2016 WORLD CONGRESS ON IN VITRO BIOLOGY

LATE SUBMISSION ABSTRACTS

2016 Meeting of the Society for In Vitro Biology
June 11 – 15, 2016
San Diego, California

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

PLANT SYMPOSIA ABSTRACTS

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P-3 Evolution of an Automated Synthetic Biology Portal
Steven B. Riedmuller, *Synthetic Genomics*

ALGAE BIOTECHNOLOGY – Wednesday, June 15, 10:30 am – 12:30 pm

P-38 Massive Mutant Screens to Develop a Photosynthetic Bioproduction Platform
Benjamin E. Rubin, *University of California San Diego*

INTERACTIVE POSTER SESSION ABSTRACT

IN VITRO ANIMAL CELL SCIENCES INTERACTIVE POSTER SESSION ABSTRACT
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A-3010 The NMP1 Inhibitor NSC348884 Affects Oligomerization and Induces Cell Death in Neuroblastoma Cells
Kelly R. Vlcek, *Midwestern University, Ryan Kelsch, Nil Akgul, and Kolbrun Kristjansdottir*

ANIMAL POSTER ABSTRACTS

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A-3000 Isolation of Fibroblast-like Cells after 42 Days of Postmortem Storage of Cattle Skin in a Refrigerator
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CELL BIOLOGY

A-3001 Nucleophosmin (NPM1) Localization in Neuroblastoma Cells Is Disrupted By $TmPyP_4$
Jeffery Greenland, *Midwestern University, Sarah Veen, Matthew Pytynia, Kelly R. Vlcek, Joshua Gasiorowski, and Kolbrun Kristjansdottir*

A-3002 Evaluating Blueberry Extracts as Nutraceutical Additives for Future Fish-feed Formulations Using a Fish Intestinal Epithelial Cell Line
V. Oberoi, *University of the Fraser Valley, G. S. Rai, G. Mahil, N. C. Bols, and L. E. J. Lee*

CELLULAR AND MOLECULAR TOXICOLOGY

A-3003 Evaluation of Bioactivity of Blueberry Extracts from Organic and Conventional Cultivars Using Fish Cell Lines
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A-3004 An Assessment of the Sensitivity of the Bovine Lens Assay as a Model for Ocular Toxicity
David J. McCanna*, University of Waterloo, Manlong Xu, and Jacob G. Sivak*

A-3005 Evaluation of Sub-lethal Effects by Neonicotinoids in Fish Cell Lines
K. Moghrabi, *University of the Fraser Valley, V. Oberoi, G. S. Rai, H. Sidhu, and L. E. J. Lee*

DEVELOPMENTAL BIOLOGY

A-3006 Development of a New Cell-based Assay Using Human ES/iPS Cell-derived Neural Stem Cells for Developmental Neurotoxicity (DNT) Testing
Mika Suga, *National Institutes of Biomedical Innovation, Hiroaki Kii, Takayuki Uozumi, Yasujiro Kiyota, and Miho K. Furue*

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A-3007 A Novel Technique to Quantify Medium- to High-throughput Live Cell Transgene Expression Over Time
S. C. Veen, *Midwestern University, and J. Z. Gasiorowski*

ONCOLOGY

A-3008 Cul5 Knockdown Increases the Viable Cell Number in MDA-MB-231 Breast Cancer Cells: Implications for Hsp90 Inhibitor Chemotherapy
Lauren A. C. Alt, *Midwestern University, Parvaneh Akbari, Shubha Mathur, Joseph Rojas, Erik Mersereau, Kolbrun Kristjansdottir, and Michael J. Fay*

A-3009 Osteogenic Marker Gene Expression of Osteoblasts Cultured in Two and Three Dimensional Environments
Iman Mohamed, *Midwestern University, Elisha Pendleton, and Nalini Chandar*

SIGNAL TRANSDUCTION

A-3011 NPM1 Protein-Protein Interactors in Neuroblastoma: Decoding the Domains
Alejandro Alvarez, *Midwestern University, Jaclyn Campbell, Tyler Johnson, Collin Hickey, Andy Truman, and Kolla Kristjansdottir*

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A-3012 Development and Evaluation of Loop Mediated Isothermal Amplification (LAMP) for Rapid Detection of *Neisseria gonorrhoeae*
Shazia Shaheen Mir*, Jamia Millia Islamia, Uzair, Tanzeel, Asif, and Arif Ali*

EDUCATION POSTER ABSTRACT

E-3000 Training the Next Generation-Y Through Innovative and Experiential Science Curricula, and Professional Development
Osagie Idehen, *Tuskegee University, Marceline Egnin, Gregory Bernard, Steven Samuels, Desmond Mortley, Franklin Quarcoo, Conrad Bonsi, Olga Bolden-Tiller, iBREED Students, and Craig Yencho*

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BIOTECHNOLOGY

P-3000 Media Optimization for *In Vitro* Bacoside - A Production in *Bacopa monnieri* (L.) Wettst
Mahima Bansal, *Thapar University, Anil Kumar, and M.S. Reddy*

P-3001 Targeted Gene Insertion Through Genome Editing
Rachael Barron, *J. R. Simplot Company, Manmeet Singh, Hui Duan, and Troy Weeks*

P-3002 Alternative Splicing of a Calmodulin Gene Family Member in Tomato Generates a Novel Nuclear-targeted Isoform
Daniel R. Bergey, *University of Wyoming Research & Extension Center, M. Dutt, Viji Sitther, Isaac Quarterman, and S. A. Dhekney*

P-3003 Efficient Evaluation of Physical and Molecular Plant Immune Responses to Root-knot Nematode Infection in Selected Sweetpotato Cultivars
Gregory C. Bernard, *Tuskegee University, Marceline Egnin, Conrad Bonsi, Desmond Mortley, William Witola, Steven Samuels, Caroline Land, and Kathy Lawrence*

P-3004 The International Association for Plant Biotechnology
Barbara Doyle Prestwich, *University College Cork*

P-3005 Application of Bean Based Transient Technology for Novel Insecticidal Protein Discovery
Matthew J. Heckert, *DuPont Pioneer, Janet Rice, Natalie Stoner, Deborah Clark, Ericka Veliz, Jim English, and Jennifer Barry*

P-3006 Genetic Engineering of Tobacco with Plant Pigmentation Genes for the Development of Novel Phenotypes
H. L. Jernigan, *University of Wyoming, N. Joshee, D. R. Bergey, V. Sitther, and S. A. Dhekney*

P-3007 Phylogenetic Analysis of *Olea europaea* L. Cultivars Using LTRs (Long Term Repeats) Retrotransposon-based Marker Systems
Ergun Kaya, *Mugla Sitki Kocman University, Emel Yilmaz-Gokdogan, and Muammer Ceylan*

P-3008 *In Vitro* Seed Germination of *Citrus* spp. – a Tool in Post-Cryopreservation Plant Development
Ergun Kaya, *Mugla Sitki Kocman University, Fernanda Vidigal Duerte Souza, Muammer Ceylan, and Maria M. Jenderek*

P-3009 Adventitious Shoot Regeneration and *Agrobacterium tumefaciens*-mediated Transient Transformation of Sweet Cherry (*Prunus avium* L.)
Mohamed Nagaty, *Michigan State University, Yunyan Kang, Gregory Lang, and Guo-qing Song*

P-3010 Oxidative Stress of Mature Pistachio (*Pistacia vera* L. 'Atlı') Shoot Tips During *In Vitro* Culture
Hülya Akdemir, *Gebze Technical University, Veysel Süzerer, Engin Tilkat, Ahmet Onay, and Yelda Özden Çiftçi*

P-3011 β-Carotene Bleaching and ABTS Cation Radical Scavenging Activities of the Extracts from Different Parts of *In Vivo* and *In Vitro* Raised *Pictacia lentiscus* L
Engin Tilkat, University of Batman, Veysel Süzerer, Ebubekir İzol, Abdulselam Ertaş, Hilal Surmuş Aşan, Mustafa Abdullah Yılmaz, and Ahmet Onay

P-3012 Developing the Thermally-tolerant Pectin Methylesterase for Improved Sugar Beet Biomass Processing
Jose C. Tovar, *Arkansas State University, Megan Cease, Jianfeng (Jay) Xu, and Brett J. Savary*

P-3013 Transgenic Banana Expressing *NH1* Gene Can Provide Resistance to Bacterial and Fungal Diseases
Jaindra Nath Tripathi, *International Institute of Tropical Agriculture, Richard O. Oduor, Pamela C. Ronald, and Leena Tripathi*

P-3014 Engineering Plant Cell Wall with Designer Glycopeptide-tagged E1 Endoglucanase for Improved Biomass Digestibility
Jianfeng Xu, *Arkansas State University, Hong Fang, Ningning Zhang, Gregory Phillips, and Brett Savary*

P-3040 Extensive Variation in Rate of Callus and Shoot Regene-ration, and *Agrobacterium*-mediation transformation, among Wild Black Cottonwood Genotypes *(Populus trichocarpa).*
Cathleen Ma, Oregon State University, and Steve Strauss

CELL BIOLOGY

P-3015 Plant Transformation Services
Hyeyoung Lee, *University of Missouri, Hien T. Bui, Yanjiao Zou, Neng Wan, Joann R. De Tar, Hanbing Li, Muruganantham Mookkan, Kaixuan Duan, Hua Liu, Michelle Folta, Phat Do, Christopher Willig, and Zhanyuan J. Zhang*

CELL AND TISSUE MODELS

P-3016 Purification of Plant Produced Asialo-rhuEPO and the Study of its Cytoprotective Effects on the Pancreatic Beta Cell Line RIN-m5F
Elena Arthur, *North Carolina Central University, Farooqahmed Kittur, Chiu-Yueh Hung, and Jiahua (Jay) Xie*

P-3017 Decellularized Hairy Roots as Potential Scaffolds for Mammalian Stem Cell Culture
Najwa Lee, *Arkansas State University, Tianhong Yang, and Fabricio Medina-Bolivar*

CELLULAR PATHOLOGY

P-3018 Characterization of Markers Linked to Resistance Motifs against Maize Lethal Necrosis Disease in Tanzanian Maize Germplasms
Inocent Ritte, *Sokoine University of Agriculture and Tuskegee University, M. Egnin, P. M. Kusolwa, G. He, O. Idehen, G. C. Bernard, and S. Samuels*

DEVELOPMENT BIOLOGY

P-3019 Screening Elite Cannabis for *In Vitro* Conservation
Suman Chandra, *University of Mississippi****,*** *Hemant Lata, Zlatko Mehmedic, Ikhlas A. Khan, and Mahmoud A. ElSohly*

P-3020 *Eucalyptus* Microgametogenesis Stages as a Tool for Biotechnological Studies
A. P. Martinelli, *University of São Paulo, S. Santa-Rosa, M. L. Rossi, and F. R. Muniz*

GENETIC ENGINEERING

P-3021 Comparison of Different Genetic Modification Techniques for the Improvement of the Economically Important W Murcott Afourer (*Citrus reticulata* Blanco) Citrus
M. Dutt, *University of Florida, and J. W. Grosser*

P-3022 Development and Application of the Proprietary Gene Editing Platform ARCUS
David Nicholl, *Precision BioSciences, Derek Jantz, Jeff Smith, Mike Nicholson, Jack Wilkinson, Seonhwa Dura, and John Salmeron*

P-3023 Functional Analysis of a Poly ADP-Ribose Polymerase Gene in Blue Potato *(Solanum andigenum*)
M. L. Orozco-Cárdenas, *University of California – Riverside, J. Venkateswari Chetty, A. Acosta-Rangel, and L. S. Santiago*

P-3024 CRISPR/Cas9-mediated Genome Editing for Conferring ALS-type Herbicide Resistance in Bahiagrass
Tina Strauss, *University of Florida, and Fredy Altpeter*

MICROPROPAGATION

P-3025 Optimizing Parameters for In Vitro Culture and Plant Regeneration of *Artemisia tridentata* ssp. *nyomingensis* (Wyoming Big Sagebrush)
Baktybek Asanakunov, *University of Wyoming, R. Kandel, B. A. Mealor, D. R. Bergey, N. Joshee, and S. A. Dhekney*

P-3026 Development of a Mass Propagation System for Emerald Ash Borer Resistant White Ash Trees Using Somatic Embryogenesis
Scott Merkle, *University of Georgia, Jessica Mitchell, Ryan Tull, and Paul Montello*

P-3027 In Vitro Establishment and Micropropagation of Two *Clusia* Species
Kaitlin J. Palla, *University of Tennessee Knoxville, and Xiaohan Yang*

P-3028 In Vitro Propagation and Ex Vitro Rooting of *Mitragyna parvifolia* (Roxb.) Korth.: A Threatened Tree of Medicinal Values
Ashok Kumar Patel, *Jai Narain Vyas University, Kheta Ram, and Narpat Singh Shekhawat*

P-3029 In Vitro Collecting and Propagation of *Hippobroma longiflora* and Successful Recovery of Shoot Tips after 18 Years of Cryostorage
Valerie C. Pence, *Center for Conservation and Research of Endangered Wildlife (CREW), and Bernadette L. Plair*

P-3030 In Vitro Propagation of *Vitex negundo* L., An Aromatic and Medicinal Woody Shrub
Kheta Ram, *Jai Narain Vyas University, Ashok Kumar Patel, and Narpat Singh Shekhawat*

P-3031 Parameters Enhancing Survival of *Agrobacterium*-transformed *Camelina sativa* Cultures
V. Sitther, *Morgan State University, O. Enitan, B. Tabatabai, D. R. Bergey, and S. A. Dhekney*

P-3032 Plant Development and Antioxidant Analysis of *Mentha x piperita* Obtained Through Micropropagation
B. Vaidya, *Fort Valley State University, N. Joshee, B. Asanakunov, D. R. Bergey, and S. A. Dhekney*

P-3033 In Vitro Microtuberization in 30 Potato Varieties
Thomas W. Zimmerman, *University of the Virgin Islands, and Kenya M. Emanuel*

MOLECULAR FARMING

P-3034 Engineering Sweetpotato [*Ipomoea Batatas (L.) Lam*] Expressing Synthetic Lytic Peptide for the Potential Inhibition of Human Immunodeficiency Virus Replication
Steven Samuels, *Tuskegee University, M. Egnin, T. Nashar, G. C. Bernard, and O. Idehen*

PHYSIOLOGY

P-3035 Role of Cytochrome P450s in Salt Tolerance of Plants
Prakash P. Kumar, *National University of Singapore, Pannaga Krishnamurthy, Wan-Jing Ho, Felicia Lok, Tit-Meng Lim, Qingsong Lin, Jian Xu, and Chiang-Shiong Loh*

SECONDARY METABOLISM

P-3036 Comparison of Total Phenolic Content and Total Antioxidant Activity of Essential Oils of Male and Female *Pistacia lentiscus* L.
Ebubekir İzol, University of Dicle, Veysel Süzerer, Serkan Yiğitkan, Engin Tilkat, Abdulselam Ertaş, Hilal Surmuş Aşan, and Ahmet Onay

OTHER PLANT IN VITRO BIOLOGY TOPICS

P-3037 Early Developmental Responses from Soybean Anther Cultures
Martina Garda, *Arkansas State University, K. Bade, B. Hale, M. Lowe, T. Sherrod, T. Sustich, N. Williams, and G. C. Phillips*

P-3038 Comparative Gene Expression and Metabolic Impacts in the Hyperaccumulator, *Pteris vittata* and Non-hyperaccumulator, *Pteris ensiformis* in Response to Arsenic Toxicity
Dana Reid, *Tuskegee University, Osagie Idehen, Marceline Egnin, Ramble Ankumah, Raymon Shange, Gregory Bernard, Steven Samuels, Alayjah Muhammad, and Kiara Bunton*

SILENT ABSTRACT

P-3039 Evolution of Photosynthetic Capacity During the Acclimatization to Ex Vitro Conditions of Micropropagated *Paulownia tomentosa* Plants
Abel Piqueras, *CEBAS(CSIC),* Pedro Díaz-Vivancos, Giuliano Sting Pechar, and José Antonio Hernández

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PLANT SYMPOSIA ABSTRACTS

AUTOMATION IN TISSUE CULTURE – Sunday, June 12, 10:30 am – 12:30 pm

P-3
Evolution of an Automated Synthetic Biology Portal. STEVEN B. RIEDMULLER. Synthetic Genomics, 11149 North Torrey Pines Road, La Jolla, CA 92037. Email: sriedmuller@syntheticgenomics.com

A discussion of the development and evolution of an Automated Synthetic Biology Portal. Democratizing the technologies developed at Synthetic Genomics to a distributed network of manufacturing portals. Covering real world challenges; including rapid development of vaccines to stop outbreaks before they cross borders, to developing DNA data storage and retrieval systems for complex machine instructions. Leveraging the customization and lessons learned from these projects to bring broadened capabilities to our synthetic biology manufacturing network.

ALGAE BIOTECHNOLOGY – Wednesday, June 15, 10:30 am – 12:30 pm

P-38
Massive Mutant Screens to Develop a Photosynthetic Bioproduction Platform. B. E. RUBIN[1], K. M. Wetmore[2], M. N. Price[2], S. Diamond[1], R. K. Shultzaberger[1], D. G. Welkie[1], R. Simkovsky[1], M. Ota[1], L. C. Lowe[1], G. Curtin[1], A. P. Arkin[2], A. Deutschbauer[2], and S. S. Golden[1]. [1]Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093 and [2]Physical Biosciences Division, Lawrence Berkeley National Lab., Berkeley, CA 94720. Email: Benemeryrubin@gmail.com

Synechococcus elongatus PCC 7942 is a model photosynthetic organism and a potential platform for the production of fuel and fossil fuel based chemicals. To identify the comprehensive set of genes and intergenic regions that impact fitness in *S. elongatus*, we created a pooled library of approximately 250,000 transposon mutants and used sequencing to identify the insertion locations. By analyzing the distribution and survival of these mutants under control conditions we identified 718 of the organism's 2,723 genes as essential for survival. The validity of the essential gene set is supported by its tight overlap with well-conserved genes and its enrichment for core biological processes. The differences noted between our dataset and these predictors of essentiality, however, have led to surprising biological conclusions. One such finding is that genes in the second half of the tricarboxylic acid (TCA) cycle are dispensable, suggesting that *S. elongatus* can survive with a non-cyclic and highly abridged TCA process. The library is also a powerful tool for identifying genes that are beneficial or detrimental under different growth conditions. With a focus on environments relevant to the use of *S. elongatus* as a bioproduction platform we have studied 42 conditions. These screens have resulted in the identification of hundreds of key genes involved in biofilm formation, predator resistance, and dark survival. This genome wide dataset of the essential and conditionally relevant loci of *S. elongatus* both improves our understanding of the organisms' basic physiology and will aid efforts to develop *S. elongatus* as abioproduction platform.

ANIMAL INTERACTIVE POSTER SESSION ABSTRACT

IN VITRO ANIMAL CELL SCIENCES INTERACTIVE POSTER SESSION – Tuesday, June 14, 1:30 pm – 2:30 pm

A-3010

The NMP1 Inhibitor NSC348884 Affects Oligomerization and Induces Cell Death in Neuroblastoma Cells. KELLY R VLCEK[1], Ryan Kelsch[2], Nil Akgul[1], and Kolbrun Kristjansdottir[1]. [1]Midwestern University, Biomedical Sciences Department, College of Health Sciences, Downers Grove, IL and [2]Midwestern University, Chicago College of Osteopathic Medicine, Downers Grove, IL. Email: kvlcek@midwestern.edu

Nucleophosmin-1 (NPM1) is a nucleolar protein involved in several cellular processes including ribosome biosynthesis, cell proliferation, apoptosis, molecular chaperoning, and centromere duplication. Dysregulation of NPM1 has been observed in several cancers including prostate, colon, and breast cancer. Recently, our lab has detected increased levels of NPM1 in neuroblastoma, a solid tumor often found in the adrenal glands of young children. Changes in NPM1 levels can correlate with tumor progression, therefore it is a potential target for cancer treatment. NSC348884, a small molecule inhibitor of NPM1, has been shown to disrupt oligomerization of NPM1 in breast, prostate, and lymphoma, resulting in inhibition of function and increasing cellular apoptosis. The focus of our study is to test the effects of NSC348884 on neuroblastoma cancer cells which have yet to be investigated. Here we report a concentration dependent decrease in metabolic activity of cells in SY5Y and WS neuroblastoma cell lines treated with 1-10 μM NSC348884 for 24 hours as measured by WST-1 assay. The use of Annexin V flow cytometry assays examines the levels of apoptosis resulting from treatment with the inhibitor. In addition, the oligomerization status of NPM1 in neuroblastoma cells was investigated through the application of Native PAGE following treatment with NSC348884. Understanding the role of NPM1 and the effect of NSC348884 on neuroblastoma cells may lead to novel treatment options for neuroblastoma patients.

ANIMAL POSTER ABSTRACTS

BIOTECHNOLOGY

A-3000

Isolation of Fibroblast-like Cells After 42 Days of Postmortem Storage of Cattle Skin in a Refrigerator. MAHIPAL SINGH and Brian Walcott. Animal Science Division, Agricultural Research Station, Fort Valley State University, Fort Valley, GA. Email: singhm@fvsu.edu

Somatic cell nuclear transfer has renewed interest in tissue storage due to its usage in reintroducing lost genetics back into the breeding pool in animal agriculture, preserving genetic diversity, and reviving endangered species. Several studies have shown an inverse relationship between cell survival and postmortem time interval. However, the limits of time interval within which live cells can be isolated from animal tissues postmortem, have not been adequately studied. The objective of this study was to assess the limits of cell survival in bovine skin stored at 4°C after the animal death. Outgrowth of cells around skin explants (2-3 mm^2) in culture dishes was used to isolate the primary fibroblasts. Ear skin was procured from the university slaughter house from nine healthy animals and stored at 4°C in the lab. Skin explants (n=90) were cultured on eighteen 60mm diameter dishes (5 explants/dish) after 0, 7, 14, 21, 28, 35 and 42 days of storage (at 4°C) in DMEM media supplemented with 10% FBS and 50 units/mL of penicillin and 50 μg/mL of streptomycin. Outgrowth of fibroblast-like cells around the explants was scored after 10 days of culture in a CO_2 incubator. Out of 462 explants that adhered to dish surface, 282 (61.04%) exhibited outgrowth. Our results showed outgrowth of cells in all the time points studied including 8% explants in 42 days of postmortem storage time interval. In general, the number of outgrowing cells decreased with increasing postmortem storage time interval. To test the cytogenetic status of the cells derived from postmortem tissues stored for 42 days, we established secondary cultures from primary cells at p4 level. Cytogenetic analysis of 42 dpm tissue derived cells performed on 20 G-banded metaphase cells revealed a normal female karyotype (60,XX[20]). These cultures have been passaged upto 25 times till date and exhibit comparable morphology to controls; however, they exhibit reduced growth. These results suggest that live usable cells can be recovered from bovine skin tissues upto 42 days of postmortem, if the skin is stored at 4°C.

CELL BIOLOGY

A-3001

Nucleophosmin (NPM1) Localization In Neuroblastoma Cells Is Disrupted By $TmPyP_4$. JEFFERY GREENLAND, Sarah Veen, Matthew Pytynia, Kelly R. Vlcek, Joshua Gasiorowski, and Kolbrun Kristjansdottir. Midwestern University, Department of Biomedical Science, 555 31st Street, Downers Grove, IL 60515. Email: kkrist@midwestern.edu

Neuroblastoma is a pediatric cancer with the high-risk form of the disease correlated with amplification of MYCN and a low 5-year survival rate. Our lab has previously found that high MYCN levels correlate with high NPM1 gene and protein levels in neuroblastoma cell

lines. NPM1 is a multi-functional protein with roles in synthesis and assembly of ribosomes and DNA replication. NPM1 is primarily localized to the nucleolus, but shuttles from the nucleolus to the nucleus and cytoplasm. NPM1 protein expression has been found to be elevated in breast, prostate and gastric cancers and in some cases is correlated with advanced disease status. Mutations in NPM1 have been identified in leukemia that alter the localization of NPM1 from nucleolar to cytoplasmic and result in more favorable overall survival. No such mutations have been identified in neuroblastoma. However, localization of wild-type NPM1 has been shown to be altered in leukemia cells after treatment with a competitive inhibitor of NPM1, $TmPyP_4$, along with an increase in cell death. The goal of this study is to examine whether $TmPyP_4$ also causes altered localization of NPM1 and cell death in neuroblastoma cells. We show that WS neuroblastoma cells treated with $TmPyP_4$ for 24-hours had a dose-dependent decrease in metabolically active cells measured using a WST-1 assay. We examined endogenous NPM1 localization via indirect immunofluorescence using anti-NPM1 antibodies and DAPI stain. A GFP-NPM1 construct was transfected into neuroblastoma cells and fluorescence microscopy used to monitor localization of exogenous NPM1 protein. NPM1 was predominantly localized to the nucleolus in both endogenous and exogenously expressed NPM1. FIJI-ImageJ was used to quantify localization of NPM1 before and after treatment with $TmPyP_4$. Preliminary results indicate that in WS neuroblastoma cells treatment with 4 μM $TmPyP_4$ for 24-hours disrupts nucleolar localization of NPM1. We are currently testing the effect of $TmPyP_4$ on mutant and truncated forms of NPM1.

A-3002

Evaluating Blueberry Extracts as Nutraceutical Additives for Future Fish-feed Formulations Using a Fish Intestinal Epithelial Cell Line. V. OBEROI[1], G. S. Rai[1], G. Mahil[1], N. C. Bols[2], and L. E. J. Lee[1]. [1]University of the Fraser Valley, Department of Biology, Abbotsford, BC, CANADA and [2]University of Waterloo, Department of Biology, Waterloo, ON, CANADA. Email: Vishesh.Oberoi@student.ufv.ca

The cost of fish feed is the single most expensive component in aquacuture practices and inexpensive alternative sources/ingredients are being sought. Value-added plant components are being explored as additives to provide for example, antimicrobial activity to reduce antibiotic use and to stimulate the immune system, or to enhance fillet quality and/or storability, as well as to increase value/desirability of product. Blueberries (*Vaccinium* spp.) contain health-promoting bioactive compounds such as anthocyanins (delphinidin, malvidin), flavanols (epicatechin), flavonols (quercetin), ascorbic acid, among many other compounds desirable in the food and nutraceutical industries. Evaluating the effectiveness of dietary ingredients using whole fish is costly and time consuming thus alternative models are being sought. For the testing of novel dietary components in humans and domestic animals, cell lines have been instrumental, but a similar approach has seldomly been performed for aquacultured fish species. The salmon aquaculture industry is looking for nutraceuticals to maintain healthy stocks and supplement the dietary needs of cultured fish inexpensively. In this study, bioactive compounds present in blueberries (eg. malvidin, delphinidin, quercetin, epicatechin) as well as crude blueberry extracts from five local cultivars (Bluecrop, Liberty, Duke, Draper, Eureka) are evaluated for their stimulatory or inhibitory effects on various cellular functions using RTgut-GC, an intestinal cell line derived from rainbow trout. Cell viability, monolayer integrity, phagocytosis, migration ability and collagen synthesis were evaluated to assess differences in bioactivity since fruit quality, antioxidant capacity and flavonoid content have been reported to vary among these cultivars.

CELLULAR AND MOLECULAR TOXICOLOGY

A-3003

Evaluation of Bioactivity of Blueberry Extracts from Organic and Conventional Cultivars Using Fish Cell Lines. G. MAHIL[1], V. Oberoi[1], K. Moghrabi[1], G. S. Rai[1], N. C. Bols[2], and L. E. J. Lee[1]. [1] University of the Fraser Valley, Department of Biology, Abbotsford, BC, CANADA and [2]University of Waterloo, Department of Biology, Waterloo, ON, CANADA. Email: Gaganjeet.Mahil@student.ufv.ca

Fish feed (based on costly fish meal components) and antibiotics are the most expensive factors affecting the viability of salmonid aquaculture practices. Inexpensive plant-based feed components are being sought to add to fish feed as nutraceuticals, that is, not only for its nutritional needs for the fish but also as pharmaceutical components to enhance the health of fish (and also for the consumers, ie humans) as well as for marketability purposes (enhance taste, color, value). For humans and land-based animals, the nutritional and/or pharmacological value of food extracts have been routinely evaluated using *in vitro* methods employing intestinal cell lines. A similar approach for evaluating feed components for aquacultured species have not been reported. In this study, we explore a non-traditional plant product for fish feed: Blueberry extracts, as an inexpensive source of nutraceuticals for fish. Blueberries are rich in antioxidants and micronutrients, thus are valued for human and animal consumption. We evaluate organic vs conventionally grown blueberries to

assess differences in bioactivity since the fruit quality, antioxidant capacity and flavonoid content have been reported to vary depending on the crop cultivation method. Furthermore, the persistence of systemic pesticides (pesticides that are taken up by plants and remain active within the plant tissues) such as neonicotinoids (NN), questions not only the nutritional value but the safety of conventionally grown blueberries that are regularly exposed to insecticides. Effects on cellular functions, antioxidant activity and cell integrity using RTgut-GC, a rainbow trout intestinal cell line, and other relevant fish cell lines will be presented.

A-3004

An Assessment of the Sensitivity of the Bovine Lens Assay as a Model for Ocular Toxicity. DAVID J. MCCANNA[1], Manlong Xu[2], and Jacob G. Sivak[2]. [1]University of Waterloo, Centre for Contact Lens Research, Optometry and Vision Science, Waterloo, ON, CANADA and [2]University of Waterloo, Optometry and Vision Science, Waterloo, ON, CANADA. Email: djmccann@uwaterloo.ca

Purpose: The maximum tolerated dose in the eye of an ophthalmic chemical is affected by the concentration of the chemical in the eye drop, the duration of contact and the total dose instilled. Using the bovine lens, an *in vitro* model that is capable of evaluating both single and multiple-dose exposures was developed. Methods: Bovine lenses were exposed to benzalkonium chloride (BAK) , 0.01% in 0.9% sodium chloride solution, for 15 min. Each bovine lens was then rinsed, placed into fresh medium and incubated at 37°C in a CO_2 incubator. The single-dose group was maintained in culture medium after initial exposure for 96 hours. The multiple-dose group was exposed for 15 min. to BAK (0.01%) solution 24, 48, 72 hours after the initial exposure. On the fourth day of incubation of the lenses, the metabolic activity was assessed using the alamarBlue viability assay. In addition, the optical quality of the lenses was determined using a laser scanner. Results: Single exposure of 0.01% BAK caused the metabolic activity of the bovine lens to significantly decrease 33% ($p < 0.05$) four days after the initial exposure when compared to untreated control lenses. Treating the bovine lens with multiple treatments of 0.01% BAK caused an even greater drop ($p < 0.05$) in metabolic activity to less than 73% of the control. Using the laser scanner the toxicity of the BAK treatments was also detected as both the single and multiple exposed lens groups showed significantly reduced optical quality compared to the control lenses ($p < 0.05$). This drop in metabolic activity also occurs after exposing cultured human ocular cells to BAK. Conclusion: BAK is a chemical that has been shown *in vivo* to be toxic to the eye at 0.01%. Using this *in vitro* assay we were able to demonstrate that BAK toxicity could be determined. In addition, a repeat exposure to 0.01% BAK over the course of three days caused increased toxicity and demonstrates that this assay can be used to measure the toxicity of ophthalmic chemicals that are used with multiple installations over the course of many days.

A-3005

Evaluation of Sub-lethal Effects by Neonicotinoids in Fish Cell Lines. K. MOGHRABI, V. Oberoi, G. S. Rai, H. Sidhu, and L. E. J. Lee. University of the Fraser Valley, Department of Biology, Abbotsford, BC, CANADA. Email: Kamal.Moghrabi@student.ufv.ca

Novel pesticides have gained regulatory acceptance as they are touted to be specific to target pests through known modes of action. However, non-lethal and non-specific effects could occur in non-target animals through as yet undescribed mechanisms. Neonicotinoids (NNs) have become the most widely used insecticides in agricultural practices worldwide, and although, advertised as safe for non-target animals and specific against insect pests, chronic and sub-lethal effects are beginning to become apparent in mammals, birds and aquatic animals. Unlike previous generations of pesticides, NNs are taken up within plant tissues and are long lived not only systemically within treated plants or animals, but also can persist in soil and water bodies for several years. This persistence in the environment and its ability to permeate within the tissues of plants and animals, exposes vertebrates to cumulated doses that could have detrimental effects. Since significant amounts end up in water bodies, effects in freshwater and/or estuarine fish are specifically worrisome. Evaluating various fish species at the whole organismal level is costly and prohibitive thus representative fish cell cultures could provide initial insights into the mode of action of NNs. Cell cultures are excellent investigative tools to study biological functions in isolation without compounding effects of physiological processes within whole organisms. Cell lines from various fish species: trout, eel, walleye, killifish, fathead minnow, were evaluated for effects on cell migration, cell proliferation and wound closure using 'scratch assays' that are easy to perform and score. For all cell lines, inhibition of cell migration with increasing NN doses were observed.

DEVELOPMENTAL BIOLOGY

A-3006

Development of a New Cell-based Assay Using Human ES/iPS Cell-derived Neural Stem Cells for Developmental Neurotoxicity (DNT) Testing. MIKA SUGA[1], Hiroaki Kii[2], Takayuki Uozumi[2], Yasujiro Kiyota[2], and Miho K. Furue[1]. [1]National Institutes of Biomedical Innovation,

Health and Nutrition (NIBIOHN), Osaka, JAPAN and [2]Nikon Corporation, Tokyo, JAPAN. Email: mikasuga@nibiohn.go.jp

Human pluripotent stem cells, including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, could be a promising new tool for in vitro developmental neurotoxicity (DNT) testing. Several DNT testing methods based on human ES cells have been proposed with "3R" concept; refining, reducing and replacing the DNT tests on vertebrata. However, animal-derived components such as Knockout Serum Replacement (KSR), B27 supplement, or Matrigel, are still used to derivate neuronal cells from human ES cells in the methods. Recent studies have suggested that evaluation using the comprehensive transcriptomics analysis is applicable to detect gene expression response to chemical exposure. However, more cost-effective, rapid and simple screening method would be expected. To address these issues, we are developing a new ES/iPS cell based assay for DNT testing under completely chemically defined culture condition without any animal-derived components using an imaging analyzer. We used an antiepileptic drug, Valproic acid (VPA), which is known to cause neural tube defects, to verify the results measured by our method for DNT testing. The effects of VPA on the commercially available neural progenitor cells or neural stem cells differentiated from ES/iPS cells in our new culture conditions were assessed by immunohistochemial analysis or phase-contrast imaging. The imaging analysis data have provided more detail information about the effect of VPA. Our cell-based-assay could be useful for cost-effective, rapid and simple DNT testing.

GENE THERAPY

A-3007

A Novel Technique To Quantify Medium- to High-throughput Live Cell Transgene Expression Over Time. S. C. VEEN and J. Z. Gasiorowski. Midwestern University, Department of Biomedical Sciences, 555 31st Street, Downers Grove, IL 60515. Email: scveen@midwestern.edu

Despite improvements in methodology and equipment, there are still several well established, but limited, traditional techniques that are commonly used today to study and quantify transgene expression. One of these techniques focuses on using microscopes to measure fluorescent reporter transgene expression. Although this technique can be useful in observing single cell transgene expression, large cell populations cannot be observed as a whole unless time-consuming stitch image acquisition is performed. Other existing methods involve the use of secreted reporter genes, but they typically require multi-step assays (fluorescence/absorbance/lumi-nescence) to be carried out to measure the expression level. While these methods can deliver results, they can also be strenuous and easily subjected to user bias. To improve upon these existing techniques, the aim of our research was to test if a macro-scale fluorescence photo-imager would allow us to non-invasively acquire sensitive, quantitative, single snapshots of an entire population of cells in a multi-well plate for reporter analysis. Normally, a macro-scale fluorescence photo-imager is designed to detect mm-sized bands on agarose or polyacrylamide gels, however, with appropriate light sources and filters, we used it to image multi-well tissue-culture plates seeded with a range of cell densities. The fluorescence reporter expression of the entire population of cells was then tracked at various time points between 4-72 hours. We observed that the transgene expression level demonstrated a linear trend in relation to the number of cells. This model can serve as a powerful, efficient tool to quantify live cell transgene expression over time in a medium to high-throughput fashion and allow us to study the expression of transgenes or transfection reagents on a macro-scale level.

ONCOLOGY

A-3008

Cul5 Knockdown Increases the Viable Cell Number in MDA-MB-231 Breast Cancer Cells: Implications for Hsp90 Inhibitor Chemotherapy. LAUREN A. C. ALT, Parvaneh Akbari, Shubha Mathur, Joseph Rojas, Erik Mersereau, Kolbrun Kristjansdottir, and Michael J. Fay. Midwestern University, Department of Biomedical Sciences, 555 31st Street Downers Grove, IL 60515. Email: mfayxx@midwestern.edu

The *Cul5* gene is located on a region of chromosome 11 that is associated with LOH in breast cancer, and as a result, Cul5 is implicated in breast tumorigenesis. Inhibitors of HSP90 are being evaluated as novel chemotherapeutic agents. The mechanism of action of these drugs involves the recruitment of Cul5 to the HSP90 complex which results in the degradation of HSP90 client proteins by the ubiquitin-mediated proteasome pathway. One such client protein that Cul5 degrades is the oncogenic epidermal growth factor receptor HER2/ErbB2. Triple negative breast cancer is an aggressive form of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR) or HER2/ErbB2. The goal of this project is to determine the effects of Cul5 knockdown on the MDA-MB-231 triple negative breast cancer cell line, and to determine if MDA-MB-231 cells are responsive to the HSP90 inhibitor 17-AAG. Cul5 knockdown was performed by transfecting MDA-MB-231 cells with Cul5 targeting siRNA pool or a

non-targeting negative control siRNA pool. Cul5 knockdown was verified 72 hrs post transfection by Western blot analysis, and cells counts were performed using trypan blue and the BIO-RAD TC20™ automated cell counter. Cells were also treated for 72 hrs with vehicle control (DMSO), or with 0-1,500 nM 17-AAG, and cell counts were performed. Knockdown of Cul5 expression at the protein level was successful as demonstrated by Western blot analysis. The Cul5 knockdown samples demonstrated an increase in the number of viable cells compared to the non-targeting negative control siRNA samples. The 17-AAG treated MDA-MB-231 breast cancer cells demonstrated a dose-dependent decrease in the number of viable cells without a change in the percent viability. Since MDA-MB-231 cells do not express HER2/ErbB2, we are currently performing experiments to identify the degraded HSP90 client proteins that are contributing to the chemotherapeutic effect of 17-AAG in triple negative breast cancer. We are also performing experiments to determine if Cul5 knockdown attenuates the chemotherapeutic effect of HSP90 inhibitors.

A-3009

Osteogenic Marker Gene Expression of Osteoblasts Cultured in Two and Three Dimensional Environments. IMAN MOHAMED, Elisha Pendleton, and Nalini Chandar. Midwestern University, Department of Biochemistry, 555 31st Street, Downers Grove, IL. Email: imohamed88@ midwestern.edu

Osteosarcoma is the most common primary malignant bone tumor in adolescents and young adults with tumors occurring in areas where the bone is growing rapidly. Loss of differentiation in osteoblasts holds prognostic significance in osteosarcoma patients .Osteosarcomas are frequently deficient in classic tumor suppressors such as p53 and retinoblastoma (Rb).Understanding the normal process of osteoblast differentiation and the role Rb plays in its progression helps us understand the consequences of deviating from it. We have utilized MC3T3E-E1, a murine osteoblast like cell line that has been extensively used to study osteogenesis in vitro. We have cultured these cells in a three-dimensional environment, and analyzed their differentiation capacities. We have also studied the loss of Rb function on differentiation capacity in 2 and 3D cultures using the MC3T3-E1 cell line with a stable knockdown of Rb expression. Levels of known osteogenesis markers such as Runx2, Osterix, BSP and Osteocalcin have been analyzed using realtime PCR. For mineralization changes, a quantitative Alizarin Red assay was utilized. Growth characteristics of cells grown on collagen beads was also studied. Our data shows that culturing osteoblasts in 3D demonstrated differentiation earlier and at a higher level as determined by the expression of markers of bone differentiation. Runx2, Osterix, BSP and osteocalcin showed the expected response to a differentiation promoting media, while loss of Rb either reduced or changed the pattern of expression of these markers. There was roughly a three- fold increase in calcium deposits in wild type cells when compared to their mineralization in 2D cultures. With Rb loss, mineralization capacity was reduced 4 fold when compared to controls. In conclusion our results show that growth in 3D hastens osteoblast differentiation and expression of bone markers when compared to their traditional 2D growth in cultures. Rb deficiency in osteoblasts causes a decrease in expression of bone markers in both 2D and 3D cultures when compared to controls.

SIGNAL TRANSDUCTION

A-3011

NPM1 Protein-Protein Interactors in Neuroblastoma: Decoding the Domains. ALEJANDRO ALVAREZ[1], Jaclyn Campbell[1], Tyler Johnson[1], Collin Hickey[1], Andy Truman[2], and Kolla Kristjansdottir[1]. [1]Midwestern University, Department of Biomedical Sciences, Downers Grove, IL and [2]University of North Carolina, Department of Biological Sciences, Charlotte, Charlotte, NC. Email: kkrist@midwestern.edu

NPM1, also known as nucleophosmin, is a nucleolar ribonucleoprotein with multiple functions within cells including involvement with ribosome biogenesis, molecular chaperoning, centrosome duplication and genomic stability. NPM1 consists of multiple domains including the N-terminal domain, which is involved in oligomerization and molecular chaperoning, and the C-terminal domain, which is involved in DNA/RNA binding. NPM1 dysregulation has been shown to be involved in human cancers, such as ovarian and gastric cancer as well as leukemias. In some cases, elevated levels of NPM1 correlate with advanced disease. Neuroblastoma is a childhood cancer arising from neural crest cells, which most often presents as a tumor on the adrenal gland. We have found elevated levels of NPM1 protein in high risk (MYCN positive) neuroblastoma cell lines. The high risk form of neuroblastoma has poor survival rates for patients, indicating a need for new treatments. NPM1 is known to interact with a number of different proteins in order to carry out its functions within the cell, but these interactions have not been studied in neuroblastoma. To investigate protein-protein interactors of NPM1 in neuroblastoma a yeast two-hybrid screen was done using full length NPM1 protein as the bait and a human fetal kidney library of proteins as prey. Hits from the screen were re-transformed back into yeast in order to perform β-galactosidase assays to determine the strength of the interaction. Selected hits from the screen using the full

length NPM1 were also assayed against truncated forms of NPM1. These truncated forms had one or more domains of NPM1 removed. Interaction strengths determined by the β-galactosidase assays were normalized to the strength of interaction using the full length NPM1. Through this method we are able to correlate protein binding of NPM1 interactors to domains within the protein and quantitate binding strength.

A-3012

Development and Evaluation of Loop Mediated Isothermal Amplification (LAMP) for Rapid Detection of *Neisseria gonorrhoeae*.SHAZIA SHAHEEN MIR, Uzair, Tanzeel, Asif and Arif Ali. Department of Biosciences, Jamia Millia Islamia, New-Delhi, INDIA-110025. Email: shaziamir29@gmail.com, ali.arifali@ gmail.com

Nucleic acid amplification tests (NAATs) have gradually taken over the conventional methods for detection of *N. gonorrhoeae* (Edward T et al. 2014). However, all these NAATs have intrinsic disadvantages of requiring a specialized instrument for amplification or an elaborated and complicated method for detection of amplified products. Thus, for settings with minimal facilities, there is a need for a simple and cost-effective test that would permit rapid and reliable screening of *N. gonorrhoeae* (WHO, 2006). In this study potential of LAMP has been exploited to develop a technique for the detection of *N. gonorrhoeae. Opa* gene and *porA* pseudogene based LAMP assay were developed and clinically evaluated in a preselected patient population separately**.** A total of 388 samples were collected and evaluated, out of which 142 were male samples and 246 were female samples. Four of the 11 discrepant samples were sequenced and confirmed. Samples which were positive by Culture/or 2 gene PCRs/or sequencing were considered as "True positives" (n= 80). LAMP showed concordance (99.2%) with PCR and very high sensitivity, specificity and accuracy. With Cohan's Kappa statistics, LAMP and PCR showed substantial agreement (99.7%) and moderate chance agreement (67.4%) with each other. Detection of the end product with the naked eyes, using SYBR Green I and Hydroxy Napthol Blue (HNB) dyes made LAMP feasible for field use also. This LAMP assay offers a highly sensitive and specific assay not only for detection, but also confirmation of *N. gonorrhoeae,* thereby saving cost and time.

EDUCATION POSTER SESSION

E-3000

Training the Next Generation-Y Through Innovative and Experiential Science Curricula, and Professional Development. Marceline Egnin[1], OSAGIE IDEHEN[1], Gregory Bernard[1], Steven Samuels[1], Desmond Mortley[1], Franklin Quarcoo[1], Conrad Bonsi[1], Olga Bolden-Tiller[1], iBREED Students[1], and Craig Yencho[2]. [1]Tuskegee University, College of Agriculture, Environment and Nutrition Sciences, Tuskegee, AL 36088 and [2]North Carolina State University, College of Agriculture and Life Sciences, Department of Crop Science and Production, Raleigh, NC 27695. Email: megnin@mytu.tuskegee.edu

Engaging minority students with *invitro* sciences experts was the realm of the "Innovative Plant molecular Breeding Research and Experiential Education" (iBREED) project as a contribution to training in response to the "Dying Breed" concept of students shying away from plant sciences, identified by USDA-NIFA in this big data era. In a discovery, creative, and imaginative manner, The Tuskegee University iBREED program helps students envision themselves as the next generation of plant breeders, changing the world one genome at a time. A cohort of eighteen iBREED students were brought together in a collaborative environment working in teams of peers. Student mentees carried out hands-on plant-based research and gained first-hand knowledge from distinguished scientists in true conventional research as well as modern molecular breeding techniques including tissue culturing methods, bacterial and plant transformation, seed development, genetics and plant biotechnology. In addition, students benefitted from visits and interactive engagement with experts in CRISPR technology, government agencies and agricultural corporations. Modest financial incentives played an important role in recruiting students, sustaining their drive to master the iBREED training and molding professionals in precision breeding and invitro plant science. The success of this project in near-peer mentors role helped overcome students' indifference and leverage research opportunity for their next generation career; thus, fostering a new appreciation for the meaning and relevancy of plant breeding and related precision sciences. *Work supported by: USDA-NIFA 1890-CBG funded iBREED project to Tuskegee University Plant Biotech Lab, GWCAES-CAENS.*

PLANT POSTER SESSIONS

BIOTECHNOLOGY

P-3000

Media Optimization for *In Vitro* Bacoside - A Production in *Bacopa monnieri* (L.) Wettst. MAHIMA BANSAL, Anil Kumar, and M. S. Reddy. Thapar University, Department of Biotechnology, Patiala 147001, INDIA. Email: mahima.bansal8@gmail.com

Bacosides, well-known brain tonic and restorative, are the active principle extracted from *Bacopa monnieri*. Bacosides

finds their applications in various pharmaceutical preparations for the treatment of mental problems and improvement of cognition (Singh and Dhawan 1997). For the sustained supply of bacosides for the pharmaceutical industry, there is a need to develop the alternative biotechnological methods for the production of bacosides. This will help in the conservation of *B. monnieri* in the wild. Plant cell, tissue and culture are promising biotechnological approaches for the production of a range of secondary metabolites (Alfermann and Petersen 1995; Fowler and Scragg 1998). Compared to whole plants, cell and organ culture offer the advantage of faster growth and metabolic rate with a condensed biosynthetic cycle. However, the lower yield of secondary metabolites in cell culture is a major bottle neck for commercial exploitation of this technology. Optimization of medium components has received much attention as an effectual measure to improve production of secondary metabolites (Ravishankar and Venkataraman 1993; Dixon 1999). The aim of the present work was to optimize medium composition and other conditions for the production of bacosides using cell suspension culture and hairy root culture. Firstly, factors were studied using one factor at a time approach. Subsequently, optimization was carried out using Plackett-Burman (PB) design and final optimization by response surface methodology (RSM).

P-3001

Targeted Gene Insertion through Genome Editing. RACHAEL BARRON, Manmeet Singh, Hui Duan, and Troy Weeks. J. R. Simplot Company, Boise ID. Email: Rachael.barron@Simplot.com

The development of commercial transgenic crops with improved traits is challenging. However, advances in genome editing technologies such as transcription activator-like effector nucleases (TALENs) will help to minimize these challenges. We have developed a method using TALENs to insert a silencing cassette into a defined region of the potato (*Solanum tuberosum*) genome, selected for insertion and derived consistent silencing of genes of interest. Ranger Russet stem explants were co-transformed using *Agrobacterium* harboring two binaries. One binary contained a vector for the transient expression of TALENs engineered to cleave within the endogenous 5' UTR intron following the polyubiquitin (*Ubi7*) promoter. The second binary contained a promoter-less acetolactate synthase (ALS) gene cassette that confers resistance to the selective agent imazamox. Linked to the ALS marker was a cassette for the tuber-specific silencing of polyphenol oxidase 5, vacuolar acid invertase and asparagine synthase 1. Following transformation and regeneration under selection, the development of shoots suggested that the endogenous *Ubi7* promoter was driving the expression of the ALS gene. Of shoots rooting in the presence of selection, 45% were determined by molecular analyses to have an insertion of the marker/silencing cassette at the targeted site. Field trial results for selected lines having a single, targeted insertion showed consistent silencing of the genes of interest and similar yield among the lines. We believe that site-directed integration will reduce the number of independent events required for field trials, enable consistent trait efficacy and facilitate the regulatory process.

P-3002

Alternative Splicing of a Calmodulin Gene Family Member in Tomato Generates a Novel Nuclear-targeted Isoform. DANIEL R. BERGEY[1], M. Dutt[2], Viji Sitther[3], Isaac Quarterman[4], and S. A. Dhekney[1]. [1]University of Wyoming Research & Extension Center, 3401 Coffeen Ave., Sheridan, WY 82801; [2]University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850; [3]Morgan State University, Dept. of Biology, 1700 E. Cold Spring Lane, Baltimore, MD 21251; and [4]Sheridan College, 3104 Coffeen Ave., Sheridan, WY 82801. Email: bergeyd1@gmail.com

Calmodulin (CaM) is a highly conserved, cytosolic, calcium-binding protein that senses and transduces changes in intracellular Ca^{++} levels to regulate diverse cellular responses. Comprehensive screening a tomato cDNA library to identify CaM gene family members revealed the presence of two unique CaM sequence variants derived from the same genomic sequence. One cDNA variant coded for the standard cytosolic CaM isoform, but the other variant coded for a novel CaM isoform harboring an additional exon-derived, C-terminal region consisting of a consensus nuclear localization signal (NLS). Exhaustive genomic database searches revealed the presence of homologous CaM-NLS variants in over 30 other diverse plant species, indicating strong selection pressure in plants for nuclear CaM activity. Nuclear targeting potential of the additional NLS domain was confirmed via *Agrobacterium*-mediated transformation of grapevine somatic embryos with nuclear and cytosolic tomato CaM variants fused to the green fluorescent protein (GFP) coding sequence. Fluorescence confocal microscopy revealed intense accumulation of GFP in nuclei of plants expressing the CaM-NLS/GFP fusion gene, and these plants displayed normal development. In contrast, embryogenic cultures and somatic embryos expressing the CaM/GPF fusion gene accumulated GFP in the cytosol only, and displayed highly abnormal development. The widespread presence of functional, nuclear-targeted CaM variants in the plants poses intriguing questions regarding potential nuclear roles for CaM, and provides a useful experimental model for

investigating molecular mechanisms affecting evolutionary change, such as alternative splicing, exon shuffling, gene duplication, etc. Future studies will include determining functional roles for nuclear CaM, identifying nuclear target sequences, and evaluating the potential of using engineered CaM-NLS variants to modify developmental processes and plant responses to various biotic and abiotic stresses.

P-3003

Efficient Evaluation of Physical and Molecular Plant Immune Responses to Root-knot Nematode Infection in Selected Sweetpotato Cultivars. GREGORY C. BERNARD[1], Marceline Egnin[1], Conrad Bonsi[1], Desmond Mortley[1], William Witola[2], Steven Samuels[1], Caroline Land[3], and Kathy Lawrence[4]. [1]Tuskegee University, College of Agriculture, Environment and Nutrition Sciences, Tuskegee, AL 36088; [2]University of Illinois, Urbana – Champaign, College of Veterinary Medicine, Department of Pathobiology, Urbana, IL 61801; [3]University of Florida, College of Agriculture, Department of Plant Pathology, Gainesville, FL 32611; and [4]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849. Email: gbernard4673@mytu.tuskegee.edu

Sweetpotato is the 6th most important food crop. However, there is limited information on the genetic mechanisms of resistance to a major pathogen in sweetpotato production, the root-knot nematode. The first objective of this study involved the efficient evaluation of host responses to root-knot nematode infection in five genotypically distinct cultivars of sweetpotato under controlled settings. Significant differences ($P < 0.05$) were shown among sweetpotatos in root weights, necrosis and galling index values, *inplanta* egg counts and nematode soil counts. To identify important molecular responses to nematode infection, a genome-wide expression profiling analysis was performed on resistant, susceptible and uninoculated sweetpotato cultivars using RNA-Seq. Significant differences ($P < 0.05$) in defense-related gene expression were observed among cultivars on days 14 and 46 post-infection. The resistant cultivar was distinguished from susceptible and untreated control plants primarily by elevated transcription of pathogenesis proteins, transcriptional regulators, resistance gene protein receptors bearing the characteristic NBS-ARC-LRR motif, peroxidases, proteinase inhibitors and other important components of plant immunity. Protein alignments of putative resistance protein DRL23 between resistant and susceptible plant samples revealed polymorphisms in the NBS domain which may have affected gene expression in the susceptible cultivar. Using protein database tools, putative resistance proteins DRL7 and DEF22 were predicted to interact with other defense-related proteins involved in the plant immune response. To date, this study represents the first genome-wide expression profiling analysis of sweetpotato under root-knot nematode challenge. Our findings have identified new intermediately resistant sweetpotato cultivars and have provided information on the discreet transcriptional events in sweetpotato during root-knot nematode infection. *Work supported by Tuskegee University, GWCAES, NIFA-EVANS-ALLEN-T-CAP and UC DAVIS-TU CREATE-IGERT*

P-3004

The International Association for Plant Biotechnology. BARBARA DOYLE PRESTWICH. University College Cork, School of Biological Earth and Environmental Science, North Mall Campus, Distillery Fields, Cork, IRELAND. Email: b.doyle@ucc.ie

The IAPB formerly known as the IAPTC (International Association of Plant Tissue Culture) was founded in 1963. The first congress was held in University Park, Pennsylvania.Since then there have been twelve meetings. In his paper on the history and evolution of the IAPB, Vasil (2008) provides a summary of the historical activities of the organisation. He says ' *In particular, the congress proceedings serve as time capsules, providing a wealth of information about the best of science and the most prominent scientists of the time. The history of the IAPB is indeed the history of plant biotechnology* '. The name was changed in 1998 from IAPTC (via IAPTC&B) to IAPB, which was thought to better reflect the changes in the research landscape Vasil (2008). At the end of each congress, a new President is appointed whose role is to manage the virtual IAPB office and host the next quandrennial symposium. The current President is Dr. Barbara Doyle Prestwich, UCC, Ireland (2015 to 2018). The next quadrennial meeting will be held in Ireland. The IAPB congress was last hosted in Europe in Florence, Italy in 1994 where > 1300 delegates attended. The meeting prior to that was held in Amsterdam, The Netherlands in 1990, where > 2000 delegates participated. Ireland is an ideal location for the next symposium, offering as it does, a gateway between America and Europe. The congress in 2018 (http://www.iapb2018.com) will provide an opportunity for world class scientists to congregate over a period of six days in a state-of-the-art conference centre and present their work on the main issues in Plant Biology and Biotechnology. The topics will include (but not limited to) food security, micronutrient deficiency, biodiversity, sustainability, phytopharmaceuticals and scientific communication. The latest advanced tools in plant biotechnology will be presented to include sessions on genome editing, synthetic biology and associated regulatory issues. Save the date for IAPB, Dublin Ireland

(August 19th to 24th) 2018 and join us for what promises to be a memorable scientific and social IAPB program

P-3005

Application of Bean Based Transient Technology for Novel Insecticidal Protein Discovery. MATTHEW J. HECKERT[1], Janet Rice[2], Natalie Stoner[2], Deborah Clark[2], Ericka Veliz[2], Jim English[1], and Jennifer Barry[3]. DuPont Pioneer, [1]4010 Point Eden Way, Hayward, CA 94545; [2]Experimental Station - 353, 200 Powder Mill Road, Wilmington, DE 19803; and [3]7000 NW 62nd Avenue, Johnston, IA 50131. Email: matt.heckert@pioneer.com

Among methods employed to confirm and characterize potential novel insecticidal protein candidates, *In Planta* transient expression techniques proved enabling and valuable where certain other heterologous expression systems showed recalcitrance. Here, we present examples of how agro-infiltration of *Phaseolus vulgaris* leaves was used for confirmation of insecticidal activity of newly discovered genes encoding potential insecticidal proteins. This transient system was also used to assess the potency and insect activity spectrum of those newly discovered proteins. Our results demonstrate the unique utility of transient technology in novel trait discovery.

P-3006

Genetic Engineering of Tobacco with Plant Pigmentation Genes for the Development of Novel Phenotypes. H. L. JERNIGAN[1], N. Joshee[2], D. R. Bergey[3], V. Sitther[4], and S. A. Dhekney[3]. [1]University of Wyoming, Undergraduate student of Biology, Laramie, WY 82071; [2]Fort Valley State University, 1005 State University Drive, Fort Valley, GA 31030; [3]University of Wyoming, Sheridan Research and Extension Center, Sheridan, WY 82801; and [4]Morgan State University, Department of Biology, Baltimore, MD 21251. Email: hjerniga@uwyo.edu

Flavonoids produced by plants are responsible for the specific coloration observed in leaves and flowers. In the current study, anthocyanin biosynthesis-related genes were used to produce transgenic tobacco plants with unique coloration. The grapevine *VvMybA1*, maize Leaf Color (LC) and citrus RUBY genes were isolated and individually placed along with a NPTII gene under the control of a constitutive CaMV35S promoter. Tobacco leaf discs of cultivar 'Samsun' were obtained from in vitro germinated seedlings and co-cultivated with *Agrobacterium* containing the genes of interest for 72 hours in the dark. Co-cultivated leaf discs were then transferred to MS medium containing 4.44 μM BAP and 0.5 μM NAA along with the addition of carbenicillin, cefotaxime and kanamycin antibiotics (MSTcck medium) to inhibit bacterial growth and permit specific growth of transgenic cells. Shoots obtained from co-cultivated explants were transferred to MS medium containing 0.5 μM NAA for the production of roots. Fully developed plants were transferred to sterilized potting mix and hardened under conditions of high relative humidity, prior to being transferred to a greenhouse. Transient gene expression was evidenced by the presence of dark red spots on explants after 3-5 days of co-cultivation. Transgenic shoots exhibiting intense red pigmentation were observed after 3-4 weeks of growth on MSTcck medium. The frequency of transgenic shoot production ranged from 25-33%. Approximately 75-85% of the shoots produced roots following transfer to rooting medium. Transgenic plants exhibited diverse patterns of red pigmentation in shoot and root tissues. We are currently studying the patterns of leaf development using scanning electron microscopy. The development of plant lines with varied levels and patterns of pigment production could be potentially used as cultivars with novel phenotypes.

P-3007

Phylogenetic Analysis of *Olea europaea* L. Cultivars Using LTRs (Long Term Repeats) Retrotransposon-based Marker Systems. ERGUN KAYA, Emel Yilmaz-Gokdogan, and Muammer Ceylan. Mugla Sitki Kocman University, Faculty of Science, Molecular Biology and Genetics Department, 48000, Kotekli-Mugla-TURKEY. Email: ergunkaya@mu.edu.tr

The great number of Mediterranean basin olive varieties require an efficient method of genetic analysis for the improvement of conservation management strategies for the genetic resources and for the protection of the commercial varieties quality label. In addition, there is the problem arising from the existence of homonyms and synonyms. This makes cultivar identification very difficult and complex. In this study, fourty-six clones belong to eight Turkish (each of them has five clones) and two Italian (each of them has three clones) olive cultivars were investigated for phylogenetic analysis using retrotransposon-based marker systems which are Inter-retrotransposon amplified polymorphism (IRAP) with 13 long terminal direct repeats (LTRs) primers and retrotransposon-microsatellite amplified polymorphism (REMAP) combined with 13 LTR and 10 inter simple sequence repeat (ISSR) primers. PCR reactions were performed in a 25 ml reaction volume containing PCR Buffer (1x final concentration), 2,5 mM $MgCl_2$, 0.4 mM each dNTP, 0,4 mM primer, 50 ng genomic DNA, and 2 unit Taq DNA polymeras. PCR circumstances were initial denaturation at 95 ^{0}C for 3 min and 35 cycles at 95 ^{0}C for 15 sec, and then 55 ^{0}C for 30 sec, a ramp to 72 ^{0}C for 3 min, followed by 10 min at 72 ^{0}C and indefinite soak at 4 ^{0}C. Amplicons were separated on 1.5% agarose gel and

monitorized by lighting under UV light. DNA fragments of IRAP and REMAP PCR reactions were scored by their existence (1) or nonexistence (0), and the ones at low intensities were scored only if they were reproducible in both the PCR runs. Cluster analysis was performed to construct dendrograms, with the unweighted pair-group method by arithmetic means (UPGMA) from the similarity input matrices using Jaccard's coefficient. We obtained 368 total band profiles, 358 of them were polymorphic (97,28 % polymorphism) and all ten cultivars divided into three big clusters and each of them had different branches however each clones of different cultivars were in the same group.

P-3008

In Vitro Seed Germination of *Citrus* spp. – a Tool in Post-Cryopreservation Plant Development. ERGUN KAYA[1], Fernanda Vidigal Duerte Souza[2], Muammer Ceylan[1], and Maria M. Jenderek[3]. [1]Mugla Sitki Kocman University, Faculty of Science, Molecular Biology and Genetics Department, 48000, Kotekli-Mugla-TURKEY; [2]Embrapa Cassava & Fruits, Caixa Postal 007, Cruz das Almas, BA 44380-000, BRAZIL; and [3]USDA-ARS National Laboratory for Genetic Resources Preservation, Fort Collins, CO 80526. Email: ergunkaya@mu.edu.tr

Citrus is one of the most ancient horticultural species domesticated by humans and is an important fruit crop in the world. Traditionally, citrus germplasm is conserved in orchards and in greenhouses. In both environments, the plants are subjected to potential losses due to pests, diseases and climatic hazards. Conservation of citrus cultivars is the best accomplished by preservintg vegetative propagules, cryopreservation of seeds might contribute to a long-term conservation of *Citrus* spp. genetic resources. Cryogenic technique of "slow-cooling" and more recently developed "vitrification/one-step freezing" procedures have been successfully applied to cryopreservation of citrus shoot tips and buds. Our objective was to test the possibility of cryopreserving seeds of mandarin (*Citrus reticulata* Blanco), sweet orange [*Citrus limon* (L.) Burmf] and lemon [*Citrus sinensis* (L.) Osbeck] using seed desiccation and direct immersion in LN_2 (-196°C). Seeds were sterlized (20% commercial bleach for 20 min, rinsed twice in sterile water), desiccated in a laminar flow hood to 17.2 - 19.2 % moisture content and directly immeresed into LN_2. After 24-48 hours of LN_2 exposure, the seeds were germinated *in vitro* on semi-solid MS medium supplemented with 0.1 µM gibberelic acid, 20 gL^{-1} sucrose, 1.5 gL^{-1} phytagel, and 3.5 gL^{-1} agar (pH 5.8) in Petri dishes. The cultivation was done in a growth chamber 27±2 °C, at 16-h photoperiod, under white cool fluorescent light of 50 $\mu mol^{-1}m^{-2}s^{-1}$. All 11 genotypes tested (5 accessions of mandarin, 3 accessions of lemon and 3 accessions of sweet orange), showed a high post-cryopreservation seed germination ranging from 68.1 to 82.2 %. The seedlings derived from the cryopreserved seeds developed roots and typical plants in *ex vitro* conditions. Our study showed that seeds of the three citrus species might be successfully stored in liquid nitrogen, germinated *in vitro* and develop true to type plants in *ex vitro* environment.

P-3009

Adventitious Shoot Regeneration and *Agrobacterium tumefaciens*-mediated Transient Transformation of Sweet Cherry (*Prunus avium* L.). MOHAMED NAGATY[1,2], Yunyan Kang[1], Gregory Lang[1], and Guo-qing Song[1]. [1]Michigan State University, Plant Biotechnology Resource and Outreach Center, Department of Horticulture, East Lansing, MI 48824 and [2]Suez Canal University, Plant Production Department, Faculty of Environmental Agricultural Sciences, El Arish, North Sinai, EGYPT. Email: songg@msu.edu

To develop protocols for *Agrobacterium tumefaciens*-mediated stable transformation of sweet cherry (*Prunus avium* L.)., *in vitro* shoot cultures were derived from one mature embryo of sweet cherry cultivar Selah. Leaves with removed petiole and three partial cuts made transversely and equidistant through the midrib area were cultured on Woody Plant Medium (WPM: Lloyd and McCown, 1980) supplemented with different plant growth regulators. Of eight treatments, 2.95 µM IBA and 4.54 µM TDZ yielded the highest regeneration frequency at 32.5% with 2.8 shoots per regenerated explant; 2.27 µM TDZ gave a regeneration frequency of 7.5 % and 5.0 shoots per regenerated explant. The shoot regeneration scheme using 2.95 µM IBA and 4.54 µM TDZ was subsequently linked with inoculation with *A. tumefaciens* strain EHA105 harboring an intron interrupted β-glucuronidase (GUS) gene (*gus*A). Co-cultivation and selection were performed using our optimized conditions for cherry rootstocks. After 4 days of co-cultivation and 16 days of selection, 59% of explants showed blue staining and intense blue was observed at wounded sites. Taken together, regeneration of adventitious shoot was achieved and transient GUS expression studies suggest an efficient gene delivery mediated by *A. tumefaciens*.

P-3010

Oxidative Stress of Mature Pistachio (*Pistacia vera* L. 'Atlı') Shoot Tips During *In Vitro* Culture. HÜLYA AKDEMİR[1], Veysel Süzerer[1,2,3], Engin Tilkat[4], Ahmet Onay[5], and Yelda Özden Çiftçi[1]. [1]Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, TURKEY; [2]Department of Medical Services and Techniques, University of Bingöl, Bingöl, TURKEY; [3]Department of Botany, University of İstanbul, İstanbul, TURKEY;

[4]Department of Biology, University of Batman, Batman, TURKEY; and [5]Department of Biology, University of Dicle, Diyarbakır, TURKEY. Email: pinarakdemir@gmail.com; beyso1985@gmail.com

Explant preparation involves wounding of the tissues which is known to cause oxidative stress both in culture initiation and in subculture. In this study, a nondestructive assay for hydroxyl radicals, using DMSO as a radical trap, was used to determine hydroxyl radical formation during tissue culture of mature P. vera 'Atlı' shoot tips. For this purpose, 250 mg/l desferoxamine (Desf) or 1 nmol 4-hydroxynonenal (HNE) was included to Murashige and Skoog (MS) medium supplemented with 1 mg/l BAP and %1 DMSO while 1 mg l-1 BA and %1 DMSO containing MS medium served as control. Formation of methane gas was analyzed by gas chromatography during 1, 3, 5, 7 and 9th day of culture while influences of these chemicals on proliferation of explants were also determined after 30 days of in vitro culture. Hyroxyl radical formation via methane gas formation was determined especially in the 1st and 3rd days (6.1 and 4.3 ppm) of in vitro culture period in control. Moreover, ethylene, although in low amount, was also detected in the tested control group. In the presence of Desf, hydoxyl radical formation was relatively lower than control in the 1st day of culture as expected, but then started to increase in 5th and 7th days of culture whereas inclusion of HNE to the medium resulted in increase of radical formation in the 3rd day of culture, however, the lowest methane production (3.14 ppm) was detected on 9th day of culture in this medium. As regards shoot proliferation, inclusion of HNE or Desf did not affect the proliferation frequency in comparison with the control, however, number of shoots proliferated per explant was declined especially in the presence of Desf in the medium. Overall results showed that pistachio shoot tips were subjected to oxidative stress especially in the initiation period of in vitro culture.

P-3011

β-Carotene Bleaching and ABTS Cation Radical Scavenging Activities of the Extracts from Different Parts of *In Vivo* and *In Vitro* Raised *Pictacia lentiscus* L. ENGİN TİLKAT[1], Veysel Süzerer[2,3,4], Ebubekir İzol[5], Abdulselam Ertaş[5], Hilal Surmuş Aşan[6], Mustafa Abdullah Yılmaz[5], and Ahmet Onay[6]. [1]University of Batman, Department of Biology, Batman, TURKEY; [2]University of Bingöl, Department of Medical Services and Techniques, Bingöl, TURKEY; [3]University of İstanbul, Department of Botany, İstanbul, TURKEY; [4]Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, TURKEY; [5]University of Dicle, Department of Pharmacognosy, Diyarbakır, TURKEY; and [6]University of Dicle, Department of Biology, Diyarbakır, TURKEY. Email: etilkat@gmail.com; beyso1985@gmail.com

The current study presents information concerning β-carotene bleaching and ABTS of the different extracts from *in vivo* and *in vitro* raised *Pistacia lentiscus* L. As the source of the research material, the fresh samples (leaf, stem and root) of in vivo grown male and female trees, *in vitro* grown female seedling samples and exocarp (hull), endocarp (shell) and seeds coats of mature fruits were obtained around Çiftlikköy, Çeşme, Izmir. The air-dried and powdered samples were extracted three times with 100 mL of ethanol for 24 hours at RT. The antioxidant properties of these 12 ethanol extracts were determined by β-carotene bleaching and ABTS methods. According to the ABTS method, all the samples exhibited very high antioxidant activity. The IC_{50} values of in vitro grown six samples were determined lower than 10 (IC_{50} of < 10). However, the extracts from *in vitro* grown stem parts was the only sample determined to be the IC_{50} of < 10. *In vitro* leaf extracts, showing the highest antioxidant activity IC_{50}:22.95±0.95 were determined to be more active from the *in vitro* roots extracts IC_{50}:35.42±1.18. According to the ABTS method, it is determined that the fruit extracts IC_{50}:352.87±3.83 and the extracts from testa prepared from the seeds showed low antioxidant activity IC_{50}:708.71±6.93, while the shell extract had higher activity IC_{50}:48.53±1.58. Among different seed extracts, the exocarp IC_{50}:352.87±3.83 and seed coat IC_{50}:708.71±6.93 displayed low antioxidant activity, whereas the endocarp showed good antioxidant properties IC_{50}:48.53±1.58. Extracts from stem sections of *in vitro* samples in both female IC_{50}:85.21±0.68 and male IC_{50}:42.38±0.25 samples were determined to be more active than the extracts from the roots and leaf sections according to the β-carotene method. It was also determined that the roots extracts were found to be more active than the leaf extracts. In brief, the prepared extracts from all *in vivo* samples displayed very low activity IC_{50} of < 10.

P-3012

Developing the Thermally-tolerant Pectin Methylesterase for Improved Sugar Beet Biomass Processing. JOSE C. TOVAR[1,2], Megan Cease[2,3], Jianfeng (Jay) Xu[2,4], and Brett J. Savary[2,4]. [1]Molecular Biosciences Graduate Program; [2]Arkansas Biosciences Institute; [3]College of Science and Mathematics; and [4]College of Agriculture and Technology; Arkansas State University, Jonesboro, AR. Email: jose.tovar@smail.astate.edu, bsavary@astate.edu (corresponding author).

Beet sugar production is an energy intensive process with high greenhouse gas emissions. Up to a third of total thermal energy consumed in a beet factory is used to dry

beet pulp, the pectin-rich biomass left after sucrose extraction. We are investigating a *Citrus* fruit thermally-tolerant pectin methylesterase (TT-PME) for application to reduce energy inputs for beet pulp processing. We hypothesize that processive PME action can modify cell wall structure to improve water separation from beet pulp prior to drying through a calcium cross-linking mechanism. Our objectives are to: 1. determine if PME action can reduce water binding in beet pulp, 2. establish specific antibodies for TT-PME detection, and 3. demonstrate TT-PME recombinant expression *in planta*. Using a quantitative benchtop assay, we determined PME treatment reduced water binding in beet pulp by 25% over untreated controls. These results provided proof of concept that TT-PME expression in sugar beet roots may confer a novel processing benefit. As a tool for detecting TT-PME in transgenic plants, we produced antibodies using a sequence-specific peptide antigen that differentially detected TT-PME. Towards developing an expression strategy for sugar beets, different TT-PME structural constructs were transiently expressed in *Nicotiana benthamiana*. TT-PME's native signal peptide yielded high recombinant expression levels comparable to the well-established patatin signal peptide, and active TT-PME expression required the PRO region. Results from these expression studies will direct future strategies for TT-PME expression in sugar beet taproots. Biotech beets expressing TT-PME is envisioned to deliver an innovative processing output trait providing economic and environmental benefits for beet sugar processing.

P-3013

Transgenic Banana Expressing *NH1* Gene Can Provide Resistance to Bacterial and Fungal Diseases. JAINDRA NATH TRIPATHI[1,2], Richard O. Oduor[2], Pamela C. Ronald[3], and Leena Tripathi[1]. [1]International Institute of Tropical Agriculture, Nairobi, KENYA; [2]Department of Biotechnology and Biochemistry, Kenyatta University, Nairobi, KENYA; and [3]Department of Plant Pathology, UC Davis, Davis, CA. Email: j.tripathi@cgiar.org

Bananas (*Musa* sp.) provide an essential food and revenue for more than 100 million small holder farmers in east and central African countries. The crop has potential to feed tropical countries in Africa as well as whole world. Banana Xanthomonas wilt (BXW) and Fusarium wilts are the two most destructive diseases of banana in tropical and subtropical regions. Genetic engineering is one of the most important tools to control these diseases due to lack of resistance in banana varieties against bacterial and fungal pathogens. In this study, the rice *NH1* gene has been inserted into the embryogenic cell suspension of "Sukali Ndiizi" cultivar through *Agrobacterium*-mediated transformation in an effort to develop transgenic banana resistant to bacterial as well as fungal pathogens. About 100 transgenic lines harbouring the *NH1* gene were generated. Molecular characterization was performed by PCR and Southern analysis to confirm presence and integration of transgene into the banana genome. Twenty independent transgenic lines were evaluated for resistance to BXW disease by artificial inoculation by *Xanthomonas campestris* pv. *musacearum* (Xcm)of potted plants under glasshouse conditions. Majority of lines showed significantly higher resistance to BXW in comparison to non-transgenic plants. Two out of twenty lines tested showed complete resistance with no BXW disease symptom development.These transgenic lines were further evaluated against *Fusarium oxysporum* f. sp. *cubense* race 1 using potted plants bioassay. Four transgenic lines showed tolerance to Fusarium wilt disease with significantly lower disease symptoms on leaves and corms in comparison to non-transgenic plants. Our results show that expression of the *NH1* gene in banana transgenic plants boost resistance to both bacterial and fungal diseases.

P-3014

Engineering Plant Cell Wall with Designer Glycopeptide-tagged E1 Endoglucanase for Improved Biomass Digestibility. JIANFENG XU[1,2], Hong Fang[2], Ningning Zhang[1], Gregory Phillips[2], and Brett Savary[1,2]. [1]Arkansas Biosciences Institute and [2]College of Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: jxu@astate.edu

Reengineering plant cell walls with cell wall-modifying (CWM) enzymes represents a promising solution to overcome the recalcitrance for lignocellulosic biomass efficient conversion to liquid biofuels. This project investigated innovative application of hydroxyproline (Hyp)-O-glycosylation to engineer novel designer glycopeptide tags for heterologous CWM enzymes to maximize their hydrolytic functions in cell walls without significantly affecting the plant phenotypes. A thermostable CWM enzyme, E1 endoglucanase from *Acidothermus cellulolyticus* was used in this study. The E1 holoenzyme and its catalytic domain (E1cd) was each engineered into tobacco plants with or without a $(SP4)_{18}$ tag that consists of 18 tandem repeats of "Ser-Pro-Pro-Pro-Pro" motif and directs Hyp-O-glycosylation with oligo-arabinosides in planta. While the over-expression of E1cd and $(SP4)_{18}$-tagged E1cd did not change the phenotypes of the transgenic plants, engineering E1 holoenzyme or $(SP4)_{18}$-tagged E1 delayed the flowering time of the plants and produced variegated leaves after flowering, though the harvest biomass yields were not significantly impacted. The saccharification analysis suggested the engineering of enzyme E1cd, $(SP4)_{18}$-E1cd and E1 in planta contributed to increased biomass

saccharification efficiency by 2-fold, 4.2-fold and 3.5-fold, respectively, compared to wild type plants. This study has demonstrated the functionality of Hyp-O-glycosylated designer glycopeptides as a molecular carrier in facilitating the glycohydrolase deposition and stabilization in the cell wall matrix, which significantly improved the biomass saccharification efficiency without loss of biomass accumulation.

CELL BIOLOGY

P-3015

Plant Transformation Services. Hien T. Bui, HYE-YOUNG LEE, Yanjiao Zou, Neng Wan, Joann R. De Tar, Hanbing Li, Muruganantham Mookkan, Kaixuan Duan, Hua Liu, Michelle Folta, Phat Do, Christopher Willig, and Zhanyuan J. Zhang. University of Missouri, Plant Transformation Core Facility, 007A & B Ernie & Lotti, Sears Plant Growth Facility, Columbia, MO 65211. Email: zhangzh@missouri.edu

University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art plant transformation services over the past 14 years. The facility is aiming at fostering plant science research by providing transformation services worldwide. The services are on fees for cost recovery only, not for profit. The facility staff is dedicated to providing various types of transformation services with a focus on maize (*Zea mays*), soybean (*Glycine max*), switchgrass (*Panicum virgatum)*, sorghum (*Sorghum bicolor*), wheat (*Triticumaestivum*), rice (*Oryza sativa*), alfalfa (*Medicago truncatula*), as well as *Setaria viridis*. The service categories include both standard and customized transformation. Transformation systems for all crops utilize *Agrobacterium*-mediated approaches and somatic embryogenesis processes except for soybean and *Medicago*. The *Agrobacterium*-mediated cot-node transformation system coupled with organogenesis regime is employed for soybean and *Medicago* transformation. The facility is also ready to take on new service projects to transform new plant species as user's requests. Research activities are geared towards developing high-throughput transformation systems, effective small RNA-mediated gene silencing, gene stacking through coordinated transgene expression, and precise genome modifications to meet the needs of crop improvement and genome discoveries. More details on the facility can be found at http//www.plantsci.missouri.edu/muptcf.

CELL AND TISSUE MODELS

P-3016

Purification of Plant Produced Asialo-rhuEPO and the Study of Its Cytoprotective Effects on the Pancreatic Beta Cell Line RIN-m5F. ELENA ARTHUR, Farooqahmed Kittur, Chiu-Yueh Hung, and Jiahua (Jay) Xie. Department of Pharmaceutical Sciences, Biomanufacturing Research Institute and Technology Enterprise, North Carolina Central University, Durham, NC. Email: earthur1@eagles .nccu.edu

Diabetes has evolved into a high priority global epidemic. The current treatment involves timely injection of insulin to maintain glucose homeostasis. However, alternate holistic treatments that directly target the repair of beta cells to stimulate endogenous insulin production are desperately needed. Erythropoietin (EPO) is a glycoprotein hormone produced by the kidneys that plays an invaluable role in blood cell production. Surprisingly, however, it has also been shown to protect brain, heart and pancreatic tissues from various injuries. Despite its exceptional tissue-protective activities in animal models, it has not found use in humans because of its adverse side effects. Asialo-erythropoietin (asialo-rhuEPO), an EPO derivative lacking sialic acid residues was reported to exhibit excellent tissue-protective activities as EPO but with no side effects. Unfortunately, its costly and limited production from commercially available EPO prevents its use in clinical settings. In our lab, a plant-based expression system was established to produce asialo-rhuEPO in the tobacco plants. In the present study, a two-step process was developed to purify asialo-rhuEPOfrom plant leaf extracts. Up to 33% of the soluble asialo-rhuEPO was recovered from plant leaf extracts. To investigate its protective effects on pancreatic β-cells, purified protein was tested on insulin-producing β-cell line RIN-m5F. In an in vitro model of chemically induced apoptosis, plant-produced asialo-rhuEPOprovidedup to 40% protection to RIN-m5F cells from staurosporine(STS)-induced cell death.

P-3017

Decellularized Hairy Roots as Potential Scaffolds for Mammalian Stem Cell Culture. NAJWA LEE[1], Tianhong Yang[2], and Fabricio Medina-Bolivar[1]. [1]Department of Biological Sciences, Arkansas State University, Jonesboro, AR; [2]Molecular Biosciences Graduate Program, Arkansas State University, Jonesboro, AR; and [3]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR. Email: najwa.lee@smail.astate.edu, fmedinabolivar@astate.edu

Bioengineering strategies to address the shortage of human organs rely on the use of human tissues as scaffolds for the development of human organs. This project is aimed at providing an alternative approach by utilizing plant tissues as human organ scaffolds. "Hairy roots" developed via genetic transformation of plant cells with *Agrobacterium rhizogenes* show diverse phenotypes and could offer distinct types of decellularized scaffolds to support growth and

proliferation of stem cells. In this project, previously established hairy roots from *Nicotiana tabacum* (tobacco), *Arachis hypogaea* (peanut), *Bixa orellana* (annatto), *Scutellaria lateriflora* (American skullcap) and *Vitis rotundifolia* (muscadine grape) were selected for decullarization studies because of their distinct branching and hairiness. Hairy roots were exposed to different concentrations of detergent for up to 2 weeks to decellularize the root tissue. The effectiveness of this decellularization treatment was evaluated by determining the amount of protein remaining in the tissue after treatment. Our results show that detergent treatments are effective in decellularizing selective hairy root tissues. These tissues may offer potential alternative scaffolds that could be tested for growth of human stem cells.

CELLULAR PATHOLOGY

P-3018

Characterization of Markers Linked to Resistance Motifs Against Maize Lethal Necrosis Disease in Tanzanian Maize Germplasms. INOCENT RITTE[2], M. Egnin[1], P. M. Kusolwa[2], G. He[1], O. Idehen[1], G. C. Bernard[1], and S. Samuels[1]. [1]Tuskegee University, College of Agriculture, Environment and Nutrition Sciences, Tuskegee, AL 36088 and [2]Sokoine University of Agriculture, Department of Crop Science and Production, Morogoro-TANZANIA. Email: Iritte8222@mytu.tuskegee.edu, megnin@mytu .tuskegee.edu

Among the biological constraint facing maize production in Tanzania is the severe occurrence of maize lethal necrosis disease (MLN) raising an urgent need for application of new approaches. A pool of 22 Tanzanian maize genotypes that included promising landraces and susceptible breeding lines selected under artificial inoculation, CIMMYT and US lines with known MLN background were subjected to AFLP screening. Eleven AFLP primer combinations were screened and resulted in identification of 1025 polymorphic AFLP allelic fragments. The genetic similarity matrix was mined using Numerical Taxonomy System (NTSYSpc) software. On the basis of AFLP DNA marker polymorphism data, genetic similarities among the selected Tanzanian maize landraces and other maize lines in the study were estimated by Unweighted Pair Group of Arithmetic Mean (UPGMA) to develop a dendrogram that revealed three clusters grouping genotypes according to their reaction to MLN disease. Promising resistant and tolerant genotypes were grouped in cluster I and susceptible genotypes in cluster II and III. Also landraces were grouped according to agro-ecological locations where they were collected. Unambiguous polymorphic AFLP fragments were eluted purified and sequenced. Sequencing and nucleotide BLAST showed similarity of the loci, which are associated to disease resistance genes such as pathogenesis related proteins, Serine/threonine kinase proteins, rust resistance protein *rp3-1*, receptor kinases and *Zea mays* putative zinc finger proteins. Other important loci that are responsible for plant response to stress were also identified. *Work supported by: USAID-funded iAGRI project to Ohio State University, Tuskegee University, Tanzania Sokoine University of Agriculture.*

DEVELOMENTAL BIOLOGY

P-3019

Screening Elite Cannabis for *In Vitro* Conservation. Suman Chandra[1], Hemant Lata[1], Zlatko Mehmedic[1], Ikhlas A. Khan[1,2], and MAHMOUD A. ELSOHLY[1,3]. [1]University of Mississippi, National Center for Natural Products Research, School of Pharmacy, University, MS 38677; [2]University of Mississippi, Department of Pharmacognosy, School of Pharmacy, University, MS 38677; and [3]Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677. Email: suman@ olemiss.edu

As a plant, Cannabis is a complex species. It belongs to family *Cannabaceae*. Nomenclature of cannabis is a matter of ongoing debate and of divided opinion among the research community. Few groups recognize cannabis as one species with many varieties or sub species whereas, others consider it as a group of two to three species. Cannabis is an annual, dioceous (occasionally monoecious), wind pollinated plant. Due to its allogamous nature it is very difficult to maintain its potency and chemical profile if grown from seeds. Therefore, screening elite female mother plants with desired chemical profile and their multiplication using vegetative propagation and/or *in vitro* propagation is a suitable way to maintain the quality and efficacy of biomass product. Thus, it is important to monitor plant to plant variation in cannabinoids profile and content with developmental stages of growth. In this regard, *Cannabis* plants were grown from seeds under outdoor conditions. Male plants were removed from the field to avoid pollination and only female plants were kept for further cultivation. Among these plants, few randomly selected healthy female plants were periodically analyzed for Δ^9-THC and other phytocannabinoids content through the different stages of growth (from seedling to harvest) using GC/FID. In general, THC content increase with plant age up to a highest level during budding stage, where the THC content reached a plateau for about a week before the plants were harvested. The change in the concentration of other cannabinoids follows a similar pattern in some cases but show more variability depending on the individual plant. Based upon chromatographic analysis, a significant plant to plant variation in Δ^9-THC content was observed in

these plants. In this presentation, these variations will be discussed. Plant germplasm of selected elite female clones (based on their chemical profile) were preserved using *in vitro* techniques for the future use.

P-3020

Eucalyptus Microgametogenesis Stages as a Tool for Biotechnological Studies. A. P. MARTINELLI[1], S. Santa-Rosa[1], M. L. Rossi[1], and F. R. Muniz[2]. [1]University of São Paulo, CENA, Av. Centenario, 303, Piracicaba, SP, 13416-930, BRAZIL and [2]Fibria Celulose SA, Rodovia General Euryale Jesus Zerbini, Km 84, Jacareí, SP, 12340-010, BRAZIL. Email: adriana.martinelli@usp.br

Eucalyptus is an important woody species for the production of cellulose and other wood products. A detailed characterization of pollen development can contribute to biotechnological studies and breeding programs. This study aimed to characterize microgametogenesis in *Eucalyptus grandis* and *Eucalyptus urophylla*, determining the key developmental stages and correlating these with the flower bud size. This is important as a quick explant selection method for further biotechnological studies. Flower buds in different sizes were collected from the indoor breeding orchard at Fibria, these were measured and classified according to the longitudinal length in six groups. Anthers were collected from flower buds from each group and processed for observations under light microscopy, through fixation, dehydration, infiltration and embedding in Spurr, sectioning and staining with toluidine blue for general histological observations. Fresh anthers were also stained with DAPI for microspore nucleus observations. Six stages were defined for both species according to the predominant developmental events: 1. male archesporial cell; 2. microspore mother cell (MMC); 3. MMC in meiosis, tetrads and free microspores; 4. vacuolate microspore; 5. vacuolate microspore to bicellular pollen grain; 6. bicellular pollen grain. These stages were observed in flower buds varying from ≤ 9.5 mm to 12.2 mm. Ideal stages for *in vitro* anther and microspore culture initiation should be done preferably with explants obtained from flower buds on stages 4 to 5 for better results. Our preliminary results indicate that individual microspore cultures might be more suitable for *in vitro* culture due to the diminute size of the anthers and difficulties to determine if callus formation initiates in the microspores or anther tissues. Results presented here are part of ongoing biotechnological studies in *Eucalyptus* species.

GENETIC ENGINEERING

P-3021

Comparison of Different Genetic Modification Techniques for the Improvement of the Economically Important W Murcott Afourer (*Citrus reticulata* Blanco) Citrus. M. DUTT and J. W. Grosser. Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: manjul@ ufl.edu

The W Murcott Afourer mandarin is an attractive and easy to peel mandarin that originated in Morocco. This cultivar has gained considerable popularity in recent years and is now cultivated in the United States as a fresh market cultivar. Citrus, however is threatened by *Candidatus* Liberibacterasiaticus (*CLas*), an invasive bacterial species that causes the disease Huanglongbing (HLB) and has become endemic to some of the citrus production areas of the United States. This disease has drastically reduced citrus cultivation (mainly in Florida) due to the unavailability of HLB resistance in the commercially cultivated cultivars. Genetic transformation to incorporate an anti-microbial transgene into the citrus genome can potentially allow a citrus cultivar to successfully resist *CLas* infection and remain productive. Mandarins, however are recalcitrant to conventional epicotyl mediated transformation techniques and it is difficult to successfully transform W. Murcott Afourer. A comparative study between four genetic modification techniques – three utilizing juvenile tissue (protoplast, callus and epicotyls) and mature tissue revealed that callus transformation was the most efficient method of transforming W Murcott Afourer, followed by the protoplast mediated transformation technique. Both epicotyl mediated as well as mature tissue transformation resulted in very few positive transformants. A high rate of contamination in mature tissue explants (mainly from endophytic bacteria and fungi) reduced the overall transformation efficiency. We employed a rapid micro grafting technique on tender rootstocks to quickly propagate our transgenic lines. The time required to harden off the transgenic plants was dependent on the transformation process (ranging from 3 months following juvenile epicotyl transformation to 8 months after protoplast transformation). Molecular analysis of the regenerated plants confirmed the presence of the transgene in the citrus genome.

P-3022

Development and Application of the Proprietary Gene Editing Platform ARCUS. DAVID NICHOLL, Derek Jantz, Jeff Smith, Mike Nicholson, Jack Wilkinson, Seonhwa Dura, and John Salmeron. Precision BioSciences, 302 E. Pettigrew St., Durham NC, 27709. Email: david.nicholl@precisionbiosciences.com

Gene editing is a rapidly expanding field in biotechnology. The ability to make direct edits in genes of animals and plants is opening up new avenues of research for the improvement of human health and agriculture. Precision BioSciences has developed a novel gene editing platform called ARCUS. The cornerstone of this technology is the synthetic ARC nuclease. This was developed by engineering a small homing endonuclease discovered in *Chlamydomonas* called I-CreI with altered sequence specificity and dna-binding affinity. Our ARC nuclease shares many of the positive attributes of this homing endonuclease, such as small size and unparalleled sequence specificity, but it can more easily be evolved into a custom gene editing tool that recognizes a DNA sequence of our choosing. This proprietary technology can be used to insert, delete or edit DNA. It has been shown to be effective in animal cells, human cells and many plant species. At Precision BioSciences, we're using ARCUS genome editing in an effort to overcome cancers, cure genetic diseases, and enable the development of safer, more productive food sources. Here we will present the key modifications to the enzyme to create this technology and exhibit some of its successful applications in plants. In addition we will highlight ongoing efforts to develop our in house plant gene editing capabilities.

P-3023

Functional Analysis of a Poly ADP-Ribose Polymerase Gene in Blue Potato *(Solanum andigenum*). J. Venkateswari Chetty, A. Acosta-Rangel, L. S. Santiago, and M. L. OROZCO-CÁRDENAS. University of California – Riverside, Plant Transformation Research Center, Botany and Plant Sciences Department, Riverside, CA 92521. Email: venkatej@ucr.edu

Poly ADP-Ribose polymerase (PARP) is a family of enzymes involved in DNA repair, programmed cell death (PCD), and post-translational modification of various nuclear proteins by transferring the ADP-ribose moiety of NAD^+ to Lys residues (ribosylation). *Solanum andigenum* (Blue potato) was found to have three genes (PARP2, PARP2A and PARP3) in the PARP family. To further understand PARP function, the cDNA encoding PARP2 was amplified from immature fruit of blue potato and the PARP2 antisense construct that targets the 5'-end of the PARP2 transcript was made and used to transform the blue potato. Molecular characterization by PCR, RT-PCR and Southern blot analyses of the primary transformants confirmed presence and down-regulation of the transgene. The mutant plants showed increases in plant fresh weight, root mass and micro-tuber formation under *in vitro* conditions. These results are similar to those obtained in a previous study when the PARP chemical inhibitor 3-Methoxybenzamide was applied in the medium of *in vitro* micropropagated wild type plants. Furthermore, water deficit resistance was evaluated by comparing a progressive dehydration treatment and post recovery in wild type and transgenic plants and also by measuring chlorophyll fluorescence (Fv/Fm), water content, gas exchange and water potential in leaves. The cumulative results show that PARP2 plays an important role in growth, root development, micro-tuber formation and abiotic stress tolerance in blue potato.

P-3024

CRISPR/Cas9-mediated Genome Editing for Conferring ALS-type Herbicide Resistance in Bahiagrass. TINA STRAUSS and Fredy Altpeter. University of Florida, Agronomy Department, Genetics Institute, Plant Molecular and Cellular Biology Program, Gainesville, FL. Email: tstrauss@ufl.edu, altpeter@ufl.edu

In Florida bahiagrass (*Paspalum notatum*) is the most important forage grass and grown on more than 2.5 million acres in support of the beef and dairy cattle industry. Its drought tolerance and resistance against most insects and diseases makes it the preferred turfgrass in low-maintenance areas, like roadsides, and large residential lawns. The lack of herbicide-selectivity against undesired grassy weeds and the low tolerance of bahiagrass to commercially available grass herbicides makes the weed management challenging. Our goal is to develop a superior apomictic bahiagrass cultivar with an herbicide-resistance trait that allows the suppression of weeds and other (bahia)grasses which germinate from the seedbank in the soil. Our approach is based on the CRISPR/Cas9 technology that contains a nuclease which can be used for introducing a double strand break in a specific target locus. The co-delivery of a repair template with the desired nucleotide alteration will result in a modified *ALS* (acetolactate synthase) gene that confers herbicide resistant. Biolistic transfer of the genome editing tools into bahiagrass cells, selection and regeneration of putative events will be described. The regenerated bahiagrass will be analyzed for the modification in the ALS gene by PCR, restriction and sequencing analyses. The evaluation of herbicide resistance will be carried out under greenhouse conditions.

MICROPROPAGATION

P-3025

Optimizing Parameters for In Vitro Culture and Plant Regeneration of *Artemisia tridentata* ssp. *wyomingensis* (Wyoming Big Sagebrush). BAKTYBEK ASANA-KUNOV[1], R. Kandel[1], B. A. Mealor[1], D. R. Bergey[1], N. Joshee[2], and S. A. Dhekney[1]. [1]University of Wyoming, Sheridan Research and Extension Center, Sheridan WY

82801 and [2]Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030. Email: b.asanakunov@gmail.com

Forb and shrub species are critical in land reclamation for improving habitat diversity. *Artemisia tridentata* ssp. *nyomingensis* (Wyoming big sagebrush) is an important shrub species used in reclamation projects. Sagebrush seed availability (mostly collected from wild plants) is hampered by weather variation and loss of stands to invasive species and fire. Sagebrush micropropagation is a viable alternative for rapid multiplication of vigorous plants. In this study, factors influencing in vitro culture were studied to optimize protocols for sagebrush plant regeneration. Shoots were obtained from sagebrush shrubs growing in native habitats and nodes were used as explants for culture initiation. Explants were surface-sterilized with commercial bleach solution at 5-20% concentrations for varying periods of time followed by 3 washes in sterile distilled water. Explants were placed on media treatments containing MS or C2D salts with varying concentrations of BAP. Shoot proliferation was recorded by counting the number of shoots produced by explants after 4-5 weeks. Resulting shoots were transferred to rooting media treatments containing MS or C2D salts containing varying levels of auxins. Fully developed plants were transferred to sterile potting mix and acclimated under conditions of high humidity prior to transfer to a greenhouse. Explants treated with 20% bleach solution for 10 min exhibited maximum survival on shoot proliferation medium. Half-strength MS and C2D salts exhibited lower browning and higher shoot proliferation compared to full strength salts. Although a high degree of variability was observed among treatments, the maximum number of shoots (4.6) was observed on ½ strength C2D medium containing 6.6 µM BAP. The highest percentage of rooting (52.8%) was observed in shoots cultured on ½ strength MS medium containing 2.7 µM NAA. We are currently studying additional treatments to improve plant regeneration. The development of an efficient micropropagation protocol will enable rapid multiplication of healthy plant material for use in reclamation efforts.

P-3026

Development of a Mass Propagation System for Emerald Ash Borer Resistant White Ash Trees Using Somatic Embryogenesis. SCOTT MERKLE, Jessica Mitchell, Ryan Tull, and Paul Montello. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602. Email: smerkle@uga.edu

White ash (*Fraxinus americana*) is both an important forest species and a highly-valued landscape tree. Ash wood, which is strong, straight-grained and dense, is used for a variety of products, including tool handles, baseball bats, furniture, flooring and cabinets. Unfortunately, all North American ash species are under threat of extirpation from their native ranges by the emerald ash borer (EAB; *Agrilus planipennis)*, an exotic wood-boring beetle that has already destroyed millions of ash trees in 15 US states and Canada. "Lingering" ash trees are healthy trees growing in areas where EAB infestation has caused almost complete mortality of the ash population. The fact that these lingering ash trees have survived infestation may indicate genetically-based resistance or tolerance to EAB. Culture initiation experiments using seed and zygotic embryo explants from lingering ash parents in Michigan and cultured on a modified Woody Plant Medium, showed that seed collection date and explant type (naked zygotic embryo versus whole seed) significantly affected embryogenesis induction, while, 2,4-D concentration (2 or 4 mg/L) and explant length did not. Embryogenesis induction ranged as high as 37.5% for some genotypes and collection dates. White ash embryogenic cultures grown in suspension culture, size fractionated and plated on nylon mesh overlaid on basal medium, produced somatic embryos that were used in germination experiments, which tested the effects of activated charcoal and gibberellic acid (GA3) on germination. Combining 0.5 g/L activated charcoal and 10 mg/ L GA3 produced the most vigorous somatic seedlings in the shortest time. Several white ash somatic seedlings survived transfer to ex vitro conditions and grew rapidly in the greenhouse. Clonally propagating lingering ash trees through somatic embryogenesis will facilitate testing for genetically-based resistance and may make possible mass propagation of trees that are naturally resistant to EAB.

P-3027

In Vitro Establishment and Micropropagation of Two *Clusia* Species. KAITLIN J. PALLA[1] and Xiaohan Yang[2]. [1]The Bredesen Center for Interdisciplinary Research and Graduate Education, University of Tennessee – Knoxville, Knoxville, TN and [2]Oak Ridge National Laboratory, Knoxville, TN. Email: kpalla@vols.utk.edu

Crassulacean acid metabolism (CAM) is a photosynthetic pathway with an inherently high water use efficiency. Found largely in plants adapted to hot, arid climates, CAM offers a strategy to meet the future challenges of climate change and efficient land usage. Understanding the genetics behind CAM can inform efforts to engineer C3 plants to express CAM features, enhancing drought tolerance in crop plants grown for food or fuel without sacrificing yield. Facultative CAM plants like *Clusia pratensis* hold the key to genes involved in a reversible switch between C3 metabolism and environmentally induced CAM photosynthesis. Currently, no attempt has been

made to get species within the *Clusia* genus into tissue culture. Here, we present the first report of developing an in vitro micropropagation protocol for *C. minor* and *C. pratensis*. Using juvenile greenhouse material, a surface disinfestation protocol was determined. Plant growth was evaluated on Murashige and Skoog medium (MS), ½ MS, woody plant medium (WPM), and Driver and Kuniyuki Walnut medium. WPM was found to sustain significantly healthier shoot explants. The effect of 6-benzylaminopurine (BAP), BA + thidiazuron (TDZ), and BA + 1-Naphthalenacetic acid (NAA) is being evaluated for ability to stimulate reliably proliferating shoot cultures. Adventitious shoot regeneration is also being attempted for *C. minor* and *C. pratensis*. Mature *C. minor* leaf explants showed no response to MS or WPM supplemented with BA, NAA, or BA + NAA after 6-8 weeks in light or dark culture. Young leaf explants are being evaluated for organogenic potential on WPM supplemented with BA, NAA, or BA + NAA under light and dark conditions. Petiole and shoot segments, along with root segments are also being evaluated.

P-3028

In Vitro Propagation and Ex Vitro Rooting of *Mitragyna parvifolia* (Roxb.) Korth.: A Threatened Tree of Medicinal Values. ASHOK KUMAR PATEL, Kheta Ram, and Narpat Singh Shekhawat. Jai Narain Vyas University, Biotechnology Unit, Department of Botany, UGC – Centre of Advanced Study (CAS), Jodhpur – 342 001, Rajasthan, INDIA. Email: ashpatel47@gmail.com

Mitragyna parvifolia (Roxb.) Korth. (Rubiaceae), commonly known as "Kadam", is mainly distributed in Indian subcontinent and known for its numerous medicinal properties in Ayurvedic system of medicine. The bioactive constituents of plant are mitraphylline, isomitraphylline, pteropodine, isopteropodine, speciophylline and uncarine F. This plant is commercially important in timber and paper industry. The quality of wood is equal to the teak and used in making of furniture, constructions materials and agricultural appliances. Owing to its extensive medicinal and commercial applications, this species has been over-exploited and presently facing a high risk of threats. Naturally this plant propagates through its very minute seeds (≈10,000 per gram), but germinated seedlings are very delicate and get washed away with rainy water. This abstract reports an efficient micropropagation system for *M. parvifolia* using nodal explants of a mature tree. For axillary shoot proliferation, BAP (3.0 mgL^{-1}) proved the best and produced 5.3±0.82 shoots. The shoots were further multiplied by repetitive transfer of mother explant and by subculturing. The maximum numbers (13.4±1.26) of shoots were produced on MS medium having a combination of BAP (0.5 mgL^{-1}), Kin (0.25 mgL^{-1}) and IAA (0.1 mgL^{-1}). For rooting under ex vitro conditions, shoot's base pulse-treated with IBA (500 mg L^{-1}, for 5 min) generated the maximum number (8.5±0.97) of roots. The rooted plantlets were successfully acclimatized in a greenhouse and transferred to nursery with ≈80% transplant survival rate. The protein contents of "in vitro growing" and "ex vitro established" plants were run over SDS-PAGE to compare the amount of total proteins and expression levels of different polypeptides in these two different conditions.

P-3029

In Vitro Collecting and Propagation of *Hippobroma longiflora* and Successful Recovery of Shoot Tips after 18 Years of Cryostorage. VALERIE C. PENCE and Bernadette L. Plair. Center for Conservation and Research of Endangered Wildlife (CREW), Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

In vitro methods and shoot tip cryopreservation are critical components of the toolbox needed to address the ex situ conservation of species classified as "exceptional" or not adaptable to seed banking. While shoot tips of many species have been cryopreserved, there have been few reports of their longevity, particularly of shoot tips of wild species. In 1995-97, in vitro collecting methods were used in a series of experiments to collect ethanol-sterilized leaf discs and stem tissues (extracted with a hypodermic needle) from wild *Hippobroma longiflora* plants in Trinidad. Several lines of shoot-propagating cultures were initiated from these collections, from which shoots were rooted and plants were acclimatized. In addition, shoot tips from these cultures were isolated and cryopreserved using the encapsulation dehydration method and banked in liquid nitrogen in CREW's CryoBioBank. After 17-18 years in cryostorage, shoot tips were removed from LN, thawed, and recovered on the same medium used for recovery and maintenance at the time of banking: MS salts and organics, 3% sucrose, 0.5 mg/L BAP, 0.05 mg/L NAA, and .25% gellan gum. Post-storage survival ranged from 20 to 90 % in samples of the 3 banked genotypes, with an average survival of 63.3%, compared with an average pre-storage survival of 62% overall for this species. An average of 44% of post-storage shoot tips went on to develop leaves and shoots, demonstrating an ability to re-establish shoot-propagating cultures. This high post-storage survival suggests little or no loss of viability over the time of storage and indicates that longer studies would be fruitful. While *H. longiflora* is not a rare or an exceptional species, it serves to demonstrate the effectiveness of bringing the in vitro methods of collecting, propagation, and tissue cryopreservation together to provide methods for ex situ conservation that do not depend on traditional seed-based

propagation or preservation. (Post-storage research supported by grant # LG-25-12-0595 from the Institute of Museum and Library Services.)

P-3030

In Vitro Propagation of *Vitex negundo* L., An Aromatic and Medicinal Woody Shrub. KHETA RAM, Ashok Kumar Patel, and Narpat Singh Shekhawat. Jai Narain Vyas University, Biotechnology Unit, Department of Botany, Jodhpur 342 001, Rajasthan, INDIA. Email: ramkheta@gmail.com

Vitex negundo L. (Verbenaceae) is a valuable aromatic plant and known for its extensive medicinal properties in Indian system of medicine. Plant's leaves contain active constituents such as betulinic acid, ursolic acid and ß-sitosterol which shows anti-cancer, anti-HIV and angiogenic properties, respectively. Natural propagation through seeds is affected due to poor germination and conventional methods like vegetative cuttings, root suckers are quite slow and not efficient to meet the present days demand. This abstract presents an efficient in vitro propagation of *Vitex negundo* using nodal shoot segments of a mature plant. Multiple shoots were optimally differentiated on MS medium containing BAP (2.0 mg L^{-1}), IAA (0.1 mg L^{-1}) and additives. Further shoot multiplication was achieved by repeated transfer of original explants and through subculturing. The maximum number of shoots (29.2±2.34) were produced after 5th subculture on MS medium supplemented with BAP (0.5 mg L^{-1}), Kin (0.1 mg L^{-1}), IAA (0.1 mg L^{-1}), and additives. The shoot cultures exhibited in vitro flowering, if subculturing period was delayed beyond 5-6 weeks. About 90% of the micropropagated shoots rooted in vitro on quarter strength MS medium supplemented with IBA (5.0 mg L^{-1}) and 0.02% of Activated Charcoal. In order to minimize the cost, time and labour, ex vitro rooting techniques was attempted. More than 95% of micropropagated shoots were rooted ex vitro on pulse-treatment of IBA (500 mg L^{-1}) for 5 min. The rooted plantlets by both the methods were successfully acclimatized in a greenhouse. The hardened plants were transferred to the nursery with more than 80% transplant survival rate. The micropropagated plants were morphologically similar to the mother plant.

P-3031

Parameters Enhancing Survival of *Agrobacterium*-transformed *Camelina sativa* Cultures. V. SITTHER[1], O. Enitan[1], B. Tabatabai[1], D. R. Bergey[2], and S. A. Dhekney[2]. [1]Department of Biology, Morgan State University, Baltimore, MD 21251 and [2]University of Wyoming, Sheridan Research and Extension Center, Sheridan, WY 82801. Email: viji.sitther@morgan.edu

Camelina sativa, a flowering plant in the family Brassicaceae, produces 30-40% seed oil making it a valuable renewable feedstock for the biofuel industry. Genetic transformation for varietal improvement requires efficient in vitro regeneration and gene delivery systems to generate large numbers of transformants from which improved individuals can be selected. Although our laboratory has developed successful *Agrobacterium*-mediated transformation methods using the GFP and NPTII genes in *C. sativa* cv. PI650159, explant survival after transformation has posed challenges. In this study, an improved protocol for enhancing survival rate of transformed explants was established. Optical density of *Agrobacterium* cultures adjusted to 0.2, 0.4, and 0.6 at 600 nm revealed a significant increase in explant survival at 0.2. Reduction of co-cultivation period from three to two days improved transformation efficiency of explants. While a three day wash period after co-cultivation completely killed the transformants, elimination of this step resulted in survival of explants. In addition, an increase in cefotaxime concentration from 50 mg L^{-1} to 100 mg L^{-1} at five days enhanced survival of cultures, presumably by further inhibiting *Agrobacterium* growth. Shoots from transformed explants transferred to Murashige and Skoog medium supplemented with 0.2 mg L^{-1} NAA resulted in rooting, leading to acclimatization for seed production. Optimization of these parameters has led to the establishment of an improved and efficient protocol for the generation of putative transgenic lines in *C. sativa* cv. PI650159. In future studies, value-added traits for enhancing oil production will be incorporated in this promising biofuel crop.

P-3032

Plant Development and Antioxidant Analysis of *Mentha x piperita* Obtained Through Micropropagation. B VAIDYA[1], N. Joshee[1], B. Asanakunov[2], D. R. Bergey[2], and S. A. Dhekney[2]. [1]Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030 and [2]University of Wyoming, Sheridan Research and Extension Center, Sheridan WY 82801. Email: vaidyab@fvsu.edu

Mentha is an important essential oil yielding crop used in the food, cosmetics and pharmaceutical industries. In the current study, shoot proliferation and plant regeneration of *Mentha x piperita* (peppermint) 'Black Mitcham' cultivar was compared in solid and liquid culture systems. Shoot tips from field-grown plants were surface sterilized and adventitious shoot proliferation response was tested on either MS or C2D medium containing varying levels of BAP, kinetin and 2iP. The treatment exhibiting the highest shoot proliferation in solid culture was to study growth in liquid medium using Liquid Lab Rocker (LLR) vessels. After six weeks, shoot numbers were recorded and rooting

of microshoots was induced on MS or C2D medium containing varying levels of IBA and NAA. Plant regeneration was estimated after 4 weeks. Plant development using SEM and biochemical analyses including Total Polyphenol Content (TPP) and antioxidant capacity measurement, and estimation of total flavonoid content were done to compare plants obtained from micropropagation with greenhouse-grown plants. The maximum number of shoots were obtained on C2D medium containing 4.0 µM BAP followed by MS medium containing 4.0 µM BAP. Significantly higher number of shoots were produced in liquid culture compared to those grown on the solid medium. Among the various rooting treatments, MS medium containing 1.0 µM IBA produced the maximum number of roots. SEM showed differences in leaf surface morphology between the greenhouse grown and micropropagation-derived plants in trichome types and density. Antioxidant potential of plants indicated higher TPP, TEAC values and flavonoid content in dried leaf extracts compared to fresh leaves. A higher level of TPP and flavonoid content was observed in micropropagation-derived plants while greenhouse-grown plants registered higher TEAC values. This study indicates that liquid culture system can enhance in vitro plant production with cost reduction and air-dried herbs can be used as a supplement for antioxidant activity without any loss of activity.

P-3033

In Vitro Microtuberization in 30 Potato Varieties. THOMAS W. ZIMMERMAN and Kenya M. Emanuel. University of the Virgin Islands, RR#1 Box 10,000, Kingshill, VI 00850. Email: tzimmer@uvi.edu

Potatoes, *Solanum tuberosum*, regularly grown in Jamaica and St Kitts, are planted in October. Harvest of potatoes stateside is September through October. However, freshly harvested potatoes require 2-3 months of cool storage, vernalization, to break dormancy to sprout and grow. The objective was to induce microtubers in tissue cultured potato that could be harvested and vernalized year-round for planting in the Virgin Islands. Virus-free potato germplasm, 30 varieties, was obtained from the USDA as in vitro plants. Tissue culture medium was prepared with 10% sucrose and short nodal sections of potato stems placed on the gelled medium. Data was collected weekly on microtuber formation. After four weeks, ten varieties started to form microtubers but at a low percentage per variety. Within eight weeks, 18 varieties had formed microtubers. The microtuberization rate was between 10 – 33% per variety. Microtubers can be induced from potato cuttings for 60% of the varieties tested after eight weeks in vitro on 10% sucrose. Further studies will be developed to enhance microtuberization efficiency incorporating reduced light and plant growth regulators. This research was supported by USDA-NIFA-Multistate Hatch and USDA-NIFA-Insular Tropical Grant funds.

MOLECULAR FARMING

P-3034

Engineering Sweetpotato [*Ipomoea Batatas (L.) Lam*] Expressing Synthetic Lytic Peptide for the Potential Inhibition of Human Immunodeficiency Virus Replication**.** STEVEN SAMUELS[1], M. Egnin[1], T. Nashar[2], G. C. Bernard[1], and O. Idehen[1]. Tuskegee University, [1]College of Agriculture and Nutrition Sciences, Department of Agriculture, Plant Biotech & Genomics Research Lab and [2]College of Veterinary Medicine, Nursing & Allied Health, Department of Pathobiology, Tuskegee, AL 36088. Email: ssamuels6952@mytu. tuskegee.edu

Treatments of infectious diseases in humans and animals have traditionally been targeted by chemically synthesized drugs, with the majority of the burden of cost falling on the individual in need of treatment. With the new revolution of producing therapeutic compounds, such as peptides in plant based systems, the cost of production is dramatically decreased. Antimicrobial peptides have also been found to target intracellular molecules, such as DNA/RNA or enzymes. Synthetic lytic peptides *jc41n* and *jc41nd,* capable of inhibiting the progression of HIV have been developed at Tuskegee University and expressed in sweetpotato. Synthetic lytic peptides gene constructs, were engineered into sweetpotato resulting in seven transgenic plantlets, which were subjected to molecular analysis to confirm stable integration and expression of the transgene. Transgenic sweetpotato tested positive for the presence of the transgene using primers specific for the *jc* genes and 35S promoter and NOS terminator. Quantitative PCR on genomic DNA and cDNA from positive transformants, parental non-transformed control, and JC plasmids confirmed gene insertion number and stable integration of the transgenes, and determined relative levels of transcript expression. Upon successful screening, protein extract containing the expressed JC protein was analyzed for cell cytotoxicity, molecularly cloned Env-pseudotyped virus neutralization, and reduced pseudovirus production to elucidate possible mechanisms of action. Successful development and approval of sweetpotato expressing this novel therapeutic compound can be both a powerful tool in treatment of the HIV epidemic. Current HIV therapies although effective, require expensive input cost, which generates retail cost that to many are unaffordable. A treatment regime developed in a plant system will drastically decrease input cost lowering the need for high retail cost. *Work supported by Tuskegee University, GWCAES,*

NIFA-EVANS-ALLEN-T-CAP and UC DAVIS-TU CREATE-IGERT.

PHYSIOLOGY

P-3035

Role of Cytochrome P450s in Salt Tolerance of Plants. PRAKASH P. KUMAR, Pannaga Krishnamurthy, Wan-Jing Ho, Felicia Lok, Tit-Meng Lim, Qingsong Lin, Jian Xu, and Chiang-Shiong Loh. National University of Singapore, Department of Biological Sciences, 10 Science Drive 4, SINGAPORE 117543. Email: dbskumar@nus.edu.sg

The tropical mangrove tree *Avicennia officinalis* can exclude ~95% salt at the roots which is facilitated by enhanced hydrophobic root barriers (Casparian bands and suberin lamellae). In an RNA Seq project, we identified several cytochrome P450 (CYPs) genes that were differentially regulated under salt stress in the seedling roots of *A. officinalis*, which was further validated by qRT-PCR and induction of expression in the seedling roots by NaCl. From this group of CYPs, CYP86B1 that may regulate suberin biosynthesis was characterized using *Arabidopsis CYP* mutant, *Atcypb6b1* as the heterologous experimental plant system. Reduced suberin lamellae and Casparian bands were seen in the roots of *Atcypb6b1* compared to the wild-type roots. Ectopic expression of *A. officinalis CYP86B1* coding sequence in *Arabidopsis* resulted in partial rescue of the reduced suberin phenotype, indicating a role for CYP86B1 enzyme in controlling suberin biosynthesis. Further, the regulation of *CYPs* by various WRKY transcription factors was studied in order to understand the molecular regulation of suberin biosynthesis. Our data suggest that CYPs play an important role in salt tolerance of plants (in both glycophytes and halophytes), and this information could help in developing strategies to impart salt tolerance to crop plants.

SECONDARY METABOLISM

P-3036

Comparison of Total Phenolic Content and Total Antioxidant Activity of Essential Oils of Male and Female *Pistacia lentiscus* L. EBUBEKiR İZOL[1], Veysel Süzerer[2,3,4], Serkan Yiğitkan[5], Engin Tilkat[6], Abdulselam Ertaş[1], Hilal Surmuş Aşan[7], and Ahmet Onay[7]. [1]Department of Pharmacognosy, University of Dicle, Diyarbakır, TURKEY; [2]Department of Medical Services and Techniques, University of Bingöl, Bingöl, TURKEY; [3]Department of Botany, University of İstanbul, İstanbul, TURKEY; [4]Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, TURKEY; [5]Department of Pharmaceutical Botany, University of Dicle, Diyarbakır, TURKEY; [6]Department of Biology, University of Batman, Batman, TURKEY; and [7]Department of Biology, University of Dicle, Diyarbakır, TURKEY. Email: izolebubekir@gmail.com; beyso1985@gmail.com

Mastic tree (*Pistacia lentiscus* L.) is a perennial plant species in the form of evergreen shrub that 2-3 meters in height can grow up to 5 meters, and belongs to Anacardiaceae family. *P. lentiscus* var. Chia (Duham) has economically grown especiallyin the Greek Island of Chios in the eastern Mediterranean, and produces transparent colorless resin after drying returning to the ivory, known as the aromatic gum resin. Different parts of *P. lentiscus* L. contain various types of phytochemical constituents like terpenoids, phenolic compounds, fatty acids, and sterols. The aim of the present study is to determine total phenolic contents and antioxidant properties of the samples determined by four different analytical methods: CUPRAC (cupric reducing antioxidant capacity) β-carotene bleaching, DPPH (2,2-diphenyl-1-picrylhydrazyl) free and ABTS cation radical scavenging methods, respectively. Fresh leaf and stem samples taken from female and male *P. lentiscus* L trees grown in the vicinity of Çeşme county are divided into branches and leaves. Then, they were chopped into small pieces. Volatile oil was obtained by hydrodistillation method with 300 ml of water from 100 gr. weighed sample. In terms of the phenolic content, especially both the leaves and the branches of the male samples were richer than the female samples. According to the method β-carotene, all four essential types displayed low CUPRAC scavenging activity. With this method, none of the samples displayed biological activity, but the ABTS method showed a good level of activity for all samples. According to the ABTS method, the highest activity (IC50: 72,123±1,104) were determined in the oil obtained from the male stem sections.

OTHER PLANT IN VITRO BIOLOGY TOPICS

P-3037

Early Developmental Responses from Soybean Anther Cultures. MARTINA GARDA[1,2,3], K. Bade[1], B. Hale[1], M. Lowe[1], T. Sherrod[1], T. Sustich[2], N. Williams[1], and G. C. Phillips[1,3]. [1]Arkansas State University, College of Agriculture and Technology, P. O. Box 1080 State University, AR 72467; [2]Arkansas State University, College of Mathematics and Science, P. O. Box 1030, State University, AR 72467; and [3]Arkansas State University, Arkansas Bioscience Institute, P. O. Box 639, State University, AR 72467. Email: martina.garda@smail.astate.edu

Soybean androgenesis is challenging and has not advanced in over 10 years. Our research has explored alternative triggers not yet reported in the literature which may influence androgenetic responses in soybeans. We identified three trigger factors (11 ^{0}C incubation, 40 mg/L

2,4-D, nitrogen starvation medium) following overnight shock of donor plants at 4 ^{0}C which increase the expression of putative gametic calli (PGC) in soybean anther cultures. We have also observed several unexpected responses that were present at an 80-90% rate while scoring anther cultures after 1 month incubation. These responses included water-like droplet structures emerging from anthers; engorgement of the central portion of anthers; and release of some microspores from anthers. These early responses declined over time and PGC were not apparent until the second or third month of incubation. The results presented here provide additional descriptors that may facilitate development of a model androgenesis system among soybeans and legumes.

P-3038

Comparative Gene Expression and Metabolic Impacts in the Hyperaccumulator, *Pteris vittata* and Non-hyperaccumulator, *Pteris ensiformis* in Response to Arsenic Toxicity. Osagie Idehen, DANA REID, Marceline Egnin, Ramble Ankumah, Raymon Shange, Gregory Bernard, Steven Samuels, Alayjah Muhammad, and Kiara Bunton. Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL. Email: dreid9326@ mytu.tuskegee.edu, megnin@mytu.tuskegee.edu

Arsenic is a metalloid that is present in numerous regions of the environment. It is highly toxic in its inorganic form and is a major environmental issue as it is prime contaminant in soils and ground water. Long-term exposure to inorganic arsenic can lead to chronic arsenic poisoning resulting in serious health issues such as skin lesions and cancer. In the environment it is typically present in the form of arsenite (AsIII) and arsenate (AsV) with arsenate having a more dominant presence than arsenite in an aerobic environment. *Pteris vittata* (Chinese brake fern) has a unique ability of tolerating arsenic containing soils as well as hyper-accumulating arsenic and the majority of the arsenic accumulates in the fronds as AsIII. Several arsenic-tolerant genes (*ACR1*, *ACR2*, *ACR3*, *ACR3,1*) have been identified in *P. vittata*. Although the mechanism has still not been definitively elucidated, it is believed that *ACR3* plays a role in arsenite being sequestered in the vacuole. Additionally, levels of antioxidants such as ascorbate and glutathione were found at much higher levels than in *P. ensiformis* and are believed to be regulated by arsenic-tolerant genes. Characterization of these genes are therefore critical in better understanding the molecular mechanisms involved in plant growth in an arsenic-contaminated environment. In this study, a comparative gene expression analysis will be conducted on samples from two ferns: arsenic-hyperaccumulator, *P. vittata* and arsenic-sensitive *P. ensiformis*, propagated in arsenic-contaminated soils in order to determine differential gene expressions of arsenic-tolerant and oxidative stress genes in the root and fronds of both plant systems. Work supported by: USDA-NIFA 1890-CBG funded iBREED project to Tuskegee University Plant Biotech Lab, GWCAES-CAENS.

PLANT SILENT ABSTRACT

P-3039

Evolution of Photosynthetic Capacity During the Acclimatization to Ex Vitro Conditions of Micro-propagated *Paulownia tomentosa* Plants. A. PIQUERAS, Pedro Díaz-Vivancos, Giuliano Sting Pechar, and José Antonio Hernández. CEBAS (CSIC) Department of Plant Breeding, Murcia 30100, SPAIN. Email: Piqueras@cebas .csic.es

During the transfer to *ex vitro* conditions, *in vitro* plants are exposed to light intensities higher than those used under *in vitro* conditions, resulting usually in photoinhibition. In addition, the high differential vapour pressure between *in vitro* and *ex vitro* condition can induce water stress.These stresses may lead to an imbalance between light energy absorption and light energy utilization in acclimatized plants and ultimately to the formation of ROS. Chlorophyll a fluorescence analysis by *Pulse-Amplitude (PAM) fluorometry* has been used to monitor the physiological changes to in vitro-propagated Paulownia tomentosa plants during the process of acclimatization to ex vitro conditions. The technique allows a precise and nonintrusive assessment of photosynthesis and provides data about overall photosynthetic quantum yield and capacity. PAM Chlorophyll a fluorescence measurements were taken at 0 (in vitro), 7, 14, 21, and 28 d of acclimatization of the micropropagated Paulownia tomentosa plants. With the appearance of new leaves, higher photosynthetic capacities were observed and light saturation point increased (days 14 and 21). *F*v:*F*m decreased directly after transplantation of the micropropagated plantlets, afterwards, a recovery was observed, The photochemical quenching qP coefficient increased gradually during the first two weeks of the acclimatization. A progressive rise in ETR (Electron transport rate), which was not detected in leaves formed in vitro, could be observed in new ex vitro developed leaves.The close association between both qP (a measure of the trapped energy that is used in photochemical events such as carbon fixation) and ETR during the evolution of acclimatization means that some selected chlorophyll a fluorescence parameters may be used as a surrogate for photosynthetic rates, and therefore the chlorophyll fluorescence technique could be used as an effective, non-destructive and practical tool for the evaluation of the physiological status of micropropagated paulownia

plants during acclimatization and to control the quality of the process.

BIOTECHNOLOGY

P-3040

Extensive Variation in Rate of Callus and Shoot Regeneration, and *Agrobacterium*-mediation transformation, among Wild Black Cottonwood Genotypes *(Populus trichocarpa)*. CATHLEEN MA and Steve Strauss. Oregon State University, Department of Forest Ecosystems and Society, Corvallis OR 97331. Email: Caiping.ma@ oregonstate.edu, steve.strauss@oregonstate.edu

The capacity for plant regeneration and transformation (RT) is notoriously variable among species and genotypes of plants. In many cases, transformation is impossible or impractical. The reasons for this extraordinary biological variation, however, are largely unknown. As part of a major project to use GWAS (genome wide association studies) to map genes controlling RT in poplar, we are studying variation in RT among resequenced wild genotypes of black cottonwood—for which low levels of linkage disequilibrium facilitate GWAS-based gene identification. To estimate the extent of variation in RT, we first studied 20 wild genotypes for their levels of callus, shoot, and root regeneration based on our previously published protocol (Ma C, Strauss SH, Meilan R. 2004. Agrobacterium-mediated transformation of the genome-sequenced poplar clone, Nisqually-1 (*Populus trichocarpa*). Plant Molecular Biology Reporter 22:1-9.). We tested both direct and indirect regeneration pathways using two different types of explants, petioles and leaves from greenhouse-grown plants. We found that indirect regeneration, where callus proliferation preceded shoot induction, strongly promoted shoot regeneration, but that the effect varied widely between petiole and leaf explants. Among tested genotypes, five gave a rate of 60-100% of petiole explants regenerating shoots, and five showed a complete lack of shoot meristem differentiation. While six genotypes showed 60-95% leaf explants regenerating shoots, three gave no bud differentiation at all. The average number of shoots from leaf explants are higher (3.7) than petiole fragment (2.9). The rate of transient GFP expression following *Agrobacterium* cocultivation was high, but varied among genotypes (range of 39-71% per genotype). However, the rate of GFP expression decreased to 1.5-24.5% after 3 months on shoot induction medium (SIM) with 100mg/L kanamycin. Therefore our efforts focused on studying the factors that affect the rate of stable transformation using *in vitro* growing plants. First, using five genotypes we tested effects of pre-culture prior to *Agrobacterium* infection on transient GFP expression using four different calli induction media (CIM). The results showed pre-culture one day on CIMs greatly increased transient GFP expression, from 0-4% to 40-66% for leaf explants, and from 0-1% to 23-44% for stem and petiole explants. Transient GFP expression rates varied among genotypes from 40-66% for leaf and 24-45% for stem explants when pre-culture was employed; in contrast rates were 0-4.3% for leaf and 0-1% for stem without pre-culture. Co-cultivation in a medium rich in auxin doubled the rates of stable GFP expression, with rates varying from 23%-48% for leaf explants. This is likely because actively dividing cells in S-phase are significantly increased by auxin treatment, and such cells are more able to integrate foreign DNA (Leandro Pena et al. 2004. Early Events in *Agrobacterium*-mediated Genetic Transformation of Citrus explants. Annals of Botany 94:67-74). The effect of acetosyringone (AS) in an auxin-rich medium during co-cultivation was studied in five genotypes. AS in CIM during co-cultivation enhanced GFP expression, both during transient and stable transformation phases, in both leaf and stem explants of all tested genotypes. We will also report on studies of inoculation times, and recovery of transgenic shoots.

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