™ Bioreactor. F. MEDINA-BOLIVAR and L. Nopo-Olazabal. Arkansas Biosciences Institute and Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401. Email: fmedinabolivar@astate.edu

Hairy roots obtained via *Agrobacterium rhizogenes*-mediated transformation have attracted much attention for secondary metabolite production because of their genetic stability, rapid growth, and ability to synthesize the same metabolites as in planta. More recently, these systems have been also exploited for expression of recombinant proteins such as antibodies and therapeutics. Both, secondary metabolites and proteins, can be secreted into the hairy root culture medium reducing labor and cost associated with the purification of these molecules. Scale-up of hairy root biomass have been often associated with high cost and complex bioreactors. To address these issues, we have tested the simple-to-operate Liquid Lab™ (Southern Sun Biosystems, Inc.) rocker reactor for growth of hairy roots of *Hyoscyamus muticus* and *Nicotiana tabacum* (tobacco) and production/secretion of sesquiterpenes and recombinant green fluorescent protein (GFP5). Medium in the bioreactor vessel was exchanged every 2 weeks and pH and conductivity measurements were recorded. To minimize chances of contamination, the biocide PPM™ (plant preservative mixture) was added at a concentration of 0.02% after the first 2 weeks of culture. Concentrations of PPM™ higher than 0.1% inhibited growth of the roots. Sesquiterpene production in hairy roots of *H. muticus* was studied by elicitation with 600 µM of copper sulfate. Cultures were elicited every 2 weeks and the sesquiterpenes were analyzed in the medium by thin layer chromatography. Secretion of GFP5 was studied in hairy root of tobacco engineered with the "super promoter", patatin signal peptide and GFP5. The content of GFP5 in the roots and medium was determined by ELISA. Our studies indicate that the liquid lab reactor system is a low-cost scalable alternative for production and secretion of secondary metabolites and recombinant proteins from hairy roots.

P-3018

Micropropagation of *Spilanthes acmella* L. and Evaluation of Its Larvicidal Activity Against Malarial Vectors *Culex quinquefasciatus* and *Anopheles stephensi* Liston. VIBHA PANDEY¹, Veena Agrawal¹, Kuldeep Sharma¹, S. P. Singh² and K. Raghavendra². ¹Department of Botany, University Delhi, Delhi-110007 and ²Malaria Research Centre, 22, Sham Nath Marg, Delhi-110054, INDIA. Email: drveena_du@yahoo.co.in

Spilanthes acmella L. (Asteraceae), commonly known as 'akarkara' is a valuable medicinal herb containing strong antimalarial, antibacterial, antifungal and larvicidal activity. The plant owes its

activity to the bioactive compound spilanthol and a number of immune stimulating alkylamides. In vitro regeneration of this potential herb has been achieved through leaf explants excised from in vitro raised shoots, on MS medium augmented with a variety of growth regulators such as auxins (2,4-D, NAA & IBA) and cytokinins (BA & Kn) tried either alone or in combination. MS + 1 µM NAA + 10 µM BA proved optimum for differentiating an average of 12.90 ± 0.32 shoot buds per responding culture in 60% explants. An elevation in average shoot length (2.53 ± 0.45) along with further proliferation of shoot buds (15.7 ± 0.70) was observed when the shoots were subcultured on MS + 10 µM BA. Such buds on transfer to MS + 1 µM NAA + 1 µM BA showed significant enhancement in shoot length (5.27 ± 1.0) in 100% cultures. For rhizogenesis, MS (½) + 0.1 µM IBA proved optimum, where 100% shoots developed an average of 19 ± 0.56 roots within 15 days. The plants were successfully transferred to greenhouse after initial hardening. 100% of the plants showed normal flowering and set seeds without any morphological variation. The bioefficacy of these in vitro regenerated plants were tested against late III/ early IV instar larvae of *Culex quinquefasciatus* and *Anopheles stephensi* where 100% mortality was achieved at a concentration of 3.75 ppm in *Culex quinquefasciatus* and at 7.5 ppm in *Anopheles stephensi* from the crude extract of root.

P-3019

In Vitro Propagation of the Medicinal Plant *Aristolochia indica* L. through Nodal Explants. V. SELVAKUMAR* and T. Balakumar. Centre for Plant Biochemistry and Molecular Biology, The American College, Madurai 625 002, INDIA and *present address: Department of Plant Pathology, University of Florida, FL. Email: selvak@ufl.edu

Aristolochia indica L. belonging to family Aristolochiaceae, the whole plant contains 1-2-nonacosinic acid and its roots contain an essential oil having phenanthrene derivatives like aritolic acid, aristolochic acid, alkaloid 1-curine (the alkaloid aristolochin) etc.,. Aristolochic acid is used for stimulating phagocytosis in infectious diseases in combination with antibiotics. Because of an important medicinal properties in this plant we selected and standardized a protocol for in vitro propagation of the medicinal plant *Aristolochia indica* L. (Aristolochiaceae) using nodal explants. Kinetin (KN), gibberellic acid (GA₃), benzyl adenine (BA) and adenine sulphate (AdS) were used individually as well as in combinations (KN+GA₃, KN+BA and KN+AdS) with 2.46 µM IBA incorporated in the basal Murashige and Skoog's (1962) medium. Induction of shoot proliferation was promoted by AdS (27.1 µM) alone and also in combination with other PGRs. Compared to the other combinations KN (23.25 µM) and AdS (13.5 µM) evoked high frequency of bud break. IBA resulted in the enhancement of root formation, inclusion of IAA showed only callusing in the younger explants. NAA elicited no response. Using our protocol, from one twig of *A. indica* (10-12 responsive nodal explants), within a period of three months, 10-12 plantlets could be raised with 70% transplantation success.

P-3020

Carbohydrate Concentration of Medium Influences Rooting and Acclimatization in Cassava. THOMAS W. ZIMMERMAN, Jay Wiltshire, and Jacqueline A. Kowalski. University of the Virgin Islands Agricultural Experiment Station, RR1 Box 10,000, Kingshill, St. Croix, VI 00850. Email: tzimmer@uvi.edu

Micropropagation of cassava is relatively straightforward and shoots from microcuttings root without plant growth regulators in

in vitro. However, acclimatizing in vitro rooted shoots can result in high mortality. Cassava roots are prone to deterioration following wounding or bruising causing great losses during acclimatization. Direct rooting was conducted from cassava shoots pretreated by growing them in vitro on 0% to 10% sucrose. Sucrose levels over 6% caused shorter plant growth in vitro. Shoots, 1.5 cm in length from the varying sucrose media were rooted in vitro in medium grade vermiculite or in situ in ProMix™ potting medium. A success rate of over 95% was obtained following rooting in vermiculite followed by transfer and acclimatization in a ProMix™ potting medium. The friable vermiculite was easily removed from the cassava roots reducing the damage normally occurring during the rinsing and removal of adhering gelled medium. However, the developing storage roots were distorted and abnormally forming as a result of the circular in vitro growth pattern. Direct rooting of in vitro shoots using a plastic dome cover for one week was most successful from shoots grown on 4% to 8% sucrose. Direct rooting of in vitro cassava shoots resulted in root development most near to that in situ from stakes and the success rate is influenced by the carbohydrate content of the tissue culture medium.

PLANT TRANSFORMATION

P-3021

Disease Resistant Transgenic Grapevine Constitutively Expresses *Vitis vinifera* Thaumatin-like Protein. S. A. DHEKNEY, Z. T. Li, M. Van Aman, M. Dutt, J. Tattersall, K. T. Kelley, and D. J. Gray. University of Florida/IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703-8504. Email: sadanand@ifas.ufl.edu

Grapevine produces a number of pathogenesis-related (PR) proteins in response to biotic stresses. For example, *Vitis vinifera* thaumatin-like protein (VVTL-1) is induced in grapevine after exposure to fungal infection and/or toxin. To evaluate constitutively expressed VVTL-1, its coding sequence was cloned from grapevine by PCR and placed with an EGFP/NPTII fusion gene under the control of a CaMV35S-derived bidirectional duplex promoter complex. Somatic embryos of *V. vinifera* 'Thompson Seedless' were transformed with *Agrobacterium* and 71 independent transgenic lines were recovered based on kanamycin resistance and GFP expression. Transgenic plant lines were screened for resistance to powdery mildew (*Uncinula necator*) in a greenhouse along with non-transformed 'Thompson Seedless' as a susceptible control and *Vitis* hybrid 'Tampa' as a resistant control. Individual plants were rated three times a week by recording the number of lesions on leaves and stem and by assessing plant growth. Susceptible control plants developed severe disease symptoms 7 days after onset of the first visible lesions. Among transgenic plants tested, 6% of individual lines exhibited a 7-10 day delay in symptom development compared to susceptible controls. High disease pressure in the greenhouse was indicated by the resistant control plants, which exhibited mild disease symptoms 17 days after susceptible controls. Selected transgenic lines are being propagated, repeatedly re-screened and will be tested under field conditions. In addition, transgenic VVTL-1 lines of *V. vinifera* 'Merlot' and *Vitis* hybrid 'Seyval Blanc' are being screened.

P-3022

In Vitro Propagation and Transformation System Development in *Selaginella moellendorffii*. B. HALL¹, J. Banks², D. Wright³, J. Townsend³, and E. Vollbrecht¹. ¹Iowa State University, 2282 Molecular Biology Building, Ames, IA 50011; ²Purdue University, Lilly Hall, 915 West State Street, West Lafayette IN. 47907; and ³Iowa State University, 1043 Carver Co-Lab, Ames, IA 50011. Email: bdhall@iastate.edu

An In Vitro propagation and transformation system is being developed for the lycopod (Lycopodiophyta) *Selaginella moellendorffii*. Rapid propagation using half-strength MS with sucrose at pH6.5 has provided a continuous source of axenic plant material for *Agrobacterium*-mediated transformation experiments. Transient expression assays demonstrate *Selaginella* is susceptible to Agrobacterium infection. Experiments are underway to optimize conditions of cocultivation, *Agrobacterium* strains, vectors, and selectable markers. Once stable T-DNA integration and transgene expression have been confirmed, experiments utilizing a homologous recombination system will be initiated. Up to date research results will be presented.

P-3023

Production of Biologically Active Human Secretory Leukocyte Protease Inhibitor (SLPI) in Transgenic Tobacco and Rice Biomass. Mariam Sticklen, HESHAM ORABY, and Chuansheng Mei. Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824. Email: stickle1@msu.edu

The largest component of the HIV treatment is drug costs. For a typical HIV patient in 2004, the treatment cost ranged from \$5,500-\$13,600/patient/year. Currently, T-20 (fuzeon) is the most expensive anti-AIDS drug in the market, at \$20,000/patient/year. Regardless of the costs, none of these drugs are naturally human-based products, and therefore patients in certain cases develop allergic reactions. Human Secretory Leukocyte Protease Inhibitor (SLPI) is an anti-HIV protein naturally produced in the human body in regions with high levels (saliva) and/or individuals with high levels of this protein have a notably reduced acceptance to HIV transmission. At present, SLPI is sold for \$215/100 microgram, translating into \$2.15 M/gram. This extreme high cost has prohibited conducting large-scale human trials, and certainly has prohibited the use of this protein in a drug regimen. The main reasons for such high costs is the fact that production of its non-glycosylated cationic protein form in *E. coli* requires very extensive denaturation and renaturation to refold this disulfide-rich protein in its biologically active form. A plant-specific vector containing SLPI gene, polyhistidine tag, β -glucuronidase (*gus*) and bar herbicide resistance selectable marker genes was used to develop transgenic plants. Molecular and histochemical GUS analyses of T1 rice and T2 tobacco plants confirmed presence and expression of the transgenes. Production of the protein had no apparent deleterious effects on plant growth and development. Work is in progress to test the biological activity of plant-produced SLPI. Should it be biologically active, a series of clinical trials will be performed with collaboration of Mayo Clinic HIV Research Center. The plant-produced human SLPI protein in transgenic plants could be marketed as low as \$5000/kilogram in the future.

SILENT ABSTRACTS

P-3024

Investigating the Efficiency of Plant Cell-based Cultures to Achieve Bioproduction. BIPUL BISWAS, N. Joshee, A. Yadav, and A. K. Yadav. Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030-4313. Email: biswasb@fvsu.edu

Exploring tools of plant cell culture to develop suitable protocols to produce bioproducts has great significance for biotechnology. Plant products are naturally synthesized during specific season and their production is localized and/or tissue based. However, access to those bioactive compounds poses various limitations for us. Therefore, to make them readily available, plant cell culture based investigations seem quite appropriate. It will not only help us to produce them all year round, but will also help us carry out other

studies on these important compounds. Although efforts are being made by some researchers but they are not sufficient. A few successful reports showed that this is possible and the failures may be mainly due to the user ignorance or lack of proper tools. Our present study towards in vitro production of bioproducts is mainly to explore through our experiences of tissue culture and traditional knowledge based on a specific medicinal plant and its uses for the benefit of human kind. Neem (*Azadirachta indica* A. Juss.) is an important medicinal tree of Indian origin. Different plant parts of neem have various significant uses for storing food grains, treating skin diseases, fever, and diabetes. A good number of neem products including neem oil, neem cake, soap, cream, toothpaste, insecticides, etc. are available in the market. Scientists have found that azadirachtin is the key plant compound in neem for all its important activities. It has been found that the azadirachtin production varies from plant to plant and from tissue to tissues on the same trees. The present investigation was conducted to determine the activities of plant cell culture based bioproducts and the production of azadirachtin.

P-3025

Agrobacterium Mediated Transformation in Cucumber (*Cucumis sativus* L.). P. SURESHKUMAR¹, N.Selvaraj², Kasturiiregan³, and A. Ganapathi³. ¹Department of Biotechnology, School of Engineering and Technology, Bharathidasan University, Tiruchirappalli-24, INDIA; ²Department of Botany, E.V.R College, Tiruchirappalli-24, INDIA; and ³Department of Biotechnology, Bharathidasan University, Tiruchirappalli-24, INDIA. Email: sureshbiotech2003@yahoo.co.in

Cucumber (*Cucumis sativus* L) belonging to family Cucurbitaceae is an important horticultural plant. It is mainly cultivated for its fruit, which are used for slicing, pickling and juice extraction. Cucumber suffers from a number of viral, fungal diseases and pests. Transformation of *Cucumis sativus* cv poinsett 76 was achieved using *Agrobacterium* strain EHA 105 driven by a 35S CaMV contains a cointegrated vector with selectable kanamycin marker npt-II (neomycin phospho transferase), bar and reporter gene GUS (β -glucuronidase). Cotyledon explants were inoculated with a bacterial suspension (108 Cells.ml⁻¹) cocultivated for 24-72 hrs and placed on MS medium augmented with BA (1.0 mg/l), AdS (25 mg/l) and glutamine (50 mg/l) along with different concentration of kanamycin (0,25,50,75,100,150 and 200 mg/l). The cocultivated explants were selected in medium containing kanamycin (100mg/l). Transient GUS expression was observed in the meristematic region of cotyledon explants immediately after 24 hrs of co-cultivation. Transformation of the agro strains EHA 105 was confirmed by the presence of nptII gene in the putative transgenic plants by PCR & southern analysis. The amplified bands corresponding with expected size (690bp) of primers indicate the integration of nptII in to plant Transformation efficiency was studied with and without acetosyringone. The transformation efficiency 5.2% in EHA105 strains without acetosyringone and when acetosyringone (25mM) was added the transformation efficiency was it enhanced by almost twice in EHA 105 from 5.2% to 9.4% respectively. This protocol may be adopted for transferring any pest resistance genes of agronomic interest.

P-3027

Efficient Recovery of Transgenic Plants of Alfalfa (*Medicago sativa*) Through High Frequency Shoots Organogenesis from Leaf Derived Callus. HARI P SINGH, Kaye Knowles, Seema Dhir, and Sarwan Dhir. Center for Biotechnology, Fort Valley State University, Fort Valley, Georgia 31040. Email: singhh@fvsu.edu

Alfalfa (*Medicago sativa*), also known as Purple Medick and Trefoil, is a perennial flowering plant cultivated as an important forage crop. However, alfalfa sprouts are used as a salad ingredient in the United States and Australia and tender shoots are eaten in some places as a leaf vegetable. Alfalfa has the potential to be the most prolific of all leaf vegetable crops, processed by drying and grinding into powder, or by pulping to extract leaf concentrate. This versatile herb is also a folk remedy for arthritis, diabetes, asthma, hay fever, and is reputed to be an excellent appetite stimulant and overall tonic. Excellent source of nutritive properties with minerals, chlorophyll and vitamins. Being such an important constituents of dietary habit alfalfa has been targeted to incorporate special genes into the plants to generate novel proteins which will be candidate vaccines for specific diseases. Unlike traditional "injection" method edible vaccines can be used in a more humane and economical way. The regeneration or growth of transgenic plants is often an acute problem in such experiments. To address this issue the possibility of using organogenesis culture to efficiently recover transgenic plants of alfalfa has been studied in detail. The initial data showed that use of 6-benzylaminopurine (BAP) in combination with 2, 4-dichlorophenoxyacetic acid (2, 4-D) has significantly enhanced percentage of callus formation. The maximum callus formation was observed with Murashige and Skoog medium (MS) medium supplemented with 0.50 and 0.25 mg l⁻¹ of 2, 4-D and BAP respectively. Callus regeneration or organogenesis was observed by transferring yellowish callus to MS medium supplemented with various concentrations (0.25, 0.50, 1.0, 1.5, 2.0, and 2.5 mg l⁻¹) of BAP, thidiazuron (TDZ) or kinetin (KN) alone or in combination with 1-naphthaleneacetic acid (NAA) (0.25 mg l⁻¹). The results on the performance of the treatments and further testing for rooting and transformation are underway.

P-3028

Bioreactor Technology to Producing Biopharmaceutical in Roots. G. SIVAKUMAR*, L. Bacchetta* and K. Y. Paek**. *Biotech-Gen, ENEA, Casaccia, Via anguillarese 301 00060, Rome, ITALY and ** HortTech, Chungbuk National University, Cheongju, SOUTH KOREA. Email: sivakumar@libero.it

Bioreactor technology will be a challenging novel approach for higher production of nutraceutical and biopharmaceutical bioactive compounds. Ginseng, hazelnut and Brassica sps. are important industrial crops in developed countries, which are having higher medicinal properties to one's health and beauty. We established bioreactor technology for higher production of safety and bioactive nutritional as well as medicinal ginsenosides, α -tocopherol and sulforaphene from above said biofactory adventitious root culture. We will discuss new developments and efforts in pilot-scale bioreactor technology artificial adventitious roots in biopharmaceuticals.