2018 IN VITRO BIOLOGY MEETING

LATE SUBMISSION ABSTRACTS

2018 Meeting of the Society for In Vitro Biology
June 2 – 6, 2018
St. Louis, Missouri

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

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A-2 In Vitro Studies of Gastrointestinal Epithelial Cell Differentiation and Reprogramming (Paligenosis)
Megan D Radyk, *Washington University School of Medicine, Zhifeng Miao, and Jason C Mills*

A-3 Regulatory Networks Specifying Cortical Interneurons from Human Embryonic Stem Cells Reveal Roles for CHD2 in Interneuron Development
Kristen L. Kroll*, Washington University School of Medicine, Kesavan Meganathan, Emily M. A. Lewis, Paul Gontarz, Shaopeng Liu, Ed G. Stanley, Andrew G. Elefanty, James E. Huettner, and Bo Zhang*

CELL AND MOLECULAR ENGINEERING USING PROGRAMMABLE DNA BINDING PROTEINS – Tuesday, June 5, 3:30 pm – 5:00 pm

A-15 Genome Editing and 3D-cell Culture to Create Novel Human Cell-based Assays for ADME/Tox Applications
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A-3000 Evaluation of Seeding Techniques by Monitoring Cell Distribution Using Label-free Live Cell Imaging
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CELL AND TISSUE MODELS

A-3001 Expression of a Dominant Negative Mutant of NPM1 in Neuroblastoma Cells Results in Altered Localization of NPM1
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A-3002 Using Mass Spectrometry to Identify Proteins Involved in Enhanced and Directed Growth of Dorsal Root Ganglion Grown on Nano-sized Topography
Anton Uhlen*, Midwestern University, Harsh Sharthiya, Sarah C. Veen, Kelly Keeler, Joshua Z. Gasiorowski, Michele Fornaro, and Kolla Kristjansdottir*

CELLULAR AND MOLECULAR TOXICOLOGY

A-3003 Use of RNA-Seq to Identify Cadmium-dysregulated MicroRNAs in NRK-52E Rat Proximal Tubule Epithelial Cells
Ribhi Salamah*, Midwestern University, Lauren A.C. Alt, Zyaria Stubbs-Russell, Prem Patel, Aatmiya Patel, Joshua R. Edwards, Walter C. Prozialeck, and Michael J. Fay*

CHEMICAL CARCINOGENESIS

A-3004 Investigating a Role for Chemokines in Head and Neck Cancer
Travis Parker, *Midwestern University, Zhang Zhong, and Annette Gilchrist*

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A-3005 Development of a High Throughput-high Content Assay to Identify the Teratogenic Potential of Organophosphates
J. Garcia, *California State University San Bernardino, T. M. Ubina, J. Argueta, A. Ashour, and N. Bournias-Vardiabasis*

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A-3006 Dissecting the Role of Adhesion Kinase FAK in Mediating CAP1 Regulation of ERK and Breast Cancer Cell Functions
Rokib Hasan*, Arkansas State University, Joshua Gray, Faith Allen, Thomas Kelly, and Guolei Zhou*

ANIMAL SILENT ABSTRACTS

A-3007 Modified Plant Virus as a Basis for Vaccine Design Against Pathogen of Bacterial Nature
O. A. Kondakova, *Lomonosov Moscow State University, E. A. Trifonova, E. M. Ryabchevskaya, M. V. Arkhipenko, N. A. Nikitin, and O. V. Karpova*

A-3008 The Design of Rotavirus Recombinant Antigen for Novel Candidate Vaccine Based on Modified Plant Virus
E. Trifonova, *Lomonosov Moscow State University, N. Nikitin, E. Ryabchevskaya, O. Kondakova, J. Atabekov, and O. Karpova*

PLANT POSTER ABSTRACTS

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Jeffrey Adelberg*, Clemson University, Jacqueline Nyalor-Adelberg, Rabia El-Hawaz, and Robert Eisenreich*

BIOTECHNOLOGY

P-3000 Herbicide-induced Gene Expression System
Maren Arling*, E I Du Pong De Nemours and Company, K. McBride, K. Lowe, W. Gordon-Kamm, A. Anand, N. Wang, K. Snopek, B. Lenderts, C. Hastings, G. Hoerster, J. Farrell, and B. Vickroy*

P-3001 Characterization of Selected cDNA-AFLP Transcripts Involved in Sweetpotato Storage Root Development
Adrianne Brown, *Tuskegee University, Sy Traore, Frieda Sanders, Marceline Egnin, Gregory C. Bernard, Osagie Idehen, Foazi Bukari, Desmond Mortley, and Conrad Bonsi*

P-3002 Establishment of Hexaploid Sweetpotato Protoplast as a Systems for Gene Expression, Genome Editing and Elucidation of Gene Functions
Foaziatu Bukari*, Tuskegee University, Sy Traore, Marceline Egnin, Osagie Idehen, Gregory C. Bernard, Yi-Lang Lee, Stanton Gelvin, Adrianne Brown, Desmond Mortley, Conrad Bonsi, and Deloris Alexander*

P-3003 Developing *In Vitro* Tools to Enable Quinoa (*Chenopodium quinoa* Willd.) Biotechnology
S. Elizabeth Castillo, *Donald Danforth Plant Science Center, Jose C. Tovar, Anastasia Shamin, Paige Pearson, Raj Deepika Chauhan, Nigel Taylor, and Malia Gehan*

P-3004 Producing Fully Functional Human Sulfatase Enzyme Replacement Therapeutics in Plants by Co-producing the Human Sulfatase-modifying Factor SUMF1
Shivakumar Devaiah and Kassandra Riley, *BioStrategies LC, Varun Katta, Walter Acosta, Jorge Ayala, Carole L. Cramer, and David N. Radin*

P-3005 Understanding the Hydroxyproline-O-Glycosylation Pathway to Enhance Recombinant Therapeutic Protein Production
Oliver Dozier, *Arkansas State University, Kelin Key, Jianfeng Xu, and Maureen C. Dolan*

P-3006 In Vitro Micrografting of Tomato (*Solanum* Spp.)
Dora Janeth García-Jaramillo*, Universidad de Caldas, Lucía Atehortúa Garcés, Jairo Castaño Zapata, and Nelson Ceballos Aguirre*

P-3007 *In Vitro* Seed Germination and Establishment of Hairy Root Cultures of a Peruvian Peanut Cultivar as a Bioproduction Platform of Stilbenoids
Maria Elena Gonzalez Romero*, Arkansas State University, and Fabricio Medina-Bolivar*

P-3008 Use of Non-integrating WUS for Recovering Transgenic Events Via Direct Embryogenesis in Maize
George Hoerster*, DuPont Pioneer, Keith Lowe, Ajith Anand, Ning Wang, Kevin McBride, and Bill Gordon-Kamm*

P-3009 Precision Gene Editing in Agriculture
Javier Narvaez*, Cibus, Greg Gocal, Christian Schöpke, Mark Knuth, Dave Songstad, Steve Sanders, Noel Sauer, and Peter Beetham*

P-3010 Engineering Tobacco BY-2 Cell with CRISPR/Cas9 Technology for Reduced Proteolytic Activities
Kiera O'Keefe, *Arkansas State University, and Jianfeng Xu*

P-3011 Deciphering the Function of Phased Small Interfering RNAs from Protein Coding Genes in *Arabidopsis*
Suresh Pokhrel*, University of Missouri, and Blake C. Meyers*

P-3012 Investigating Molecular Differences in Sweetpotato Beta-Carotene From Dark-to-White Colored Flesh Cultivars
Sy Traore*, Tuskegee University, Foaziatu Burkari, Marceline Egnin, Adrianne Brown, Osagie Idehen, Gregory C. Bernard, Yi-Lang Lee, Stanton Gelvin, Conrad Bonsi, and Desmond Mortley*

P-3013 Regulation of Cell Wall Strength and Salt Tolerance by the *Arabidopsis* MUR4
Omar Zayed, *Purdue University, and Chunzhao Zhao*

CELLULAR AND MOLECULAR TOXICOLOGY

P-3014 Elucidating the Molecular Mechanisms of Arsenic Accumulation in *Pteris Vittata*
Osagie Idehen*, Tuskegee University, Marceline Egnin, Ramble Ankumah, Raymon Shange, Sy Traore, Gregory C. Bernard, Conrad Bonsi, Foaziatu Burkari, and Adrianne Brown*

CELLULAR PATHOLOGY

P-3015 Potential Nematicidial Activity of Silver Nanoparticles Against Root-knot Nematode (*Meloidogyne incognita*)
***Jacob Fitch**, Tuskegee University, Gregory C. Bernard, Byungjin Min, Naresh Shahi, Marceline Egnin, Sy Traore, Osagie Idehen, Foziatu Bukari, Willard Collier, and Conrad Bonsi*

CYTOKINES

P-3016 High-yield Secretion of Human Interleukin-12 from Plant Cell Cultures for Therapeutic Uses
***Colton Batten,** Arkansas State University, Ningning Zhang, and Jianfeng Xu*

GENETIC ENGINEERING

P-3017 A New and Robust *Agrobacterium* Strain for Citrus Transformation
***Diaa Alabed,** USDA-ARS, and James Thomson*

P-3018 The Future is Here: Developing New Corn Products Through Direct Genome Editing of Elite Inbreds
***Melissa A Rahe**, DuPont Pioneer, Todd J. Jones, Keith Lowe, Deping Xu, Nathalie Sanyour-Doyel, Chenna Peng, and Kristen Carpenter*

P-3019 Development of Site-specific Recombinase Technology for Targeted Integration with Marker Removal
***James Thomson**, USDA, and Roger Thilmony*

P-3020 Plant Transformation and Genome-modification Services at Donald Danforth Plant Science Center
***Veena Veena,** Donald Danforth Plant Science Center, Preethi Vallal, Todd Finley, and Dave Busby*

P-3021 Expression of the Arabidopsis WRINKLED 1 Transcription Factor Leads to Higher Accumulation of Palmitate in Soybean
***Pamela A. Vogel, Shen Bayon De Noyer,** University of Nebraska-Lincoln, Hyunwoo Park, Hanh Nyugen, and Tom Clemente*

METABOLIC ENGINEERING

P-3022 Ectopic Expression of a Peanut Prenyltransferase Gene in Muscadine Grape Hairy Roots to Produce the Bioactive Prenylated Stilbenoid Arachidin-2
***Mohammad Azim,** Arkansas State University, Parker Knapp, Tianhong Yang, and Fabricio Medina-Bolivar*

P-3023 Peanut Hairy Roots: A Bioproduction Platform for Elucidating the Biosynthesis of Prenylated Stilbenoids
***Tianhong Yang,** Arkansas State University, Lingling Fang, Sheri Sanders, Srinivas Jayanthi, Gayathri Rajan, Ram Podicheti, Thallapuranam Krishnaswamy Suresh Kumar, Keithanne Mockaitis, and Fabricio Medina-Bolivar*

MICROPROPAGATION

P-3024 Improved Statistical Analysis for Projecting Mineral Nutrient Ranges
***Meleksen Akin,** Igdir University, Charles Hand, Ecevit Eyduran, and Barbara M. Reed*

P-3025 The *In Vitro* Propagation of *Pteris vittata* and *Pteris ensiformis* Using Selective Media
***Capri Charleston,** Tuskegee University, Osagie Idehen, Marceline Egnin, Sy Traore, Gregory C. Bernard, Adrianne Brown, Foaziatu Bukari, Caitlin Skinners, and Dominique Jiles*

P-3026 In Vitro Micropropagation of Commercial and Non-commercial Tomato Genotypes
***Dora Janeth García-Jaramillo,** Universidad de Caldas, Lucía Atehortúa Garcés, Jairo Castaño Zapata, and Nelson Ceballos Aguirre*

MOLECULAR FARMING

P-3027 Hydroxyproline-O-glycosylation Tag for Increasing Recombinant Protein Production Using the Transient Plant Expression Platform
***Cristofer Calvo,** Arkansas State University, Jianfeng Xu, and Maureen C. Dolan*

PRODUCT APPLICATIONS

P-3028 Identification of Cellulase Inhibitors Using Corn-seed Produced Enzymes
***Hong Fang**, Arkansas State University, Gurshagan Kandhola, Kalavathy Rajan, Kendall R. Hood, and Elizabeth E. Hood*

SECONDARY METABOLISM

P-3029 Elicitation of Prenylated Stilbenoids in Hairy Root Cultures of Three Cultivars of Peanut
***Abbas Karouni,** Arkansas State University, Tianhong Yang, and Fabricio Medina-Bolivar*

P-3030 Bioproduction and Purification of Bioactive Prenylated Stilbenoids from Hairy Root Cultures of Peanut
***Sepideh Mohammadhosseinpour,** Arkansas State University, Lingling Fang, Tianhong Yang, and Fabricio Medina-Bolivar*

P-3031 Assessment of Antioxidant Activity of Purified Stilbenoids and Stilbenoid-enriched Extracts from Peanut Hairy Roots
Patrick Roberto, *Arkansas State University, Maria Elena Gonzalez Romero, and Fabricio Medina-Bolivar*

SOMATIC CELL GENETICS

P-3032 Exploring Somatic Hybrid Production in Sweetpotato Between a Nematode-Resistant Orange-flesh and a Susceptible White and Dry Flesh Cultivars in Next Generation Breeding
Larencia Williams*, Tuskegee University, Foaziatu Bukari, Marceline Egnin, Caitlin Skinner, Gregory C. Bernard, Sy Traore, Osagie Idehen, Adrianne Brown, Desmond Mortley, and Conrad Bonsi*

PLANT SILENT ABSTRACTS

P-3033 Complexes Consisting of Genetically Modified TMV Virions and Immunogenic Proteins: Characterization by Immunological and Physico-chemical Methods
Tatiana Gasanova, *Lomonosov Moscow State University, and Peter Ivanov*

P-3034 The Role of the C-terminus of the Tobacco Mosaic Virus Coat Protein in Its Structural Characteristics and Ability to Interact with TMV Movement Protein
N. O. Kalinina, *Lomonosov Moscow State University, A. V. Makhotenko, V. V. Makarov, and S. S. Makarova*

P-3035 Some Features of Vegetative Propagation of *Origanum vulgare* L.
Elena Myagkih*, Research Institute of Agriculture of Crimea, Olga Yakimova, and Alexandr Mishnev*

P-3036 Clonal Micropropagation In Vitro of *Melissa officinalis* L.
Olga Yakimova, *Research Institute of Agriculture of the Crimea, Natalia Yegorova, Margarita Zagorskaya, and Irina Stavtzeva*

ANIMAL SYMPOSIA ABSTRACT

FRONTIERS IN TRANSLATIONAL TISSUE MODELING – Monday, June 3, 10:30 am – 12:30 pm

A-2

In Vitro Studies of Gastrointestinal Epithelial Cell Differentiation and Reprogramming (Paligenosis). MEGAN D RADYK[1], Zhifeng Miao[1,2], and Jason C Mills[1,2,3]. [1]Division of Gastroenterology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO; [2]Department of Surgical Oncology and General Surgery, The First Affiliated Hospital of China Medical University, Shenyang, CHINA; and [3]Departments of Developmental Biology and Pathology & Immunology, Washington University School of Medicine, St. Louis, MO. Email: meganradyk@wustl.edu, jmills@wustl.edu

Adult stem cells work to maintain the highly organized epithelial units of the stomach by dividing and giving rise to more differentiated, mature cell types. This linear progression of stem cell to mature cell changes when the stomach is injured, as recent publications have shown that mature cell types in the stomach can give rise to less differentiated cells. For example, differentiated zymogenic "chief" cells are normally post-mitotic, yet they undergo regenerative plasticity upon tissue damage such as the death of acid-secreting parietal cells. Chief cells dedifferentiate/reprogram in a systematic, step-wise process to assist in damage repair: 1. downscaling and removal of differentiation-associated proteins by autophagy, 2. expression of progenitor and embryonic genes, and 3. entry to the cell cycle. We have found these same reprogramming steps to be conserved in differentiated cells that become regenerative in many organs (stomach, pancreas, kidney, liver) and, because this regenerative sequence seems to be an evolutionarily conserved cellular process (like mitosis or apoptosis), we have given it a set term: Paligenosis. The pathological ramifications of chief cell paligenosis is that it results in Spasmolytic Polypeptide-Expressing Metaplasia (SPEM), a potentially precancerous lesion most often observed in humans infected with the bacterium *Helicobacter pylori*. While SPEM is generally a transient tissue state to aid in recovery after injury, prolonged SPEM is a known risk factor for the development of gastric adenocarcinoma; thus further study of paligenosis may guide advances in cancer biology. *In vitro* methods to study epithelial cells the stomach and other gastrointestinal organs have been drastically improved since the discovery of 3D organoid culture and continued advances with gastrointestinal cell lines. We discuss *in vitro* systems in the Mills Lab used to complement *in vivo* studies by providing specific signaling mechanisms and cell responses involved in gastrointestinal epithelial cell differentiation and reprogramming (paligenosis).

A-3

Regulatory Networks Specifying Cortical Interneurons from Human Embryonic Stem Cells Reveal Roles for CHD2 in Interneuron Development. KRISTEN L. KROLL[1], Kesavan Meganathan[1], Emily M. A. Lewis[1], Paul Gontarz[1], Shaopeng Liu[1], Ed G. Stanley[3], Andrew G. Elefanty[3], James E. Huettner[2], and Bo Zhang[1]. [1]Department of Developmental Biology[1] and [2]Department of Developmental Cell Biology, Washington University School of Medicine, 660 S. Euclid Avenue, Saint Louis, MO 63110 and [3]Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, AUSTRALIA. Email: kkroll@wustl.edu

The mammalian cerebral cortex consists of two main categories of neurons, excitatory neurons that convey information to distant neurons within and outside the cortex and GABAergic cortical interneurons (cINs) that provide local inhibitory inputs to modulate excitatory neuronal activity. Interneuron loss or dysfunction during development alters the balance between neuronal excitation and inhibition, contributing to many neurodevelopmental disorders, including epilepsy. During fetal development, many cINs derive from the medial ganglionic eminence (MGE), a transient ventral forebrain structure. While altered cIN development contributes to neurodevelopmental disorders, the inaccessibility of the developing human fetal brain has hampered efforts to define molecular networks controlling this process. Therefore, here we refined protocols for directed differentiation of human embryonic stem cells into MGE-like progenitors and cIN-like neurons, obtaining efficient, accelerated production of mature cIN subtypes with the expected functional properties. We used this model to define transcriptome changes accompanying MGE specification and cIN differentiation and direct targets of the NKX2-1 transcription factor in this process. NKX2-1 acts at the apex of gene regulatory networks controlling cIN development, with loss in mice resulting in MGE-derived cIN deficits, acquisition of alternate fates, and spontaneous seizures, while human NKX2-1 mutations cause chorea, hypotonia, and dyskinesia, likely involving similar cIN disruption. However, NKX2-1 targets during in human cIN development were unknown. Therefore, we integrated NKX2-1-associated genes in MGE-like progenitors with the MGE- and cIN-enriched transcriptome to define NKX2-1 direct target genes encoding known and novel regulators of cIN migration, differentiation, and function. This work identified suites of transcriptional and epigenetic regulators that may comprise a gene regulatory network mediating human MGE specification. Among these was CHD2, a chromatin remodeling enzyme. Human mutations resulting in *CHD2* haploinsufficiency cause pediatric epilepsies involving refractory seizures, cognitive decline, and poor prognosis. As *CHD2* expression increased during cIN differentiation, we examined its requirements for this process, determining that NKX2-1 regulates *CHD2* expression by binding a cis-regulatory element (CRE) in the *CHD2* gene promoter, while CHD2 and NKX2-1 co-bind some of the same CREs, and CHD2 promotes NKX2-1-dependent target gene transactivation. Accordingly, CHD2 knockdown and knockout revealed requirements in cIN differentiation, and CHD2-dependent transcriptional programs including regulation of human epilepsy genes during this process. Together, these data provide a foundation for beginning to define the molecular basis of human cIN specification and differentiation and to identify how its dysregulation may contribute to neurodevelopmental disorders.

CELL AND MOLECULAR ENGINEERING USING PROGRAMMABLE DNA BINDING PROTEINS – Tuesday, June 5, 3:30 pm – 5:00 pm

A-15

Genome Editing and 3D-cell Culture to Create Novel Human Cell-based Assays for ADME/Tox Applications. G. PEGG, M. Bourner, L. Marshall, J. Pratt, T. Steiner, J. Blasberg, M. Angeles, and D. Thompson. MilliporeSigma, 2909 Laclede Avenue, St. Louis, MO. Email: gene.pegg@sial.com

A major challenge to preclinical drug toxicology safety testing is the correct identification of cell transporters, nuclear receptors and xenobiotic enzymes involved in drug metabolism. Understanding the mechanism of drug uptake and clearance will reduce costs of drug development and improve drug safety. Recent advances in gene editing technology have allowed the creation of knock-out/knock-in drug metabolizing cell lines representing liver, intestine and kidney tissues. This presentation will provide an overview on the gene editing targets and toxicity testing approaches across multiple cell lines. As small molecule inhibitors of transporters are often non-specific, and siRNA yields incomplete knock-down of targeted genes, studies utilizing these gene edited cell clones provide a definitive result of the mechanism by which specific drugs are imported, exported and metabolized. Additionally, these cell lines can be utilized in 3D-models which have been reported to be more physiologically relevant for some investigations.

ANIMAL POSTER ABSTRACTS

BIOTECHNOLOGY

A-3000

Evaluation of Seeding Techniques by Monitoring Cell Distribution Using Label-free Live Cell Imaging. MIHO K. FURUE, Hiroaki Kii, Atsushi Kitajima, Tetsuomi Takasaki, Tomoro Dan, Takayuki Uozumi, and Yasujiro Kiyota. Stem Cell Business Development Department, Technology Solution Sector, Healthcare Business Unit, NIKON Corporation. Yokohama Plant, 471, Nagaodai-cho, Yokohama, Kanagawa 244-8533, JAPAN. Email: Miho.Furue@nikon.com

Human and animal derived cultured cells are now widely used as a tool for biomedical and pre-clinical research, and also as materials for cell based therapy. Accordingly, a thorough understanding of the characteristics of the cells is essential to be used, and also quality control for the cells is required. However, individual techniques for expanding and maintaining cells have been regarded minor in importance. To grow good quality cells, good culture techniques are required. Among the process of culturing cells, passage procedure is one of the most critical steps. Whether the passage is properly done or not, are judged by observing how the cells are growing under the microscope. In this study, we monitored distribution and growth of the cells seeded by individuals by label-free live cell imaging. In particular, an

immortalized mesenchymal stem cell line, UE7T-13 was seeded by a beginner and an expert on a 6-well-plate, and images of the cells were automatically acquired every 12 hrs in a cell culture observation system, Biostation CT. Then, from the images, cell distribution and number on each well and cell was measured by an imaging analysis software, CL-Quant. Distribution of the cells seeded by beginners was unevenly whereas that by expert was evenly. Cell growth of the cells seeded by beginners was slower compared with that by expert. We suggest that label-free live cell monitoring method can evaluate individual seeding techniques. This method would be valuable to standardize best method for seeding cells and expected to be used for quality control of cell cultures in manufacturing biological products.

CELL AND TISSUE MODELS

A-3001

Expression of a Dominant Negative Mutant of NPM1 in Neuroblastoma Cells Results in Altered Localization of NPM1. SAAD MOHIUDDIN, Jaclyn Campbell, Kelly Keeler, and Kolla Kristjansdottir. Biomedical Sciences Department, Midwestern University, 555 31st Street, Downers Grove, IL 60515. Email: smohiuddin16@ midwestern.edu

Neuroblastoma is a solid tumor of the sympathetic nervous system found most commonly in the adrenal glands of children less than 5 years of age. High-risk neuroblastoma is associated with low 5-year survival rates and few targeted therapies are available for this subset of patients. We previously found NPM1 protein levels to be elevated in high-risk neuroblastoma cells. Nucleophosmin-1 (NPM1) is a nucleolar protein and molecular chaperone that is known to be upregulated or mutated in many cancers including colon cancer and acute myeloid leukemia (AML). A dominant negative mutation in NPM1 found in AML disrupts the C-terminal nucleolar localization signal of NPM1 and generates a new nuclear export signal, which alters the normal balance in nuclear-cytoplasmic NPM1 shuttling, causing cytoplasmic localization. Preliminary studies of this mutant in AML show better response to chemotherapy and better overall survival. However, there is debate as to whether this improvement is due solely to this dominant negative mutation in NPM1 or other underlying factors. To study the role of NPM1 in neuroblastoma we have transiently expressed the dominant negative mutant of NPM1 with a GST-tag in WS neuroblastoma cells. The NPM1 localization is altered resulting in cytoplasmic staining of NPM1 as visualized by confocal microscopy. We are currently studying the effects of this mutant NPM1 on proliferation and migration of WS neuroblastoma cells using Wound Healing Assays.

A-3002

Using Mass Spectrometry to Identify Proteins Involved in Enhanced and Directed Growth of Dorsal Root Ganglion Grown on Nano-sized Topography. ANTON UHLEN[1], Harsh Sharthiya[2], Sarah C. Veen[2], Kelly Keeler[2], Joshua Z. Gasiorowski[2], Michele Fornaro[2,] and Kolla Kristjansdottir[1]. [1]Department of Biomedical Sciences and [2]Department of Anatomy, Midwestern University, 555 31st St, Downers Grove, IL. Email: auhlen39@midwestern.edu

The limited recovery seen in victims suffering from Peripheral Nerve Injury continues to be a problem among trauma related injuries. Recent findings have shown that regeneration of nerves is possible *in vivo*, however this process is transient and slow, thereby, limiting the healing process. Regeneration of completely severed nerves has been met with limited success especially when the gap between nerve stumps is large. Many studies focused on enhancing nerve regeneration have been performed *ex vivo* and *in vitro*. Previous results from our research group have shown that nano-sized ridged and grooved topographical surfaces can enhance and direct axonal growth of *ex vivo* grown mouse Dorsal Root Ganglia (DRG) nearly threefold when compared to flat surfaces. The proteins associated with this enhanced and directionalized regrowth have yet to be identified. Therefore, we conducted a proteomic analysis of proteins in DRGs grown on topographical surfaces and compared them to DRGs grown on flat surfaces. Using Mass Spectrometry 182 proteins targets common to both samples were identified and the expression levels compared. Two protein targets, GST-mu and Rab1A, were found to be elevated in DRGs grown on ridged and grooved topographical surfaces and chosen for follow-up analysis. We show that GST-Mu is detected in DRGs using immunofluorescence on a confocal microscope and quantitative analysis of GST-Mu expression in DRGs is currently being performed. We have also used immunofluorescence to visualize Rab1A and GST-mu in an *in vitro* model with rat pheochromocytoma cells, PC12 cells that have been differentiated and grown on topographical surfaces.

CELLULAR AND MOLECULAR TOXICOLOGY

A-3003

Use of RNA-Seq to Identify Cadmium-dysregulated MicroRNAs in NRK-52E Rat Proximal Tubule Epithelial Cells. RIBHI SALAMAH[1], Lauren A. C. Alt[2], Zyaria Stubbs-Russell[1], Prem Patel[1], Aatmiya Patel[1], Joshua R. Edwards[2], Walter C. Prozialeck[2], and Michael J. Fay[1,2]. [1]Department of Biomedical Sciences, Midwestern University, Downers Grove, IL and [2]Department of Pharmacology, Midwestern University, Downers Grove, IL. Email: mfayxx@ midwestern.edu

Cadmium (Cd) is a nephrotoxic environmental pollutant that is currently ranked 7th on the 2017 Agency for Toxic Substances and Disease Registry (ATSDR)/EPA list of hazardous substances. The nepthrotoxic effects of Cd are well characterized; however, the molecular mechanisms of Cd-induced nephrotoxicity have not been completely elucidated. The goal of this study was to use RNA-Seq analysis to identify Cd-dysregulated microRNAs (miRNAs) in NRK-52E rat proximal tubule epithelial cells. Sub-

confluent NRK-52E cells were treated for 24 h with 10 μM $CdCl_2$ or saline control. This dose of Cd caused cell-cell separation without overt cell death. Total RNA from Cd-treated and saline control samples was used for RNA-Seq analysis to identify differentially expressed miRNAs. In the Cd-treatment group, 46 miRNAs demonstrated significantly increased expression and 51 miRNAs demonstrated significantly decreased expression compared to the saline control (t-test, $p \leq 0.05$, n=3 per group). With these significantly dysregulated miRNAs in the Cd-treatment group, 23 upregulated miRNAs and 20 downregulated miRNAs demonstrated a 2-fold or greater change in expression. Target prediction of differentially expressed miRNAs was performed using two computational target prediction algorithms (TargetScan 50 and Miranda 3.3a) to identify miRNA binding sites. The results of this research demonstrate that Cd alters the miRNA expression profile in NRK-52E rat proximal tubule epithelial cells. These dysregulated miRNAs may play an important role in Cd-induced kidney injury by inhibiting gene expression at the port-transcriptional level. Experiments will be conducted to confirm altered miRNA expression, and to validate predicted targets.

CHEMICAL CARCINOGENESIS

A-3004

Investigating a Role for Chemokines in Head and Neck Cancer. TRAVIS PARKER[1], Zhang Zhong[2], and Annette Gilchrist[2]. [1]Midwestern University, College of Dental Medicine, Downers Grove, IL 60615 and [2]Midwestern University, Chicago College of Pharmacy, Downers Grove, IL 60615. Email: tparker42@midwestern.edu

Head and neck cancer (HNC) is the sixth leading cancer by incidence globally. The malignancy is associated with high morbidity and mortality. The more advanced the disease, the greater the risk metastasis foreshadowing a poor patient prognosis.Head and neck carcinomas are histologically and clinically heterogeneous. For example, squamous cell carcinomas (SCC) are characterized by lymphogenous spread, while adenoid cystic carcinomas (ACC) disseminate preferentially hematogenously.Muller *et al* (Int. J. Cancer: 118, 2147–2157 (2006)) suggested that ACC and SCC express distinct sets of chemokine receptors that subsequently influence the metastatic behavior. Chemokines and their receptors have been implicated in tumor metastasis for a number of cancers including HNC. We focused on CCR1 and CCR5 as Chuang *et al* (Journal of cellular physiology. 2009 Aug;220(2):418-26) found that CCL5 increased the migration of human oral cancer cells through CCR5, and Silva *et al* found CCR1 may have a role in the spread of oral squamous cell carcinoma to the lymph nodes (Oncology reports. 2007 Nov;18(5):1107-13). We sought to determine if chemokine receptor antagonists could alter proliferation of WSU-*HN6, a*tongue-derived squamous carcinoma cell line. Twelve compounds were examined (AZD4818, BX471, CCX354, CP481715, J113863, Maraviroc, MLN3897, PS415767, PS31291, and PS899877, TAK 779, UCB 35625). Of these 8 are specific for CCR1, 1 is selective for CCR5 (Maraviroc), 2 are enantiomers targeting CCR1, CCR2, CCR3, and CCR5 (J113863/UCB 35625), 1 inhibits CCR2 and CCR5 (TAK 779), and 1 inhibits CCR1 and CCR5 (PS415767). We hope that by understanding the role of chemokine receptors in HNC proliferation we may ultimately provide a novel approach for this devastating disease.

DEVELOPMENT BIOLOGY

A-3005

Development of a High Throughput-high Content Assay to Identify the Teratogenic Potential of Organophosphates. J. GARCIA, T. M. Ubina, J. Argueta, A. Ashour, and N. Bournias-Vardiabasis. California State University San Bernardino, Department of Biology, 550 University Pkwy San Bernardino, CA 92407. Email: garcj499@coyote.csusb.edu

The U.S. NTP estimates every year approximately 2,000 new chemicals are introduced with an estimated backlog of 30,000 untested chemicals. This behooves the need for fast and reliable methods to screen newly introduced chemicals for developmental toxicity. *Drosophila melanogaster* is a preferred model for the study of genetics and development. It is a model organism for mechanistic studies of toxicants and for predicting toxicant damage already present. *Drosophila* have highly conserved genes and pathways controlling development, stress responses and xenobiotic metabolism. In addition, they are robust, easy to maintain, and have high fecundity; making them an excellent candidate for this assay. Previous work utilizing *D. melanogaster* embryonic stem cell (dESC) cultures has shown to be an accurate predictor of developmental toxicity potential. Homogenized gastrula-staged embryos from *D. melanogaster* predominately differentiate into neuronal clusters (NC) and myotubes (MT) after 24 hours and can be scored. MTs have a tube-like structure with multiple nuclei and are analogous to muscular system development. NCs are a cluster of about 16 neurons with multiple axons and are analogous to nervous system development. A high throughput-high content (HT/HC) protocol using dESC cultures was developed and a step-by-step procedure was created. With the ImageXpress Micro XL System, a HT/HC image analyzer, we aimed to create a HT/HC system that can detect toxicity of several organophosphates (OPs) in a relatively short period of time. dESC cultures were treated with several OPs, including chloropyrifos and merphos. Preliminary feeding trials in adults revealed several OPs to be toxic at 1000ug/ml. We are currently working with transgenic strains containing Hsp22 and Hsp70 reporters to determine if we can detect toxicants that cause a reduction in NC and MT numbers, but also toxicants that have a subtler effect on the stress levels of these cells. In addition to dESC cultures, we will also be testing *D. melanogaster* embryonic cell lines with the intent of transfecting them with bio-reporters.

ONCOLOGY

A-3006

Dissecting the Role of Adhesion Kinase FAK in Mediating CAP1 Regulation of ERK and Breast Cancer Cell Functions. ROKIB HASAN[1], Joshua Gray[2], Faith Allen[2], Thomas Kelly[3,4], and Guolei Zhou[1,2]. [1]Molecular Biosciences Program, Arkansas State University; [2]Department of Biological Sciences, Arkansas State University; [3]Department of Pathology, University of Arkansas for Medical Sciences; and [4]Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences. Email: mdrokib.hasan@smail.astate.edu

We recently identified a novel role for the actin-regulating protein CAP1 (Cyclase-Associated Protein 1) in controlling both the invasiveness and proliferation of breast cancer cells through ERK (External signal-Regulated Kinase). However, CAP1 as a cytoskeletal protein is unlikely to regulate ERK directly; unravelling the signaling molecules that may link CAP1 to ERK will provide mechanistic insights into normal cell functions of CAP1 as well as its roles in human cancer. We reported that FAK (Focal Adhesion Kinase) physically interacts with CAP1, and knockdown of CAP1 in Hela and breast cancer cells also led to alterations in FAK activity and cell adhesion. Moreover, FAK has been reported to also regulate ERK, making it an attractive candidate that may link CAP1 to ERK and the invasiveness and proliferation of cancer cells. To test FAK's role, we are using a combination of approaches including RNAi silencing of FAK, as well as inhibition of its kinase activity using chemical inhibitors, to determine if these manipulations rescue the elevated ERK activity and enhanced proliferation and invasiveness in metastatic breast cancer cells caused by CAP1 knockdown. Our preliminary results suggest that FAK likely indeed mediates CAP1 regulation of ERK. This study carries important translational implications.

ANIMAL SILENT ABSTRACTS

A-3007

Modified Plant Virus as a Basis for Vaccine Design Against Pathogen of Bacterial Nature. O. A. KONDAKOVA, E. A. Trifonova, E. M. Ryabchevskaya, M. V. Arkhipenko, N. A. Nikitin, and O. V. Karpova. Department of Virology, Lomonosov Moscow State University, 1-12 Leninskie gory, 119234, Moscow, RUSSIA. Email: olgakond1@yandex.ru

The present work is devoted to the design of new generation vaccine against pathogen of bacterial nature - *Bacillus anthracis*. Existing vaccines have shown their effectiveness, but their use is associated with certain risks, serious side effects and contraindications. Therefore, the development of safe recombinant vaccine with controlled components ratio and lower reactogenicity is an extremely urgent task. Plant viruses are promising tools in different fields of biotechnology including candidate vaccines development. They are essentially safe because mammals and plants have no common pathogens. The novel candidate vaccine contains recombinant protective antigen (PA) of *B. anthracis* and spherical particles generated by thermal transition of tobacco mosaic virus rod-like virions. In our previous studies we showed that SP is a promising platform for antigen presentation. SPs are biosafe and biodegradable, RNA-free, highly immunogenic, stable to different external factors and have unique adsorption properties. Furthermore, we demonstrated that SPs possess effective adjuvant properties. Here we assembled the complex of SP with recombinant PA. PA is a major target of the humoral B-cell mediated immune response during *B. anthracis* infection, and induces neutralizing antibodies. The formation of "SP-PA" complexes was detected by method of immunofluorescence microscopy. The ability of PA adsorbed on the SPs surface to specifically react with anti-*B. anthracis* PA antibodies was shown. Presumably, in new candidate vaccine SPs will be act as effective adjuvant and also as a platform that stabilizes PA on its surface. The work was funded by the Russian Science Foundation (grant № 18-14-00044).

A-3008

The Design of Rotavirus Recombinant Antigen for Novel Candidate Vaccine Based on Modified Plant Virus. E. TRIFONOVA, N. Nikitin, E. Ryabchevskaya, O. Kondakova, J. Atabekov, and O. Karpova. Department of Virology, Lomonosov Moscow State University, Moscow, RUSSIA. Email: trifonova@mail.bio.msu.ru

Rotaviruses induce an infectious disease that is the main cause of children severe diarrhea all over the world and one of the factors that determines the level of child mortality. Currently only attenuated vaccines are used for vaccination against rotavirus. Despite the effectiveness of attenuated rotavirus vaccine, it is important to note that live vaccines contain replicating virus and can reverse to the virulent form. Furthermore there is a risk of intussusception from rotavirus vaccination. Thus the development of new safe and effective non-replicating vaccine against rotavirus is highly desirable. Rotavirus capsid proteins VP7 and VP4 elicit neutralizing antibodies *in vivo* and are the main targets for the development of new generation vaccines. The present work is focused on the design of rotavirus recombinant antigen for further usage in novel candidate vaccine based on modified plant virus as an adjuvant. The recombinant antigen was constructed on the base of pQE-30 plasmid vector and composed of 6 His residues at the N-terminus and six tandem repeats of 21 amino acid sequence. This sequence contains neutralizing epitope of VP4 (aa 1-10) and neutralizing epitope of VP7 (aa 142-152). VP4 M1-L10 epitope is conservative in all rotavirus P-genotypes, whereas VP7 M142-M152 epitope is a consensus sequence of the most widespread rotavirus A genotype – G1. Recombinant rotavirus antigen (RV AG) was expressed in E. coli and purified. The Western blot analysis showed that RV AG interacted with commercial polyclonal antiserum to the rotavirus. As an effective adjuvant and platform for presentation of RV AG in novel candidate vaccine against rotavirus, spherical particles (SPs) obtained by thermal remodeling of tobacco mosaic virus will be used. The

formation of RV AG complex with SPs was demonstrated by fluorescent microscopy. The work was funded by the Russian Science Foundation (grant № 14-24-00007).

PLANT POSTERS

MICROPROPAGATION INTERACTIVE POSTER SESSION – Monday, June 4, 1:30 – 2:30 pm

P-2048

In Vitro Mineral Nutrition Affects Growth and Flowering of Echinacea in Greenhouse and Nursery. JEFFREY ADELBERG[1], Jacqueline Nyalor-Adelberg[1], Rabia El-Hawaz [1], and Robert Eisenreich[2] .[1]Clemson University, Clemson, SC and [2]Ball Horticultural, West Chicago, IL. Email: jadlbrg@clemson.edu

A high quality *in vitro* Echinacea plantlet should thrive in greenhouse acclimatization and flower in the same season in the container nursery. This research determined optimal *in vitro* mineral composition for state III media of *Echinacea* x Sombrero® "Salsa Red" shoot culture. Combinations of Murashige and Skoog (1962) nutrient salts were generated over different ranges (x=MS concentration); (0.125-1.5x) NH_4NO_3, (0.5-1.5x) KNO_3, (0.5-1.65x) meso-nutrients, (0.5-2x) micro-nutrients, (0.5-1.5x) iron chelate, with plant densities of 3-9 plants per Magenta vessel using response surface methods. Transferred plants were acclimatized under mist or in a fog tunnel for four weeks, then transferred to outdoor container culture in 1 L pots for 10 weeks. The highest number of leaves *in vitro* was 4.4±0.9 leaves with high plant density, 1.5x NH_4NO_3, 1.65x mesos, 1.5x iron, 2x micro, and 0.6x KNO_3. Greener leaf color required reducing meso-nutrients to 0.5x and KNO_3 to 0.9x with high NH_4NO_3, iron, and micronutrients. After 4 weeks in the greenhouse, 97.9±0.1% of plant survived in either the mist or tunnel conditions. Plants acclimatized in the tunnel flowered at 6±0.6 weeks and plants acclimatized in mist flowered in 7±0.9 weeks. Plants from the high plant density cultures, high minerals and with 0.25x iron flowered faster. Increasing NH_4NO_3 and KNO_3 to 1.5x and micros to 2x with meso-nutrients reduced to 0.5x and iron to 0.25x increased the number of flowers to 17±3.5 flowers/plant under tunnel and to 13±3.5 flowers/plant under the mist (after 10 weeks). A more concentrated mineral media was developed for high plant density tissue culture preferred by industry. Greener, leafier *Echinacea* plugs were not related to enhanced flower development.

BIOTECHNOLOGY

P-3000

Herbicide-induced Gene Expression System. MAREN ARLING, K. McBride, K. Lowe, W. Gordon-Kamm, A. Anand, K. McBride, N. Wang, K. Snopek, B. Lenderts, C. Hastings, G. Hoerster, J. Farrell, and B. Vickroy. E I Du Pong De Nemours and Company, Pioneer Hi-Bred International Inc., 8305 NW 62nd Ave, Johnston, IA, 50131. Email: maren.arling@pioneer.com

Chemically induced expression systems are useful both commercially and academically. The tetracycline repressor (TetR) is the basis for the most robust gene switch systems in eukaryotes. However, its use in plants is impractical since the ligands are antibiotics and light sensitive. TetR binds to the tet operator in a promoter, repressing expression of any gene. Binding of tetracycline to TetR creates a conformational change in the repressor, releasing it from the *tet* operator allowing transcription of the de-repressed gene to occur. Several rounds of gene shuffling of the tetracycline repressor (TetR) gene created mutants that bind sulfonylurea (SU) herbicides instead of tetracycline, creating an Ethametsulfuron Repressor (ESR) (Lassner, Michael, Loren L. Looger, Kevin E. McBride, and Brian McGonigle. SULFONYLUREA-RESPONSIVE REPRESSOR PROTEINS. E I Du Pong De Nemours and Company, Pioneer Hi-Bred International Inc, assignee. US8877503). This ESR system was tested by de-repressing DSRED in maize and de-repressing the transcription factors *Wuschel* and *Babyboom* in rice.

P-3001

Characterization of Selected cDNA-AFLP Transcripts Involved in Sweetpotato Storage Root Development. ADRIANNE BROWN, Sy Traore, Frieda Sanders, Marceline Egnin, Gregory C. Bernard, Osagie Idehen, Foazi Bukari, Desmond Mortley, and Conrad Bonsi. College of Agriculture, Environment & Nutrition Sciences, Department of Agriculture and Environmental Sciences, Tuskegee University, Tuskegee, AL. Email: abrown9633@tuskegee.edu, megnin@tuskegee.edu

Sweetpotato (*Ipomoea batatas* L.) is a palatable dicotyledonous root crop known for its high starch and beta-carotene content accumulated in its edible tuberous root. Globally, this crop produces approx. 130 million tons annually and is known to thrive in tropical and subtropical environmental conditions. Sweetpotato crop yield is partially determined based on the development of the storage root per plant which is the primary resource used for human consumption. However, limited information has been obtained in understanding the molecular mechanisms of sweetpotato storage root initiation mainly due to its polyploidy complexity. This study focused on the identification of transcriptionally regulated genes involved in storage root initiation and development using complementary DNA-amplified fragment length polymorphisms (cDNA-AFLP). Sweetpotato cultivar NCC-58 was grown in Nutrient film technology (NFT) as hydroponic and *Invitro* conditions. The developing storage roots and fibrous roots were collected from 7-42 days post planting in NFT and 28-90days in Vitro, and at 21 days for leaf tissues. Total RNA was extracted for cDNA-AFLP analyses. Transcript derived fragments (TDFs) were eluted purified and sequenced. Sequence analysis using BLASTX revealed the identification of several gene families including the pentaricopeptide repeat superfamily, potassium channel blocker (KK1) protein family, transcription factors and uncharacterized hypothetical proteins. Identifying the

full sequence of putative root initiation, bulking and development, followed by their functional characterization will assist researchers in understanding harvest index. *Work supported by USDA-NIFA Grant #2017-38821-26414; Tuskegee University CAENS-GWCAES-NIFA-EVANS-ALLEN and iBREED.*

P-3002

Establishment of Hexaploid Sweetpotato Protoplast as a Systems for Gene Expression, Genome Editing and Elucidation of Gene Functions. FOAZIATU BUKARI[1], Sy Traore[1], Marceline Egnin[1], Osagie Idehen[1], Gregory C. Bernard[1], Yi-Lang Lee[2], Stanton Gelvin[2], Adrianne Brown[1], Desmond Mortley[1], Conrad Bonsi[1], and Deloris Alexander[1]. [1]College of Agriculture, Environment & Nutrition Sciences, Tuskegee University, Tuskegee, AL and [2]Department of Biological Science, Purdue University. West Lafayette, IN. Email: fbukari0632@tuskegee.edu, megnin@tuskegee .edu

Target modification of plant genome is vital for elucidating and manipulating gene functions in plant research and agriculture in general. CRISPR-Cas systems have emerged as the preferred SSNs for research purposes due to its simplicity, efficiency and versatility. However, effective delivery of gene-editing apparatus into plant systems for high efficiency targeted genome modification remains a challenge for most plant due to their complexity and moreover their high ploidy. Recently, many researchers have successfully applied CRISPR/Cas9 genome editing system on high ploidy genome plants such as wheat, tobacco etc. However, the system is yet to be applied to sweetpotato genome. Our study focused on testing the feasibility of expressing genes in sweetpotato protoplast as a system for efficient genome editing and regeneration. Freshly isolated leaf protoplasts were transfected with high expression of yellow fluorescence (YFP) vectors (pSATI-Venus-vird2, pSATI-Venus- GSR and pSATI-Venus-PiP2) utilizing PEG/Calcium mediated transfection to investigate nuclear, cytoplasmic and plasma-membrane localization respectively in hexaploid sweetpotato. Strong YFP fluorescence signals were observed throughout the three cellular compartments indicating the ability of using sweetpotato protoplast as a system for gene expression and systematic characterization of gene functions. Co-transfection of sweetpotato protoplast with Cas9 and guide RNA targeting the *Ipomoea batatas* (Ib)phytoene desaturase (*pds*) gene for modification of the hexaploid genome was employed to examine the efficacy of the system as a toolkit for delivery CRISPR/Cas9 apparatus for high efficient genome modification. PCR amplification and T7 Endonuclease-I assays of the transfected products revealed CRISPR/Cas9 mediated activities at the ib*pds* locus of sweetpotato genome, further consolidating the efficacy of the protoplast system for gene expression and manipulation. *Work supported by USDA-NIFA Grant #2017-38821-26414; Tuskegee University CAENS-GWCAES-NIFA-EVANS-ALLEN and iBREED.*

P-3003

Developing *In Vitro* Tools to Enable Quinoa (*Chenopodium quinoa* Willd.) Biotechnology. S. ELIZABETH CASTILLO, Jose C. Tovar, Anastasia Shamin, Paige Pearson, Raj Deepika Chauhan, Nigel Taylor, and Malia Gehan. Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132. Email: ecastillo@danforthcenter.org(presenting), mgehan@danforthcenter.org(corresponding)

Quinoa is a highly nutritious crop that is adapted to a wide range of temperatures (-2 to 38°C), making it an excellent model to study temperature stress tolerance. We are developing *in vitro* regeneration and transformation methods for quinoa to functionally test genes, and *in vitro* pollen germination methods to study the effects of temperature on pollen viability. We successfully transformed one genotype of quinoa calli at an efficiency of 52%. We used a diluted 2-day *Agrobacterium tumefaciens* GV3101 culture in Linsmaier and Skoog medium, pH 5.8, and vacuum-infiltrated 2-week old quinoa calli from cotyledon or hypocotyl explants of genotype PI 614886. Transformation efficiency was assessed by GUS-stained calli after 2 days of co-culture and 2 weeks in regeneration media. We are currently developing a regeneration protocol for quinoa. Thus far, we have used four quinoa genotypes, in a two-stage protocol, with varying hormone concentrations. We obtained 2.8% regeneration efficiency with one genotype with both hypocotyl and cotyledon explants. Our current focus is improving regeneration, to enable quinoa transformation. To develop a pollen germination protocol for quinoa we tested four media types containing varying concentrations of sucrose with or without H_3BO_3 and $Ca(NO_3)_2$. We obtained 41% pollen germination in liquid media with 32% sucrose, 0.01% H_3BO_3, and 0.01% $Ca(NO_3)_2$, pH 5.5. At this pollen germination efficiency we are ready to test our pollen germination protocol under stress conditions and to use it in pollen magnetofection transformation methods.

P-3004

Producing Fully Functional Human Sulfatase Enzyme Replacement Therapeutics in Plants by Co-producing the Human Sulfatase-modifying Factor SUMF1. SHIVAKUMAR DEVAIAH[1], KASSANDRA RILEY[1], Varun Katta[1], Walter Acosta[1], Jorge Ayala[1], Carole L. Cramer[1,2], and David N. Radin[1]. [1]BioStrategies LC, P.O. Box 2428, State University, AR, 72467 and [2]Arkansas Biosciences Institute at Arkansas State University, State University, AR 72467. Email: shiva@biostrategies-lc.com (corresponding), kriley@biostrategies-lc.com

Plant-based transient expression systems and cultured plants cells are being developed as bioproduction platforms for human replacement enzymes (ERTs) for rare genetic diseases such as the lysosomal storage disorders. Sanfilippo A syndrome (MPS IIIA) is a lysosomal disease caused by defects in the gene encoding N-sulfoglucosamine sulfohydrolase (SGSH), which is responsible for degrading

heparan N-sulfate. MPS IIIA is characterized by relatively mild somatic features but severe neurological manifestations leading to dementia and death during puberty or early adulthood. Development of effective ERTs for MPS IIIA is hindered by the challenge of enzyme delivery to the central nervous system. BioStrategies LC is exploring the use of the plant RTB lectin (non-toxic B-subunit of ricin) to effectively deliver human enzymes to a broad array of cell types including "hard-to-treat" organs such as brain. Constructs encoding human SGSH and SGSH:RTB fusions were expressed by *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana*. Although full-length products were detected in plant extracts, no sulfamidase enzymatic activity was detected. Human sulfatases carry a unique active-site amino acid, Ca-formylglycine (FGly), required for catalytic activity. This post/co-translational modification is mediated by formylglycine-generating enzyme (encoded by *SUMF1*) in the ER. Mass spectrometry analyses indicated that FGly was absent in plant-derived SGSH products. SUMF1 constructs were developed for plant-based expression and, when co-expressed with SGSH or SGSH:RTB constructs, yielded fully active plant-made sulfamidase with specific activities higher than that of mammalian-cell-derived SGSH. *In vitro* efficacy of SGSH:RTB in correcting the cellular disease phenotype was demonstrated in MPS IIIA patient fibroblasts. Challenges of co-expression strategies and recent approaches to develop a "more-scalable" human sulfatase platform will be discussed. Supported by NIH/NINDS Phase II SBIR grant (R44NS083230)to BioStrategies LC.

P-3005

Understanding the Hydroxyproline-O-Glycosylation Pathway to Enhance Recombinant Therapeutic Protein Production. OLIVER DOZIER[1], Kelin Key[2], Jianfeng Xu[3], and Maureen C. Dolan[1,2,3]. [1]Department of Biological Sciences, [2]Molecular Biosciences Program, and [3]Arkansas Biosciences Institute, Arkansas State University, P. O. Box 639, State University, AR 72467. Email: oliver.dozier@smail.astate.edu, mdolan@astate.edu

Therapeutic proteins make up a rapidly growing market of the pharmaceutical industry. Currently, there is a need for platforms producing complex proteins for use in human and animal health. Plants offer a promising system, however, a general problem with the production of therapeutics is producing them in a way that combats their innate instability. To circumvent this issue, our lab has developed two technologies: transient plant expression and HypGP technology. By utilizing the HypGP technology, we can leverage a unique, plant-specific O-glycosylation process called hydroxyproline (Hyp)-O-glycosylation. The HypGP technology involves engineering novel designer HypGP sequence tags to a therapeutic protein gene sequence. These HypGP tags consist of the tandem dipeptide repeat of serine-proline; proline residues are subjected to hydroxylation and subsequent O-glycosylation by attaching branched arabinogalactans. By utilizing this technology and attaching sugar molecules to the therapeutic, the problems associated with protein instability and secreted protein yield can be curbed. While the pathways and mechanisms of N-glycosylation and O-glycosylation are largely understood, little is known about the plant-specific Hyp-O-glycosylation process. My project goal is to begin to define the pathway this glycosylation process employs within the endomembrane system of plants. Whole plant transient expression of constructs encoding EGFP, a HypGP tag of 32 serine-proline repeats $(SP)_{32}$, and +/- KDEL endoplasmic reticulum retrieval tag were designed. In addition to determining the relative expression and stability of these two variants of EGFP, relative sub-cellular distribution will be compared by several laboratory methods, including differential centrifugation, to allow the study of this glycosylation pathway. Better understanding of this glycosylation pathway will contribute to improved production of therapeutic proteins using HypGP fusion tags and accelerate the plant-based production platform for recombinant protein production.

P-3006

In Vitro Micrografting of Tomato (*Solanum* Spp.). DORA JANETH GARCÍA-JARAMILLO[1], Lucía Atehortúa Garcés[2], Jairo Castaño Zapata[1], and Nelson Ceballos Aguirre[1]. [1]Department of Agricultural Producction, Universidad de Caldas, Manizales, Caldas, COLOMBIA and [2]Faculty of Natural Sciences, Universidad de Antioquia, Medellín, Antioquia, COLOMBIA. Email: doraj.garcia@ucaldas.edu.co, nelson.ceballos@ucaldas.edu.co

The vascular wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most limiting factors of large-scale of tomato production in Colombia and in the word. This problem is principally due to the absence of commercial genotypes resistant, the lack of genetic improvement programs, abrupt climate changes and the absence of appropriate technology for better management of production systems. Therefore, the aim of this study was to establish the methodology for the micrografting of tomato using non-commercial Cherry tomatoes as a rootstock as a resistant variety and commercial Chonto tomato as a scion. Two methods of micrografting were used: the conventional Inverted T cleft method and the Bisel cut method. The experiment was submitted to five evaluations in weekly intervals. Shoot tips mayors a 0,2 cm culture on MS solid media supplemented with 3% sucrose and B5 vitamins and inverted T method possessed the highest micrografting success values in comparison to others treatments. Statistical differences were found between the combinations evaluated. Also, from all the grafting combinations tested grafted plants were produced at different rates of success, the best results were obtained from IAC412/CAR and IAC391/CAR combinations with 75% and 65% grafting success rates, respectively. The use of these tomato micrografts can contribute to the control of vascular wilt of tomato and increase the quality and production in the crop. However, this result should be automatize in the near future, to be able

to get generate massive micrografting materials to commercialize these varieties.

P-3007

In Vitro Seed Germination and Establishment of Hairy Root Cultures of a Peruvian Peanut Cultivar as a Bioproduction Platform of Stilbenoids. MARIA ELENA GONZALEZ ROMERO and Fabricio Medina-Bolivar. Arkansas Biosciences Institute and Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Emails: mgonzalez@astate.edu; fmedinabolivar@astate.edu

Peanut (*Arachis hypogaea L.*) produces stilbene-type phytoalexins such as resveratrol and the prenylated stilbenoids arachidin-1 and arachidin-3. In addition to their role in plant defense, these compounds show potential beneficial effects in human health. For instance, arachidin-1 and arachidin-3 exhibit anticancer and antiviral properties, respectively. Previously, we have shown that hairy root cultures of different commercial cultivars of peanut grown in North America produce stilbenoids upon treatment with elicitors. However information about stilbenoid production by hairy root cultures of South American peanut cultivars is lacking. To this end, the aim of this research was to establish hairy root cultures of a Peruvian peanut cultivar PI 262128. Seeds were surface-sterilized and germinated on Murashige and Skoog medium with modified nitrates. Full development of seedlings was observed after 15 days of culture. Excised leaves from one-month-old *in vitro* seedlings were wounded and inoculated with *Agrobacterium rhizogene*s strain ATCC 15834. Callus and hairy root initiation were observed 12 and 19 days after inoculation, respectively. Hairy roots were excised from the inoculation site and cultured on semi-solid and liquid media to establish different hairy root lines. High performance liquid chromatography analysis of the hairy roots is being conducted to characterize the types of stilbenoids produced in these hairy root cultures.

P-3008

Use of Non-integrating WUS for Recovering Transgenic Events via Direct Embryogenesis in Maize. GEORGE HOERSTER, Keith Lowe, Ajith Anand, Ning Wang, Kevin McBride, and Bill Gordon-Kamm. DuPont Pioneer, Johnston, IA 50131. Email: george.hoerster@pioneer.com

The transcription factor WUSCHEL has been shown to aid maize transformation. However, when integrated into the genome, constitutive expression of WUS results in a deleterious pleiotropic effects (Lowe et al The Plant Cell (2016), 28: 1998-2015). Excision systems have been developed to excise WUSCHEL from random agro transformation events, but an ideal system would capture the power of WUSCHEL without the need for excision. We have successfully tested two different approaches to address this issue. The first method mixes together two agro solutions, one containing WUS and no selectable marker and one with a "trait" and selectable marker. The second method places the WUS expression cassette outside of the left border of the t-DNA that contains the trait genes, thus delivering a transient pulse WUS. Both methods have been successfully used to efficiently produce single-copy integration of the trait-containing T-DNA into the maize genome without concomitant integration the WUS expression cassette.

P-3009

Precision Gene Editing in Agriculture. JAVIER NARVAEZ, Greg Gocal, Christian Schöpke, Mark Knuth, Dave Songstad, Steve Sanders, Noel Sauer, and Peter Beetham. Cibus, 6455 Nancy Ridge Dr., San Diego, CA 92121. Email: jnarvaez@cibus.com

The potential benefits of precision gene editing are profound and widespread with applications across bacterial, fungi, mammalian and plant systems. This talk will focus on the many ways in which precision gene editing is revolutionizing agriculture by accelerating trait development and breeding in plants. Cibus has developed a suite of advanced non-transgenic plant breeding techniques called the *Rapid Trait Development System*™ (*RTDS*™) that precisely and predictably make small defined DNA changes within the plant genome to obtain a desired phenotype/trait. At the core of this technology is the Gene Repair OligoNucleotide (GRON). The GRON is a chemically synthesized oligonucleotide specifically designed to be used by the plant's native DNA repair machinery as a template to produce one or a few targeted nucleotide changes of interest within the plant's DNA. The result is the development of a non-transgenic plant with a desired phenotype/trait. These novel plant traits range from increased nutritional value, improved disease resistance, and the ability to grow in challenging changing environments. With an ever-increasing population, these enhanced plant traits will enable us to meet future global food demands.

P-3010

Engineering Tobacco BY-2 Cell with CRISPR/Cas9 Technology for Reduced Proteolytic Activities. KIERA O'KEEFE[1] and Jianfeng Xu[2]. [1]Department of Biological Science and [2]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401. Email: kiera.okeefe@smail.astate.edu

Nicotiana tabacum cv. Bright yellow 2 (BY-2) cell has been widely used for recombinant protein production due to its appealing features including being fast-growing, robust and readily undergoing *Agrobacterium*-mediated transformation and cell cycle synchronization. However, major challenges still exist including low protein productivity and non-mammalian glycosylation. Glyco-engineered BY-2 lines that produce foreign proteins with humanized N-glycoforms were recently created through genome editing. The objective of this project is to apply the CRISPR/Cas9 genome editing tool to engineering new BY-2 cell lines with reduced proteolytic activities for enhanced production of therapeutic proteins. Plant genomes encode several hundred proteases that can be distinguished four major classes based on the

active site residues for catalysis: serine, cysteine, aspartic and metallo-types. As a proof of concept, we designed gRNAs to knock out two protease genes encoding a serine- and metallo-type protease, respectively, in the genome of BY-2 cells. These two classes of proteases were found responsible for the rapid proteolytic degradation of recombinant human interleukin-12 (hIL-12) in BY-2 cell culture. The constructed gRNA and Cas9 gene were stably transformed into both the wild type BY-2 cells and the BY-2 cells expressing hIL-12. The BY-2 colonies with target gene knockout in genome were selected based on DNA sequencing. The protease activity inside the cultured BY-2 cells and in medium will be compared before and after genome editing.

P-3011

Deciphering the Function of Phased Small Interfering RNAs from Protein Coding Genes in *Arabidopsis*. SURESH POKHREL[1,2] and Blake C. Meyers[1,2]. [1]Division of Plant Sciences, University of Missouri, Columbia, MO and [2]Donald Danforth Plant Science Center, St. Louis, MO. Email: spokhrel@danforthcenter.org, bmeyers@danforthcenter.org

Plant small RNAs range from 20-24 nucleotides in length and are mainly classified into microRNAs (miRNAs), heterochromatic small interfering RNAs (het-siRNAs), and phased, secondary small interfering RNAs (phasiRNAs). Among these, phasiRNAs are generated by the action of miRNA triggers on mRNA derived from protein-coding genes or non-coding genes and subsequently processed by RNA-dependent RNA polymerase 6 (RDR6), and Dicer-like proteins DICER-LIKE 4 (DCL4) and 5 (DCL5; previously known as DCL3b) to produce 21 and 24 nucleotide duplexes respectively. In *Arabidopsis*, there are total ~ 30 protein coding genes that produce 21-nucleotide phasiRNAs, and the majority of these are *PENTATRICOPEPTIDE REPEAT (PPR)* genes. To produce phasiRNAs, the *PPR* genes are targeted by miR161 and miR400, along with *TAS1*- and *TAS2*-derived tasiRNAs. *TAS1* and *TAS2* transcripts are targeted by miR173 to produce tasiRNAs, thus the PPR-phasiRNA network comprises three miRNAs and two *TAS* genes in *Arabidopsis* – yet the functional importance of this regulation is unknown. Therefore, the objective of my research was to identify functional roles of this regulation. The knockout of three MIRNA genes (miR173, miR161 and miR400) were made by using CRISPR/Cas9. RNA-seq and small RNA-seq of leaves of three knockout mutants, wild type (Columbia-0), and *rdr6-15* are being performed to measure the mRNAs and small RNAs levels particularly of phasiRNAs spawning PPR genes. We are aiming to under how phasiRNAs from *PPR* genes regulate expression of its own gene family members. And this regulation is a useful model of how phasiRNAs function to regulate protein coding genes in many other plants – like *NB-LRRs* in legume species, or *MYB* transcription factor-encoding genes in other eudicots.

P-3012

Investigating Molecular Differences in Sweetpotato Beta-Carotene from Dark-to-White Colored Flesh Cultivars. SY TRAORE[1], Foaziatu Burkari[1], Marceline Egnin[1], Adrianne Brown[1], Osagie Idehen[1], Gregory C. Bernard[1], Yi-Lang Lee[2], Stanton Gelvin[2], Conrad Bonsi[1], and Desmond Mortley[1]. [1]College of Agriculture, Environment & Nutrition Sciences, Tuskegee University, Tuskegee, AL and [2]Department of Biological Science, Purdue University. West Lafayette, IN. Email: straore@tuskegee.edu, megnin@tuskegee.edu

Sweetpotato (*Ipomoea batatas* L.) is an herbaceous perennial vine plant; a root staple crop farmed in tropical regions around the world. The edible root is highly rich in carbohydrates, dietary fibers and anti-oxidants. Besides these nutritional qualities, sweetpotato is also rich in beta-carotene. The content of beta-carotene in sweetpotato root determines the color of the flesh, as dark orange flesh cultivars are richer in beta-carotene compared to light-colored flesh. Therefore, their cultivation has been promoted in areas around the world to solve pro-vitamin A deficiency. Our group is interested in understanding the difference of beta-carotene content between dark and light-colored flesh of sweetpotato. Phytoene desaturase (*pds3)*, one of the key genes involved in beta-carotenoid biosynthesis pathway, were amplified, cloned and sequenced from several sweetpotato cultivars including white, yellow, orange and purple flesh. Sequence alignment using the BLAST tools showed polymorphism between the *pds* genes of the different sweetpotato cultivars, resulting in amino acid changes of these pds proteins. Further functional study of the *pds* gene using CRISPR/Cas9 technology will elucidate the role of *pds* in the difference of beta-carotene content in white and dark colored flesh sweetpotato cultivars. *Work supported by USDA-NIFA Grant #2017-38821-26414; Tuskegee University CAENS-GWCAES-NIFA-EVANS-ALLEN and iBREED a USDA USDA-NIFA Grant#2014-38821-22448.*

P-3013

Regulation of Cell Wall Strength and Salt Tolerance by the *Arabidopsis* MUR4. OMAR ZAYED and Chunzhao Zhao. [1]Horticulture and Landscape Architecture Department, Purdue University, West Lafayette, IN. Email: ozayed@purdue.edu

Salinity is one of the widely-studied plant abiotic stresses that substantially limits crop yield and production. Salts reduce plant cell wall flexibility by increasing cell wall content of cross-linked structures. Maintaining cell wall architecture and integrity is an important criterion for enhancing plant stress tolerance. The presence of wall arabinose-containing polymers had been suggested to be the key structural component responsible of the unique abiotic stress tolerance characteristic of plants. To identify different genetic loci that are involved in salt stress response, we screened for Arabidopsis mutants that showed relatively growth defect in NaCl medium compared with wild type. *mur4* showed hypersensitivity to salt but behave normally under other

osmotic stress condition. MUR4 encodes a UDP-xylose 4-epimerase, the key pathway of arabinose biosynthesis in plant. Arabinose accounts for 5–10 % of cell wall saccharides in Arabidopsis and is mainly found in arabinan, CLE, extensin, arabinoxylan, AGP and RGII. From these downstream structures, we found different contribution from each of these components in cell wall integrity and salt tolerance. Salt reduce arabinan, AGP and extensin contents in the cell wall. Reactive oxygen species (ROS) and hydrogen peroxide H2O2 were also over-accumulated in *mur4* plants under salt stress compared to the wild type. PLAT are plant-stress proteins that are suggested to promote stress tolerance. Nevertheless, little information is known regarding their specific functions. It is suggested that PLAT regulate salt tolerance by affecting the catalytic activity and substrate specificity of membrane proteins. However, the mechanism and the partner proteins are still unknown. Both MUR4 and PLAT1, which are induced by salt stress, positively regulate salt tolerance. Split-LUC and yeast two hybrid assay showed that MUR4 directly interacts with PLAT1. Altogether, our data suggest that arabinose content is an important factor in salt tolerance and PLAT1 is involved in the regulation of MUR4 activity.

CELLULAR AND MOLECULAR TOXICOLOGY

P-3014

Elucidating the Molecular Mechanisms of Arsenic Accumulation in *Pteris Vittata.* OSAGIE IDEHEN, Marceline Egnin, Ramble Ankumah, Raymon Shange, Sy Traore, Gregory C. Bernard, Conrad Bonsi, Foaziatu Bukari, and Adrianne Brown. College of Agriculture, Environment and Nutrition Sciences, Tuskegee University, Tuskegee, AL. Email: oidehen1985@tuskegee.edu, megnin@tuskegee.edu

Bioremediation has emerged as an important process used to treat contaminated agricultural fields including water, soil and subsurface material. The process consists of introducing different forms of life such as microorganisms and plants, to break down environmental pollutants; therefore, clean up polluted sites. *Pteris vittata* (*P. vittata*) has been identified as an arsenic hyper-accumulator with a potential for use in bioremediation of arsenic contaminated environment. *P. vittata* is known to produce root exudates, which may influence arsenic bioavailability, hence, its effects on the microbial community structure and enzyme activity in its rhizosphere. Moreover, *Pteris vittata* compared to *Pteris ensiformis* (*P. ensiformis*), has a unique ability of tolerating arsenic containing soils and accumulating arsenic in the vacuole. However, the molecular mechanisms by which *P. vittata* tolerates arsenic is not elucidated. In this study, *P. vittata* and *P. ensiformis* were exposed to different concentration of Arsenic (0, 50, and 250 mg As kg^{-1} soil); root and leaf samples were collected at different time points 3, 6, 9 and 12 days post treatment. Ionome analysis revealed a high concentration of arsenic in *P vittata* compared to *P. ensiformis* as indicated in previous reports. Moreover, transcriptome analysis showed the up regulation of several genes families including arsenic tolerant and stress related genes. Furthermore, gene expression profiling experiments have shown a high expression of several genes including arsenic reductase, transporter and receptor genes. Functional study of these genes will elucidate the molecular mechanism by which *P. vittata* is tolerant to Arsenic. Work supported by: USDA-NIFA Grant#2014-38821-22448 (iBREED), GWCAES-CAENS.

CELLULAR PATHOLOGY

P-3015

Potential Nematicidial Activity of Silver Nanoparticles Against Root-knot Nematode (*Meloidogyne incognita*). JACOB FITCH[1], Gregory C. Bernard[1], Byungjin Min[2], Naresh Shahi[2], Marceline Egnin[1], Sy Traore[1], Osagie Idehen[1], Foziatu Bukari[1], Willard Collier[1], and Conrad Bonsi[1]. [1]Department of Agriculture & Environmental Sciences and [2]Department of Food and Nutritional Sciences, Tuskegee University, Tuskegee, AL. Email: gbernard@tuskegee.edu

Plant-parasitic nematodes (PPNs) are incredibly damaging pests, which cause significant losses in crop yields worldwide. One of the most prevalent PPNs is the root-knot nematode (*Meloidogyne* spp.) ranks number one on the most economically devastating list of pests and thus scientifically important PPNs. Recently, the use of chemical nematicides for root-knot nematode management has decreased due to governmental restrictions; which necessitates the development and identification of alternative nematicidial active products. In this study, we evaluated the use of silver nanoparticles (AgNPs) as a potential biopesticide under in-vitro conditions. AgNPs were synthesized utilizing chitosan ad's reducing agent through microwave irradiation. When J2-stage nematodes were exposed to 0.05µg of AgNPs, 100% of nematodes became inactive within 24 and 48 hrs. During a 15 min exposure, over 95% of nematodes where immobilized. Significant mortality was also observed at 1min of exposure. The nematicidial effects of chitosan was not observed in controls during the treatments; thus, the primary nematicidial effect was attributed to the effect of AgNPs, which has been correlated with induction of oxidative stress in nematodes. Our preliminary study has demonstrated a potential non-chemical alternative for the management of the root-knot nematodes. Future experiments will include concentration optimizations of AgNPs and its reducing agent potentials prior to applying to greenhouse and field trials for efficacy determination on nematode burdens during plant cultivation. *Work supported by Tuskegee University iBREED, a USDA USDA-NIFA Grant#2014-38821-22448; Tuskegee University CAENS-GWCAES, NIFA EVANS ALLEN, Department of Food and Nutritional Sciences, Plant Biotech & Genomics Lab and Molecular Plant Pathology Lab.*

CYTOKINES

P-3016

High-yield Secretion of Human Interleukin-12 from Plant Cell Cultures for Therapeutic Uses. COLTON BATTEN[1],

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Interleukin 12 (IL-12) is a cytokine that is naturally produced by cells in response to bacteria and plays a primary role in the generation of cellular immunity. IL-12 is a T cell-stimulating factor and thus is directly linked with cell-mediated immunity. Due to this, IL-12 has several therapeutic uses including but not limited to protective immunity to intracellular bacteria, promotion of natural killer cells and T lymphocyte production, and several other autoimmunity advantages. Plant cell culture is proposed to be a safe, cheap, and efficient high-yielding alternative production platform for human IL-12. Due to the low yield expression of plant cell culture technology, we designed a plant-specific hydroxyproline (Hyp)-O-glycosylation code for the engineering of Hyp-O-glycosylated peptides (HypGPs) that function as a molecular carrier to facilitate the secretion of IL-12. My project focuses on expressing IL-12 in tobacco cells with a HypGP tag consisting of 20 tandem repeats of "Ala-Pro" dipeptide, or $(AP)_{20}$. The recombinant $(AP)_{20}$-tagged IL-12 was characterized in terms of secreted protein yields, Hyp-O-glycosylation, and biological functions in stimulating the production of T cells and autoimmunity to intracellular bacteria.

GENETIC ENGINEERING

P-3017

A New and Robust *Agrobacterium* Strain for Citrus Transformation. DIAA ALABED and James Thomson. USDA-ARS, Crop Improvement and Genetics Research, Western Regional Research Center, Albany, CA. Email: Diaa.Alabed @ars.usda.gov, James.Thomson@ars.usda.gov

Citrus is one of the most important fresh fruit crops in the world. However, many challenges are affecting crop maintenance, production and quality such as diseases, abiotic and biotic stresses.Most recently, there is an urgent demand for developing new traits in citrus through genetic transformation via *Agrobacterium* in citrus. Only one strain (EHA105) has been widely reported and proven to be efficient in a number of varieties. Many wild *Agrobacterium* strains exist that have not been tested for their efficiency in transforming citrus. We have screened 45 wild type *Agrobacterium* strains isolated from different plant species grown in various geographical regions. Eight strains were competent to transfer T-DNA into citrus cells when using a control vector pCTAGV3, carrying *DsRed* marker gene and *NptII* selectable marker gene. Out of the eight strains, four were found to transform citrus tissue and chosen for further characterizing and sequencing. Strains D13 and D41 were the most efficient in transferring the *DsRed* gene into *Carrizo* epicotyl explants. However, strain D41 was found to produce numerous galls (78%) in infected tissues and was considered the most effective. Interestingly, no *Agrobacterium* overgrowth or tissue necrosis was observed. Of interest was the fact that transgenic shoots regenerated both directly from transformed tissues and indirectly from galls. Mortality rate was exceptionally low in all replicates; the transformed tissues looked as healthy, it proliferated and regenerated transgenic shoots similar to unselected regenerating tissue. Based on *DsRed* expression comparison, the highest transformation rate was obtained from *Agrobacterium* strain D41 (88%), followed by EHA105 (84%), D13 (23%), D20 (6%), and D8 (3%). Finally, we have identified a new and robust *Agrobacterium* strain D41 for citrus transformation. The strain is currently in the process of being sequenced and disarmed after which a comparison will be made to commonly used strain EHA105 for stable transformation frequency.

P-3018

The Future Is Here: Developing New Corn Products Through Direct Genome Editing of Elite Inbreds. MELISSA A. RAHE, Todd J. Jones, Keith Lowe, Deping Xu, Nathalie Sanyour-Doyel, Chenna Peng, and Kristen Carpenter. Applied Science & Technology, DuPont Pioneer, Johnston IA. Email: melissa.rahe@pioneer.com

In the corn tissue culture world, we have long relied on model inbreds that could be transformed with great ease. However, these inbreds were often less desirable in the field as they possessed agronomic shortcomings or did not represent modern germplasm. With recent advances in morphogenic gene manipulation as described in Lowe K, et al. (2016) (Morphogenic regulators Baby boom and Wuschel improve monocot transformation. The Plant Cell 28:1998-2015), and cutting edge "QuickCorn" technology, using unfavorable inbreds is a thing of the past. We are now able to directly transform any inbred, including our most elite germplasm. We have demonstrated this principle in over 20 elite inbred lines to date. We believe this technology has the potential to revolutionize the way we approach tissue culture, transformation and genome editing, not only in corn, but potentially in many other crops as well.

P-3019

Development of Site-specific Recombinase Technology for Targeted Integration with Marker Removal. JAMES THOMSON and Roger Thilmony. Crop Improvement and Genetics Unit, Western Regional Research Center, USDA, Albany, CA. Email James.Thomson@ars.usda.gov

Recombinase-mediated genetic engineering provides a favorable direction for enhancing the precision of biotechnological approaches. Technology is rapidly expanding the way genetic engineering can be accomplished, the questions asked and the applications that can be attained. With these possibilities in genetic manipulation the interest in metabolic engineering requires the addition of many genes into the host cell for proper expression has been renewed. However, the combination of required genes and control elements is often empirically determined and thus requires multiple rounds DNA manipulation. While genetic

engineering can be accomplished by nuclease or host cell mediated homologous recombination, the efficiencies for precisely inserting large DNA sequences is low and tends to contain errors. Recombinase-mediated engineering offers a solution for sequential rounds of DNA stacking at high rates of integration and low (to nonexistent) levels of error introduction. Another issue is the limitation of available selectable markers for multiple rounds of engineering. Recombinase technology, when properly designed allows the removal of selection marker genes from the system while integrating the next gene(s) of interest. This in turn allows marker gene recycling between successive rounds of DNA insertion. Due to concerns over the presence of antibiotic resistance genes in the food supply and their escape into the environment, the ability to efficiently remove marker genes prior to marketing or release is highly desirable. Previous studies have documented how site-specific recombination can produce transgenics with stable gene expression over multiple generations and also resolve multicopy transgene inserts, initially silenced for expression, to a single functional genomic copy. Research in this lab addresses the need for publicly available recombinase technology. Data for a practical combinatorial approach for transgene integration and DNA stacking combined with subsequent excision of the selectable marker will be presented.

P-3020

Plant Transformation and Genome-modification Services at Donald Danforth Plant Science Center. VEENA VEENA, Preethi Vallal, Todd Finley, and Dave Busby. Donald Danforth Plant Science Center, Saint Louis, MO. Email: vveena@danforthcenter.org

The Plant Tissue Culture and Transformation Facility is an integral part of the core research facilities at the Donald Danforth Plant Science Center. The facility plays an important role towards the Danforth Center's goal to improve the human condition through plant science. We are enabling progress in plant research by the development and optimization of genome modification and transformation technologies in both model and crop plant systems. We train researchers and provide access to the equipment (self-service) and high-quality lab space to the internal as well as external researchers from both non-profit and for-profit entities. Over the years of its existence, the facility has produced transgenic plants from tobacco, petunia, tomato, Arabidopsis, soybean, Indian mustard, maize, cassava, sweet potato, potato, and most recently green foxtail (*Setaria viridis*), an emerging model system for the plant biology research in monocots. We also provide full-service transformation research and consulting services to academic institutions and commercial entities, delivering transgenic events and cell cultures utilizing our state-of-the-art equipment

P-3021

Expression of the Arabidopsis WRINKLED 1 Transcription Factor Leads to Higher Accumulation of Palmitate in Soybean. PAMELA A. VOGEL[1*], SHEN BAYON DE NOYER[1*], Hyunwoo Park[1], Hanh Nyugen[2], and Tom Clemente[1]. [1] Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588 and [2] Center for Biotechnology, University of Nebraska-Lincoln, Lincoln, NE 68588. **These authors contributed equally to this work*

Soybean (*Glycine max* L. Merr) is a commodity crop highly valued for its protein and oil content. The high percentage of polyunsaturated fatty acids in soybean oil results in low oxidative stability, which is a key parameter for usage in baking, high temperature frying applications, and extended shelf life of packaged products containing soybean oil inclusion. Introduction of a seed-specific expression cassette carrying the Arabidosis transcription factor WRINKLED1 (AtWRI1) into soybean, led to seed oil with levels of palmitate up to apprioximatly 20%. Stacking of the AtWRI1 transgenic allele with a transgenic allele harboring the mangosteen steroyl-ACP thioesterase (GmFatA) resulted in oil with total saturates up to 30%. The creation of a triple stack in soybean, wherein the AtWRI1 and GmFatA alleles were combined with a FAD2-1 silencing allele led to the synthesis of an oil with 28% saturates and close to 60% oleate. Constructs were then assembled that carry a dual FAD2-1 silencing element/GmFatA expression cassette, alone or combined with a AtWRI1 cassette. These plasmids are designated pPTN1289 and pPTN1301, respectively. Transgenic events carrying the T-DNA of pPTN1289 displayed an oil with stearate levels between 18% to 25%, and oleate in the upper 60%, with reduced palmitate (<5%). While events combined with the AtWRI1 (pPTN1301) had similar levels of stearic and oleate levels, but containing wild type levels of palmitate. The modified fatty acid composition results in increased oil quality with higher oxidative stability, and helps circumvent the need of partial hydrogenation processes.

METABOLIC ENGINEERING

P-3022

Ectopic Expression of a Peanut Prenyltransferase Gene in Muscadine Grape Hairy Roots to Produce the Bioactive Prenylated Stilbenoid Arachidin-2. MOHAMMAD AZIM[1], Parker Knapp[1], Tianhong Yang[1], and Fabricio Medina-Bolivar[1,2]. [1]Arkansas Biosciences Institute and [2]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: mohammad.azim@smail.astate.edu, fmedinabolivar@astate.edu

Stilbenoids are phenolic compounds that accumulate in response to biotic and abiotic stresses in a small number of plant species including muscadine grape and peanut. These compounds not only serve as phytoalexins but also exhibit biological activities important to human health, including anticancer, anti-inflammatory and neuroprotective properties *in vitro*. However, some stilbenoids, such as the non prenylated stilbenoid resveratrol, have poor bioavailability limiting their application *in vivo*. Recently, we identified a stilbenoid-specific prenyltransferase in peanut which can convert resveratrol to the prenylated stilbenoid

arachidin-2, a potentially more bioavailable stilbenoid than resveratrol. Therefore, our goal was to express this peanut stilbenoid prenyltransferase in muscadine grape to produce arachidin-2 and ultimately enhance its health benefits. Hairy roots of muscadine grape were developed via transformation with an engineered *Agrobacterium rhizogenes* harboring a peanut stilbenoid prenyltransferase. Four muscadine grape hairy root lines showed the presence of the peanut prenyltransferase gene. Enzyme assays using resveratrol as substrate and protein extracts from the transgenic hairy roots confirmed the presence of arachidin-2 as the enzyme reaction product. To study the production of arachidin-2, wild-type and transgenic muscadine grape hairy roots were co-treated with the elicitors methyl jasmonate, cyclodextrin, hydrogen peroxide, and magnesium chloride for 96 hours and then extracts from the tissue and medium were analyzed by high performance liquid chromatography. Arachidin-2 was present only in the transgenic hairy roots and its identity was confirmed by mass spectrometry. Our results demonstrated the successful production of the prenylated stilbenoid arachidin-2 in the transgenic muscadine grape hairy roots and the potential to leverage this metabolic engineering strategy to develop muscadine grape fruits with enhanced health benefits.

P-3023

Peanut Hairy Roots: A Bioproduction Platform for Elucidating the Biosynthesis of Prenylated Stilbenoids. TIANHONG YANG[1,2], Lingling Fang[1], Sheri Sanders[3], Srinivas Jayanthi[4], Gayathri Rajan[5], Ram Podicheti[5], Thallapuranam Krishnaswamy Suresh Kumar[4], Keithanne Mockaitis[3,6], and Fabricio Medina-Bolivar[1,7]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR; [2]Molecular Biosciences Graduate Program, Arkansas State University, Jonesboro, AR; [3]Pervasive Technology Institute, Indiana University, Bloomington, IN; [4]Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR; [5]School of Informatics and Computing, Indiana University, Bloomington, IN; [6]Department of Biology, Indiana University, Bloomington, IN; and [7]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: tianhong.yang@smail.astate.edu, fmedinabolivar@astate.edu

Prenylated stilbenoids are phenolic compounds produced in a very limited number of plants such as peanut (*Arachis hypogaea*) to counteract biotic and abiotic stresses. In addition to their role in plant defense, they also exhibit diverse biological activities with potential application in human health. Despite their importance, the biosynthetic pathways of prenylated stilbenoids remain to be elucidated. Previously, we developed an elicitor-controlled prenylated stilbenoid bioproduction system using hairy root cultures of peanut. By leveraging this bioproduction platform with a combined targeted transcriptome and metabolome approach we have discovered prenyltransferase genes involved in the biosynthesis of prenylated stilbenoids. Two of these genes were functionally characterized in a transient expression system of *Agrobacterium*-infiltrated leaves of *Nicotiana benthamiana*. One of the prenyltransferases, AhR4DT-1, catalyzes the prenylation of resveratrol at its C-4 position to form arachidin-2, while another, AhR3'DT-1, adds the prenyl group to C-3' of resveratrol. Fluorescence microscopy studies confirmed the subcellular location of these enzymes to the plastid. Our studies highlight the reliability of hairy root cultures as ideal biological systems for the elucidation of specialized metabolic pathways.

MICROPROPAGATION

P-3024

Improved Statistical Analysis for Projecting Mineral Nutrient Ranges. MELEKSEN AKIN[1], Charles Hand[2], Ecevit Eyduran[3], and Barbara M. Reed[4]. [1]Igdir University, Agricultural Faculty, Department of Landscape Architecture, Igdir-TURKEY; [2] Oregon State University, Department of Horticulture, OR 97331; [3]Igdir University, Agricultural Faculty, Department of Animal Science, Biometry Genetics Unit, Igdir-TURKEY; and [4]USDA-ARS-Retired, National Clonal Germplasm Repository, 33447 Peoria Rd, Corvallis, OR 97333. Email: akinmeleksen@gmail.com

Some of the problems confronting tissue culture studies are mineral salt precipitation and plant toxicity due to high nutrient concentrations. The classification and regression tree (CART) algorithm is able to define the lowest factor amount required for an optimal response within an experimental design. The CART data mining algorithm constructs visual, easy to interpret binary tree diagrams by dividing heterogeneous data into homogeneous subgroups. All input variables are considered when subgrouping to build the tree. Data partitioning is performed based on minimizing the variance within a group and maximizing the variance between groups. CART was employed to predict the exact minor-mineral nutrient ranges for optimal growth of *Corylus avellana* L. cultivars. Driver and Kuniyuki Walnut medium (DKW) minor salts (H_3BO_3, $CuSO_4{\cdot}5H_2O$, $MnSO_4{\cdot}H_2O$, $Na_2MoO_4{\cdot}2H_2O$ and $Zn(NO_3)_2{\cdot}6H_2O$) were varied from 0.5× to 4× DKW with 41 treatments in a response surface methodology (RSM) optimal design. $NiSO_4{\cdot}6H_2O$ was also a factor within the design tested between 0-6 μM. Shoot quality was affected by genotype, B and Mo. The required mineral ranges for improved quality were 2.215< B 4, 0.5 Mo 2.495. Shoot length depended on genotype B and Mo. Longer shoots required 1.665< B 4 and 0.5 Mo Cu 2.355, 0.5 B 2.6, 1.875< Zn 4 were required for improved multiplication. The common mineral nutrient concentration requirements for better quality, shoot length and multiplication of the tested hazelnut genotypes could be defined as: B 2.3× DKW, Cu 0.5×, Mo 0.5× and Zn 1.9×. These concentrations were more precise than the earlier analysis using RSM alone.

P-3025

The *In Vitro* Propagation of *Pteris vittata* and *Pteris ensiformis* Using Selective Media. CAPRI CHARLESTON, Osagie Idehen, Marceline Egnin, Sy Traore, Gregory C. Bernard,

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The *Pteris vittata* (Chinese brake fern) and *Pteris ensiformis* (slender brake fern), native to tropical regions, are commonly grown in Southern California and Southeastern region of the United States and the Eastern Hemisphere. Research shows propagations of fern spores and vegetative parts can be challenging. This study evaluated the *in vitro* propagation of these two important ferns modified Murashige and Skoog (MSO) and Hoagland (HS) media. The objective of this study was to evaluate the physiological responses to the different media variations and the factors affecting the *in vitro* propagation of the *Pteris ensiformis* and *Pteris vittata*. In this study, data such as: germination rate and percent yield were obtained to determine sterilization method and the media variation best suited for the growth of the *Pteris vittata* and *Pteris ensifromis*. Preliminary results show higher germination rate in half strength MSO without glucose. Incubating the spores in treated water for 3days improved the spore germination rates. Laminar gametophyte development started 21days seeding in media. The present study contributes to the understanding of factors affecting micropropagation of ferns for molecular research in phytoremediation and for ornamental uses. *Work supported by Tuskegee University iBREED, a USDA USDA-NIFA Grant#2014-38821-22448; CAENS-GWCAESNIFA-EVANS-ALLEN.*

P-3026

In Vitro Micropropagation of Commercial and Non-Commercial Tomato Genotypes. DORA JANETH GARCÍA-JARAMILLO[1*], Lucía Atehortúa Garcés[2], Jairo Castaño Zapata[1], and Nelson Ceballos Aguirre[1]. [1]Department of Agricultural Producction, Universidad de Caldas, Manizales, Caldas, COLOMBIA and [2]Department of Biotechnology, Universidad de Antioquia, Medellín, Antioquia, COLOMBIA. Email: doraj.garcia@ucaldas.edu.co, nelson.ceballos@ucaldas.edu.co

Tomato *(Solanum lycopersicum L.),* has a great economic potential due to its high nutritional and medical value. It is a vegetable with a high demand due to its importance as productive and protective food for its content rich in minerals and vitamins; in addition, it is a rich source of lycopene, β-carotenes, flavonoids and vitamins A and C. The present study describes an efficient methodology for disinfection, germination and micropropagation of 10 genotypes of commercial and non-commercial tomatoes. A combination of nine treatments was evaluated in the sterilization and germination; after 15 days the contamination and germination rate was determined. To select the best treatment by genotype was applied an selection index over physiological variables such as contamination and germination rate, length of the seedling and best morphogenesis. For micropropagation three culture media with different hormone combination was evaluated. The use of hypochlorite as a disinfectant was appropriate for seeds superficial sterilization. The selection of the best treatment for sterilization and germination depends on the genotype; additionally the evaluated genotypes responded positively to micropropagation in MS basal medium supplemented with 2% of sucrose, vitamin B5, and hormones free. This methodology giving access to biotechnological applications in the genetic improvement of the tomato specie such as selection of resistant genotypes, clonal propagation, micrografting, somatic embryogenesis, synthetic seeds and genetic engineering.

MOLECULAR FARMING

P-3027

Hydroxyproline-O-glycosylation Tag for Increasing Recombinant Protein Production Using the Transient Plant Expression Platform. CRISTOFER CALVO[1,2], Jianfeng, Xu[2], and Maureen C. Dolan[1,2]. [1]Department of Biological Sciences and [2]Arkansas Biosciences Institute, Arkansas State University, P. O. Box 639, State University, AR 72467. Email: mdolan@astate.edu

Plant-based recombinant protein production is emerging as a promising approach with significant advantages in cost and safety over other eukaryotic and prokaryotic expression systems. One of the leading plant-based platforms for recombinant protein production is a transient *Agrobacteria*-mediated expression system in *Nicotiana benthamiana*. Despite the advantages of plant recombinant proteins, the most important bottleneck that limits the commercialization is the low protein yields. Plants have a unique type of O-glycosylation that has potential to enhance the stability and solubility of recombinant proteins expressed using plant platforms. Specifically target gene sequences are fused with a sequence to code for hydroxyproline-O-glycosylated peptide (HypGP) tags. These tags serve to modify the recombinant expressed protein with protective sugars to improve physicochemical stability during purification as well as employment and delivery of the recombinant protein. Therefore, the overall goal of this project is to understand how these HypGP tags affect protein expression, purification and bioactivity using tobacco transient expression system as a biofactory. We have targeted the expression of two model proteins to characterize this HypGP technology: a very unstable rainbow trout interleukin 22 (IL-22) and a readily-monitored enhanced GFP (eGFP). The "sugar coated" IL-22 expression is significantly enhanced over the untagged protein and can be successfully purified. Data showing bioactivity of HypGP-tagged IL-22 to confirm this tag does not interfere with the function of this cytokine will be presented. To understand the underlying mechanism of HypGP modification process of recombinant expressed proteins, we explored the impact of plant stressors such as drought for increasing expression and recovery of sugar coated eGFP in an effort to optimize the Hyp-O-

glycosylation technology for the production of recombinant proteins on plant production platforms.

PRODUCT APPLICATOINS

P-3028

Identification of Cellulase Inhibitors Using Corn-seed Produced Enzymes. HONG FANG[1], Gurshagan Kandhola[2], Kalavathy Rajan[3], Kendall R. Hood[4], and Elizabeth E. Hood[5]. [1]Molecular Biosciences, Arkansas State University, Jonesboro, AR; [2]Biological & Agricultural Engineering, University of Arkansas, Fayetteville, AR; [3]Biosystems Engineering and Soil Science, University of Tennessee. Knoxville, TN; [4]Infinite Enzymes, Jonesboro, AR; and [5]Arkansas State University Biosciences Institute and College of Agri. & Tech., Arkansas State University, Jonesboro, AR. Email: hong.fang@smail.astate.edu

Lignocellulosic biomass has been regarded as a sustainable feedstock for advanced biofuel production. However, multiple difficulties remain in the conversion of this feedstock to sugars and subsequent fuels and thus impede the development of advanced biofuels. One of the most serious problems is low cellulase efficiency due to inhibition by breakdown products involved in feedstock conversion. The maize seed expression system has been used to produce economical and efficient cellulases, however, the inhibition of these enzymes by both exogenous inhibitors (generated during the pretreatment of cellulosic feedstock) and endogenous inhibitors (derived from maize expression system) prevents the highly efficient conversion of lignocellulosic feedstock. In order to eliminate potential inhibition, inhibitory compounds must be identified. Our approach is to utilize three recombinant cellulases (endocellulase E1, exocellulase CBHI and exocellulase CBHII expressed in transgenic corn seeds) to identify inhibitory compounds derived either from pretreated pine logging residues, which are potential source for biofuel production or from native corn seeds. Two enzymatic analysis systems were used to test inhibition. We have demonstrated that oligomers composed of xylan, galactan and mannan from pinewood were the most inhibitory compounds to our recombinant cellulases.

SECONDARY METABOLISM

P-3029

Elicitation of Prenylated Stilbenoids in Hairy Root Cultures of Three Cultivars of Peanut. ABBAS KAROUNI[1], Tianhong Yang[1], and Fabricio Medina-Bolivar[1,2]. [1]Arkansas Biosciences Institute and [2]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: abbas.karouni@smail.astate.edu, fmedinabolivar@astate.edu

Prenylated stilbenoids are inducible defense compounds found in a few plant species such as peanut that have potential applications in human health as anticancer, antiviral and anti-obesity agents. In order to study the biosynthesis of these compounds, hairy roots cultures are ideal biological systems because they reproduce the biosynthetic potential of the parental plant. To this end, hairy root cultures of three commercial cultivars of peanut, i.e. Hull, Andru II and Georgia Green, were established via *Agrobacterium rhizogenes*-mediated transformation. To induce the production of prenylated stilbenoids, the cultures were co-treated with the elicitors methyl jasmonate, cyclodextrin, hydrogen peroxide and magnesium chloride. Prenylated stilbenoids were extracted from the culture medium with ethyl acetate after 0, 48, 96, 144 and 192 hours of elicitor treatment and then analyzed via reverse-phase high performance liquid chromatography. The hairy root cultures of the three cultivars showed the presence of the non-prenylated stilbenoid resveratrol and prenylated stilbenoids arachidin-1, arachidin-2, arachidin-3, arachidin-5 and an unknown arachidin-5 derivative. Arachidin-1 and arachidin-3 where the most abundant prenylated stilbenoids and the highest levels were found in cultivar Hull. Whereas arachidin-5 derivative levels were higher in cultivar Andru II. The different levels of stilbenoids observed among the distinct peanut cultivars will be useful to select particular hairy root lines for production of specific types of bioactive prenylated stilbenoids.

P-3030

Bioproduction and Purification of Bioactive Prenylated Stilbenoids from Hairy Root Cultures of Peanut. SEPIDEH MOHAMMADHOSSEINPOUR[1,2], Lingling Fang[2], Tianhong Yang[2], and Fabricio Medina-Bolivar[2,3]. [1]Molecular Biosciences Graduate Program, [2]Arkansas Biosciences Institute, and [3]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: sepideh.mohammad@smail.astate.edu, fmedinabolivar@astate.edu

Prenylated stilbenoids are phenolic compounds produced as self defense mechanisms against biotic and abiotic stresses in peanut plants. In addition to their role in plant defense, they have potential applications in human health due to their anticancer and antiviral properties. Hairy root culture of peanut is an ideal biological system to study the biosynthesis of prenylated stilbenoids because the production of these compounds can be controlled with elicitors and the prenylated stilbenoids secrete into the culture medium. A previously established hairy root line of peanut cv. Hull was co-treated with four different elicitors, i.e. methyl jasmonate, cyclodextrin, hydrogen peroxide and magnesium chloride. The stilbenoids were extracted from the culture medium with ethyl acetate after 198 hours of elicitation treatment and analyzed by reverse-phase high performance liquid chromatography (HPLC). The stilbenoids identified included the non-prenylated stilbenoid resveratrol and the prenylated stilbenoids arachidin-1, arachidin-2, arachidin-3 and arachidin-5. Two unknown prenylated stilbenoids, arachidin-2 derivative and arachidin-5 derivative, were also present in the medium. The most abundant prenylated stilbenoids were arachidin-1 and arachidin-3 with yields higher than 300 mg/L. To purify the prenylated stilbenoids, ethyl acetate extracts were separated by high performance counter current

chromatography (HPCCC) and semi-preparative HPLC. The purified prenylated stilbenoids are being used in different anticancer assays. We conclude that the combined hairy root culture-based bioproduction and HPCCC/semi-preparative HPLC purification systems are effective for obtaining high levels of purified bioactive prenylated stilbenoids.

P-3031

Assessment of Antioxidant Activity of Purified Stilbenoids and Stilbenoid-enriched Extracts from Peanut Hairy Roots. PATRICK ROBERTO[1], Maria Elena Gonzalez Romero[1], and Fabricio Medina-Bolivar[1,2]. [1]Arkansas Biosciences Institute and [2]Department of Biological Sciences, Arkansas State University, Jonesboro, AR. Email: patrick.roberto@smail.astate.edu; fmedinabolivar@astate.edu

Reactive oxygen species (ROS) are reactive chemical species that cause oxidative stress in organisms by oxidizing important biomolecules in the cell such as lipids, proteins, and DNA. In humans, oxidative stress is associated with several illnesses including cancer and age-related diseases. To counteract ROS, organisms have evolved elaborate enzymatic and non-enzymatic antioxidant systems. For instance, the peanut plant produces the antioxidant stilbenoid resveratrol to counteract oxidative stress. Interestingly, along with resveratrol, several prenylated stilbenoids are also produced. However, the antioxidant properties of these compounds remain to be characterized. To this end, the goal of this study was to produce different types of stilbenoids using a peanut hairy root culture system and characterize their antioxidant properties. Hairy root cultures of peanut were co-treated with the elicitors methyl jasmonate, cyclodextrin, hydrogen peroxide, and magnesium chloride to induce the biosynthesis of stilbenoids. After different periods of elicitation, stilbenoids were extracted from the culture medium and analyzed by high performance liquid chromatography (HPLC). The most abundant stilbenoids were the non prenylated stilbenoid resveratrol and the prenylated stilbenoids arachidin-1 and arachidin-3. Arachidin-2 and arachidin-5 were also present but at a lower concentration. The stilbenoids were further purified by high performance counter current chromatography and semi-preparative HPLC. The antioxidant capacity of the extract and purified stilbenoids was determined using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) [ABTS] assay. The results indicated that the extracts from the culture medium enriched in prenylated stilbenoids exhibit higher antioxidant activity when compared to the non prenylated stilbenoid resveratrol. Our studies suggest that the peanut hairy root extracts are a good source of antioxidant compounds.

SOMATIC CELL GENETICS

P-3032

Exploring Somatic Hybrid Production in Sweetpotato Between a Nematode-Resistant Orange-flesh and a Susceptible White and Dry Flesh Cultivars in Next Generation Breeding. LARENCIA WILLIAMS, Foaziatu Bukari, Marceline Egnin, Caitlin Skinner, Gregory C. Bernard, Sy Traore, Osagie Idehen, Adrianne Brown, Desmond Mortley, and Conrad Bonsi. College of Agriculture, Environment and Nutritional Sciences, Plant Biotech and Genomics Research Lab, Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL 36088. Email: lwilliams5588@ tuskegee.edu, megnin@ tuskegee.edu

Somatic Hybridization is a process in the development of hybrid plants through the fusion of somatic protoplasts from sexually incompatible species and genomes. This process has opened avenues in plant improvement to overcome the major limitations of sexual hybridization. Sexual incompatibility in sweetpotato pose a genetic manipulations challenge, hindering research progress and overall improvement. Hence, protoplast hybridization presents a potential method to overcome these sexual barriers. Sweetpotato is a genotypically diverse and a nutritional powerhouse for beta-carotene, vitamin E, potassium, and calcium; however, its breeding is besetted by poor seed setting and incompatibility, which limit the introgression of beneficial traits; thus, serves as a candidate for agronomic and disease resistant somatic breeding. For this study, fused somatic protoplasts were induced between a nematode resistant cultivar, Nugget (orange flesh), and a nematode susceptible cultivar, DMO1 (high dry matter white flesh), in hopes of regenerating new plantlets to overcome these problems. Sweetpotato leaf explant protoplasts were harvested following enzyme digestion. Polyethylene Glycol (PEG)-Calcium mediated treatment was employed in protoplast fusion of the two cultivars. The fused cells were then further treated to help buffer and stabilize osmosis within the hybrid cells. The Three-stage somatic embryogenesis protocol was utilized to develop hybrid calli for plantlet regeneration. Microscopic screening of the fused cells exhibited characteristics of both parent protoplasts. Heterokaryotic fusion frequency was about 8%; however, the frequency of calli regenerating was around 10%. Further molecular analysis of the hybrid embryos and plantlets will help characterize the nature of newly regenerated heterozygous lines as well as the fate of the nematode resistant gene. *Work supported by Tuskegee University iBREED, a USDA USDA-NIFA 1890 Grant#2014-38821-22448; Tuskegee University CAENS-GWCAES-NIFA-EVANS-ALLEN.*

PLANT SILENT ABSTRACTS

P-3033

Complexes Consisting of Genetically Modified TMV Virions and Immunogenic Proteins: Characterization by Immunological and Physico-chemical Methods. TATIANA GASANOVA and Peter Ivanov. Lomonosov Moscow State University, Faculty of Biology, Department of Virology, Moscow, RUSSIA. Email: tv.gasanova@gmail.com, pivanov @genebee.msu.ru

Recently we obtained complexes between genetically modified Tobacco Mosaic Virus (TMV) particles and proteins carrying conserved influenza antigens such as M2e epitope, hemagglutinin fusion peptide (fp) as well as core M1 protein. Viral vector TMV-NtK was constructed by insertion of 4 amino acid residues (including chemically active lysine) into the exposed N-terminal part of the coat protein. *Nicotiana benthamiana* plants were agroinjected and TMV-NtK particles were purified from non-inoculated leaves. The efficiency of chemical conjugation employing TMV-NtK particles, DHFR-M2e, DHFR-fp, M1 proteins expressed in *E.coli* and EDC-NHS bioconjugate reaction was high, which was confirmed by Coomassie staining and Western blotting (Gasanova and Ivanov, 2017). For additional characterization we used phosphotungstic acid contrasting and conventional electronic microscopy. The diameter of the complexes did not differ significantly from the initial TMV-NtK virions, but rod-shaped particles with dense "grains" on the surface formed highly organized and extensive network. Dynamic light scattering (DLS) demonstrated that the single peaks, reflecting the complexes TMV-NtK/DHFR-M2e, TMV-NtK/DHFR-fp, TMV-NtK/M1 were significantly shifted relative to the control TMV-NtK virions. The indirect enzyme-linked immunosorbent assay (ELISA) with TMV-, DHFR-M2e- (Petukhova et al., 2013) and M1-specific antibodies showed that the complexes retain stability during overnight adsorption on plastics followed by numerous washing steps. The ratio of anti-TMV antibodies reacting with the carrier and the conjugated complexes was 1:2; the ratio between DHFR-M2e and TMV-NtK/DHFR-M2e complex was 1:1; similarly, the ratio between M1 and TMV-NtK/M1 was 1:1. Thus, it can be concluded that the significant part of the complexes' surface is coated with the target protein.

P-3034

The Role of the C-terminus of the Tobacco Mosaic Virus Coat Protein in Its Structural Characteristics and Ability to Interact with TMV Movement Protein. N. O. KALININA[1], A. V. Makhotenko[2], V. V. Makarov[1], and S. S. Makarova[2]. [1]Belozersky Institute of Physico-Chemical Biology and [2]Biological Department, Lomonosov Moscow State University, Leninskie Gory, Moscow, 119991, RUSSIA. Email: stmakarova@gmail.com

Previously we demonstrated that deletions in the intrinsically disordered C-terminus of TMV CP dramatically affected viral infection: deletions more then 3 amino acid residues (a.a) (CPΔPAT), namely 6a.a. (CPΔTSG) and 20 a.a. (CPΔYNR) abolished viral infection. In this work we studied structural characteristics of the recombinant CPs and their ability to interact with TMV movement protein (MP). We used circular dichroism (CD) spectroscopy in the far UV region to compare optical properties of recombinant CPs and showed that deletion of even 3 C-terminal a.a. (CPΔPAT) led to the serious structural rearrangements of the TMV CP molecule, accompanied by a transition of alpha-structural protein (wild type TMV CP) into protein with an increasing number of beta elements. Deletions in the CP C-terminus also changed surface charge (zeta potential) and increased aggregation ability of mutant CPs.The wt TMV CP and CPΔPAT but not CPΔTSG and CPΔYNR were able to interact with TMV MP in vitro (Far-Western assay). These data are in agreement with the results obtained in vivo during simultaneous agroinfiltration constructs expressing genes of GFP-MP and mRFP-CP into leaves of *Nicotiana benthamiana* plants. Both proteins were colocalized in cell wall plasmodesmata. We suggest that theTMV CP C-terminus contains functional determinant/determinants involved in previously assumed interaction with TMV MP and cell-to-cell transport and likely takes part in virion assembly. This work was supported by the Russian Science Foundation project No 14-24-00007.

P-3035

Some Features of Vegetative Propagation of *Origanum vulgare* L. ELENA MYAGKIH, Olga Yakimova and Alexandr Mishnev. Research Institute of Agriculture of Crimea, Simferopol, RUSSIA. Email: origanum.science@mail.ru

Origanum vulgare L. is a perennial herb. It is widely used in the perfumery, cosmetics, food and pharmaceutical industries, characterized by the content of essential oil carvacrol – a valuable phenol, which has a strong antibacterial effect. For the accelerated reproduction of new varieties and genotypes, we developed methods for vegetative propagation of O. vulgare, which ensure the preservation of economically useful properties of the genotype: the technology of clonal micropropagation of oregano in vitro and the propagation technique of O. vulgare by the method of green cuttings in conditions of finely dispersed moistening. As a result of the research, it was found that by micropropagation in vitro of some genotypes, the coefficient index reached 1: 94.9 pieces per cycle of reproduction. It makes possible to obtain up to 1131654.0 pieces of adapted microclones from one explant in six months. To reduce the cost of seedlings, we recommend to use the method of propagation of O. vulgare by the method of green cuttings. This method's coefficient of plant reproduction, depending on the genetic characteristics of the sample without the use of root stimulants, ranged from 1:44 to 1: 539 pieces as compared to the coefficient of plant reproduction by the traditional method of dividing the bush – from 1: 5 to 1:12 pieces.

P-3036

Clonal Micropropagation In Vitro of *Melissa officinalis* L. OLGA YAKIMOVA, Natalia Yegorova, Margarita Zagorskaya, and Irina Stavtzeva. Research Institute of Agriculture of the Crimea, Simferopol, RUSSIA. Email: olyyakimova@yandex.ru

Lemon balm (*Melissa officinalis* L.) – a perennial herbaceous medicinal and essential oil plant, widely used in medicine. To increase the efficiency of breeding with this valuable plant, it is expedient to apply biotechnological methods, one of which is clonal micropropagation. The aim of study was to

investigate the influence of some factors on lemon balm cultivars propagation *in vitro*. As a primary explants meristems and segments of the stem with a node of three cultivars were used. The studies revealed that genotype, composition of nutrient medium, type of explant, conditions and duration of cultivation had influence on the efficiency of *M. officinalis* propagation. The conditions for explants cultivation at the main stages of micropropagation were optimized. When introduced into the culture *in vitro* the maximum number of shoots and their length for cultivar Sobornaya were on Murashige and Skoog (MS) nutrient medium supplemented with BAP (1.0 mg/l) and GA_3 (0.5 mg/l) and for cultivar Citronella – on MS medium with kinetin (0.5 mg/l) or BAP (1.0 mg/l) and GA_3 (0.5 mg/l). At the second stage of propagation *in vitro* the MS medium with 0.5 ml/g BAP was optimum for cultivars Sobornaya and Krymchanka (multiplication index were 19.9 and 17.6, respectively), and for cultivar Citronella – MS medium with 1.0 mg/l kinetin (multiplication index 19.3). Maximum frequency of rooting for Citronella and Sobornaya cultivars (75.5 and 93.3%, respectively) was noted on nutrient medium with the addition 0.5 mg/l NAA, for Krymchanka (90.9%) – with 1.0 mg/l of IBA. The conditions of adaptation *in vivo*, providing 85.7-100.0% survival of multiplied microplants, were optimized. Results of the study were the basis for developing methods of *M. officinalis* micropropagation.

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