2017 IN VITRO BIOLOGY MEETING

LATE SUBMISSION ABSTRACTS

2017 Meeting of the Society for In Vitro Biology
June 10 – 14, 2017
Raleigh, North Carolina

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

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***Nicholas Smith,** Midwestern University, Mae J. Ciancio, Matt Pytynia, Marc Scheetz, Vanessa Leone, and Christian C. Evans*

A-3014 RTgut-GC and RTS11-GFP Cell Lines as In Vitro Models to Study Fish Gut Physiology and Enteritis
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EDUCATION SILENT ABSTRACT

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P-3029 Direct Embryogenesis from Maize Embryos and Leaves
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Kathryn Bumila, *Worcester Polytechnic Institute, and Susan Roberts*

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P-3044 Comparison of Cell Culture System and Adventitious Root Culture System in *Panax ginseng* and *Panax quinquefolius*
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P-3047 Dandelion *In Vitro* Response to Different Culture Strategies for Triterpenes Production
M. Martinez, *Pontifical Catholic University of Valparaíso, L. Jorquera, R. Chamy, D.Prüfer, C. Schulze Gronover, and P. Poirrier*

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Angelina Song, North Carolina State University, and Deyu Xie

ANIMAL SYMPOSIA ABSTRACT
IN VITRO AND SILICO DATABASES AND ANALYSIS - Monday, June 12, 3:30 pm – 5:00 pm

A-10

Biomarker Discovery in Human Trials Through the Analysis of Metabolomic, Proteomic, and RNA-seq Data. MATTHEW WAMPOLE. Clarivate Analytics. 1500 Spring Garden St, Ste 400, Philadelphia, PA 19130. Email: matthew.wampole@clarivate.com

Renal failure is just one type of organ failure that can occur in patients with sepsis, and can lead to lifelong dependence on dialysis even after surviving the infection. Early detection of these diseases and their associated complications through the use of biomarkers can help save lives and prevent lifelong disabilities. Using MetaCore, a manually curated database of interactions and signaling pathways for omics analysis, we will to analyze the multi-omics data from the Community Acquired Pneumonia and Sepsis Outcome and Diagnostics(CAPSOD) study (https://clinicaltrials.gov/ct2/show/NCT00258869). These study patients with sepsis of community associated pneumonia were monitored reduced kidney function and blood samples were collected. Pathway analysis of the metabolomic, proteomic, and mRNA sequencing data was performed to uncover biological relationships between these multi-omic datasets. Using the results reported[2] from the CAPSOD study data (http://www.ncbi.nlm.nih.gov/pubmed/25993322), we will look to answer these two questions. What metabolic relationships can we find between the metabolomic and RNA-seq data? What changes metabolites and proteins concentrations in the patients plasma could be biomarkers for disrupted processes?

ANIMAL POSTER ABSTRACTS
APOPTOSIS

A-3000

Asialoerythropoietin Suppresses MST1 Activation In Pancreatic Beta-cells. ELENA ARTHUR[1], Farooqahmed Kittur[1], Lin Yuan[1,2], Chiu-Yueh Hung[1], and Jiahua (Jay) Xie[1]. [1]Department of Pharmaceutical Sciences, Biomanufacturing Research Institute & Technology Enterprise, North Carolina Central University, Durham, NC and [2]School of Basic Medical Sciences, Ningxia Medical University, Yinchuan, CHINA. E-mail: earthur1@eagles.nccu.edu

Loss of pancreatic beta-cell function and mass leads to impaired insulin secretion and dysfunctional glucose homeostasis which are the basis for Type I and Type II diabetes. Therapeutics to impede or even reverse pancreatic beta-cell failure and apoptosis are urgently needed. Asialo-rhuEPO, an enzymatically desialylated form of recombinant human erythropoietin (rhuEPO), has been shown to have cardioprotective and neuroprotective functions with no adverse effects like that of sialylated rhuEPO. Heretofore, the anti-apoptotic effect of asialo-rhuEPO[P] on pancreatic beta-cells has not been reported. Since no available method for the expression of asialo-rhuEPO exists, we and others have used plant-based expression systems to produce recombinant human asialo-EPO (asialo-rhuEPO[P]). In the present study, we used staurosporine(STS)-induced apoptosis in the pancreatic beta-cell as a model to study the cytoprotective effects of asialo-rhuEPO[P] and determine the involvement of MST1 in asialo-rhuEPO[P]-mediated cytoprotection. In the beta-cell apoptosis signaling pathway, MST1 acts both as an activator as well as a target of caspases-3. Our study revealed that asialo-rhuEPO[P] protects pancreatic beta-cells from STS-induced apoptosis by preventing both MST1 and caspase-3 activation. Considering the many advantages of plant-based expression, asialo-rhuEPO[P] could be developed as a novel therapeutic drug to treat diabetes.

CELL AGING

A-3001

Phosphoinositide 3-Kinase Inhibitors and Nicotinamide Suppress Expression of Senescence-specific Phenotypes in Cultured Normal Human Cells, an *In Vitro* Aging Model. KOOZI MATUOKA[1], Keiko Sasaki[1], and Kuang Yu Chen[2]. [1]Faculty of Pharmacy, Chiba Institute of Science, Choshi, Chiba, JAPAN and [2]Department of Chemistry and Chemical Biology, Rutgers, State University of New Jersey, Piscataway, NJ. Email: kmatuoka@cis.ac.jp

Aging is an irreversible phenomenon that most, if not all, living things undergo during their passage through development, maturation and life time. In order to elucidate its mechanisms, it is of powerful use to utilize cell systems cultured *in vitro* in terms of experimental simplification, reproducibility and respect of life. Some of the aging-related parameters of those cells have been shown to well correlate with those observed *in vivo*. A number of *in vivo* studies have reported that energy-metabolizing systems, including AMP kinase/sirtuins and phosphoinositide 3-kinase (PI3K)/Akt, play critical roles in the acceleration of aging, prompting us to examine whether such a system(s) work at the cellular level. In the present study, normal human foreskin fibroblast BJ cells were capable to dividing up to population doubling level (PDL) 70-80 and gradually changed during serial cultivation: old (late-passaged) cells were morphologically enlarged, slowly moving and growing and exhibiting high SA-gal expression. We tested cellular responses to various reagents modifying their activities and found that nicotinamide (NAM, an NAD precursor) suppressed expression of those senescence-related phenotypes in old cells. Further analyses also revealed that some PI3K inhibitors exerted similar effects to those of NAM. Since PI3K comprises various subtypes, they may play distinct, individual roles in the aging. qPCR determination showed tendencies that class II and class III messages, but not those of class I, are higher in old cells. Exposure of BJ cells to NAM elevated expression of most PI3K subtypes. Additionally, telomerase-expressing BJ cells escaping from replicative senescence were also analyzed. By utilizing normal human cells as an *in vitro* aging model, the possibility has been presented that the process of aging most likely involves energy-metabolizing systems which include PI3K and NAM and that one could manipulate the aging by modulating those molecules. Our experiments aiming at PI3K subtypes would be of potential use to pinpoint a pivotal molecule(s) in the aging phenomena.

CELL BIOLOGY

A-3002

Live Cell Imaging System for Cell Culture Evaluation by Cell Culture Observation System. MIHO K. FURUE[1,2], Mika Suga[2], Hiroaki Kii[1], Hiroko Eimori[1], Tomoro Dan[1], and Yasujiro Kiyota[1]. [1]Stem Cell Business Development Section, Microscope Solution Business Unit, NIKON Corporation, Yokohama Plant, Kanagawa, JAPAN and [2]Stem Cell Laboratory, National Institute of Biomedical Innovation, Nutrition and Health, Osaka, JAPAN, Email: Miho.Furue @nikon.com

Cell culture is evolving rapidly into an effectively and widely used research tool for basic research, pharmaceutical study and cell-based therapy. Accordingly, a thorough understanding of the characteristics of the cells is essential to be used, and quality control for the cells is required. Cell morphology is an important characteristic used as a criterion in the evaluation of cell quality. Morphology-based evaluation method using live cell imaging can provide real-time information on cell conditions without damaging cells, allowing cells to be continuously used for further application. Here, we have developed live cell imaging system to evaluate cell cultures by a cell culture observation system, Biostation CT. This system was validated by cultures of human pluripotent stem cells, demonstrating that cell numbers, cell growth, cell conditions, and culture skills were evaluated. This system is expected to be used for quality control of cell cultures in manufacturing biological products.

A-3003

Bromodomain Proteins as Novel Therapeutic Targets in Triple Negative Breast Cancer. YUAN YOU and Michael J. Rossi. Department of Biology & Environmental Science, University of New Haven, West Haven, CT. Email: yyou1@unh.newhaven.edu, mrossi@newhaven.edu

Triple negative breast cancers (TNBC) are particularly aggressive and more likely to recur than other subtypes of breast cancer because there are no targeted therapies. Bromodomain and extraterminal domain (BET) inhibitors have shown efficacy in several models of cancers by inhibiting BET's function at gene regulation. JQ1 is a specific bromodomain inhibitor believed to function as a competitive inhibitor, with IC_{50} values from 92-112 nM. In this study, mesenchymal like cells (MDA-MB-231, Hs578T) and basal like cells (Hcc1143) were used to examine the inhibition by JQ1. A 3D culture system was built to explore whether this 3D culture system will cause the activation of an epithelial-mesenchymal transition (EMT) and whether EMT will cause cells to be more or less sensitive to JQ1 treatment. In this research, both western blot and immunofluorescence confirmed that BRD3 and BRD4 are expressed in TNBC cells in both 2D and 3D culture systems. However, EMT occurred when Hcc1143 and MDA-MB-231 cells were cultured in the 3D culture system, and they had a significantly higher bromodomain protein expression in 3D. It was likely that the presence of EMT induced the higher expression of bromodomain proteins in the 3D cell culture system. On the other hand, MTT assays were performed to determine IC_{50} for JQ1 with a range of 30~170 nM in a 2D culture system. A 3D viability assay found an IC50 for JQ1 in the 3D culture system with a range of 10~80 nM. MDA-MB-231 cells, and Hcc1143 cells were more sensitive to JQ1 in a 3D culture system than in a 2D culture system. Consequently, JQ1 inhibits the growth of TNBC cells in both systems and suggest BDR3 and BDR4 as potential therapeutic targets for TNBCs.

CELLULAR AND MOLECULAR TOXICOLOGY

A-3004

Neuroprotective Effect of the Human Adipose Stem Cell Secretome. THERESA M CURTIS[1], Joseph Hannett[1], Rebecca M. Harman[2], and Gerlinde R. Van de Walle[2]. [1]Department of Biological Sciences, State University of New York at Cortland, Cortland, NY and [2]Baker Institute for

Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY. Email: Theresa.curtis@cortland.edu

Arsenic exposure through contaminated food and water causes irreversible neural damage and affects millions of people worldwide. Several studies have demonstrated that secreted factors (secretome) from mesenchymal stem cells (MSC) can promote neural recovery after several forms of injury including stroke and neurodegenerative diseases. To determine if the secretome from adipose-derived MSC (ADSC) have a therapeutic potential in arsenic damaged neurons, the following study was conducted. Human neuroblasts (SH-SY5Y) were pre-exposed to the secretome from ADSC and then challenged with different concentrations of arsenic. After various exposure times, the extent of neuronal injury was assessed using MTT and LDH release assays and LIVE/DEAD staining. Our studies found that the ADSC secretome could protect the neurons from damage induced by arsenic. We are currently exploring the underlying mechanism for this effect in more detail. Our preliminary data suggest that a soluble factor(s) from the secretome is (are) binding to the neuroblasts causing an increase in cell spreading. Interestingly, this mechanism is independent of neuroblast differentiation, which is a novel finding compared to previous studies that described a differentiation-dependent protection mechanism.

A-3005

Inhibition of Exendin-4-induced Steatosis by Protein Kinase A in Cultured HepG2 Human Hepatoma Cells. G. HAMMEL[1], A. Y. Chen-Liaw[1,2], and George Gomez[1]. [1]Biology Department, University of Scranton, 800 Linden Street, Scranton, PA 18510 and [2] Molecular Oncology, Washington University, 660 S. Euclid Avenue, St. Louis, MO 63110. Email: gabrielle.hammel@gmail.com

Nonalcoholic fatty liver disease is a serious form of chronic liver disease characterized by the abnormal accumulation of triglycerides within the liver cells, resulting in a steatotic liver. Glucagon-like peptide 1 as well as its analog exendin-4 can ameliorate certain aspects of the metabolic syndrome by inducing weight loss and reducing hepatic triglyceride accumulation, but it is unclear whether those effects resulted from the incretin effects of glucagon-like peptide 1on the pancreas, or from direct action on the liver. This study investigates the direct action of exendin-4 on steatotic hepatocytes in culture and identifies the cellular mechanism mediating its action. HepG2 human hepatoma cells were cultured and steatosis was induced by incubation on media supplemented with 2 mM each of linoleic acid and oleic acid. Steatotic hepatocytes were then pre-incubated in the protein kinase A inhibitor H89 for 30 minutes, then treated with exendin-4 over a period of 24 hours. Lean controls were left untreated. Triglyceride content were quantified with AdipoRed staining. Our results showed that steatotic cells maintained high levels of intracellular triglycerides (80%) compared to lean controls (25%). Exendin-4 treatment caused a significant reduction in intracellular triglyceride content after 6 hours that persisted through 24 hours, while protein kinase A inhibitors abolished the effects of exendin-4. The results demonstrate the exendin-4 induces a partial reduction in TG in steatotic hepatocytes over a treatment period of 24 hours via the GLP-1 receptor mediated activation of protein kinase A. Thus, the reduction in hepatocyte triglyceride accumulation is likely driven primarily by downregulation of lipogenesis and upregulation of β-oxidation of free fatty acids.

A-3006

An in Vitro Model of Airway Fibroblast Functions to Assess Airway Remodeling in Asthma. VICTORIA L. MCQUADE[1], Jingjing Niu[2], Barbara Theriot[1], David D'Alessio[2], Mary Jane Cunningham[3], Loretta Que[1], and Jennifer L. Ingram[1]. [1]Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, Duke University, Durham, NC; [2]Department of Medicine, Division of Endocrinology, Duke University, Durham, NC; and [3]Nanomics Biosciences, Inc., The Woodlands, TX. Email: vlm12@duke.edu

Obesity increases circulating levels of pro-inflammatory/fibrotic factors that influence pathobiology in the lung. Allergic asthma is characterized by airway remodeling with fibrosis. Airway fibroblasts contribute to airway remodeling by migrating to and proliferating within the submucosa, where they secrete extracellular matrix proteins. Interleukin-13 (IL-13) is a type 2 cytokine that directs these processes. Leptin is an adipokine produced in elevated levels in the obese; while secretion of glucagon-like peptide 1 (GLP-1) is impaired with obesity. We have developed an *in vitro* system to assay human and mouse airway fibroblast invasion and migration at baseline and in response to IL-13, leptin and GLP-1. By comparing the *in vitro* fibroblast functions to clinical and physiological parameters in asthmatic human subjects as well as in mouse models of allergic airway disease and gastric bypass surgery, we endeavor to predict *in vivo* remodeling by measurement of *in vitro* fibroblast behavior. In our model, mice are fed high fat diet and concurrently exposed to house dust mite allergen or saline control for 20 weeks. Obese mice undergo vertical sleeve gastrectomy or sham surgery. Four weeks post-surgery, airway physiology is measured and lung tissue harvested. Lung fibroblasts are isolated in culture and expanded. Cells (1×10^5/well) are seeded onto Matrigel or non-Matrigel transwell inserts and incubated for 72 hrs in the presence of IL-13, leptin and GLP-1. Quantification of the numbers of invading and migrating lung fibroblasts *in vitro* is an assessment of fibroblast activity and can be correlated with airway resistance, lung compliance, airway wall thickness, peribronchial collagen deposition and circulating levels of cytokines. Using these techniques in tandem facilitates development of an *in vitro* system to predict airway remodeling in obese asthma, which will then be tested in human patients pre- and post-bariatric surgery. There is also

the potential to use this model to evaluate invasive fibroblast gene expression for identification of biomarkers and therapeutic targets.

A-3007

In Vitro Responses of Lake Sturgeon Cell Lines to a Common Lampricide TFM Are Correlated with Its Known Whole-fish Sensitivity. NGUYEN T. K. VO[1], Levi Moore[1], Katelin W. Spiteri[1], and Stephanie J. DeWitte-Orr[1,2]. [1]Department of Health Sciences and [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON, CANADA. Email: nvo@wlu.ca

Lampricides are currently being applied to streams and rivers to control the population of sea lamprey, an invasive species, in the Great Lakes. One of the most commonly used lampricide agents in the field is 3-trifluoromethyl-4-nitrophenol (TFM). Unfortunately, TFM exerts unintended toxic effects on non-target fish. One of the fish species that are sensitive to TFM is juvenile lake sturgeon (LS) (*Acipenser fulvescens*). Whether TFM toxicity can be manifested at the cellular level in the same way as it does in whole fish is not known. Here we report the establishment of five LS cell lines derived from the liver, gill, skin and intestinal tract of juvenile LS and some of their cellular characteristics. Comparative toxicity of TFM in five LS cell lines was assessed by two fluorescent cell viability dyes, Alamar Blue and CFDA-AM, in two conditions: with/without serum and 24/72 h exposure. Deduced EC_{50} values were compared among the cell lines and to the reported *in vivo* LC_{50}. Tissues sensitive to the effects of TFM *in vivo* correlated with cell lines from the same tissues being most sensitive to TFM *in vitro*. Our data suggests that whole fish sensitivity of LS to TFM is likely attributable to sensitivity at the cellular level. Thus, these cell lines can be used to screen and evaluate the toxicity of the next generation of lampricides on the LS.

CHEMICAL CARCINOGENESIS

A-3008

Role of Dietary Factors and Heat Shock Protein 70 Expression in Colorectal Cancer Development by 4-nitroquinoline-1-oxide. DANIEL OSWALD[1], Andrew Ngyuen[1], Matthew Pytynia[1], Sarah Veen[1], Alice Meyer[3], Christian C. Evans[1,2], and Mae J. Ciancio[1]. [1]Biomedical Sciences Program, [2]Physical Therapy Program, College of Health Sciences, and [3]Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove IL. Email: doswald66@midwestern.edu

Colorectal cancer is one of the deadliest and most prevalent cancers in the United States. The American Cancer Society estimates that in 2017 there will be 135,430 new cases of colorectal cancer. Although improving treatment methods continue to decrease the mortality of colorectal cancer, it remains a significant target for new therapeutic strategies. Heat Shock Protein 70 (Hsp70) is a stress-induced, cytoprotective protein. It can act as both a tumor promoter by inhibiting apoptotic pathways and a tumor suppressor by inhibiting pro-tumorigenic inflammatory cytokines. How Hsp70 and dietary fat might influence the colorectal response to the oral carcinogen, 4-ntiroquinoline-1-oxide (4NQO), was examined in this study. Using an epithelial cell specific, Hsp70 transgenic (TG) mouseline, we explored the effect of a high-fat (HF) diet and Hsp70 overexpression on the potential colon cancer response to the oral carcinogen, 4NQO. Female TG (n=19) and non-transgenic (NTG; n=19) littermates were randomly divided into HF (60 kcal%) or normal diet groups. Each group received either normal water (H_2O), 1.25% propylene glycol-water (PG/H_2O), or 50μg/μL 4NQO in PG/H_20 for 17 weeks, followed by water alone for 6 weeks. Body weights and oral tumor sizes were documented weekly. At 23 weeks, colons were examined for gross morphological and microscopic changes. Crypt length and width were measured using Image J. β-catenin and Ki-67 staining were used to detect proliferation. There was no evident 4NQO- or HF-induced dysplasia or crypt distortion. Ki-67 and b-catenin staining studies are currently being conducted. Analysis of cellular proliferation markers may allow for determination of precancerous changes that are not detectable by morphological measurements. 4NQO does not have the same profound carcinogenic effect in the colon tissues as it does in the oral cavity.

OTHER IN VITRO ANIMAL CELL SCIENCES

A-3009

Maltooligosaccharide Chemosensation by Intestinal Enteroendocrine L-Cells Regulate the Endogenous Release of Gut Hormones and Glucose Homeostasis. MARWA EL HINDAWY[1], Choon Young Kim[2], and Bruce R. Hamaker[1]. [1]Food Science, Purdue University, West Lafayette, IN and [2]Food and Nutrition, Yeungnam University, Gyeongsan, REPUBLIC OF KOREA. Email: melhinda@purdue.edu

Recent studies showed that the suppression of the hypothalamic neuropeptide expression in rats fed slowly digestible starch was concurrent with increased satiety and lower food intake compared to rats fed rapid digestible starch. This suggests that complex carbohydrates arriving to the ileum and through triggering L-cells promote these physiological effects. It was not clear, however, what carbohydrate type activates L-cells, whether it is α-amylase starch digestion products, or maltooligosaccharides (MOS), or glucose itself, or even bacterial fermentation products. We show that intestinal L-cells exhibit increased response of anorexigenic gut peptide secretion with MOS. Glucagon-like peptide-1 (GLP-1) secretion was significantly higher in STC-1 cells treated with MOS compared to glucose or propionate. Increasing the degree of polymerization of the MOS incrementally increased GLP-1 levels. Maltotriose and maltopentaose showed over 2-fold increase in GLP-1 levels (1.16 and 1.23 pM/μg protein respectively) compared to glucose (0.57 pM/μg protein) or propionate (0.38 pM/μg protein). MOS increased oxyntomodulin (OXM) levels in the human L-cells, and maltotriose promoted the most effect. A

dramatic increase (5-fold) was shown in the relative expression of the gastrointestinal peptide, cholecystokinin, in cells treated with maltotriose. Differential gene expression of STC-1 cells revealed a significant decrease in the expression of the thioredoxin-interacting protein and arrestin domain-containing protein-4 in cells treated with maltotriose compared to those treated with glucose. Therefore, MOS are not only being sensed by L-cells to stimulate endogenous release of gut hormones, but also induce signaling pathways related to cellular oxidative status and glucose homeostasis. MOS exhibits a unique effect on L-cell sensitivity and gut hormone productivity. The use of dietary carbohydrates to achieve a gut physiological response that relates to satiety and food intake could be a new approach for food for health applications.

A-3010

Anti-inflammatory Response of Mesenchymal Stromal Cells to TNF-α Stimulation. SHIVA HAMIDIAN JAHROMI , Yunqing Li , and John E. Davies. Institute of Biomaterials and Biomedical Engineering and Faculty of Dentistry, University of Toronto, ON, CANADA. Email: shiva.hamidianjahromi@mail. utoronto.ca

Tumor necrosis factor (TNF)-stimulated gene 6 (TSG-6) is a major anti-inflammatory mediator released by activated MSCs. Neonatal MSCs are considered more metabolically active than cells derived from adult tissues, and potentially less heterogeneous. We hypothesized that a TNF-α activated neonatal MSC population (human umbilical cord perivascular cells – HUCPVCs) would show an enhanced level of TSG-6 activation compared to adult bone marrow MSCs (bmMSCs). *In-vitro*, we compared the TNF-α stimulation response of HUCPVCs to both human and mouse bmMSCs, with both TNF-α dose, and exposure. HUCPVCs, hbmMSCs and mbmMSCs were seeded at 5000 cells/cm2 (n=3) and after 24 hours, stimulated with 1, 10, 50, and 100ng/mL of human recombinant TNF-α for HUCPVCs and hbmMSCs, and mouse recombinant TNF-α for mbmMSCs. HUCPVCs and mbmMSCs were pooled populations. Supernatant was collected for measurement of secreted TSG-6 protein (enzyme-linked immunosorbent assay) and total RNA of the same cells was isolated for real-time reverse transcription polymerase chain reaction. 1ng/mL TNF-α stimulation of HUCPVCs caused a significant upregulation of TSG-6 within the first 30 minutes of exposure (~11.5 fold). By contrast, hbmMSCs showed 2-fold increase by 1 hour that increased to 9.5-fold with 50ng/mL TNF-α for the same exposure time. However, mbmMSC showed a 2-fold increase after 24 hours that was independent of TNF-α concentration. Hence, both human MSC populations exhibited enhanced TSG-6 upregulation upon TNF-α stimulation compared to mbmMSCs. We also observed a TSG-6 expression level variation among different donors, as has previously been reported in human bmMSCs. Nevertheless, our data show that the neonatal cells employed exhibited both an enhanced and more rapid response to low dose (1ng/mL) TNF-α exposure. Secreted protein levels of TSG-6 supported our gene expression data. HUCPVCs showed a higher sensitivity, and more prompt response to TNF-α stimulation compared to both hbmMSCs and mbmMSCs. Hence, neonatal MSCs may be a stronger candidate when treating inflammatory diseases.

A-3011

Impact of HSP70 Expression in Preventing Male Infertility in Obese Mice. JENNIE OKO[1], Emily Hayes[1], Ketjona Deli[1], Matthew Pytynia[1], Sophie La Salle[2], and Mae J. Ciancio[1]. [1]Biomedical Sciences Program, College of Health Sciences and [2] Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL. Email: joko78@ midwestern.edu

Obesity is a multifactorial disorder that is implicated in male-factor infertility. Studies have demonstrated a reduction in sperm quality and fertility rates with diet-induced obesity (DIO), possibly due to a breakdown in the blood-testis barrier (BTB). Factors that may prevent DIO-mediated BTB compromise could have significant clinical applications. Heat shock protein 70 (HSP70) is a stress-inducible protein that is reported to protect intestinal barrier function under high fat (HF) dietary conditions. Whether HSP70 expression can maintain the BTB in DIO mice is unclear. The purpose of this study was to investigate whether epithelial specific, HSP70 overexpression can preserve integrity of the BTB and fertility factors in male DIO mice. Five-week-old, male Hsp70 transgenic (TG; n=12) and non-transgenic (NTG, n=8) littermates were individually housed and placed on either a low fat (LF; 10 kcal%) or HF (60 kcal%) diet for 14 weeks. Fertility was monitored by time to copulation, pregnancy rate, litter size, and weights. Testes were collected for histological and molecular analyses. Sperm counts, motility, and morphology were assessed. Preliminary results indicate no significant difference in time to copulation, litter size, testis weight, sperm counts or motility regardless of diet or genotype. Offspring sired by TG males were significantly smaller than those from NTG males ($P = < 0.05$). Initial sperm assessments suggest morphological changes in HF mice. Western blot and histological analyses are currently underway to evaluate testicular and BTB integrity. Although preliminary, the smaller size of offspring sired by TG males suggests that epithelial cell specific overexpression of HSP70 may impact sperm quality in ways that influence fetal development.

A-3012

Do Antibiotics or Fecal Microbiota Transplants Alter the Development of Obesity in Sedentary Mice? NICHOLAS SMITH[1], Mae J. Ciancio[1], Matt Pytynia[1], Marc Scheetz[2], Vanessa Leone[4], and Christian C. Evans[1,3]. [1]Biomedical Sciences Program, College of Health Sciences; [2]Clinical Pharmacy, Chicago College of Pharmacy; [3]Physical Therapy Program, College of Health Sciences, Midwestern University, Downers Grove, IL; and [4]Department of Medicine, The

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Intestinal microbes are vital to overall health and weight management. Studies suggest that antibiotics may lead to weight gain by inducing intestinal dysbiosis and that exercise (Ex) prevents diet-induced obesity (DIO) by altering gut microbial balance. Fecal microbiota transplants (FMT) may represent a treatment for both antibiotic- and diet-induced obesity by normalizing microbial balance. The purpose of this study was to determine the effect of oral antibiotic treatment and FMT on the development of high fat (HF) DIO in sedentary (Sed) mice. Male donor C57Bl/6 mice (n=12) were raised for 12 weeks on a HF diet (60 kcal% fat) under Sed or Ex conditions. Fecal samples were collected and processed into HF/Sed or HF/Ex slurries for FMT. Male recipient mice (n=32) received a 3-day course of neomycin (1.35mg) and polymyxin B (2.425mg) by oral gavage. Twenty mice received FMT (100 µl/mouse x 1/week) for 10 weeks of either HF/Sed or HF/Ex slurry; the remaining 12 mice continued on a LF or HF diet without FMT. Weekly body weights, food intake, glucose regulation, and body fat were measured. Fecal DNA was extracted for sequencing. A two-way ANOVA and Sidak post-hoc test were used ($p < 0.05$). Antibiotic-treated mice gained more weight on either LF or HF diet compared to control mice that didn't receive antibiotics or FMT ($p=0.003$). Among donors, Sed mice gained more weight, had poor glucose control, and more body fat than the Ex mice ($p < 0.05$). Weekly FMT from Sed or Ex donors did not alter weight gain in recipient mice. These data suggest that antibiotic treatment alone can induce weight gain and that FMT from Sed or Ex donors was unable to curb weight gain. These results suggest that short-term use of antibiotics alters gut microbiota in a manner not normalized by FMT.

A-3014

RTgut-GC and RTS11-GFP Cell Lines as In Vitro Models to Study Fish Gut Physiology and Enteritis. LUCY EJ LEE[1], Harshraj Sidhu[1], Veronica Kobes[1], Patrick Pumputis[2], and Niels C Bols[2], Departments of Biology, [1]University of the Fraser Valley, Abbotsford, BC, and [2]University of Waterloo, Waterloo, ON, CANADA. Email: lucy.lee@ufv.ca

Several in vitro models have been developed for research into human intestinal physiology and pathophysiology, but similar models for fish are lacking, although they could be very useful for many basic and practical problems in aquaculture. The intestine is an interface between nutrients and the host epithelial cells responsible for nutrient absorption and between potentially pathogenic microorganisms and the host macrophages responsible for coordinating immune responses. Human in vitro models include cultures of tumour-derived intestinal epithelial cell lines, such as Caco-2, organized as monolayers on culture inserts to study absorption or as spheroids alone or together with macrophages to study immune interactions. To develop similar in vitro models for fish, we have developed an intestinal epithelial cell line, RTgutGC, and a macrophage cell line, RTS11. RTgutGC expresses several tight junction proteins and form a sealing barrier on culture inserts. Through nucleofection, an RTS11 variant, RTS11-GFP, has been developed that continuously expresses Green Fluorescent protein (GFP). RTS11-GFP has been co-cultured with RTgutGC in monolayer and spheroid formats to study epithelial/macrophage interactions. These include intraepithelial macrophage projections and intestinal barrier functions. These co-culture systems are being developed to provide practical and reproducible culture models to investigate suitability of novel fish feed components to enhance or disrupt fish intestinal physiology and host responses that could prevent development of enteritis.

ANIMAL SILENT ABSTRACT

A-3013

Estimation of the Safety of Oligoring Insecticide on Bone Marrow Cells of Domestic Bull *Bos taurus Taurus L.* V. V. OBEREMOK.[1], K. V. Laikova[1], P. M. Nyadar[1], A. G. Barsegan[1], N. V. Gal'chinsky[1], M. N. Shumskykh[1], S. A. Nazarov[1], I. S. Kashapova[2], I. V. Chivilev[2], E. V. Kornienko [2], A. L. Arhipova [2], S. N. Kovalchuk[2], and G. Yu Kosovskiy[2]. [1]Crimean Federal V.I. Vernadsky University, 4 Vernadsky Ave., Simferopol, CRIMEA, 295007 and [2]Federal State Budget Scientific Institution Centre of Experimental Embryology and Reproductive Biotechnology, 12/4 Kostyakova St., Moscow, RUSSIAN FEDERATION, 127422. Email: genepcr@mail.ru, Corresponding author: biopalmgene@gmail.com

The need for insecticides is not questioned but many of them cause great harm to the environment, and it encourages the search for new and safe preparations. In this connection, the perspective substitution for plant protection are products based on natural polymers – nucleic acids, namely, DNA insecticides (Oberemok, 2008; Oberemok et al., 2016; Nyadar et al., 2016) and RNA preparations (Gu, Knipple, 2013). An important aspect of the development of such means of insect pest control is the evaluation of their safety for both animals and plants. In our studies to control gypsy moth (*Lymantria dispar* L.) larvae, we developed DNA insecticide, an oligoRING (5'-dCGA CGT GGT GGC ACG GCG-3') from anti-apoptotic gene IAP-3 of *Lymantria dispar* multicapsid nuclear polyhedrosis virus (LdMNPV) and IAP-Z gene of gypsy moth, which has no significant negative effect on insects (Oberemok, Skorokhod, 2014; Oberemok et al., 2015) and plants (Oberemok et al., 2013; Zaitsev et al., 2015). To estimate the cytotoxicity of the oligoRING fragment for vertebrate animals and possible use of DNA insecticides in agriculture and forestry on a large scale, studies were carried out on bone marrow mesenchymal stem cells of domestic bull *Bos taurus taurus* L. In Lipofectamine® (Invitrogen, USA) transfected mesenchymal stem cells of the domestic bull, the DNA antisense fragment (5'-dCTC CAG ATT CCC AAC ACC-3') of *B. taurus taurus* anti-apoptotic

IAP-2 gene at a concentration of 1 femtomole/cell caused a significant increase in the percentage of dead cells on the 12th day after transfection, 5.92 ± 0.18% in the oligoIAP-2 group versus 1.39 ± 0.09% in the control ($p < 0.05$). On the contrary, the control antisense fragment of the antiapoptotic LdMNPV IAP-3 gene (oligoRING-insecticide) in the same concentration showed no such effect: 1.35 ± 0.07% in the oligoRING group versus 1.39 ± 0.09% in the control ($p>0.05$).The obtained results indicate the safety of the antisense oligoRING insecticide for cells of domestic bull as representative of vertebrate animals.

EDUCATION SILENT ABSTRACT

E-3000

The Effects of Cellular Characteristics and Nutritional Factors on Dichloroacetate Resistance in Human Breast Cancer Cells. MINU KIM, Hyobin Seok, Seulki You, Minyoung Choi, and Kwangil Kang. Department of Biological Science, Korea Science Academy of KAIST, Busan, Rep. of KOREA. Email: minu.kim.yu@gmail.com

To understand the resistance of Dichloroacetate (DCA) which induce apoptosis in breast cancer cells, the characteristics and nutritional factors of cancer cell lines were studied. Human breast cancer cell lines MDA-MB-231 and MCF-7 were treated with DCA and their cell viability was examined. MDA-MB-231 cells lack estrogen receptors and form a loose aggregate, compared to MCF7 cells. In addition, the expression of HMGB1 in the cells was restricted to the nucleus before DCA treatment and was resistant to DCA – induced apoptosis. In order to understand the resistance to DCA in breast cancer cells, nutritional factors such as estrogen and steroid, 3D-Spheroid formation, HMGB1 expression and distribution were examined and compared with ratio of cell death induction of DCA. HMGB1 expression and intracellular distribution were related to the effects of DCA, and nutritional factors did not significantly affect DCA induced cell death pathway.

PLANT POSTER ABSTRACTS
BIOTECHNOLOGY

P-3000

Biosafety of Plant Research in Greenhouses and Other Specialized Containment Facilities. DANN ADAIR[1], Sue Tolin[2], Anne K. Vidaver[3], and Ruth Irwin[2]. [1]Conviron, Pembina, ND; [2]Virginia Polytechnic Institute and State University, Blacksburg, VA; and [3]University of Nebraska, Lincoln, NE. Email: dadair@conviron.com

Minimal biosafety guidance regarding plant science impacts present needs and offers little to assist emerging needs resulting from new technologies. An important distinction must be recognized: plant research focuses on environmental protection whereas traditional biosafety is about worker and subject protection. To address this need for guidance, a chapter, bearing the name of this presentation, in *Biosafety, Principles and Practices, 5th* edition, ASM Press, 2017 was created. The chapter describes agency regulations and guidelines for working with plants and related organisms grown in containment facilities. Containment related to biotechnology is seldom a regulated situation but the guidance offered in the NIH Guidelines is universally accepted. USDA-APHIS is the primary regulatory agency for plants and associated organisms. A basic understanding of what constitutes containment and examples of containment facilities and specific design elements are presented. Management practices, including the need for appropriate signage, are also illustrated. Collaboration with leading professionals (co-authors Tolin, Vidaver, and Irwin) was key to assembling the chapter. Literature reviews and consultations with relevant agencies and professionals were the basis for compiling regulatory and guidance materials. Experience on design teams and facility management contributed to the thorough understanding of specialized facility features. Discussions at an annual biosafety course indirectly solicited the community resulting in a new plant containment symbol. This new symbol serves to counter the misapplication of the universal biohazard symbol. While it is important to identify plants grown under containment, seldom is there a risk to humans. Reserving the universal biohazard symbol for the appropriate risk ensures its validity.

P-3001

Development of an Efficient Gene Editing System for Hexaploid Sweetpotato. FOAZIATU BUKARI[1], Sy Traore[2], Marceline Egnin[1], Osagie Idehen[1], Gregory C. Bernard[1], Conrad Bonsi[1], and Stanton Gelvin[3]. [1]College of Agriculture, Environment & Nutrition Sciences, Tuskegee University, Tuskegee, AL; Department of Biology, Auburn University, Auburn AL; and [3]Department of Biological Science, Purdue University, West Lafayette, IN. Email: fbukari0632@ mytu.tuskegee.edu

This ability to selectively alter genomic DNA sequences in vitro has opened new breeding avenues for difficult and high-ploidy crops such as sweetpotato. Sweetpotato is an important crop that presents numerous breeding challenges because of its ploidy level. Plant protoplast transfection of gRNAs-cas9 complex facilitates rapid analysis of CRISPR induced site-specific DNA double-strand breakage and resultant deletions or insertions in protoplast cells 24h after transfection. This study seeks to test the feasibility of CRISPR–Cas-mediated mutagenesis in the hexaploid sweetpotato genome and develop toolkits for precise, efficient gene targeting or editing. Multiple sgRNA constructs, with 20–22-nt targeting *pds3* gene in diploid and hexaploid sweetpotato, and *Arabidopsis* and tobacco as controls, were transiently tested in 48 hours transfected leaf-derived protoplasts to confirm that a specific sgRNA, or sets of sgRNAs, are effective in generating mutations. Subsequent analyses of the PCR amplicons by NEB T7 endonuclease I assays produced multiple digested fragments in addition to

the parental band, indicating the double-strand break introduced by CRISPR/Cas9 in *pds*3 locus of sweetpotato genome. The best sgRNA resulted in the highest percentage of the cleaved products in multiplex editing especially for the hexaploid. Colorless calli from transfected protoplasts developed in sweetpotato regeneration media further confirmed CRISPR/Cas9 mediated activities at the target site. Our results validate the proof of concept in sweetpotato gene editing. USDA-NIFA 1890 Grant#2014-38821-22448; TU-GWCAES.

P-3002

Cell-penetrating Peptides for Biomolecule Delivery Into Plant Cells. DELANEY BRAY-STONE, D. Wolyn, and A.M.P Jones. Department of Plant Agriculture, University of Guelph, Guelph, Ontario, CANADA. Email: dbraysto@uoguelph.ca.

Cell-penetrating peptides (CPPs) range between 8-35 amino acids in length and can be used for intracellular delivery of biomolecules. They are usually cationic or amphipathic and are able to translocate across the negatively charged cell membranes of various organisms, including mammals and plants, through endocytotic and putative non-endocytotic mechanisms. A wide range of cargo sizes and types can be imported into the cell through covalent and non-covalent binding to CPPs, such as nanoparticles, fluorophores, RNA, RNA-guided endonucleases, DNA and proteins. CPPs have emerged as a powerful tool for various biotechnological applications, however their use with plants remains in its infancy. In plants, the cell wall presents an additional barrier to CPP-mediated cargo delivery. This research aims to provide a comprehensive comparison of six CPPs (R9, BP100, TAT_2, R9-TAT_2, R9-BP100 and $(BP100)_2K_8$) that are described in the literature for use with plant cells. The selected CPPs were conjugated to fluorescein isothiocyanate and are being investigated for use with plant suspension cells, protoplasts and ultimately with callus and leaf tissues. Preliminary results of CPP import into viable tobacco BY-2 cells and protoplasts will be presented, such as confocal imaging of localization to the cell surfaces, cytoplasm and organelles. Long-term objectives of this research are to optimize intracellular delivery of bioactive proteins and plasmids through non-covalent binding with CPPs.

P-3003

A Soybean Glycinin Promoter Drives High Cotyledon-enhanced Expression in Both Somatic and Zygotic Embryos. ERIC A DEAN and John J Finer. Department of Horticulture and Crop Science, OARDC, The Ohio State University, Wooster, OH. Email: dean.1063@ buckeyemail.osu.edu

In order to precisely and continuously study expression of soybean seed storage proteins during embryo development, *in vitro* systems are needed. *In vitro* embryo development methods have been developed both as a means to recover interspecific hybrids following wide crosses, and as a tool for plant regeneration from embryogenic cultures. While these *in vitro* soybean embryo development systems have been used for plant recovery, they are not often used to study gene expression or factors that influence seed composition. Somatic and zygotic embryos, cultured *in vitro*, could provide a rapid system for modelling compositional changes or results from modification to seed storage protein genes. For this research, our goals were to further normalize soybean somatic embryo development, as well as provide additional methods for *in vitro* studies of zygotic embryo development by monitoring cotyledon growth and expression of a late-development cotyledon-enhanced promoter. A soybean glycinin promoter, designated GmSeed5, was cloned upstream of the *green fluorescent protein* coding region and introduced into soybean. Non-transformed cotyledons from zygotic embryos were also used in embryo development studies. Embryos were cultured on MSM6, SHaM, and a B5 based embryo rescue medium and monitored using both manual and semi-automated image collection. Cotyledons developed on SHaM displayed an average 2.3-fold increase in area over a 180-hour time period while cotyledons on MSM6 and the B5 media showed a 1.7-fold increase in cotyledon size, with no statistical difference between the either treatment. Immature zygotic embryos developed on SHaM for two weeks prior to desiccation germinated with 100% efficiency. MSM6-developed embryos germinated with 94% efficiency, and B5 embryos germinated at 68%. GFP expression, regulated by the glycinin promoter increased in cultured somatic embryos after 25 days on solid SHaM. This *in vitro* embryo development system is a valuable tool to study embryo development, embryo- or cotyledon-enhanced promoters and changes to genes associated with seed composition.

P-3004

Key Roles for Soil Microbiota in *In Vivo* and in *In Vitro* Plant Systems. BARBARA DOYLE PRESTWICH, Saoirse O Neill , Jack Daly and Darren Heenan-Daly. School of Biological Earth and Environmental Sciences, Butler building, Distillery Fields, North Mall, Cork, IRELAND. Email: b.doyle@ucc.ie

The importance today of the soil microbiota is undeniable. Today, many areas of plant science research engage with soil microorganisms particularly bacteria. A search of google scholar using the search term 'soil bacteria' returned over two million hits (March 2017). In a world where human population growth and changes in climate are putting pressure on food security, plus changes in the availability of crop-protectant chemicals, scientists are seeking alternative strategies for crop growth and protection. Malnutrition (hidden hunger) is an issue facing many people. It is estimated that ~ two billion people suffer from malnutrition caused by a lack of basic nutrients. Our laboratory in UCC (https://www.ucc.ie/en/bees/staff/), the current virtual office of the IAPB (http://www.iapbhome.com/) is engaged

with the use of soil microbes in the area of disease control (we were previously engaged in South America in VALORAM on this topic http://valoram.ucc.ie/), tissue culture of plants and biofortification. We are interested in the volatilome of soil isolates (rhizosphere) and the impact of these low molecular weight organic compounds on plant growth and health. Of 120 isolates taken from selected sites in the south west of Ireland, nearly 40 % performed better than *B. subtilis* FZB (control) in antagonism plate tests against *R. solania* with the same number being able to solubilise phosphate and about 10 % HCN producers. In terms of microbial volatile organic componds, our isolates produced a range of compounds including 1,3- Butanediol, 1-Decanol, 1-Undecene, 1-Nonanol amongst others. In terms of tissue culture, the impact of isolated potato endophytes (courtesy of AIT http://www.ait.ac.at/en/) on growth of Irish potato cultivars Golden Wonder and Maris Piper and the pre-famine variety Lumper is currently under investigation as is the role of selected microbes in zinc and iron mobilisation. Our colleague based at AIT is one of our keynotes at IAPB 2018 (http://iapb2018.com/) where the importance of the soil microbiota in global food security will feature as one of the topics.

P-3005

The Importance of Professional Organisations Such as the International Association for Plant Biotechnology (IAPB) in Supporting the Industry and in Communicating Science. BARBARA DOYLE PRESTWICH[1], Eoin Lettice[1], Ewen Mullins[2], and Norma Cotter[1]. [1]School of Biological Earth and Environmental Sciences, Butler building, Distillery Fields, North Mall, Cork, IRELAND and [2]Teagasc Oakpark Research Centre, Carlow, IRELAND. Email: b.doyle@ucc.ie

The International Association for Plant Biotechnology is over half a century old. Founded in 1963 to serve scientists working in the area of plant tissue culture research, the inaugral congress was held in Pennsylvania . However, formal establishment of the organisation wasn't until 1970 at the meeting in Strasbourg France. Eleven quadrennial symposia have been held, alternating between different countries and continents (http://www.iapbhome.com /about) in the intervening period. During half a century, many advances have been made in the area of tissue culture and biotechnology-related activities. The first genetically modified plant wasn't commercialised until over twenty years after the society had been founded. The society has played and continues to play an important role in networking and in promoting scientific excellence through the organisation of the quandrennial congress and the publication of its international peer-reviewed journal, *In vitro* Cellular and Developmental Biology - Plant (http://www.springer.com /life+sciences/plant+sciences/journal/11627), the publiccation and dissemination of the society newsletter and the continual recruitment and communication with colleagues in 52 countries globally. Find us through website (http://www.iapbhome.com/); twitter (https://twitter.com/iapbhome); Facebook (https://www.facebook.com/International-Association-for-Plant-Biotechnology-125261657163/) and LinkedIn (https://www.linkedin.com/groups/2233720). The next congress (the 14th quandrennial symposium) will be held in Dublin, Ireland (http://iapb2018.com/) after a break of almost 25 years. Professional societies not only serve professional members but also have a role to play in communicating science to the public. In Ireland, where the virtual office is currently located (2015-2018) we educate and recruit members from the next generation of scientists i.e undergraduate students and school children as part of our outreach activities.

P-3006

Genetically Modified TMV Particles May Serve as a Carrier for Chemical Conjugation of Influenza Antigens to Produce Multivalent Nanovaccines. 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

The recombinant TMV-NtK viral vector based on the tobacco mosaic virus TMV-U1 genome was created by inserting mutations into the N-terminal portion of the coat protein (CP) gene. According to X-ray analysis, this region is exposed on the surface of viral particles. Mutagenesis of the CP suggested the insertion of additional amino acid residues (ADFK), including reactive lysine; their nucleotide sequence was optimized for expression in plants. *Nicotiana benthamiana* plants were infiltrated via *Agrobacterium* coding for TMV-NtK. Modified TMV-NtK virions were purified from non-inoculated leaves either with PEG or by ultracentrifugation (100.000 g). TMV-NtK preparation was analyzed by SDS-PAGE/Coomassie staining; main protein with electrophoretic mobility of 21 kDa was detected. Electronic microscopy confirmed the stability of modified particles. Chemical conjugation of TMV-NtK virions and target influenza antigens expressed in *E. coli* included conversion of free carboxyl groups of antigens into the activated ester of the carboxylic acid with 5mM N-Cyclohexyl-N-(2-morpholinoethyl)carbodiimide-metho-p-toluenesulfonate (analogue of EDC) followed by conversion to the intermediate ester under the action of 1mM N-hydroxysuccinimide (NHS). Then intermediate ester reacts with amino groups representing N-terminal lysine on the surface of the TMV-NtK particles. Proteins DHFR (21.5 kDa) and DHFR-M2e (25.5 kDa) (Petukhova et al, 2013) were conjugared with TMV-NtK under the following conditions: 15', 4°C at adding the EDC then 10' at room temperature (r.t.); 15', r.t. at adding the NHS; 2 hours, r.t. after adding the TMV-NtK. SDS-PAGE analysis revealed complexes formed by recombinant proteins and modified particles. Western blots proved the presence of viral CP and target antigens in the complexes. The results obtained open the possibility of creating multivalent broad spectrum influenza vaccines based on TMV platform. In addition to

conserved M2e epitope, hemagglutinin (HA), protein M1 and fusion peptide (fp) can be used as candidate target antigens.

P-3007

Direct Cryopreservation of Florida Native Orchid Seed. BENJAMIN A. HUGHES and Michael E. Kane. University of Florida, Environmental Horticulture Department, 2043 IFAS Research Drive, Gainesville, FL, 32611. Email: benhughes@ufl.edu

There are approximately One-hundred and twenty orchid species native to Florida, seventy-seven of which have been classified as endangered, threatened, or commercially exploitable. Seed banking is a valuable conservation tool that allows for storage of a large gene pool at reduced cost of *in situ* seed conservation. Ultra-low temperature storage at -196 °C in liquid nitrogen can theoretically maintain seed viability indefinitely and has been successful for numerous orchid species. The effect of direct seed cryopreservation on the germination and subsequent development of nine Florida native orchid species was examined. When compared to unfrozen controls, the germination of cryopreserved seed was not significantly different in *Bletia purpurea*, *Encyclia tampensis*, *Epidendrum amphystomum*, *Epidendrum nocturnum*, *Epidendrum rigidum*, *Eulophia alta*, and *Prosthechea cochleata*. Cryopreserved seed of *Calopogon tuberosus* germinated at a higher rate (41%) than the unfrozen control (35%), whereas cryopreserved seed of *Cyrtopodium punctatum* germinated at a lower rate (57%) than the unfrozen control (76%). Surface sterilization prior to cryopreservation in *B. purpurea*, *C. punctatum*, *E. tampensis*, and *E. nocturnum* resulted in a significant reduction in post-cryopreservation germination in all species when compared to surface sterilization after cryopreservation. Based on the results, direct cryopreservation offers significant *ex situ* conservation potential for Florida native orchid seed banking.

P-3008

Gene Stacking into an Engineered "Safe-Harbor" Locus of Sugarcane. RATNA KARAN, Yang Zhao, Georgina Sanahuja, Jae Yoon Kim, Je Hyeong Jung, and Fredy Altpeter. Agronomy Department, University of Florida - IFAS, Gainesville, FL 32611. Email: rkaran@ufl.edu

Sugarcane is a high biomass producing C4 crop used for commercial production of table sugar and biofuel. Biotechnology has tremendous potential for crop improvement. However, the complex and highly polyploid sugarcane genome compromises the predictability of expression level and stability of randomly integrated transgenes. Genome editing has emerged as tool for targeted genome modification in the genome of crop plants including sugarcane. CRISPR /Cas9, TALEN and other designer nucleases allow the induction of double-strand breaks (DSBs) at specific sites. The repair of these targeted genome lesions through cellular repair processes including homologous recombination (HR) or non-homologous end joining (NHEJ) pathways offers opportunities for targeted insertion of transgenes. However, proper sites for gene integration hereby named "safe harbor loci" must be localized in non-coding regions and possess high gene expression. Knowledge of such sites in sugarcane is lacking. Their identification is complicated by the highly redundant and not fully sequenced sugarcane genome. Therefore, we have generated target sugarcane lines with engineered single copy target locus flanked by insulators resulting in superior transgene performance. We will describe efforts employing CRISPR-Cas9 or TALEN technology to precisely knock-in the gene of interest into this pre-engineered locus in sugarcane.

P-3009

GWAS Identification of Loci Associated with Rooting in POPULUS. CATHLEEN MA[1], Anna C. Magnuson[1], Christine Zawaski[2], Wellington Muchero[3], Jialin Yuan[4], Yuan Jiang[4], Fuxin Li[5], Jonathan R. Cumming[2], Steve P. DiFazio[2], and Steven H. Strauss[1]. [1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis OR; [2]Department of Biology, West Virginia University, Morgantown WV; [3]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge TN; [4]Department of Statistics, Oregon State University, Corvallis OR; and [5]Department of Electrical Engineering and Computer Science, Oregon State University, Corvallis OR. Email: Caiping.Ma@oregonstate.edu; Steve.Strauss@oregonstate.edu

Dormant cuttings of black cottonwood (Populus trichocarpa) were taken from a fully resequenced population of up to 537 wild trees for two independent experiments, one in Oregon and one in West Virginia, to study genetic variation and Quantitative Trait Nucleotides (QTNs) using genome wide association studies (GWAS). This project is part of a larger NSF-funded effort to study genes affecting in vitro response in poplar. Cuttings were rooted in water (Oregon) or 0.5 mM CaNO3 solution (West Virginia) and root growth measurements were recorded. Three weeks post-planting, pictures were taken of each of the cuttings and scored for root density and shoot growth. The images were also analyzed digitally using color-based clustering to segment the cuttings into root, shoot, and stem areas, producing data on average stem diameter and root area. The data also were decomposed into principal components, which were measured along with the underlying traits. ANOVA showed that rooting traits had highly statistically significant variation, with heritabilities varying from 4% to 15%. The population of black cottonwood has a panel of 29 million SNPs representing a marker every 17-base across the genome and rapidly decaying linkage disequilibrium that falls below 0.2 within 3Kb. Efficient mixed model association (EMMA), accounting for kinship, was used to correlate a panel of 8.2 million SNPs to phenotypic variation, where several dozen SNP loci were found to have strong associations with one or more rooting traits at a false discovery rate (FDR) threshold of 0.05. For example, one of the strongest SNPs mapped to a gene homologous to a plant UBX Domain-containing

protein 1 (PUX1), whose loss of function was associated with accelerated root and inflorescence growth. We will report on further phenotypic and GWAS results. We thank the National Science Foundation Plant Genome Research Program for support (IOS # 1546900, Analysis of genes affecting plant regeneration and transformation in poplar), as well as the Department of Agriculture (National Institute for Food and Agriculture contract 2014-67013-21657).

P-3010

Improved Direct Transformation via Particle Bombardment of Split-immature Embryos in Soybean (*Glycine max*). Siva Chennareddy[1], Toby Cicak[1], Katherine Effinger[1], TEJINDER MALL[1], Dayakar Pareddy[2] and Rodrigo Sarria[2]. [1]Dow AgroSciences LLC, 1281 Win Hentschel Blvd., West Lafayette, IN 47906 and [2]Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268. Email: tkmall@dow.com

Embryogenic callus and suspension cultures intiated from immature zygotic embryos have been used as tissue targets for direct transformation in soybean. Such methods, however, generally require a prolonged tissue culture period to prepare explants and are often associated with somaclonal variation. We developed an improved direct transformation method via particle bombardment of split-immature zygotic embryos with intact embryonic axis. This method involves cold treatment and plasmolysis of explants prior to bombardment. Transgenic events were produced using hygromycin and glufosinate selection. Transgenic somatic embryos developed within as little as 4 weeks after bombardment of explants. Transgenic plants were regenerated 4-5 months after bombardment and the entire process from bombardment to T1 seed production took 7-9 months. Transformation frequencies of 5.4% and 2% (based on the number of bombarded split-immature embryo explants) were observed with hygromycin- and glufosinate selection, respectively.

P-3011

The Use of Morphogenic Regulators BABY BOOM and WUSCHEL to Mediate Transformation of Recalcitrant Maize Inbred B73 and Sorghum P898012. MURUGANANTHAM MOOKKAN[1], Kimberly Nelson-Vasilchik[2], Joel Hague[2], Maria A. Moreno[3], Stephen Dellaporta[3], Zhanyuan J. Zhang[1], and Albert Kausch[2]. [1]Plant Transformation Core Facility, Division of Plant Sciences, University of Missouri, Columbia, MO 65211; [2]Department of Cell and Molecular Biology, University of Rhode Island, RI 02892; and [3]Department of Molecular, Cellular & Developmental Biology, Yale University, New Haven, CT 06511-2106. Email: mookkanm@missouri.edu; zhangzh @missouri.edu; apkausch@uri.edu

We have investigated the use of morphogenic regulators to overcome tissue culture restrictions to standard genetic transformation of plants in maize (Zea mays L.) inbred B73 and sorghum (Sorghum bicolor (L.) Moench) cv P898012. Current standard transformation systems are significant bottlenecks for genetic, genomic and crop improvement studies (Altpeter et al. 2016). We have examined the use of variable co-expression of maize transcription factors BABY BOOM and WUSCHEL2 linked to a drought inducible Cre/lox excision system to facilitate recovery of stable transgenic recalcitrant maize inbred B73 and sorghum P898012 without reliance on a chemical selectable marker. The pHP78891 expression cassette contains CRE driven by the maize RAB17 promoter which is drought inducible. When induced by desiccation stress, the CRE, BBM and WUS genes, which are bracketed by loxP sites, are excised. A constitutive UBI promoter directs a ZF Green GFP expression cassette as a reporter outside of the excision sites and provides transient, transgenic and developmental analysis. This system is coupled with evidence for molecular integration and analysis of stable integration and desiccation inducible CRE mediated excision. Agrobacterium-mediated transgenic introduction of this vector showed transient expression of GFP and induced stable somatic embryogenesis in maize B73 and sorghum P898012 explants. Subjection to a desiccation stress in tissue culture, enabled the excision of CRE, BBM and WUS, leaving the UBI: GFP cassette, allowing subsequent plant regeneration and GFP expression analysis. Stable GFP expression was observed in early and late somatic embryos, young shoots, vegetative plant organs, and pollen of T0 plants and in embryos of T1 plants. Transgene integration and expression of GFP positive T0 plants were also analyzed using PCR and Southern blots. This approach enables a significantly increased frequency of transformation from 0 % to 14.9% for maize B73 and to 6 % for sorghum P898012 genotypes without the use of selection agents.

P-3012

Genetic Transformation Approaches to Improve Citrus Greening Tolerant/Resistance in Citrus Trees. AHMAD A. OMAR[1, 2]; Abdullah M. Shohael[3]; and Jude W. Grosser[1]. [1]University of Florida/IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850; [2]Zagazig University, College of Agriculture, Biochemistry Department, Zagazig 44511, EGYPT; and [3]Jahangirnagar University, Department of Biotechnology & Genetic Engineering, Savar, Dhaka-1342, BANGLADESH. Email: Omar71@ufl.edu, Jgrosser@ufl.edu

Citrus greening, also called Huanglongbing (HLB) or yellow dragon disease, is one of the most serious diseases of citrus. HLB, caused by *Candidatus Liberibacter* asiaticus, is a phloem-limited fastidious pathogen transmitted by the Asian citrus psyllid, *Diaphorina citri*, and appears to be an intracellular pathogen that maintains an intimate association with the psyllid or the plant throughout its life cycle. Genetic transformation approaches to achieve tolerant/resistance to HLB is one of the most important tools as a long term solution. Over-expression of the citrus β-1,3-glucanase gene

using constitutive and phloem-specific promoters was performed to achieve this goal. Citrus β-1,3-glucanase cDNA (1011 bp) (GenBank AN AJ000081) was amplified from Valencia leaf and embryogenic callus using PCR with adding a cMyc tag (to facilitate subsequent western analysis). Transformation vector designated as p35SBG3 (constitutive) and pSuc2BG3 (phloem specific). Using a modified Agrobacterium-mediated transformation protocol, more than 60 transgenic lines of sweet orange (Valencia, OLL8, OLL20, and Vernia) and 10 'Duncan' grapefruit were regenerated containing p35SBG3 and 30 containing pSuc2BG3 to over-express the citrus β-1,3-glucanase gene. PCR analysis of these transgenic plants is showing the specific band for the BG gene. Molecular analysis for these plants including Southern, Western blot analysis and RT-PCR are showing the integration and expression of the β-1,3-glucanase gene in the regenerated plants. All transgenic lines have been micro propagated producing 4-5 replicates of each clone to expedite their evaluation under HLB high-pressure condition. The first batch of these developed transgenic plants has been planted in USDA farm at Ft Peirce, FL in July 2015. Transgenic plants in the field were screened using qRT-PCR for the present of HLB pathogen in May 2016 and all of the transgenic plants are showing negative results for HLB. A second screening will be performed in two months and the results will be presented at the meeting.

P-3013

Cell Fusion: A New Method for Transferring Cytoplasmic or Nuclear Traits Between Plants. VLADIMIR SIDOROV, Chuck Armstrong, Thomas Ream, Xudong Ye, and Annie Saltarikos. Monsanto Company, St. Louis, MO. Email: vladimir.a.sidorov@monsanto.com1

The methods alternative to sexual hybridization can be used as powerful tools for crop improvement. Both plastid and nuclear traits have been transferred via protoplast fusion (Sidorov et al., Planta 152, 1981; Gleba et al., Theor. Appl. Genet. 76, 1988); however, regeneration of plants from protoplasts remains difficult for many economically important plant species. Recently, a new technique based on grafting of plants was reported (Thyssen et al., PNAS USA 109, 2012). This asexual method was used for horizontal transfer of cytoplasmic (Stegemann et al., PNAS USA 109, 2012) and nuclear genomes between plants (Fuentes et al., Nature 511, 2014). For plastid or nuclear genome transfer we developed new technology based on wounding of a mixed population of cells of two parents growing in vitro as callus, and defined as "cell fusion". In our proof-of concept experiments, model Nicotiana species were used. Non-organized growing tissue (callus) from tobacco var. Samsun, carrying the nuclear marker genes nptII and uidA (GUS), and tobacco var. Petite Havana, carrying aadA and gfp genes in the plastid genome, were mixed together, wounded with a razor blade and placed for regeneration on selection medium containing both spectinomycin (aadA) and kanamycin (nptII). Plants with aadA and gfp positive plastids and nptII plus uidA positive nuclear background were produced. Molecular analysis confirmed the presence of all four genes in these plants. Morphology and ploidy level analysis confirmed the production of "diploid" plants similar to var. Samsun possessing transformed plastids from var. Petite Havana. Reciprocal crosses between the experimentally produced plants and wild type tobacco confirmed maternal inheritance of aadA and gfp and Mendelian inheritance of nptII and uidA. For transfer of nuclear traits between plants we used two nuclear-transformed parents with different selectable markers; one with nptII (kanamycin resistant), and another with nuclear aadA (spectinomycin resistant). Plants resistant to both antibiotics which also had different visible markers were produced.

P-3014

Seed and Pollen Sterility via *Eco*RI Expression in Genetically Engineered Rice. TAMMY L. STACKHOUSE, Reginald J. Millwood, Francisco J. Palacios, Yi Sang, and C. Neal Stewart Jr. University of Tennessee, Department of Plant Sciences, Knoxville, TN 37996. Email: tstackho@vols.utk.edu

Depending on species, the release of genetically engineered crops into the environment may lead to pollen- and seed-mediated gene flow to wild and weedy relatives, which is of potential regulatory importance. Bioconfinement technologies could be used to prevent gene flow through the expression of ablation agents within pollen or seed. In tobacco, 99 % sterile pollen was produced through the expression of the endonuclease *Eco*RI driven by a pollen-specific promoter from tomato, LAT52. To translate this approach in monocots, we selected 9 pollen- or seed- specific promoters driving *Eco*RI expression. The promoters are from various species with an emphasis on monocots, including maize, rice, barley, switchgrass, and wheat. *Agrobacterium*-mediated transformation of embryogenic rice callus has been performed and forty-two transgenic plants have been PCR confirmed for the *Eco*RI gene. Plants have been regenerated from eight of the nine vectors. The ninth vector used in transformation never yielded transgenic plants, which may be an off-effect from leaky expression in callus. Bioconfinement will subsequently be assessed by progeny analysis from selfing and outcrossing studies. Given the wide array of monocot-derived promoters used in this study, we expect to find a suitable combination that yields a high degree of tissue-specific *Eco*RI expression. Furthermore, we envisage this system will be translatable to other monocot species.

P-3015

Development of a High-throughput Protoplast Transformation System for Rapid Screening of Transgenes and Regulatory Elements in Soybean (*Glycine max* L.). MST SHAMIRA SULTANA[1], Taylor P. Frazier-Douglas[1], Reginald J. Millwood[1], Scott C. Lenaghan[2], and C. Neal Stewart Jr[1]. [1]Department of Plant Sciences, University of Tennessee, Knoxville, TN and [2]Department of Food

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Genetic engineering of soybean (*Glycine max* L.) offers the potential to increase crop yields and to develop disease, pest, and herbicide resistant varieties. To date, several transformation methods have been established in soybean to create stable transgenic varieties; however, these methods are limited, with low transformation efficiencies and long regeneration times. In this study, a high-throughput screening system was developed in soybean protoplasts, which allows for rapid analysis of putative genes of interest, as well as, functional genetic elements such as enhancers and promoters. Cell suspension cultures for soybean cv. Williams 82 were established from leaf callus initiated on MS basal media supplemented with 12 μM 2,4-dichlorophenoxyacetic acid. Protoplasts were isolated in high quantities ($1.07 \pm 0.257 \times 10^6$ viable protoplasts per 1 ml packed cell volume) using food grade enzymes. PEG-mediated transfection was carried out using a plasmid containing the cauliflower mosaic virus (CaMV) *35S* promoter to drive the expression of the gene for the *mEmerald* green fluorescent protein (GFP). The overall transformation efficiency achieved using the methods developed in this work was ~23 ± 2%. In conclusion, a reliable, low-cost, and rapid protocol for transient gene expression in soybean was developed. This system can be amended for use on a robotic platform for high-throughput screening of novel genetic components, including synthetic and/or inducible soybean promoters, which will facilitate the development of novel soybean vectors, enhancing current soybean transformation efforts.

P-3016
A Rapid Transformation Method for Switchgrass Using Culm Tissue NING WANG[1], Keith Lowe[1], Wayne Parrott[2], and William Gordon-Kamm[1]. [1]DuPont Pioneer, 8305 NW 62nd Ave, Johnston IA and [2]The University of Georgia, Athens, GA. Email: ning.wang@pioneer.com

Switchgrass (*Panicum virgatum*) is a North American perennial grass and is a C4 species adapted to a wide range of environments. Due to its potential as a bioenergy crop, there is strong interest to develop increasingly more versatile genetic transformation methods to introduce biotechnology traits to improve biomass productivity, quality, and amenability for biofuel production. The use of two maize morphogenic genes, WUS and OPD2, effectively stimulates tissue culture response in many maize inbreds and other crop species, such as sorghum, wheat, and rice (Plant Cell 28:1998-2015, 2016). In this study, we used our *Agrobacterium*-based maize morphogenic gene transformation system to rapidly transform switchgrass. Readily available culm tissue from plantlets grown *in vitro* served as explants for T-DNA delivery and subsequent callus induction. The transformed callus tissues were selected based on embryogenic morphology and all embryogenic callus was GFP positive. Embryogenic callus readily regenerated into transgenic plants. The morphogenic genes were flanked by loxP sites, and thus could be excised following natural or induced expression of the CRE recombinase gene. This system can generate transgenic plants in 12 weeks, which is considerably shorter than current methods.The University of Georgia provided materials for this study.

P-3017
Plant Cell Secreted Growth Factor Tailored to Hematopoietic Stem Cell Application. XIAOTING WANG and Jianfeng Xu. Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401. Email: Xiaoting.Wang@smail.astate.edu, jxu@astate.edu

Generation of red blood cells from hematopoietic stem cells (HSCs) used for blood transfusion represents one of the focus in regenerative medicine. Stem cell factor (SCF) is a key growth factor for proliferation and differentiation of HSCs that could differentiate into red blood cells (RBCs). However, generation of RBCs requires significant quantity and high quality of growth factors including SCF, making manufacturing at large scale cost prohibitive. Plant cell culture is proposed to be a promising bioproduction platform for growth factors based on the advantages of safety, fast growing, no risk of human pathogen, and easy downstream separation and purification. However, low protein productivity is a common bottleneck towards commercialization of this production platform. These bottlenecks will be addressed by the designer molecular carrier engineering technology (Hydroxyproline-O-glycosylated peptides molecular engineering technology) that may dramatically increase the secreted yields of expressed growth factors in plant cell culture. The SCF was expressed in tobacco BY-2 cells with a molecular carrier either at N-terminus or C-terminus. The secreted protein yields were determined. The biological activities of the secreted SCF was assayed for their function in stimulating the proliferation of human TF-1 cells. Our research may provide a novel and promising plant cell-based platform to produce large quantity of hematopoietic growth factors that facility the stem cell research and clinical applications.

P-3018
Trait Research: Gene Discovery and Validation at Bayer Crop Science. TRAIT RESEARCH GROUP, BAYER CROP SCIENCE, 3500 Paramount Parkway, Morrisville, NC 27560. Email: allan.wenck@bayer.com

Trait Research's goal is to create industry leading gene-based solutions to improve food, feed and fiber crops that benefit farmers and society. We do this through identifying or creating new versions (induced mutations) of native genes and/or introducing novel genes from other plant and organism to improve specific traits. One of the foundations for this work is our MiDAS trait discovery platform. This platform contains the industries' largest collection of sequenced microbes with >120,000 sequenced bacterial

species isolated from environmental samples throughout the world. Use of this collection has led to the isolation of hundreds of novel genes for use not only in production of plants with new traits, but also for other portions of the Bayer CropScience. Our gene discovery and validation workflows will be highlighted.

P-3019

Changes in Soil Microbial Community Diversity in Response to Agrochemical Selective Pressure in a Packed-bed Reactor System. HUNTER D. WHITTINGTON[1], M. Andrea Azcarate-Peril[2], Mahatam Singh[3], and Jose M. Bruno-Barcena[1]. [1]Department of Plant and Microbial Biology, NC State University, Raleigh, NC; [2]Department of Medicine, UNC-Chapel Hill, Chapel Hill, NC; and [3]BASF, Research Triangle Park, NC. Email: hdwhitti@ncsu.edu, Corresponding Author: jbbarcen@ncsu.edu

One of the more esoteric aspects of the use of agricultural chemicals is their transient effect on the soil microbial population, and conversely the impact that soil microbes have on these compounds. Surveying the vast soil microbial community has recently become more tangible with the decline in price of high-throughput sequencing. Furthermore, advancements in chemical analytics have allowed for detection of labeled compounds even in a chemically complex medium. In this study, we use a combination of next-generation sequencing and liquid chromatography followed by tandem mass spectrometry to examine both aspects of this chemical-microbe interaction in an *in vitro* packed-bed reactor system. Upon inoculation of a packed-bed reactor with a soil sample treated with a fungicide, and establishing a chemostat using a complex medium containing said agrochemical, we found that the microbial community responded to variations in the dilution rate with significant changes at the order level. The Burkholderiales were dominant at low dilution rates and levels of residual fungicide were minimal. However, at higher dilution rates the Clostridiales were the dominant organisms and levels of residual fungicide were significantly higher. We believe that these *in vitro* systems can be used to simulate degradation of agrochemicals in the soil, allowing for a cleaner analysis and more rapid results.

P-3020

Engineering Designer Glycopeptides as a Molecular Carrier for Directing Cell Wall Depolymerizing Enzymes Accumulation in Planta. TRISTEN WRIGHT[1], Hong Fang[2], Ningning Zhang[1], Gregory Phillips[2], Brett Savary[1,2], and Jianfeng Xu[1,2]. [1]Arkansas Biosciences Institute and [2]College of Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: tristen.wright@smail.astate.edu

Engineering the plant cell wall with thermostable cell wall depolymerizing (CWD) enzymes represents a promising solution to reduce the overall cost of plant biomass processing for the production of biofuels and/or other biobased products. However, many *in planta*-expressed CWD enzymes targeted for secretion were mainly retained inside the cytoplasm membrane instead of being secreted into the cell wall matrix. This project aims to leverage an innovative strategy unique to plants − hydroxyproline (Hyp)-*O*-glycosylation − for *de novo* design and engineering of novel Hyp-*O*-glycosylated peptides (HypGPs) that can function as a molecular carrier for the heterologous CWD enzymes expressed *in planta* to maximize their functions in tailoring cell wall composition and architecture. Different designs of HypGP tags, including an extensin-based $(SP4)_{18}$ module and some arabinogalactan proteins (AGP)-based $(SP)_{32}$, $(AP)_{20}$ and $(TP)_{20}$ modules, were engineered into tobacco plants as fusion with a reporter protein (GFP) to characterize the Hyp-*O*-glycosylation and molecular carrier function of the designer HypGP modules, and to assess the phenotypic change of the transgenic plants. While the $(SP4)_{18}$ module was constitutively Hyp-*O*-glycosylated but with a rate-limiting glycosylation process, none of the engineered AGP-based modules were Hyp-*O*-glycosylated. A novel *O*-glycosylation signal peptide tag facilitating the full glycosylation of the designer modules was finally identified. To establish a proof of concept of the HypGP engineering utilized for cell wall reconstruction, a thermostable CWD enzyme, E1 endoglucanase from *Acidothermus cellulolyticus* was engineered into tobacco plants with/without a HypGP tag. The engineered HypGP tag improved the enzyme accumulation *in planta* and increased the biomass saccharification efficiency by 3.2-fold (compared with the wild type plant) without significantly affecting the biomass accumulation yields.

P-3021

Hydroxyproline-O-glycosylation: Application for Engineering Novel Designer Biopolymers In Planta for Enhanced Plant-based Production. JIANFENG XU[1,2], Ningning Zhang[1], Tristen Wright[1], Gregory Phillips[2], and Brett Savary[1,2]. [1]Arkansas Biosciences Institute and [2]College of Agriculture and Technology, Arkansas State University, Jonesboro, AR 72401. Email: jxu@astate.edu

Hydroxyproline-*O*-glycosylation involves post-translational hydroxylation of proline (Pro) to hydroxyproline (Hyp) and subsequent *O*-glycosylation, a modification that is unique to higher plants and green algae. There is evidence that strategically designed Pro-rich peptide motifs (e.g. tandem repeats of "Ser-Pro" dipeptide motifs) engineered in plants can direct extensive *O*-glycosylation on each Pro residues, and function as a "molecular carrier" in facilitating the extracellular secretion of tagged proteins. This demonstrated the feasibility of Hyp-*O*-glycosylation based biopolymer design *in planta* and triggered the applications in the following two aspects: 1) Boosting the extracellular secretion of the recombinant proteins in plant cell culture. Dramatically increased secreted protein yields up to 1500-fold were detected when the proteins, including a reporter protein GFP and many therapeutic proteins such as interferon α2, human

growth hormone, interleukin-12 and stem cell factor, were expressed in tobacco BY-2 cells with a designer biopolymer tag comprised of 32 tandem repeats of "Ser-Pro" motif or 20 repeats of "Ala-Pro" motif; 2) Engineering the plant cell wall for improved biomass processability. Expressing a thermostable endoglucanase E1 (from *Acidothermus cellulolyticus*) in tobacco plants with a designer biopolymer tag comprised of 18 tandem repeats of "Ser-Pro-Pro-Pro-Pro" motif could facilitate the deposition and stabilization of the enzyme in the cell wall matrix, leading to increased biomass saccharification efficiency by 3.5-fold compared to wild type plants. The growth and biomass accumulation of the transgenic plants was not significantly impacted.

P-3050

Application of Bean Based Transient Technology for Novel Insecticidal Protein Discovery. JIM ENGLISH[1], Matthew J. Heckert[1], Janet Rice[2], Natalie Stoner[2], Deborah Clark[2], Ericka Veliz[2], and Jennifer Barry[3]. DuPont Pioneer [1]4010 Point Eden Way, Hayward, CA 94545; [2]Experimental Station 353, 200 Powder Mill Road, Wilmington, DE 19803; and [3]7300 NW 62nd Avenue, Johnston, IA 50131. Email: james.english@pioneer.com

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

CELL BIOLOGY

P-3022

Regulation of Cell Wall Strength and Salt Tolerance by The *Arabidopsis* MUR4. OMAR ZAYED and Chunzhao Zhao. 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 are 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.

P-3023

Cytological and Biochemical Change in Plant Cell Under *In Vitro* Selection and *In Vivo* Culture of Onion (*Allium Cepa*). ABDELRAHEM A. YOUSEF[1], A.T. Abdel-Rahem[2], K. Z. Ahmed[2], and S. A. Osman[2]. [1]Field Crops Research Institute, Agriculture Research Center, Giza, Egypt and [2]Department of Genetics, Minia University, El-Minia, EGYPT. Email:Abdelrahem_yousef@live.com

The present study was undertaken to identify genetic variations occur through *in vitro* selection for *Allium* white rot disease (*Sclerotium cepivorum*) tolerance in three onion Egyptian varieties (Giza 20 (G20), Giza 6 (G6) and Beheri Red (BR)). The genetic changes detected by cytogenetical examination of mitotic index (MI), that considered as a parameter to estimate the frequency of cellular division and to detect the aberrations percentages of micronuclei studied. Micronuclei are true mutagenic aspects and lead to a loss of genetic material. The mitotic index is calculated as percentage of dividing cells of donor plants of onion genotypes (G20, G6 and BR) and their derived calli. Total dividing cell was ranged between 2.88±0.2 for G20 and 5.70±0.9 for BR. MI of donor plants of all tested cultivars was higher than derived calli. Abnormalities of micro-nuclei percentage ranged from 0.05% to 0.10% in donor plant and between 0.22% to 0.76% in calli, and abnormalities percentage of derived calli of all cultivars were higher than donor plants. The gene expiration variations detected by using isozymes as biochemical markers to characterize changes in isozyme banding patterns. The band patterns of esterase (EST) and catalase (CAT) were used to detect the genetic variability among onion sensitive plants, induced calli and regenerated somaclone plants from

calli of different genotypes. Esterase band pattern shows 5 bands, the results showed that *EST-1* band only appear in calli growing on toxic medium (MS medium supplemented with 3 mM oxalic acid) with of BR and G20 and not appear in G6. Regenerated plants from surviving calli of all tested genotypes grown on toxic media were shown two specific bands *EST-3* and *EST-4*. Respect to catalase band pattern of onion genotypes, the result showed that catalase has two major bands, one of them *CAT-2* appears in all lanes (1 through 12) except lane 4 (G20 donor plants), on the other hand specific *CAT-1* band found in all regenerated plants and calli grown on free toxic medium of BR and G20, putative calli G20 and BR.

CELLULAR AND MOLECULAR TOXICOLOGY

P-3024

Microbial Community Structure in Rhizosphere of Arsenic Hyperaccumulator *Pteris vittata* Grown on Arsenic-Contaminated Soil. OSAGIE IDEHEN, Marceline Egnin, Ramble Ankumah, Raymon Shange, Gregory C. Bernard, Conrad Bonsi, and Foaziatu Burkari. College of Agriculture, Environment and Nutrition Sciences, Tuskegee University, Tuskegee, AL. Email: oidehen1985@ mytu.tuskegee.edu

In the rhizosphere region, plant roots secrete exudates which contain a broad range of phytochemicals known to influence growth and activities of soil microbes. Plant-microbe interactions play important roles in many ecological processes including the biogeochemical cycling of nutrients in the soil; carbon sequestration; solubilization of minerals; and cycling of contaminants, like arsenic. The arsenic hyperaccumulator, *Pteris vittata* has been suggested as ecologically sustainable alternative for use in bioremediation of arsenic contaminated environment. The rhizosphere of *Pteris vittata* is a dynamic system, secreting exudates known to attract growth promoting and arsenic resistant microbes. These plant-associated microbes impact health, growth and development of *Pteris vittata* and hence its ability to phytoremediate arsenic contaminated environment. Although microbial metabolism has been associated with arsenic cycling in soil, there is very little information on the how these microbes respond to different concentration of arsenic. A full understanding of interaction between *Pteris vittata* and the microbial communities in it rhizosphere is important for the development of sustainable arsenic remediation strategies. In this study, we evaluated the diversity of microbial communities in the rhizoshpere of *Pteris vittata* grown in soil spiked with increasing concentrations of arsenic (0, 50, 100, 150, 200, and 500 mg As kg^{-1} soil) after 98 days. Rhizosphere and bulk soil samples were taken at 98-day, extracted for total DNA and subjected to paired-end sequencing (Illumina Mi-Seq) and bioinformatic analysis (Ribosomal Database Project). Preliminary results show Ascomycota as the dominant fungi phylum and the proteobacteria for the bacteria, and Crenarchaeota for the archaea. Work supported by: USDA USDA-NIFA 1890 Grant#2014-38821-22448, GWCAES-CAENS.

CELLULAR PATHOLOGY

P-3025

Evaluation of Plant Extracts on Root-Knot Nematodes (*Meloidogyne Spp*.) Under In Vitro and In Planta Conditions. GREGORY C. BERNARD[1], Marceline. Egnin[1], Willard Collier[2], Melanie Groves[1], Osagie Idehen[1], Foaziatu Bukari[1], Conrad Bonsi[1], Desmond Mortley[1], and Kathy Lawrence[3]. [1]College of Agriculture, Environment and Nutrition Sciences, Tuskegee University, Tuskegee AL; [2]College of Arts and Sciences, Tuskegee University, Tuskegee AL; and [3]Department of Entomology and Plant Pathology, Auburn University, Auburn AL. Email: gbernard4673@ mytu.tuskegee

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a staple food crop providing a significant source of energy and nutrition for consumers. Production of sweetpotato is severely decreased by the presence of root-knot nematodes (*Meloidogyne spp*.), which rank as the most economically and scientifically important plant-parasitic nematodes. Currently, there is an increasing interest in alternative measures of nematode management due to governmental restrictions of chemical pesticides and increasing nematode resistance rates. This study seeks to evaluate the nematicidial properties of ethanol extracts from plant species; Buzz button (*Acmella oleracea*), Comfry (*Symphytum officinale*), Germander (*Teucrium chamaedrys*), Chives (*Allium schoenoprasum*), and African Violet (*Saintpaulias ionantha*) against root-knot nematode *Meloidogyne incognita* under invitro and inplanta conditions. In the initial studies, the effects of plant extracts on egg hatching and survival of second stage juveniles (J2s) will be evaluated under laboratory conditions. In greenhouse-pot trials, the effects of these plant extracts will be assessed on susceptible sweetpotato and peanut varieties by phenotypic screening analyses of necrosis, egg counts, root fresh weights, plant height and soil nematode numbers. In addition, genetic expression analyses will be performed to determine the molecular responses of candidate defense-related genes in plant roots under treatment. The identification of novel anthelmintic plant extracts may contribute towards the development of plant-based products that could provide an alternative means of nematode control which is safer, more cost-effective and as efficacious as the conventional pesticides currently used for pest management in major production crops. Supported by USDA USDA-NIFA 1890 Grant#2014-38821-22448; Tuskegee University, GWCAES, NIFA-EVANS-ALLEN.

DEVELOPMENTAL BIOLOGY

P-3026

Exposing Undergraduates to Plant Cell Culture Techniques Through Designed Regeneration of *Saintpaula* Cultivars. MARCELINE EGNIN, Larencia Williams, Foaziatu Bukari, Osagie Idehen, Gregory C. Bernard, Candice Gresham, Donald Brooks, Capri Charlston, Sadiyyah Muhammad, Lashachiah Parks, Jacob Fitch, Melanie Groves, Anna Cobb, and Ibreed. College of Agriculture, Environment and

Nutrition Sciences, Tuskegee University, Tuskegee, AL. Email: megnin@mytu.tuskegee.edu

The art of plant tissue culturing provides powerful in-vitro experiential learning avenues for the next generation breeders. A stepwise hands-on tissue culture and gene editing protocol was designed for students to investigate factors affecting African Violet, *Saintpaulia ionantha* (Wendl.), regeneration in soil-grown White (WH), Deep Purple (DP), Violet (AV), Red (RD) and Pink (PK) flower color cultivars. Adventitious shoot regeneration potential of cultivars on MS basal medium supplemented with adenine sulfate, sodium phosphate, thyamine-HCl, myo-inositol, sucrose, putrescine and growth hormones, 2mg/L IAA in combination with differing levels of Kinetin or BAP was compared. Leaf position taken into consideration showed that explants isolated from apical leaves exhibited higher regeneration efficiency than those from basal portions. Shoots arose from calli throughout the surface of leaf explants more profusely on the abaxial side in induction media containing 2mg/L IAA with 2mg/L Kinetin or BAP and 20mg/L Putrescine. Regardless of genotypes or hormones, the optimized protocol resulted in regeneration frequencies from 25% to 45% for both WH and RD, 40% to 58% for PK, while AV and DP exhibited the highest rates (70% to 100%). Regeneration period of three weeks was attained only in AV with 100% rootable shoots. Addition of putrescine effectively promoted higher shoot production easily rooted on MS medium (1mg/l IAA). Hence, students gained best practices in plant tissue culture, media formulation, creating and maintaining aseptic environment. This experimental design and real time result interpretation provided novel approaches for furthering students' general understanding of the fundamental of culture production invitro. USDA-NIFA Grant #2014-38821-22448; TU-GWCAES.

P-3027

Profiling and Quantification of Phytocannabinoids for the Classification of *Cannabis sativa* L. S. CHANDRA[1], H. Lata[1], C. Gon[1], I.A. Khan[2], and M. A. ElSohly[3]. [1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677; [2]Department of Biomolecular Sciences, Division of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677; and [3]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi, University, MS 38677. Email: suman@olemiss.edu

Cannabis is one of the oldest plants cultivated for fiber, food, oil, medicinal and ritual use or as a recreational drug for millennia. As a plant, cannabis is an annual, normally dioceous and occasionally monoecious, wind pollinated species which is highly allogamous (cross fertilization) in nature. It belongs to family Cannabaceae. Whether the genus Cannabis contains one species or more has been a matter of debate. The pioneer modern taxonomist Swede Carl Linnaeus (1737) treated cannabis as a single species *Cannabis sativa*, whereas Lamarck (1785) described 'Indian cannabis strain' taxonomically different and gave it a specific name '*Cannabis indica*'. The debate continues, Cannabis is still treated as one species by some groups, while others describe it as two to four species. On other hand, Cannabis can also be classified based on their cannabinoids profile. In general, cannabis is categorized in three different popular chemotypes *i.e.* high Tetrahydrocannabinol (THC) varieties, high Cannabidiol (CBD) varieties and mix or intermediate varieties. However, Cannabis can also be selectively breed for chemotypes high in other cannabinoids such as Tetrahydrocannabivarin (THCV), Cannabichromene (CBC), Cannabigerol (CBG) and Cannabinol (CBN). In the present study, different varieties of Cannabis were grown to maturity and periodically analyzed (from vegetative to flowering stage) for different cannabinoids content using gas chromatography-flame ionization detector (GC-FID). Based on the cannabinoids profile and content, plants were screened and categorized as high THC, high CBD and intermediate (THC~CBD) varieties. Vegetative cuttings of these varieties are multiplied and conserved for the future use.

P-3028

Further Studies of Putative Gametic Calli from Soybean Anther Cultures. MARTINA GARDA[1,2,3], BRETTON HALE[1], M. Lowe[1], S. Goodling[1], N. Rao[1], K. Bade[1], and G. C. Phillips[1,3]. [1]Arkansas State University, College of Agriculture and Technology. PO Box 1080, State University. AR 72467; [2]Arkansas State University, College of Mathematics and Sciences. PO Box 1030, State University. AR 72467; and [3]Arkansas State University, Arkansas Biosciences Institute. PO Box 639, State University, AR 72467. Email: martina.garda@smail.astate.edu, brett.hale@smail.astate.edu

Soybean androgenesis has been a challenging process yet to be advanced significantly after years of attempts. Our research has explored alternative triggers not yet reported in the soybean literature which may influence androgenetic responses in soybeans, by scoring for putative gametic calli (PGCs). We have identified a few pyramidal trigger factors such as 11 ^{0}C incubation, using a nitrogen starvation medium, using sorbitol as supplemental osmoticum, and using high levels of 2,4-D (40 mg/L) which appear to stimulate the formation of PGCs in all four genotypes tested: Jack, Williams82, Thorne, and IAS-5. Recent results indicate that the best pretreatment of donor plants is a 72-hour shock of donor plants at 10 ^{0}C day/8 ^{0}C night followed by 4 ^{0}C overnight, which increased the expression of putative gametic calli (PGC) in soybean anther cultures compared to no pretreatment, 10 ^{0}C day/8 ^{0}C night alone, or 4 ^{0}C overnight alone. Jack, Williams82 and Thorne genotypes show PGC formation at such conditions using 40 mg/L 2,4-D, while the IAS-5 genotype prefers no 2,4-D. We continue to observe other responses from anthers such as:

engorgement of the central portions and the lobes of anthers, release of some microspores from anthers, as well as some apparent anther dehiscence that awaits future observations. We are in the process of comparing pH gradients to observe how these affect IAS-5. The results presented and those in progress will provide additional descriptions that may facilitate development of a future soybean and legume model androgenesis system.

P-3029

Direct Embryogenesis From Maize Embryos and Leaves. TODD JONES, Keith Lowe, George Hoerster, Ning Wang, Maurcio LaRota, Craig Hastings, and William Gordon-Kamm. DuPont Pioneer, 8305 NW 62nd Ave, Johnston IA. Email: Todd.j.jones@pioneer.com

Transformation of maize immature embryos using the combination of a phospholipid transferase promoter driving the maize Babyboom (BBM) along with a weak promoter driving the maize Wuschel2 (WUS2) results in the rapid stimulation of abundant somatic embryos arising from the scutellar epithelial layer and T0 leaf epidermis. Histological analysis indicates a single-cell origin for the somatic embryos and development closely recapitulates early zygotic embryogenesis. These somatic embryos can be directly regenerated into non-chimeric T0 plants without a callus phase. Preliminary testing indicates that this process is genotype independent and plants can be sent to the greenhouse in as little as one month after transformation. In contrast to our earlier work, T0 plants produced using this method are fertile without excision of BBM and WUS2 transgenes.

DIFFERENTIATION

P-3030

Development of an Efficient Agrobacterium Mediated Transformation Method for Octaploid Strawberry (Fragaria x ananassa Duch.). MANMEET SINGH, Eric Zinn. and Troy Weeks. Simplot Plant Sciences, Boise, ID. Email: manmeet.singh@simplot.com

Cultivated strawberry (Fragaria x ananassa Duch.) is a commercial crop of high economic value. Unlike wild species Fragaria vesca (2n=2x=14), cultivated strawberry (2n=8x=56) lacks an efficient transformation system to facilitate the introgression of genes of interest into strawberry. In our attempt to establish a highly efficient transformation system in octaploid strawberry, we examined the regeneration capacity of two octaploid genotypes, Laboratory Festival #9 (LF9) and Albion. Leaf explants from in vitro cultured plants resulted in 100% regeneration frequency in both genotypes on medium containing 2 mg L-1 TDZ [1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea] and 0.5 mg L-1 IBA (Indole-3-butyric acid). Subsequently, we employed transient GUS transformation assays to optimize various transformation parameters. A 7-day pre-culture of leaf explants followed by inoculation with Agrobacterium solution containing 200 μM acetosyringone and Tween-20 resulted in a five-fold improvement in transient GUS transformation frequency. When all of the parameters were combined along with a 5-day recovery period on medium containing 100 μM validamycin A, a transformation frequency of 84% was observed in LF9 using the neomycin phosphotransferase selectable marker gene. In addition, using a mutated potato acetolactate synthase selectable marker gene resulted in a 100% transformation frequency in LF9. In this study, we have successfully demonstrated the development of a high throughput transformation method to improve octaploid strawberry varieties through introduction of commercial traits, which will complement traditional breeding efforts.

GENETIC ENGINEERING

P-3031

Rapid and Efficient Production Maize Transformation at DuPont Pioneer. M. ARLING, T. Hu, N. Wang, A. Anand, K. Dan, W. J. Gordon-Kamm, M. Hastings, G. Hoerster, W. Hua, M. Klein, K. Lowe, J. May, J. Martinac, L. Ryan, C. Sweeny, E. Wu, J. Xu, Y. Yan, J. Chow-Yiu, L. Zhi, W. Zhu, Y. Zhu, and J. Zobrist. DuPont Pioneer, 8305 NW 62nd Ave, Johnston, IA 50131. Email: maren.arling@pioneer.com

Historically, transformation of maize has been confined to one or two genotypes, although recent developments using the morphogenic genes Babyboom (BBM) and Wuschel (WUS2) have dramatically improved this situation. Within the last few years, the use of BBM and WUS2 has been reported to both increase transformation frequencies and extend the range of inbreds that are amenable to transformation. Here we describe improved BBM and WUS2 expression cassettes which enable elimination of the traditional callus stage of plant transformation. Overexpression of BBM and WUS2 with these new cassettes results in the direct formation of somatic embryos. This embryogenic tissue is much more amenable to traditional transformation, as well as to more modern techniques such as site-specific integration and gene editing. Direct somatic embryogenesis not only reduces the transformation time frame from infection to T0 plants with well-established roots from about 80 days to about 40 days, but also significantly increases the transformation frequency. For inbreds in routine transformation, the transformation frequency (measured as the number of transgenic plants recovered per embryo infected) ranges from 15 to 44%, with the frequency of single copy intact events ranging from 2.5-4.2%. In early tests we used Cre-driven by an early embyrogenesis promoter (GLB1:PRO) to excise BBM and WUS2 and activate a selectable marker PMI. Our next generation of this system delivers marker-free plants with only the trait gene(s) in the introduced DNA. The elimination of the selectable marker and development genes is enabled by heat-inducible Cre-mediated excision cassettes.

P-3032

Advances in Gene Editing of Animals and Plants at Precision BioSciences. DAVID NICHOLL[1], D. MacLeod[1], J. Antony[1], A. Martin[1], R. Moser[2], A. Hekele[1], K. Wetzel[1], A. Brown[1], M. Triggiano[1], J. Hux[1], C. Pham[1], V. Bartsevich[1], C. Turner[1], J. Lape[1], S. Kirkland[1], C. Beard[1], J. Smith[1], M. Hirsch[2], M. Nicholson[1], D. Jantz[1], B. McCreedy[1], J. Wilkinson[1], S. Dura[1], K. Mayo[1], A. Reaves[1], M. Nguyen[1], and J. Salmeron[1]. [1]Precision BioSciences, Durham, NC 27709 and [2]Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599. Email: david.nicholl@precisionbiosciences.com

At Precision Biosciences we have developed a proprietary genome editing platform called ARCUS, with positive attributes such as small size, unparalleled sequence specificity, and programmable for custom gene editing applications. Each ARCUS reagent is optimized using a set of proprietary in silico and lab-based techniques to ensure maximum gene editing efficiency with minimum off-target activity. Here we will highlight progress to utilize this technology for the development of new plant varieties and to fight cancer. We demonstrate the editing of specific genes in specialty crops to deliver improved characteristics for farmers, processors and consumers. In healthcare recent advances in gene editing of T cells have led to successful treatment of cancers. Specifically, chimeric antigen receptor (CAR) T cell therapies has shown great response to hematological malignancies. These clinical trials have used autologous patient cells as the starting material for the T-cell gene editing. This strategy poses significant manufacturing challenges and may not be feasible for many patients due to advanced disease state or a lack of suitable numbers of CAR T cells. Alternatively, T-cells from a healthy donor can be used to produce an allogenic CAR T therapy provided you can make the cells incapable of eliciting a graft versus host response. One approach to achieve this is to eliminate the expression of the endogenous T cell receptor (TCR) through gene editing. We report a streamlined strategy for generating allogeneic CAR T cells by targeting the insertion of a CAR transgene directly into the native TCR locus using an ARC nuclease. We demonstrate that anti-CD19 T cells produced in this manner do not express endogenous TCR, exhibit potent effector function in vivo, and mediate clearance of CD19+ tumors in an in vivo mouse model.

INFECTIOUS DISEASES

P-3033

Shoot Regeneration from Leaves of Rootstock M9 and M26 and Virus Elimination in *Malus pumila*. JIN-HO KIM[1], Byung-Jin Cha[2], Daeil Kim[1], and So-Young Park[1]. [1]Department of Horticultural Science, Division of Animal, Horticultural and Food Sciences, Chungbuk National University, REPUBLIC OF KOREA and [2]Department of Plant Medicine, Chungbuk National University, REPUBLIC OF KOREA. Email: kimjh256@gmail.com, soypark7@cbnu.ac.kr

Apples are one of the most important fruit crops. However, most apple trees are infected with viruses. The migration of the viruses inside the trees is either via cell-to-cell movement through plasmodesmata or via vascular bundles. In this study, the shoot regeneration method was optimized in rootstock M9, M26, and scion 'Hongro' of *Malus pumila*. Furthermore, the effect of shoot regeneration on elimination of *Apple stem pitting virus* (ASPV) and *Apple stem grooving virus* (ASGV) using adventitious shoots regenerated from leaf segments was also studied. For this, leaf segments of *in vitro*-grown M9 and M26 were cultured on MS medium supplemented with different concentrations of thidiazuron (TDZ 0-9.08 μM), BA (0-8.87 μM), and NAA (0-1.07 μM). After 8 weeks of culture, M9 displayed a high regeneration rate (10%) and the highest number of shoots (2.0 ea/explant) on the medium containing 4.54 μM TDZ and 0.2 μM NAA. The leaf segments of M26 exhibited a shoot regeneration rate of approximately 42.5–47.5% on medium containing 2.0 μM TDZ and 0.1–0.2 μM NAA, and the highest number of adventitious shoots were formed on the medium containing 0.5 μM TDZ and 0.1 μM NAA. To evaluate the elimination of ASPV and ASGV by leaf segment culture, the virus-infected scion cultivar 'Hongro' was used. Around 5.8% of *in vitro*-grown 'Hongro' leaf segments produced adventitious shoots on 8.87 μM BA, whereas M9 and M26 showed the best results on medium containing TDZ. The two viruses (ASPV and ASGV) were analyzed from regenerated shoots of 'Hongro' using RT-PCR. These data may facilitate efforts to produce virus-free apple rootstock M9 and M26 and scion 'Hongro,' which are commercially important apple cultivars in Korea.

MICROPROPAGATION

P-3034

Modelling Ion Requirements of Hazelnuts Using Decision Tree Analyses. MELEKSEN AKIN[1], Ecevit Eyduran[2], Randall P. Niedz[3], and Barbara M. Reed[4]. [1]Igdir University, Agricultural Faculty, Department of Horticulture, Igdir, TURKEY; [2]Igdir University, Agricultural Faculty, Department of Animal Science, Biometry Genetics Unit, Igdir, TURKEY; [3]USDA-ARS Horticultural Research Laboratory, 2001 South Rock Road, FL 34945; and [4]Retired-USDA-ARS, National Clonal Germplasm Repository, 33447 Peoria Rd, Corvallis, OR 97333. Email: akinmeleksen@gmail.com

Salts are generally utilized as factors within the experimental design in tissue culture optimization studies. Using the major nutrient salts as inputs leads to ion confounding. The more appropriate approach for plant nutrition research is using ions as predictors, because the exact effect of each ion in a salt can not be determined. Computer generated D-optimal design was employed with NH_4^+, Ca^{2+}, Mg^{2+}, SO_4^{2-} and PO_4^{3-} ions as explanatory variables. K^+ and NO_3^- ions were included to make the pH neutral and were considered as inputs within the analyses. The Chi-Squared Automatic Interaction Detection (CHAID) regression tree was used to

analyze the outputs of 'Barcelona', 'Jefferson' and 'Wepster' hazelnuts. The CHAID data mining algorithm generated easy to interpret visual trees showing the significance of factors and their non-linear relationships on the shoot responses by providing the required optimal cut-off amounts of the ions. Shoot quality was affected by K^+, NO_3^- and NH_4^+. The most significant input on shoot elongation was NH_4^+ followed by Mg^{2+}, NO_3^- and Ca^{2+}. Multiplication was affected by Ca^{2+} and genotype. The optimal cut-off amounts for good shoot quality, elongation, and multiplication for hazelnut could be considered as: $NO_3^- < 88$ mM, $NH_4^+ < 20$ mM, $Ca^{2+} < 5$ mM, $Mg^{2+} > 5$ mM and $K^+ < 46$ mM.

P-3035

A System for Passive Humidity Control of *In Vitro* Culture Vessels. K. F. PIUNNO and A. M. P. Jones. Gosling Research Institute for Plant Preservation, University of Guelph, Plant Agriculture, 50 Stone Road East, Guelph, ON, CANADA. Email: kpiunno@mail.uoguelph.ca

High relative humidity can contribute to developmental problems known as hyperhydricity (vitrification) for *in vitro* plants. Forced air systems to control the headspace are costly and cumbersome. Ventilated lids are an inexpensive solution, but are neither uniform nor accurate. This project prototyped and evaluated a novel system which is placed inside the culture vessel and passively controls relative humidity. Health improvements to in vitro plants included increased chlorophyll content, increased desiccation tolerance and reduced symptoms of hyperhydricity. This system could also be used to harden plants to *ex vitro* conditions and to study the effects of relative humidity *in vitro*.

P-3036

Comparative In Vitro Growth and Corm Formation of *Sagitaria latifolia* Genotypes. PAULINA H. QUIJIA and Michael E. Kane. University of Florida, Environmental Horticulture Department, 2043 IFAS Research Drive, Gainesville, FL, 32611. Email: paulinaquijial@ufl.edu

In vitro common garden experiments were conducted using four genotypes of the wetland species *S. latifolia* established from four populations (North Carolina, South Carolina, central Florida and south Florida). In vitro growth and corm formation responses of genotypes were compared to evaluate possible ecotypic differences. Our results indicated that under in vitro culture conditions, leaf, adventitious root and leafy shoot production were not reliable indicators of ecotypic differentiation between the four genotypes. However, significant differences on in vitro corm formation were observed. The more northern genotypes produced corms under a short day photoperiod (10 hr. light/14 hr. dark) and lower temperature (15 or 20 °C). Only non-leafy rhizomes tips were produced by the more southern genotypes under the same conditions. All genotypes could be induced to produce corms in the presence of ABA under a non-inductive long-day photoperiod and higher temperature (16 hr. light/8 hr. dark, 25 °C), but its effect was genotypic specific. Maximum corm production (2.5 corms/plant) was observed in the North Carolina genotype cultured in the presence of 25 μM ABA. These results demonstrated the potential application of in vitro culture for conducting in vitro common garden studies to screen ecotypic differentiation for selecting plants adapted to specific environmental conditions.

P-3037

Regeneration of *Agave angustifolia* Plants Through Somatic Embryogenesis. JESÚS IGNACIO REYES-DÍAZ, Amaury Martín Arzate-Fernandez, and José Luis Piña Escuti. Centro de Investigación y Estudios Avanzados en Fitomejoramiento, Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, MEXICO. Email: jird.rd@gmail.com

Agave angustifolia, is the source of carbohydrate and minor components for the production of mescal, a distilled alcoholic beverage with a great demand. The process to obtain the mescal generates a main bioproducts in the form of bagasse which can be used as a fuel or pharmaceutical compounds, making an integrated agro-industrial process. The *Agave* species can be multiplied asexually, however the frequency of vegetative grown plants is very low. Under optimum environmental conditions, rarely sets viable seeds. Also, over collection of plants and seeds, as well as the habitat destruction reduce significantly the populations of this plant in its native habitat. By the above, the urgent need to stablish biotechnology techniques to mass propagate method and genetically improve several species of the genus *Agave*, like somatic embryogenesis. Using a fourth-strength Murashige and Skoog medium, we evaluated the effects of sucrose concentrations, plant growth regulators combinations, vitamins, sources of amino acids and environmental culture conditions. Thus, 271 treatments were assayed to induction of the *A. angustifolia* embryogenic callus. The embryogenic callus developed clusters of mature somatic embryo. So far, our protocol has allowed obtaining 73.4 somatic embryos per explant. The conversion frequency of somatic embryos to plantlets ranged from 95-100%. Rooted plantlets were transferred to greenhouse conditions with 100% survival of the plantlets. Factors affecting somatic embryogenesis of *A. angustifolia* were investigated. A tissue culture system for production of high quality *A. angustifolia* plants was developed to meet the demand of the mescal industry.

P-3038

Evaluation of Media Formulations for *In Vitro* Sweetpotato THOMAS W. ZIMMERMAN. University of the Virgin Islands Agricultural Experiment Station, RR#1 Box 10,000, Kingshill, VI 00850. Email: tzimmer@uvi.edu

Tissue culture of sweetpotato shoots is a way to maintain disease-free varieties. However, a medium is needed that is able to maintain and promote growth within the great diversity of sweetpotato germplasm and newly developed

varieties. The objective was to evaluated in vitro media with different salt formulations on in vitro sweet potato growth. Sweetpotato germplasm of twelve varieties with diverse skin and tuberous-root flesh color were grown from nodal segments on four salt formulations. The media formulations were DKW basal medium, Loyd and McKown WPM, MS modified by Finer and Nagasawa, and MS modified BC potato medium. All media contained 3% sucrose, 1g.L-1 Phytogel and pH adjusted to 5.8 prior to autoclaving and dispensing 20 ml in 25 mm test tubes. Nodal segments were placed in tubes sealed with Micropore surgical tape and grown in a light and temperature controlled room for seven weeks. All nodal segments rooted within a week on the media. The healthiest growth was obtained on the BC potato medium with the least chlorotic or necrotic leaves and vibrant roots. WPM resulted in aerial roots, necrotic leaves and terminals in 90% of the cultures. Shoots on DKW had the least amount of roots while WPM had the most. For extended maintenance of sweetpotato diverse germplasm in vitro, BC potato medium results in the healthiest plant growth.

P-3051

Developing a Vibrant and Sustainable Jamaican Industry with *Bambusa vulgaris:* Establishing Standard Macro and Micropropagation Protocols as a Key Part in the Process. SYLVIA MITCHELL. Medicinal Plant Biotechnology Research Group, The Biotechnology Centre, University of the West Indies, Mona Campus, Kingston 7, JAMAICA. Email: sylviamitchell.biotech@gmail.com

Bamboo has been an underutilized plant in the Caribbean even in the face of its widespread use in many industries around the world. Brought into Jamaica for river training many eons ago, it has been merely used for fence posts, yam sticks and a few craft items. There are now ~65,000 hectares of uncared, wild bamboo stands in Jamaica where once there were yam fields. Bamboo can be used in many lucrative industries – for craft, household uses, construction, food, medicine, agriculture, feed and energy. Spearheaded by the Bureau of Standards (BSJ), The Bamboo Project began in May 2014; and is being supported by public and private sector involvement, it is standards-led and market-driven. The main species of bamboo in Jamaica is *Bambusa vulgaris*. Foresighting has identified the real possibility that Jamaica will need to cultivate bamboo in the near future. The goal is establish standards – for nursery, plantation, removal and land restoration, and for value-added products such as organic charcoal and paper. For a plant not yet a crop, this requires data gathering. An MOU between UWI and BSJ is connecting academics and industry by using research to gather the data needed to establish the needed standards. Agricultural research in Jamaica is still too sparse. Through this project, relevant observations made so far will be highlighted: bamboo culms dry out very quickly so should be replanted the same day, or transported at high humidity; one or two joint bamboo culm pieces will germinate; bamboo grows as well on reclaimed bauxite land; and initiation into tissue culture has to be done within two days of cutting, using nodes with dormant buds. Variations of bamboo collected around the island are being planted in a field gene bank and future work includes DNA fingerprinting to determine their relatedness. It is expected that out of these endeavours, the foundation of the bamboo industry in Jamaica will be strong, and ready to export to the world.

PHYSIOLOGY

P-3039

Tea (*Camellia sinensis*) MYB4a Negatively Regulates Plant Phenylpropanoid Biosynthesis. MINGZHUO LI [1, 5], Yanzhi Li[1], Lili Guo[2], Niandi Gong[2], Yongzheng Pang[3] , Wenbo Jiang[3], Yajun Liu[2], Xiaolan Jiang[1], Lei Zhao[4], Yunsheng Wang[2] , De-Yu Xie[1,5], Liping Gao[2], and Tao Xia[1]. [1] State Key Laboratory of Tea Plant Biochemistry and Utilization, Anhui Agricultural University, Hefei 230036, CHINA; [2]School of Life Science, Anhui Agricultural University, Hefei 230036, CHINA; [3]Institute of Botany, Chinese Academy of Sciences, Beijing 100093, CHINA; [4]College of Horticulture, Qingdao Key Laboratory of Genetic Improvement and Breeding in Horticultural Plants, Qingdao Agricultural University, 700 Changcheng Rd, Qingdao, 266109, Shandong, CHINA; and [5]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC, 27695. Email: mli34@ncsu.edu

Green tea (*Camellia sinensis, Cs*) is highly abundant in numerous phenylpropanoid compounds. In this study, we report an R2R3-MYB transcription factor from tea leaf tissues, namely CsMYB4 belonging to the MYB4-subgroup. Transcriptional and metabolic profiling showed that the expression profile of *CsMYB4a* was negatively correlated to the accumulation of flavan-3-ols and other phenolic acids. Interaction of CsMYB4a and five promoters showed that CsMYB4a decreased five promoters' activity in the phenylpropanoid metabolism. Further ectopic expression in tobacco plants resulted in alterations of plant growth including dwarf, shrinking and yellowish leaf, and early senescence phenotypes. Transcriptomics, metabolomics, and protein-DNA interaction experiments further demonstrated that these phenotypes were associated with downregulation of numerous genes that are involved in the shikimate and phenylpropanoid pathways controlling biosynthesis of phenylalanine (Phe), lignin, and other compounds in transgenic plants. All data indicate that CsMYB4a negatively regulates the phenylpropanoid and shikimate pathways.

SECONDARY METABOLISM

P-3040

Extensive Probing of Elicited Plant Cell Cultures for Anticancer Activity. KATHRYN BUMILA and Susan Roberts. Department of Chemical Engineering, Worcester Polytechnic Institute, Worcester MA. Email: kabumila@wpi.edu1

Cancer is the second leading cause of death worldwide. In the previous 20 years, 25% of anticancer drugs prescribed were plant-derived, while another 25% were chemically altered versions of natural products, illustrating the unique ability of plants to produce chemotherapeutics and the necessity for further exploration of plants for therapeutic opportunities. Despite testing thousands of plant extracts for their potency over the last few decades, research efforts have overlooked the unique opportunity to exploit stress elicitors to induce the production of secondary compounds, or innate defense mechanisms in plants. We propose eliciting a stress response in various species of plant cell culture via multiple routes (fungal extract, methyl jasmonate, salicylic acid, etc.) and exposing cancer cells to the extract. To accomplish such an extensive screening, we collaborate with The Plant Cell Culture Library (PCCL) at UMass Amherst, the largest known collection of live plant material with over 2000 species. Preliminary work indicated that the co-culture of PCCL plant species and *Saccharomyces cerevisiae* yields interesting responses that deserve further study. We are testing the secretions of this co-culture against a subset of the National Institute of Health's NCI-60 Human Tumor Cell Line panel as well as healthy controls. MTT assays will determine potency and if the secretions are generally cytotoxic or cancer-specific. In addition, we are investigating cancer cell extracts as elicitors with the intention of promoting a cancer-specific response in plants and are developing a high-throughput elicitation-based screening platform to access potency of plant species within the PCCL.

P-3041

Improvement of Biosynthesis and Accumulation of Secondary Metabolites by Elicitation in Adventitious Root Cultures of *Echinacea purpurea*. THANH-TAM HO, Kyung-Ju Lee, Kee-Yoeup Paek, and So-Young Park. Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju 28644, REPUBLIC OF KOREA. Email: hotamqn@gmail.com, Corresponding Author: soypark7@chungbuk.ac.kr

Echinacea is an important herbal medicine used in the treatment of the common cold, and it is also a prominent dietary supplement used throughout the world. In vitro plant materials are good sources of bioactive compounds, and the elicitation technique can be used as an effective tool to improve the biosynthesis of these compounds. In this study, adventitious roots of *Echinacea purpurea* were cultured in 300 mL Erlenmeyer flasks containing 100 mL ¾-strength MS medium supplemented with 4.92 μM IBA and 3% sucrose. After 3 weeks of culture, biotic (yeast extract and chitosan) and abiotic (methyl jasmonate and salicylic acid) elicitors were applied and their influences on *E. purpurea* growth and secondary metabolite accumulation were examined. After 1 week of exposure, the abiotic elicitors caused a greater root growth inhibition than the biotic elicitors did; however, the abiotic elicitors improved secondary metabolite production from adventitious roots of *E. purpurea*. The caffeic derivatives caftaric acid, chlorogenic acid, cichoric acid, and echinacoside were analyzed by HPLC. In addition, adventitious roots of three *Echinacea* species *(E. purpurea, E. angustifolia*,and *E. pallida*) were analyzed by HPLC for comparison of caffeic derivative accumulation. Among those, *E. purpurea* had the highest content of caffeic derivatives (72.34 mg/g DW), 1.5-fold higher than that of *E. angustifolia* and *E. pallida*. These results may be useful in developing large-scale adventitious root cultures for production of biomass and bioactive compounds of *Echinacea.*

P-3042

Metabolic Profiling of Secondary Metabolites in Hairy Root Cultures of *Polygonum multiflorum* Using an HPLC and FT-IR Methods. THANH-TAM HO, Kee-Yoeup Paek, and So-Young Park. Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju 28644, REPUBLIC OF KOREA. Email: hotamqn@gmail.com, Corresponding Author: soypark7@chungbuk.ac.kr

Polygonum multiflorum is a highly acclaimed medicinal herb from Southeast Asia. Its root contains valuable secondary metabolites, including anthraquinones, stilbenes, flavonoids, tannins, and phospholipids. Among various *in vitro* plant cultivation techniques, hairy root cultures (HRCs) are characterized by fast and unlimited root growth, and have greater genetic stability than other cultivation methods. HRCs are known to accumulate phytochemical levels comparable to those of intact plants. In this study, HRCs of *Polygonum multiflorum* were established from leaf explants infected with *Agrobacterium rhizogenes* strain KCCM 11879. After 21 days of co-cultivation on a hormone-free Murashige and Skoog (MS) medium, over 60% of the explants showed hairy root induction; induced roots were confirmed by PCR using a *rolC*-specific primer. Of the 6 HRCs selected, HR-01 was found to be superior, with significantly higher root biomass (105.2 g/L of FW, 9.7 g/L of DW), which is 10-fold higher than that of non-transgenic roots on the same hormone-free medium. A significant increase was also noticed in the total phenolic (62.65 mg/g DW) and flavonoid (26.43 mg/g DW) contents of the HR-01 line, while non-transgenic roots accumulated 26.21 mg/g DW of total phenolics and 11.80 mg/g DW of flavonoids. To characterize the 6 HRC lines, 17 individual phenolics, including emodin, physcion, and quercetin, were analyzed using HPLC. Phenolic compounds in HRCs were also enhanced more than 2.5-fold by 5 days of exposure to 50 μM methyl jasmonate. In addition, metabolic profiling of hairy roots and adventitious root cultures by FR-IR analysis allowed multivariable analyses. The FT-IR spectra analysis showed discrimination of hairy roots and adventitious root cultures by bioactive accumulation in *P. multiflorum.* Thus, it is evident from this study that HRCs could be an attractive

proposition for large-scale production of root biomass and secondary metabolites of *P. multiflorum* in bioreactors.

P-3043

Biotic Elicitors Induced Differential Growth Characteristics and Ginsenoside Contents in Mutant Adventitious Root Cultures of *Panax ginseng*. KIM-CUONG LE[1], Wan-Taek Im[2], Kee-Yoeup Paek[1], and So-Young Park[1]. [1]Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju 28644, REPUBLIC OF KOREA and [2]Department of Biotechnology, Hankyong National University, 327 Chungang-no, Anseong-si, Kyonggi-do 456-749, REPUBLIC OF KOREA. Email: lekimcuong88@gmail.com, Corresponding Author: soypark7@chungbuk.ac.kr

Treatment with biotic elicitors is an important biotechnological strategy to accumulation of secondary metabolites in adventitious root cultures. In a previous study, mutant adventitious roots of *Panax ginseng* were induced by colchicine treatment, leading to accumulation of higher amounts of ginsenosides than those in the control. In this study, five gram-negative bacteria, *Mesorhizobium huakuii* (GS1035), *M. amorphae* (GS3037), *M. amorphae* (GS336), *Bradyrhizobium ganzhouense* (GS1514), and *Azotobacter beijerinckii* (EMML538), and four gram-positive bacteria *Lactobacillus plantarum* (CWH-2), *Leuconostoc* sp. (LKJ-4), *Bacillus* sp. (LHW-1), and *Bacillus* sp. (CWJ-1) were used as biotic elicitors, and their effects on production of compound K and other ginsenosides in mutant adventitious root cultures were investigated. After 5 days of treatment, all elicitors caused a reduction in root culture biomass. In particular, *M. huakuii* (GS1035) and *Leuconostoc* sp. (LKJ-4) significantly reduced the biomass of treated root cultures, by 1.5-fold (67.84 $g \cdot L^{-1}$) and 1.4-fold (72.67 $g \cdot L^{-1}$), respectively, than that of the non-treated root cultures (102.55 $g \cdot L^{-1}$). pH and electrical conductivity (EC) of the culture medium were also significantly affected by treatment with *M. huakuii* (GS1035) and *Leuconostoc* sp. (LKJ-4). The saponin content of the root cultures treated with *M. amorphae* (GS3037) was the maximum (105.58 $mg \cdot g^{-1}$dry weight) whereas that of the non-treated root cultures was 74.48 $mg \cdot g^{-1}$dry weight. Compound K production after treatment with *M. amorphae* (GS3037) increased 1.7-fold of that in the non-treated root cultures. Ginsenoside Re positively correlated with Rg_1 and Rg_2, whereas ginsenosides Rg_3, and Rg_1 positively correlated with Rh_2, and Rg_2, respectively. In conclusion, the preliminary results suggest that treatment of mutant adventitious root cultures with biotic elicitors might be an effective strategy for enhancing ginsenoside production in *Panax ginseng*.

P-3044

Comparison of Cell Culture System and Adventitious Root Culture System in *Panax ginseng* and *Panax quinquefolius*. JONG-DU LEE, Kim-Cuong Le, Kee-Yoeup Paek, So-Young Park. Department of Horticultural Science, Division of Animal, Horticulture and Food Sciences, Chungbuk National University, Cheongju 28644, REPUBLIC OF KOREA. Email: soypark7@chungbuk.ac.kr

Ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*) are commonly considered representative medicinal herbs. They are effective in the treatment of gastro-enteric disorders, diabetes, and blood circulation disorders. Today, over 30 ginsenosides in these herbs have been identified. Since in vitro culture in ginseng was first reported in 1964, both cell culture and adventitious root culture systems have been utilized for the production of biomass and secondary metabolites. In the present study, we investigated the biomass, cell division, saponin content, and ginsenoside content from each 4 lines (AM, BDS, CS, CBN) of ginseng cell culture and adventitious root culture to compare the characteristics and efficiency of both systems. Additionally, ginsenoside biosynthesis related genes were also analyzed for these cell and adventitious root lines. The results indicated that cell division was faster in cell lines than in adventitious root lines. However, adventitious root lines showed higher dry biomass and lower fresh biomass (both 1.5 to 2-fold) than did cell lines. The CS adventitious root line showed higher total saponin and ginsenoside content (10.48 mg g-1 dry weight, and 12.88 mg L-1, respectively) than did CS cell line (9.50 mg g-1 dry weight, and 2.39 mg L-1, respectively). Notably, the Rd content of CS adventitious root line was 4-fold higher than that of CS cell line. To investigate ginsenoside production, genes associated with ginsenoside biosynthesis (*SS, SE, PPDS, and PPTS*) were analyzed using real-time qPCR. The results of this study suggest that the adventitious root culture system feasible for the commercialized production of biomass and ginsenosides in the pharmaceutical, cosmetic, and functional food industries.

PLANT SILENT ABSTRACTS

P-3045

Comparative Study of Virus-Like Particles Obtained from Coat Protein of Alternanthera Mosaic Virus. E. K. DONCHENKO, T. I. Manukhova, O. A. Kondakova, N. A. Nikitin, J. G. Atabekov, and O. V. Karpova. Department of Virology, Faculty of Biology Lomonosov Moscow State University, Leninskie gory 1-12, 119234, Moscow, RUSSIA. Email: donchenko@mail.bio.msu.ru

Plant viruses are a popular object for the development of new biotechnologies, including design of medical products (vaccines and adjuvants). Thereby, study of the structure, stability and other properties of plant viruses and their virus-like particles (VLPs) is a promising and upcoming research direction. In the present work Alternanthera mosaic virus (genus *Potexvirus*, family *Alphaflexiviridae*), the closest relative of the papaya mosaic virus (PMV), was studied. Recently we have shown that AltMV coat protein (CP) polymerizes *in vitro* into RNA-free VLPs that are identical in morphology with native AltMV virions. Here we demonstrated that AltMV

virions and VLPs are stable in different conditions, including close to physiological (distilled water; 0.01 M Tris-HCl pH 7.5; 0.15 M NaCl; 0.01 M Tris-HCl pH 7.5, 0.15 M NaCl). The ability of AltMV CP to form stable VLPs at pH 7.5 and in the presence of 0.15 M NaCl significantly distinguishes it from the PMV CP, which forms stable VLPs *in vitro* only at pH 4.0. AltMV VLPs remained stable in mouse blood serum, which makes them a promising object for development of vaccines and biosafety adjuvants. For the first time the structure of AltMV virions and VLPs were compared by the CP trypsin-degradation assays designed in our lab earlier (Rodionova *et al.*, 2003). AltMV virions were trypsin-resistant, whereas VLPs were partially hydrolyzed. According to these data, AltMV virions and VLPs are similar morphologically, but have a different structure of the helical protein capsid. We also modified the procedure for AltMV isolation and significantly increased the yield of the purified virus that is necessary for the practical application of VLPs and virions. *This work was funded by the Russian Science Foundation (Grant No 14-24-00007).*

P-3046

Biolistic Delivery of the RGEN RNP into Potato Plants for Genome Editing. V. V. MAKAROV[1,2], A. V. Makhotenko[1,2], E. A. Snigir[1], A. V. Khromov[1,2], S. S. Makarova[1,2], N. O. Kalinina[1,2], and M. E. Taliansky[1,3]. [1]DokaGene Ltd, Rogachevo, RUSSIA; [2]Lomonosov Moscow State University, Moscow, RUSSIA; and [3]Cell and Molecular Sciences, The James Hutton Institute, Dundee, UNITED KINGDOM. Email: makarovvalentine@gmail.com

Rapid advances in nanotechnology have led to the development of innovative applications based on nanoparticles (NP, ultrasmall particles with sizes ≤ 100 nm). Development of applications in the field of basic and applied plant sciences promises no less revolutionary results as in biomedicine. NPs have a number of unique properties associated with their ultrasmall size and exhibit many advantages compared with existing plant biotechnology platforms for delivery of proteins, small RNAs, RNA and DNA in the plant. The first aim of this work was to develop applications for effective cleavage of DNA sequences encoding coilin and phytoene desaturase (PDS) genes (*Solanum tuberosum*). We synthesized five single guide RNAs (sgRNAs) for coilin gene based on bioinformatics prediction and only one of them demonstrated the cleavage activity *in vitro*. Of six sgRNAs synthesized for PDS gene five were active. Then we developed new NP-mediated technology for delivering the CRISPR/Cas9 system components for genome editing into potato apical meristems plants. For this thing we functionalized AuNPs with Cas9 (RNA-guided DNA endonuclease) and sgRNAs designed as described above. The resultant NPs were biolistically bombarded into apical meristems and axillary buds of the potato plants. The genome editing events (coilin and PDS genes knock out) were confirmed using the NGS of the corresponding genes and T7E1 assays. The present work was performed with the financial support from the Russian Science Foundation (grant № 16-16-04019).

P-3047

Dandelion *In Vitro* Response to Different Culture Strategies for Triterpenes Production. M. MARTINEZ[1,2], L. Jorquera[1,2], R. Chamy[1,2], D.Prüfer[3,4], C. Schulze Gronover[4], and P. Poirrier[1,2]. [1]School of Biochemical Engineering, Pontifical Catholic University of Valparaíso, Av. Brasil 2085, Valparaíso, CHILE; [2]Fraunhofer Chile Research Foundation – Centre for Systems Biotechnology (FCR-CSB), Mariano Sánchez Fontecilla 310, of. 1401, Las Condes, Santiago, CHILE; [3]Westphalian Wilhelms-University of Münster, Institute of Plant Biology and Biotechnology, Schlossplatz 8, D-48143 Münster, GERMANY; and [4]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schlossplatz 8, D-48143 Münster, GERMANY. Email: maria.martinez@fraunhofer.cl

Triterpenes are one of the main secondary metabolites responsible for the medicinal properties of *Taraxacum officinale* and is our aim was to establish an *in vitro* culture for the selective production of α-amyrin and lupeol. A tissue culture system was established for mass propagation of *T. officinale*, in which cotyledons and hypocotyls gave the best induction (100%) using sucrose as carbon source in concentrations up to 23 g/L, supplemented with NAA and BAP in combination. Triterpenes were detected after 4-6 weeks of culture and directly related to the maintenance conditions of callus. However, callus maintenance conditions were not in line with lupeol and amyrin accumulation in the cell. Particularly, carbon source concentration was an important parameter in the accumulation of these compounds. Thus, a two-stage strategy was evaluated using first a medium supplemented with low sucrose content for cell growth under NAA/BAP concentrations up to 3.0 ppm. After few weeks, callus was transferred to a medium rich in sucrose (>50 g/L) and maximum cell biomass and triterpenes yields were obtained after two months. This strategy was better rather inducing callus and maintaining cell proliferation and triterpene accumulation at higher sucrose concentration from the beginning in a single stage. Even when triterpene yields were similar for both strategies, culture time was significantly reduced using growth and triterpene accumulation separately. Nevertheless, lupeol and α-amyrin yields were lower than expected (< 0.5 mg/g dry cell), showing also different accumulation patterns. α-Amyrin yield was the lowest, showing accumulation peak at different culture time than lupeol. These observations indicated that both triterpenes cannot be produced at maximum at the same time under the proposed conditions.

P-3048

Automated Cell Counting and Viability Assessments of Canola Mesophyll Protoplasts. S. SAHAB[1], M. Ponnampalam[1], S. Georges[1], Y.Ding[1], J.Mason[1,2], G. Spangenberg[1,2], and M. Hayden[1,2]. [1]Department of Economic

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Assessments of yield and viability are routine procedures associated with protoplast isolation and their downstream applications. Haemocytometer based counting is commonly employed for quantitative assessments, however it has limited throughput and is labour intensive. Here we report the establishment of a high throughput and low labour method for counting and assessing the viability of canola mesophyll protoplasts. Our approach is based on the Cell Countess™ automated cell counting platform and uses Sytox Orange (stains dead cells) as the vital stain. The method was validated using conventional haemocytometer cell counting and Evans Blue (stains dead cells) staining. Our method was validated for untreated, PEG-treated and PEG-transfected (using GFP reporter construct) protoplasts at different time intervals (0, 24, 48, 72 hrs post transfections). Our method increases five-fold (up to 50 samples per day) the throughput that can be achieved in routine protoplasts transfection experiments. The method is applicable to other protoplasts sources.

P-3049

RNA-seq Based Comparative Transcriptomics of Red vs. Wild-type Tobacco Plants. ANGELINA SONG and Deyu Xie. North Carolina State University, Raleigh, NC. Email: aesong@ncsu.edu

Production of Anthocyanin Pigment 1 (PAP1) is a master positive regulator of anthocyanin biosynthesis in Arabidopsis thaliana. Red transgenic tobacco plants with high production of anthocyanins were obtained by the overexpression of PAP1. In our study, we performed RNA-seq for red and wild-type tobacco plants using next generation sequencing technology. As a result, we obtained 52,090,839 and 54,728,690 sequence reads with high quality for red and wild-type plants. Based on available tobacco and other model plant genomes, we assembled 249,104 contigs, most of which could be annotated a function. Different pipeline analyses, such as, TopHat and Cufflinks tool, were used to compare genome-wide transcripts between red and wild-type plants. The resulting data discovered numerous of transcriptional differentiations caused by the overexpression of PAP1. This research is supported by RJ Reynolds.

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