LATE SUBMISSION ABSTRACTS

2022 Meeting of the Society for In Vitro Biology June 4 – 7, 2022

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

ANIMAL CONTRIBUTED PAPER SESSION ABSTRACTS

IN VITRO ANIMAL CELL SCIENCES CONTRIBUTED PAPER SESSION

Monday, June 6, 5:00 pm – 6:00 pm

5:36 A-1006 LncRNA APDC, a Long Non-coding RNA, Plays Important Regulatory Roles in Metabolism of Bone and Adipose Tissues
Zoe Zhu*, Tufts University School of Dental Medicine, Yao Liu, Elissa K. Zboinski and Jake Chen*

5:48 A-1007 Developing Cell Lines from Fish and Shellfish - Cellular Aquaculture Invitromatics
Lucy E. J. Lee*, University of the Fraser Valley*

ANIMAL POSTER ABSTRACTS

INTERACTIVE POSTERS

IN VITRO ANIMAL CELL SCIENCES INTERACTIVE POSTER SESSION

Tuesday, June 7, 1:30 pm – 2:30 pm

A-3000 The Establishment of a Sponge Cell Hybridoma
Cassady Dougan*, Florida Atlantic University, Megan Conkling, Peter J. McCarthy, Amy E. Wright, and Shirley A. Pomponi*

A-3001 ECEL1, PIEZO1 and NAV2 Genes Are Altered in the Enhanced and Directed Axonal Growth in Dorsal Root Ganglia Grown Ex Vivo on Nano-scale Grooved Topographical Surfaces
Julie Ellen Tamayo, *Midwestern University, Kelly Keeler, Joshua Z. Gasiorowski, Michele Fornaro, and Kolla Kristjansdottir*

POSTERS

ADHESION FACTORS

A-3002 Changes to Plasma Membrane Composition and Loss of Communication in Osteoblasts with Rb1 Deficiency
***Elisha Pendleton**, Midwestern University, Keren Abdalah and Nalini Chandar*

CELL AND TISSUE MODELS

A-3003 Modulation of Fibroblast Growth and Senescence in Response to Sonic Hedgehog Inhibition
***Johnson Fong**, Tufts University School of Dental Medicine, Tatiana Mendez, Sunnie Kuna, Sarah Pagni, Janet Cowan, and Addy Alt-Holland*

A-3004 Delineating the Effects of Vismodgeib and Dermal Fibroblasts on Metabolic Profiles of Basal Cell Carcinoma Cells
***S. Kasraie,** Tufts University School of Dental Medicine, T. Mendez, S. Pagni, J. Baleja[2], J. Cowan, and A. Alt-Holland*

A-3005 Growth and Metabolism of Basal Cell Carcinoma Cells and Spheroids in Vismodegib-treated Cultures
***Tatiana Mendez**, Tufts University School of Dental Medicine, Arietta Rigopoulos, Sarah Pagni, James D. Baleja, Janet Cowan, and Addy Alt-Holland*

A-3006 Assays for Monitoring Cell Health in Microphysiological Systems
***Terry Riss**, Promega Corporation, D. Leippe, N. Karassina, M. Valley, and J. Vidugiriene*

A-3007 Cell Cycle Analysis of Vismodegib-treated Human Basal Cell Carcinoma Cells In-vitro
***B. Senfi**, Tufts University School of Dental Medicine, T. Mendez, M. Evers, S. Pagni, J. Cowan, A. Alt-Holland*

CELL BIOLOGY

A-3008 Isolation and Characterization of a Muscle-derived Cell Line Obtained from Australasian Snapper (*Chrysophrys auratus*)
***Georgina C. Dowd**, The New Zealand Institute for Plant and Food Research Limited, Gavril Chong, Lucy E.J. Lee, and Niels C. Bols*

CELLULAR AND MOLECULAR BIOLOGY

A-3009 Examining the Urinary Exosomal RNA Profile of Cadmium-treated Rats for Novel Biomarkers of Kidney Injury
***M. J. Fay,** Midwestern University, K. Kristjansdottir, K. Bussey, J. Edwards, and W. Prozialeck*

A-3010 Antioxidant and Cardioprotective Properties of Peanut Hairy Root-Derived Prenylated Stilbenoids in Cardiac Cells
***Rokib Hasan**, Arkansas State University, Sankalpa Chakraborty, Viswanathan Rajagopalan, and Fabricio Medina-Bolivar*

A-3011 Pro-inflammatory Signaling Is Induced in Cancer Cells by Electroporation with a Synthetic dsRNA Analog
***Loree C Heller**, University of South Florida, Amanda Sales Conniff, Guido Encalada, Shreena Patel, Manya Bhandary, and Farah Al-Takrouri*

CHEMICAL CARCINOGENESIS

A-3012 Lower Molecular Weight Polycyclic Aromatic Hydrocarbons: Carcinogenic Potential in Lung
***Alison K. Bauer**, University of Colorado Anschutz, Sabine Plottner, Melanie Wolff, Heiko U. Kafferlein, and Brad L. Upham*

GENE EXPRESSION

A-3013 Sex linked Aberrant Behavior and Hippocampal Gene Expression in *Egr1* Conditional Knockout Mice
***C. Swilley**, Virginia-Maryland College of Veterinary Medicine, and H. Xie*

INFECTIOUS DISEASE

A-3014 Biophysical Characterization of the HIV-1 Nucleocapsid Protein RNA Interaction Properties
***Anissa Belfetmi**, Université Paris-Saclay, Loussine Zargarian, Assia Mouhand, Marjorie Catala, Philippe Fossé, Carine Tisné, and Olivier Mauffret*

PRODUCT APPLICATIONS

A-3015 High-throughput Genotyping by Sequencing with the AgriSeq™ Workflow
***T. Dunbar,** Thermo Fisher Scientific, and A. Burrell*

VIRTUAL POSTERS

CELLULAR AND MOLECULAR BIOLOGY

A-3016 A Novel Approach to Bone Implant Infection Therapeutics Leveraging Proteins Derived from L. *acidophilus* and Modulating Anti-inflammatory Gene Expression
***Anouska Seal**, University of Central Florida*

CELLULAR PATHOLOGY

A-3017 Evaluation of DJ-1 and PTEN as Prognostic Biomarkers in Cutaneous Melanoma
***Ziyi Sun**, Midwestern University College of Osteopathic Medicine, and Hilal Arnouk*

CHEMICAL CARCINOGENESIS

A-3018 Per and Polyfluorinated Substances Dysregulate Gap Junctional Intercellular Communication
***Jamie E. Liebold**, Michigan State University, Lizbeth Lockwood, Joo Hye Yeo, Jinu Lee, Alison K. Bauer, Erika Lisabeth, Richard Neubig, and Brad L. Upham*

ONCOLOGY

A-3019 Evaluation of DJ-1 and PTEN Expression in Oral Squamous Cell Carcinoma
***Rey De La Torre**, Midwestern University, and Hilal Arnouk*

STEM CELLS

A-3020 Using Nanoparticle-based Magnetic Hyperthermia to Hinder Planarian Regeneration
***Thomas C. Commander**, Episcopal School of Jacksonville, and Marion Zeiner*

PLANT CONTRIBUTED PAPER SESSION ABSTRACTS

BIOTECHNOLOGY, GENOME EDITING, AND GENETIC ENGINEERING CONTRIBUTED PAPER SESSION

Monday, June 6, 5:00 pm – 6:00 pm

5:00 P-1015 Molecular Physiological Responses of *Paulownia* and Turmeric Under Various Abiotic Stress Conditions
***C. Basu**, California State University, Northridge, M. Chaires, K. Cooper, D. Gupta, N. Joshee, N. Katiyar, S. Pakala, N. Ramadoss, B. Bharadwaj, and K. Musaev*

5:15 P-1016 DNA-Free Genome Editing in Hexaploid Sweetpotato Directed by Preassembled CRISPR-Cas9 Ribonucleoprotein Complexes
***Adrianne Brown**, Tuskegee University Plant Biotechnology and Genomics Research Laboratory, M. Egnin, F. Bukari, D. Mortley, C. Bonsi, O. Idehen, D. Alexander, and G. Bernard*

5:30 P-1017 Constitutive Expression of a miR169 Gene Alters Plant Development and Enhances Drought and Salt Tolerance in Transgenic Creeping Bentgrass
***Xiaotong Chen,** Clemson University, Jason Yeung, Andrew Fiorentino, Qian Hu, Morgan Kuess, and Hong Luo*

5:45 P-1018 T-DNA-Free Gene Editing Through Transient Suppression of the *POLQ* Gene in Plants
***Heqiang Huo**, University of Florida, Guiluan Wang, and Zhanao Deng*

PLANT POSTER ABSTRACTS

INTERACTIVE POSTERS

BIOTECHNOLOGY, GENOMICS, AND PLANT PHYSIOLOGY INTERACTIVE POSTER SESSION

Monday, June 6, 1:30 pm – 2:30 pm

P-3000 A Rapid and Simplified Transformation and Genome Editing Method for Maize Inbred B104 Using Agrobacterium Ternary Vector System and Immature Embryos
***Minjeong Kang,** Iowa State University, Keunsub Lee, Todd Finley, Hal Chappell, Veena Veena, and Kan Wang*

P-3001 CRISPR/Cas9 Mediated Cell Wall Engineering of Plant Cells for Enhanced Recombinant Protein Production
***Uddhab Karki**, Arkansas State University, Hong Fang, and Jianfeng Xu*

P-3002 Post-wildfire Soil Microbiome Analysis of eDNA from the Angeles National Forest
***Savanah Senn**, Los Angeles Pierce College, Gerald Presley, and Sharmodeep Bhattacharyya*

P-3003 Advanced Plant Reporter Genes for Transient Expression
***Nathan Vorodi,** Pennsylvania State University, Natalie Thompson, David Samson, Rekha Kandaswamy, Vijay Sheri, Aliya F. Anwar, Michael Ream, Anna Filipkowski, Maia Clipsham, Sairam Rudrabhatla, and Wayne R. Curtis*

P-3004 Establishment of an Axenic Whitefly Colony for *In Vitro* Virus Transmission Studies
***Wayne R. Curtis,** The Pennsylvania State University, Natalie Thompson, David Krum, Yun-Ru Chen, Mariela Torres, Marena Trauger, Dalton Strike, Zach Weston, April Hile, and Jane Polston*

P-3005 Genome-wide Analysis of Alpha-amylase Gene Family in Major Cereal Crops
***Shabda Verma**, Punjab Agricultural University, Kanwardeep S. Rawale, Navraj Kaur Sarao, Johar Singh Saini, Gagandeep Singla, and Kulvinder S Gill*

MICROPROPAGATION/MEDICINAL PLANTS INTERACTIVE POSTER SESSION
Tuesday, June 7, 1:30 pm – 2:30 pm

P-3006 Mutation Rates in Micropropagated *Cannabis sativa* Detected Through Genotyping by Sequencing, Are SSRs Antiquated?
***Kristian Adamek**, University of Guelph, Andrew Maxwell Phineas Jones, and Davoud Torkamaneh*

P-3007 Direct Field-to-Lab Introduction of Shoot Tips and Nodal Sections from Wild Nuttal's Scrub Oak (*Quercus dumosa*) for the Purpose of *Ex Situ* Conservation Via Micropropagation
***J. Ree,** San Diego Zoo Wildlife Alliance, and C. Powell*

P-3008 Clonal Propagation of Avocado (*Persea americana*) from Adult Trees: Establishment *In Vitro*
***J. Tin**, The Huntington Library, Art Museum, and Botanical Gardens, and R. Folgado*

P-3009 Isolation and Characterization of Two Novel *Chryseobacterium sp.* Genomotypes from the Rootzone of the Medicinal Plant *Datura inoxia* by Whole Genome Sequencing
***Savanah Senn,** Los Angeles Pierce College, Kelly Pangell, Maryam Saraylou, Adrianna Bowerman, Karu Smith, and Ryan O'halloran*

P-3010 *Moringa oleifera* Leaf Extract as a Potential Plant Growth Stimulant to Enhance Salt Stress Tolerance in Wheat
***Talaat Ahmed**, Qatar University, Mustafa Elshiekh, and Muhammad Fasih Khalid*

P-3011 In Vitro Production of Adventitious Root Biomass and Important N-alkylamides Using Bioreactor Cultivation of Medicinally Important Plant *Spilanthes paniculata* Wall. ex DC
***Krishna Kant Pachauri,** Indian Institute of Technology Guwahati, and Rakhi Chaturvedi*

GENETIC ENGINEERING AND METABOLIC ENGINEERING INTERACTIVE POSTER SESSION
Tuesday, June 7, 1:30 pm – 2:30 pm

P-3012 Increased Engineering and Editing Efficiency of *Sorghum bicolor* Using Morphogene-assisted Transformation
***Kiflom Aregawi**, University of California, Berkeley, Jianqiang Shen, Grady Pierroz, Manoj K. Sharma, Jeffery Dahlberg, Judith Owiti, and Peggy G. Lemaux*

P-3013 Inducible Expression for Acceleration of Design-Build-Test-Learn Cycles in the Metabolic Engineering of Oilcane
***Moni Qiande,** University of Florida - IFAS, Dang Viet Cao, Hui Liu, John Shanklin, and Fredy Altpeter*

P-3014 Application of Developmental Regulators to Improve *In-Planta* or *In Vitro* Transformation in Plants
***Heqiang Huo,** University of Florida, Zhaoyuan Lian, Chi Dinh Nguyen, Jianjun Chen, Sandra Wilson, and Peggy Ozias-Akins*

P-3016 Protein Engineering in *Chlamydomonas reinhardtii* for Improved Production of Recombinant Proteins
***Corbin England**, Arkansas State University, and Jianfeng Xu*

P-3017 Development of an *In Vitro* Regeneration and Transformation System for Hop (*Humulus lupulus*)
***Christopher J. Willig**, Oregon State University, Michele S. Wiseman, John A. Henning, and David H. Gent, and Steven H. Straus*

POSTERS

BIOTECHNOLOGY

P-3018 Development of a Meristem-based Transformation System for Barley (*Hordeum vulgare* L.) Targeting Mature Embryo Explants
***Lucas Gontijo Silva Maia,** University of Wisconsin-Madison, Edward Williams, Mike Petersen, Ray Collier, Phil Bregitzer, Ramamurthy Mahalingam, Marcus Vinje, Shawn Kaeppler, and Heidi Kaeppler*

P-3019 Construction of Wheat Transformation System Using *GRF-GIF* Chimera Gene Cloned from Korean Wheat cv. Keumgang and 'Speed Breeding' Conditions
***Geon Hee Lee**, Kongju National University, Sang Yong Park, Tae Kyeum Kim and Jae Yoon Kim*

P-3020 Effects of Organellar Targeting of the aadA1a Selectable Marker Protein on Transformation Frequency of Meristem-based Soybean Transformation
***S. Massman,** University of Wisconsin Madison, R. Collier, E. Williams, M. Petersen, R. Harnish, B. Martinell, and H. Kaeppler*

P-3021 Optimizing *Agrobacterium*-mediated Transformation and CRISPR-Cas9 Gene Editing in the *Tropical Japonica* Rice Variety Presidio
***Marco Molina-Risco**, Texas A&M University, Michael Thomson, Mayra Faion-Molina, Oneida Ibarra, Backki Kim, and Endang M. Septiningsih*

P-3022 A Dual Recombination System for Transgene Containment and Elimination in Perennial Grasses
Xiaotong Chen, *Clemson University, Charles Henry, Annalise Enger, Qian Hu, Zhenyuan Pan, Xiaoyuan Gao, Lynda McMaster-Schuyler, Peiyu Zeng, and Hong Luo*

P-3023 Transcriptome and Metabolite Mechanisms Related to Pre-harvest Sprout (PHS) of Common Korean Wheat (*Triticum aestivum*)
Sang Yong Park*, Kongju National University, Geon Hee Lee, Woo Joo Jung, and Jae Yoon Kim*

P-3024 Plant Host Defense Peptides: Potential Tools for Disease Control
Christie Stephen*, University of Lethbridge, and Dmytro P. Yevtushenko*

P-3025 Transforming *P. hallii,* a Model for Perennial Bioenergy Grasses
Kankshita Swaminathan, *HudsonAlpha Institute for Biotechnology, Anthony Trieu, Xiaoyu Weng, Rebekah Wood, and Thomas Juenger*

P-3026 Engineering Novel Designer Biologics in Plant Cells for Oral Treatment of Inflammatory Bowel Disease
Jianfeng Xu, *Arkansas State University, Wenzheng Guo, Jonathan TrejoMartinez, and Uddhab Karki*

P-3027 Chromosomal Aberrations and Micronuclei of Somaclonal Variation in Onion Tissue Culture (*Allium cepa*)
Abdelrahem Yousef, Agriculture Research Centre, Giza, Abdel-Rahem T. Abdel-Rahem, Kasem Z. Ahmed, and Sayed Osman

CELLULAR AND MOLECULAR BIOLOGY

P-3028 Transcriptomic Analysis of Dunaliella Salina under Salt and UV Stresses
M. J. Hein*, California State University Northridge, C. Basu, and S. M. Perl*

P-3029 Genetic and Physiological Responses of Lentil Plants Under Flood Stress
Avetis Mishegyan*, California State University Northridge, Bhiolina Bharadwaj, Sanjeevi Nagalingam, Alex Guenther, Nirmal Joshee, Samantha H. Herman, and Chhandak Basu*

P-3030 Transcriptomic and Physiological Effects of Heat Stress on Turmeric (Curcuma longa)
Kirill Musaev*, California State University Northridge, and Chhandak Basu*

GENETIC ENGINEERING

P-3031 Expanding the Toolbox of Maize Promoters
Nathaniel Schleif*, University of Wisconsin – Madison. Frank McFarland, Ray Collier, and Heidi F. Kaeppler*

P-3032 The Plant Genetic Engineering Network
J. Van Eck*, Boyce Thompson Institute, H. Kaeppler, B. Gordon-Kamm, K. Lee, W. Parrott, N. Taylor, and V. Veena*

P-3049 Development of an Efficient Method for Protoplast Isolation, Transfection, and Gene Editing Form Soybean Roots
C. L. Nwoko, *Texas Tech University, A. Ojha, V. Devkar and Gunvant B. Patil*

GENOME EDITING/GENOME ENGINEERING

P-3033 Synteny Analysis: Genes Linked for Powdery Mildew Among Landraces of *Pisum sativum* and *Lathyrus sativus*
R. Bishnoi, *Agriculture University, Kota, S. Marker, and P. K. P. Meena*

P-3034 Genome Editing in Three Species of the Bioenergy Grass *Miscanthus*
Nancy A. Reichert, *Mississippi State University, Anthony Trieu, Mohammad Belaffif, Shilpa Manjunatha, Pradeepa Hirannaiah, Rebekah Wood, Yokshitha Bathula, Rebecca Billingsley, Anjali Arpan, Erik Sacks, Stephen P. Moose, Tom E. Clemente, and Kankshita Swaminathan*

P-3035 Development of Tissue Culture-free Genetic Transformation and Gene-editing Platform in Crops
Arjun Ojha Kshetry, *Texas Tech University, Vikas Devkar, Luis Herrera-Estrella, and Gunvant B. Patil*

P-3036 Phenotypic Assessment of SnRK1C Mutants in Rice (*Oryza sativa var. japonica*) cv. Kitaake
M. C. Faria Chaves*, University of Arkansas, C. Maurya, N. Soumen, S. Zhao, and V. Srivastava*

P-3037 CRISPR RNA-guided Integrase System for *Agrobacterium* Genome Editing
Ephraim Aliu*, Iowa State University, Keunsub Lee, and Kan Wang*

P-3038 Transformation of Teosinte (*Zea mays* ssp. *parviglumis*) via Biolistic Bombardment of Seedling Derived Callus Tissues
Mercy K. Azanu, *Iowa State University, Jacob D. Zobrist, Susana Martin-Ortigosa, Keunsub Lee, Qing Ji, and Kan Wang*

IN VITRO TOOLS, TECHNIQUES, AND OPTIMIZATION

P-3051 Production-scale Transformation of Poplar Hybrid Clone INRA 717-1B4, *P. tremula* x *P. alba* by Particle Bombardment for Enhanced Photosynthesis Traits
Michelle Tjahjadi*, Living Carbon, Alex Crites, Dominick Tucker, Kenneth Donsky, Gary Orr, Karli Rasmussen, Jessica Du, Li-Wei Chiu, and Matt Heckert*

MEDICINAL PLANTS

P-3039 Physiological and Transcriptomic Analysis of Turmeric plants (Curcuma longa) Under Drought Stress
Bhiolina Bharadwaj*, California State University, Northridge, and Chhandak Basu*

P-3040 Towards Genetic Engineering of Sunflower Lineage to Produce Biodiesel
F. Shaurya*, California State University, Northridge, B. Bharadwaj, and C. Basu*

PLANT GENETIC RESOURCES CONSERVATION

P-3041 Conservation of African Sweetpotato Landraces – Lessons from the Conservation of Global Vegetatively Propagated Genetic Resources Collections
David Ellis, *International Potato Center, Jan Low, Maria Andrade, Marian Dorcas Quain, Ted Carey, Matthew L.S. Gboku, Noelle L. Anglin, Godwill Makunde, Jebeh Samba, Norma Manrique-Carpintero, Rosemary Gatimu, Vania Azevedo, Charlotte Lusty, and Luigi Guarino*

P-3042 Global Plant Cryopreservation Initiative
David Ellis*, International Potato Center, Vania Azevedo, Marleen Engers, Badara Gueye, Charlotte Lusty, Nicolas Roux, and Peter Wenzel*

PLANT PHYSIOLOGY

P-3050 Assessing Climate Resilience of Different Cowpea Cultivars Through Phenotypic and Molecular Analyses.
Inocent P. Ritte, *Tuskegee University, M. Egnin, O. Idehen, D. Mortley, G. C. Bernard, and C. Bonsi*

VIRTUAL POSTERS

BIOTECHNOLOGY

P-3043 Bioinspired Superhydrophobic and Liquid-infused Slippery Coatings for Decreased Fouling, Thrombosis, and Infection of Medical Devices
E. Ozkan*, University of Georgia, A. Mondal, M. Douglass, S. P. Hopkins, M. Garren, R. Devine, R. Pandey, J. Manuel, P. Singha, J. Warnock, and H. Handa*

MEDICINAL PLANTS

P-3044 Influence of Plant Growth Regulators in Initiation of In Vitro Cell Cultures of *Tinospora cordifolia* For Biosynthesis of Silver Nanoparticles
Vartika Srivastava*, Indian Institute of Technology Guwahati, Harshajit Baruah, and Rakhi Chaturvedi*

MICROPROPAGATION

P-3045 Effect of BA on Shoot Organogenesis in Mature Leaves of *Cleome gynandra*
Aubre Joyner*, Elizabeth City State University, Gloria Payne, and Margaret Young*

SECONDARY METABOLISM

P-3046 Tissues-wide Distribution of Azadirachtin Content in Neem (*Azadirachta Indica* A. Juss.), a Miraculous Medicinal Tree Species
Rajendra Adak, *Indian Institute of Technology Guwahati, and Rakhi ChaturvedI*

P-3047 Determination of Rutin in *Musa* Leaves by High Performance Liquid Chromatography - A Step Towards Sustainability
Imnanaro Lomgkumer*, Indian Institute of Technology Guwahati, and Rakhi Chaturvedi*

PLANT SILENT ABSTRACT

P-3048 Another Successful Target in the Suborder Sternorrhyncha (Hemiptera): Green Oligonucleotide Insecticides for Aphid Control
Y. V. Puzanova, *V. I. Vernadsky Crimean Federal University, I. A. Noviko, N. A. Marochkin, E. Eken, A. K. Sharmagiy, and V. V. Oberemok*

ANIMAL CONTRIBUTED PAPER SESSION ABSTRACTS

IN VITRO ANIMAL CELL SCIENCES CONTRIBUTED PAPER SESSION

Monday, June 6, 5:00 pm – 6:00 pm

A-1006

LncRNA APDC, a Long Non-coding RNA, Plays Important Regulatory Roles in Metabolism of Bone and Adipose Tissues. ZOE ZHU, Yao Liu, Elissa K. Zboinski, and Jake Chen. Department of Periodontology, Tufts University School of Dental Medicine, Boston, MA. Email: zoe.zhu@tufts.edu

LncRNA ANRIL, a 58 kb interval on human chr9p21, has been proven to be a genetic risk factor for atherosclerosis, periodontitis, diabetes, and cancer. LncRNA APDC is an ortholog of ANRIL located on chr4 of the mouse genome. Our aim is to gain a better understanding of the regulatory role of lncR-APDC in bone cells and adipocytes metabolism. In our study, increased expression of lncR-ANRIL and lncR-APDC was found in cultured mouse bone marrow stromal cells (BMSCs) and human FOB cells during early stage of osteogenesis, respectively. Bone marrow cells harvested from lncR-APDC knockout (KO) mice were cultured and used for next generation RNA sequencing, which revealed significant decrease of bone development and mineralization. The qPCR results further confirmed that lncR-APDC deficiency impaired osteogenesis but promoted adipogenesis and osteoclastogenesis in BMSCs cells. In contrast, lncR-APDC overexpression in BMSCs stimulated osteogenesis, but inhibited adipogenesis and osteoclastogenesis. Moreover, in vivo, lncR-APDC deficiency decreased osteogenic markers in bone cells and increased adipogenic markers, such as APN, APPL1, APPL2, and AdipoR2, in adipocytes, which resulted in enhanced energy expenditure of adipocyte precursors. Cultured lncR-APDC KO BMSCs showed early appearance of p38 phosphorylation and remarkably upregulated TLR4 and MyD88 protein expression during osteoclastic differentiation, which indicates the activation of MAPK/p38 and TLR4/MyD88 signaling pathways. By tracking lncRNA-miRNA binding products with luciferase assay in co-transfected cell cultures, we identified miR-99a as a potential binding target of lncR-APDC. KDM6B, a downstream gene of miR-99a, was downregulated in lncR-APDC BMSCs KO cells and upregulated in lncR-APDC BMSCs OE cells. These results suggest that lncR-APDC exerts its osteogenic function via miR-99a/KDM6B/Hox pathway. In conclusion, lncR-APDC represents an important regulatory element in bone cells and adipocytes and possesses therapeutic potential in correcting the imbalance of bone and adipose tissue metabolism.

A-1007

Developing Cell Lines from Fish and Shellfish - Cellular Aquaculture Invitromatics. LUCY E. J. LEE. Faculty of Science, University of the Fraser Valley, Abbotsford BC, CANADA. Email: lucy.lee@ ufv.ca

As the world fisheries collapse and aquaculture ventures become non-sustainable, alternate sources of seafood are being sought-out. With the advent of "cellular agriculture", development of meat under controlled laboratory conditions is becoming an alternative venue to source meat without the ethical or environmental concerns that traditional meat sources have been subjected to. Seafood meat has been traditionally obtained from the muscle fillets of top predatory fish. However, excessive fishing, climate change and anthropogenic influences have limited the availability of desired seafood species, to the point that many are becoming extinct or are endangered. Modern cell culture techniques are allowing the culturing of muscle cells along with fibroblasts and adipocytes that constitute meat, to be readily produced under most aseptic techniques without the fear of contaminant buildup or fouling. This presentation reviews the state of current technologies in fish "invitromatics" that could make cellular aquaculture a reality. Invitromatics is a relatively new term introduced in 2017 to describe a field of research that dates back 80 years but had no distinct terminology. Since the early 1940's, scientists have been able to culture and grow cells outside an organism and maintain them for seemingly indefinite times as "cell lines". Most famous among the over 100,000 cell lines developed to date, is HeLa, the human cell line derived from a cervical cancer biopsy of an Afro-American woman over 70 years ago. HeLa was instrumental in the development of key vaccines and cancer medications, technologies for in vitro fertilization, gene mapping, cloning, among many other biotechnological applications. Although most cell lines available to date have been derived from humans, cell lines from many other organisms have been developed including fish. As of March 2022, Cellosaurus, a database cataloguing reported cell lines in the scientific literature, indicates the existence of close to 1000 fish cell lines. In this presentation I will share my almost 40 yrs experience as a fish invitromaticist.

ANIMAL INTERACTIVE POSTER ABSTRACTS

IN VITRO ANIMAL CELL SCIENCES INTERACTIVE POSTER SESSION

Tuesday, June 7, 1:30 pm – 2:30 pm

A-3000

The Establishment of a Sponge Cell Hybridoma. CASSADY DOUGAN, Megan Conkling, Peter J. McCarthy, Amy E. Wright, and Shirley A. Pomponi. Florida Atlantic University, Harbor Branch Oceanographic Institute, Ft. Pierce FL. Email: cdougan@fau.edu

Marine sponges are one of the most prolific sources of chemical compounds with human health applications. Such compounds have proven to be active against bacterial, fungal, and viral infections, as well as cancer and other diseases. Large quantities of the compounds are required for clinical development, which is challenging to obtain without overharvesting the natural population. One biological approach to the supply bottleneck is *in vitro* production of the compounds, however, not all sponge cells can be cultured. We hypothesized that the fusion of rapidly dividing cells of a sponge that does not produce a pharmaceutically relevant compound with cells of a nondividing but compound-producing sponge would result in a hybridoma that produces the compound of interest. In this study, we have established hybridomas between cells of two marine sponges, *Axinella corrugata*, which produces the antitumor compound

stevensine, and *Geodia neptuni*, which divides rapidly in a nutrient medium optimized for sponge cell culture. Successful hybridization and subsequent cell division and *in vitro* stevensine production unlock the potential for sustainable mass production of other compounds that are currently not amenable to scale-up using other approaches.

A-3001

ECEL1, PIEZO1 and NAV2 Genes Are Altered in the Enhanced and Directed Axonal Growth in Dorsal Root Ganglia Grown Ex Vivo on Nano-scale Grooved Topographical Surfaces. JULIE ELLEN TAMAYO[1], Kelly Keeler[2], Joshua Z. Gasiorowski[2], Michele Fornaro[3], and Kolla Kristjansdottir[2]. [1]Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308; [2]Biomedical Sciences Department, College of Graduate Studies, Midwestern University, Downers Grove, IL 60515; and [3]Anatomy Department, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL 60515. Email: julie.tamayo@midwestern.edu

Peripheral nerve injury can result in chronic nerve damage. Current treatment methods vary in recovery outcomes from temporary to permanent sensation loss. Peripheral nerve regeneration is a multi-factorial process, requiring both cellular and physical cues. Previous research in our laboratory demonstrated that axonal regrowth from mouse dorsal root ganglia (DRG) grown *ex vivo* can be directed and enhanced when grown on nano-scale grooved topographical surfaces. The genes and pathways that detect and respond to topographical cues are not well known. To identify genes involved in the enhanced and directed axonal regrowth, we grew mouse DRG *ex vivo* on flat or grooved topographical surfaces for six days. The DRGs were harvested and RNA was isolated. RNA samples were sent to the NUcore facility at Northwestern University for RNAseq analysis using TruSeq mRNA library prep and Illumina HiSeq Sequencing. Analysis of triplicate samples revealed 122 genes altered in abundance on grooved topography compared to flat control**.** We selected 3 genes that were altered in abundance and used RT-qPCR to validate expression levels. RNA was converted to cDNA and relative expression levels of ECEL1, PIEZO1 and NAV2 were compared to HPRT (control) using Taqman probes. Statistical analysis was performed using one-way analysis of variance (ANOVA) using Prism software and significance denoted at $p < 0.05$. Consistent with the RNASeq results, ECEL1 levels were increased while PIEZO1 and NAV2 levels were decreased in DRGs grown on nano-scale grooved topographical surfaces compared to flat surfaces. Our results suggest that ECEL1, PIEZO1 and NAV2 may be involved in controlling directionality of axonal regrowth in response to topographical cues.

ANIMAL POSTER ABSTRACTS

A-3002

Changes to Plasma Membrane Composition and Loss of Communication in Osteoblasts with Rb1 Deficiency. ELISHA PENDLETON, Keren Abdalah, and Nalini Chandar. Department of Biochemistry and Molecular Genetics, College of Graduate Studies, Midwestern University, Downers Grove, IL. Email: ependl@mid-western.edu

Osteosarcomas are characterized by inactivation of tumor suppressor genes p53 and Rb1. Recent studies have shown roles for Rb1 in maintaining cell adhesion and cell fate. In previous studies we have been able to demonstrate changes to gap junctional intracellular communication with a reduction in Rb1 expression. In this study we have attempted to understand if plasma membrane ultrastructure and other structural components contributed to the loss of communication. We studied expression of adherent junction protein cadherin 11 (Cad11), occluding junction protein, Zona occludens-1 (ZO-1), gap junction protein connexin 43 (Cn43), and the cytoskeletal protein vinculin (Vcl) in osteoblasts. Using scanning electron microscope, we found morphological alterations in osteoblasts with a stable knockdown of Rb1 expression when compared to control MC3T3 E1 osteoblasts. These normally polygonal cells appeared more elongated and fibroblastic with Rb1 loss. We have also analyzed cytoskeletal changes that were evident in the plasma membrane of these cells. A semi quantitative analyses of the different structures (filopodia, lamellipodia and pseudopodia) showed that there were more pseudopodia and lamellipodia in cells with Rb1 deficiency. Cad11, ZO-1 and Vcl protein levels were decreased in Rb1 knockdown cells when compared control. However, Cn43 expression was unaltered. Our preliminary data demonstrates that there are both morphological and molecular changes with Rb1 deficiency in the membrane of these cells. While expression of the gap junctional protein Cn43 is unaltered, alterations in other plasma membrane components may likely have impacted the overall structure and function of the cell membrane and led to a decrease in gap junctional intercellular communication.

A-3003

Modulation of Fibroblast Growth and Senescence in Response to Sonic Hedgehog Inhibition. JOHNSON FONG[1], Tatiana Mendez[1], Sunnie Kuna[1], Sarah Pagni[1], Janet Cowan[2], and Addy Alt-Holland[1]. [1]Tufts University School of Dental Medicine and [2]Tufts Medical Center, Boston, MA 02111. Email: Johnson.fong@tufts.edu

Skin basal cell carcinoma (BCC) is the most frequent cancer worldwide, with a lifetime risk of 30%. Unlike patients with sporadic BCC tumors, patients with Gorlin Syndrome can develop numerous BCCs with increasing frequency through life. The key signaling pathway that underlies the development of these tumors is the sonic hedgehog (SHH) pathway. Mutations in the tumor suppressor gene Patched (PTCH) and the proto-oncogene Smoothened (SMO) result in abnormal activation of this pathway in neoplastic basal keratinocytes. Current SHh inhibitors, such as Vismodegib, are offered as systemic treatment to these patients and aim to inhibit and abrogate the growth of tumor cells. Although this treatment modality is effective, cancer recurrence and the emergence of drug-resistant tumors after termination of the treatment suggest that additional mechanisms contribute to the development of BCC tumors. Since dermal fibroblasts play critical roles in the homeostasis of healthy and

cancerous epithelium, this study focused on the response of cultured human dermal fibroblasts to varied concentration of Vismodegib (10nM, 10μM and 100μM) or DMSO (control vehicle). Cultures were treated for seven days, and imaged daily by computer-assisted bright field microscopy. The production of β-galactosidase was used to determine cell senescence, and phosphorylation of distinct signaling molecules was determined by PathScan array. Fibroblasts retained their characteristic elongated cell shape under all culture conditions. Relative to control cultures, Vismodegib-treated cultures showed increased phosphorylation of molecules in key signaling pathways, such as ERK, P70-S6 kinase, and P-38 that are associated with pathways that control cell proliferation, motility, and response to genotoxicity. Cultures exposed to 100μM Vismodegib in particular showed growth inhibition and a marked decrease in cell senescence. While Vismodegib targets BCC cells, it can potentially modulate the growth and behavior of adjacent dermal fibroblasts, which, in turn, may influence the growth and fate of BCC tumors.

A-3004

Delineating the Effects of Vismodgeib and Dermal Fibroblasts on Metabolic Profiles of Basal Cell Carcinoma Cells. S. KASRAIE[1], T. Mendez[1], S. Pagni[1], J. Baleja[2], J. Cowan[3], and A. Alt-Holland[1]. [1]Tufts University School of Dental Medicine, [2]Tufts University School of Medicine, and [3]Tufts Medical Center, Boston, MA, 02111. Email: sima.kasraie@tufts.edu

A major clinical symptom of patients with Gorlin syndrome is the predisposition to development of numerous skin basal cell carcinomas (BCC). These tumors are dependent on abnormal activation of the sonic hedgehog (SHh) pathway, which regulates cell proliferation, organ development, and tissue repair. While the SHh inhibitor, Vismodegib, effectively inhibits BCC tumor growth in adult Gorlin syndrome patients, cancer recurrence and growth of drug-resistant tumors frequently follow treatment cessation, suggesting that additional molecular mechanisms may interact with the SHh pathway and promote BCC development. Since tumors develop in the milieu of supportive stromal cells, these mechanisms may underlie epithelial-stromal interactions and reprograming of tumor metabolism. Here, we studied the effect of a 72hr exposure to 10nM or 10μM Vismodegib on the metabolism of human fibroblasts and BCC cells (ATCC TE 354.T) in co-cultures using an insert/tray system to allow for BCC-fibroblast crosstalk via surrounding growth media. Building on our published study (Mendez et al, 2020), we used nuclear magnetic resonance and metabolomics of conditioned culture media to determine the metabolic profiles of the two cell types with an emphasis on specific energy metabolites. Data was analyzed with t-tests, one-way ANOVA with Tukey's HSD or Welch's ANOVA with the Games-Howell tests, and $p < 0.05$ was considered significant. Exposure of the cultures to Vismodegib had distinct effects on the consumption and secretion of acetate, lactate, glucose, pyruvate, glutamate, and glutamine. These effects were influenced by Vismodegib concentration and the presence of fibroblasts within the co-culture environment. Identifying biochemical pathways that are associated with the SHh pathway, influence epithelial-stromal crosstalk, and can alter the metabolic activity of BCC cells, will provide new insights into BCC tumorigenesis, response to treatment, and cancer recurrence. This, in turn, can guide the development of new and modified BCC therapies and improve outcomes for Gorlin syndrome patients.

A-3005

Growth and Metabolism of Basal Cell Carcinoma Cells and Spheroids in Vismodegib-treated Cultures. TATIANA MENDEZ[1], Arietta Rigopoulos[1], Sarah Pagni[1], James D. Baleja[2], Janet Cowan[3], and Addy Alt-Holland[1]. [1]Tufts University School of Dental Medicine, [2]Tufts University School of Medicine, and [3]Tufts Medical Center, Boston, MA 02111 Email: tatiana.mendez@tufts.edu

While basal cell carcinoma (BCC) tumors tend to grow slowly, their surgical removal can result in morbidity and disfigurement, especially in Gorlin Syndrome patients who develop numerous BCCs throughout their lives. BCCs predisposition is driven by constitutive activation of the Sonic Hedgehog (SHh) pathway. Although SHh inhibitors, such as Vismodegib, effectively reduce the BCC tumor burden in these patients, treatment cessation is linked to cancer recurrence and emergence of drug-resistant tumors. To identify additional mechanisms that contribute to the pathogenesis of BCC, we studied the effect of Vismodegib on the growth and metabolism of human BCC cells and spheroids of de-novo aggregated tumor cells organized in 3D structures. Homogenous cultures of BCC cells, and heterogeneous cultures of BCC cells and spheroids, were treated with 10nM – 1uM Vismodegib or DMSO (control) for 30 days and imaged bi-weekly by bright field microscopy. Homogeneous BCC cultures exhibited individual cells with varied sizes and shapes that formed flat and loose colony-like clusters. In heterogeneous BCC cultures, developing BCC spheroids were adhered to- and surrounded by individual BCC cells, and became opaque over time. In response to DMSO or Vismodegib treatments, new spheroids began accumulating in heterogeneous cultures 2-3 weeks earlier than in homogeneous BCC cultures. Nuclear magnetic resonance analysis and metabolomics of conditioned growth media reveled that while both culture types consumed glutamine, and secreted significant amounts of lactate and acetate, glutamate secretion and pyruvate consumption were markedly increased in homogeneous cultures. Vismodegib treatment modestly reduced acetate levels in culture with BCC cells only, and decreased glutamine levels in cultures with BCC spheroids. Further interrogation of cultured BCC cells and spheroids, which are able to grow and alter their energy metabolism in response to Vismodegib, can shed light on potential mechanisms that support tumor cell growth and survival, and the development of drug-resistant BCC tumors in vivo.

A-3006

Assays for Monitoring Cell Health in Microphysiological Systems. TERRY RISS, D. Leippe, N. Karassina, M. Valley, and J. Vidugiriene. Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711. Email: terry.riss@promega.com

Microphysiological systems (MPS) assembled using 3D organoids interconnected with medium channels are being used to create physiologically relevant multi-organ models that can predict *in vivo* responses to test compounds. Regardless of the primary assay method, it is advisable to incorporate appropriate controls, including confirming the number of viable cells at the end of the experiment. Kinetic real-time bioluminescent assays have been developed that can be multiplexed with many different primary assay methods and provide a cell number control from the same sample well. In addition to simply measuring cell number, it is important to understand the general health of cells in the MPS by monitoring key metabolites reflecting metabolic activity. We will describe a panel of bioluminescent assays for monitoring key metabolic pathways including glycolysis, the pentose phosphate pathway, fatty acid metabolism, amino acid metabolism and the TCA cycle. The assays were built using a core technology that couples metabolite-specific dehydrogenase enzymes with NAD(P)H production and generation of a luminescent signal recorded using a plate reading luminometer. The detection sensitivity and wide linear range of the bioluminescent assays allow simultaneous analysis of multiple metabolites from a small amount of culture medium removed from the MPS device.

A-3007

Cell Cycle Analysis of Vismodegib-treated Human Basal Cell Carcinoma Cells In-vitro. B. SENFI[1], T. Mendez[1], M. Evers[1], S. Pagni[1], J. Cowan[2], and A. Alt-Holland[1]. [1]Tufts University School of Dental Medicine, and [2]Tufts Medical Center, Boston, MA, 02111. Email: babak.senfi@tufts.edu

Basal cell carcinoma (BCC) is the most frequent cancer worldwide, and while the majority of BCC cases arise as slow growing sporadic tumors, patients with Gorlin Syndrome develop numerous BCCs throughout life. BCC tumor growth is dependent on aberrant activation of the sonic hedgehog (SHh) pathway. Although the SHh inhibitor Vismodegib effectively reduces BCC tumor burden in these patients, this treatment modality results in adverse side-effects, incomplete response, and regrowth of tumors upon treatment cessation. Here, we focused on cell cycle and proliferation of cultured human BCC cells in response to Vismodegib treatment. BCC cells (ATCC TE 354.T) were cultured for 21-28 days, and exposed to 10nM-10µM Vismodegib or DMSO (control) for 10 days. Thereafter, cells were trypsinized, fixed with 70% ethanol, treated with PI/RNAase solution, and analyzed using BD FACSAria II Flow Cytometer. Statistical analysis of the data was conducted using *T*-tests, and $p < 0.05$ was considered significant. Parallel BCC cultures were subjected to fluorescent immunostaining with proliferating cell nuclear antigen (PCNA) antibodies, Phalloidin, and DAPI. Flow cytometry analysis of control and Vismodegib-treated BCC cultures demonstrated that the majority of the cells (70-80%) were arrested in G0/G1 phase, while small fractions of cells were in the S (5-8%) and G2/M (12-15%) phases ($p < 0.001$). Increased drug concentrations resulted in a clear trend of an increase in cells in the G0/G1 phase and a decrease in proliferating cells in the S and G2/M phases. Additionally, only a small percentage of BCC cells demonstrated positive PCNA nuclear immunostaining. In conclusion, in established BCC cultures, only a small fraction of the cells undergo DNA replication and cytokinesis, which can contribute to the slow growth feature of these cultures in vitro, and potentially of BCC tumors in vivo. Exposure to increased concentrations of Vismodegib for a short period of time further exacerbate the growth arrest of the majority of these cells and may shed light on the tumoricidal and tumoristatic effects of this drug.

A-3008

Isolation and Characterization of a Muscle-derived Cell Line Obtained from Australasian Snapper (*Chrysophrys auratus*). GEORGINA C. DOWD[1], Gavril Chong[1], Lucy E. J. Lee[2], and Niels C. Bols[3]. [1]The New Zealand Institute for Plant and Food Research Limited, NEW ZEALAND; [2]University of the Fraser Valley, CANADA; and [3]University of Waterloo, CANADA. Email: Georgina.Dowd@plantandfood.co.nz

Chrysophrys auratus (Australasian snapper) is one of the largest, most valuable capture fisheries in New Zealand, yet few cell lines from this species have been developed, meaning the snapper invitrome is largely unexplored. We describe the invitromatics of a muscle-derived cell line initiated from snapper. Designated CAtmus1PFR (***C****hrysophrys* ***a****uratus,* **t**ail **mus**cle, **P**lant & **F**ood **R**esearch), the cell line has been passaged over 90 times in 2 years and is considered immortal. Cell line provenance has been verified by DNA barcoding and it has been confirmed free of *Mycoplasma* sp. contamination. The cell line has successfully been resuscitated following cryopreservation. CAtmus1PFR is routinely cultured in L-15 basal media supplemented with 10% fetal bovine serum (FBS) in non-vented culture flasks maintained at 18°C in ambient air. Thermal tolerance experiments indicate the cell line has a broad thermal profile with comparable proliferative growth occurring between 18°C and 30°C. Hyperthermic temperatures (>32°C) resulted in enlarged senescent cells, while hypothermic conditions (4°C) resulted in cell detachment and death. Various commercially available CO_2-independent basal media formulations were investigated for their capacity to support cell growth. Media supplemented with additional amino acids, vitamins, iron, and sugars resulted in enhanced cell line growth. CAtmus1PFR showed a dose-dependent response to varying FBS concentrations. In the absence of serum, the cell line could not replicate. Cell numbers increased as the concentration of FBS increased from 5 to 25%. The impacts of exogenous growth factors on cell morphology and proliferation were investigated. Addition of basic fibroblast growth factor to cells resulted in cells maintaining a proliferation morphology, with increased final cell numbers compared with standard culture conditions. Insulin-like growth factors also had a positive impact on cell proliferation, but did not seem to affect cell morphology. Ongoing research includes application of CAtmus1PFR in novel ways which will define its invitromatics.

A-3009

Examining the Urinary Exosomal RNA Profile of Cadmium-treated Rats for Novel Biomarkers of Kidney Injury. M. J. Fay[1], K. Kristjansdottir[2,3], K. Bussey[3], J. Edwards[1], and W. Prozialeck[1]. [1]Midwestern University, Department of Pharmacology, 555 31st

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Cadmium (Cd) is a known human toxicant that causes kidney damage at the proximal tubule. Previously we demonstrated that Cd dysregulates the microRNA expression profile in the renal cortex of 12-week Cd-treated rats with documented kidney injury. The purpose of this research was to determine if urinary exosomes isolated from Cd-treated rats versus saline controls demonstrate a differential RNA expression profile. Urine samples (24-hour) were collected from 12-week Cd-treated (N=6) and saline control (N=6) male Sprague Dawley rats and differential RNA expression was evaluated using the Exosome NGS Service from System Biosciences. Bioinformatics analysis of the data was performed using the Banana Slug Analytics platform for exosome analysis. After quality control, FASTQ files were aligned to rn5 to generate raw read counts which were then normalized. DESeq was used to determine differential expression. RNA species were defined as differentially expressed if the adjusted p-value from the negative binomial test was less than 0.05. A total of 1263 RNA species were differentially expressed when comparing exosomes from Cd-treated to saline-injected control rats. Of these, 300 were mRNAs, 136 were tRNAs, 106 were piRNAs, 4 were miRNAs, and 6 were other non-coding RNAs. All the miRNAs identified were under-expressed. Among the mRNAs, there were 144 over-expressed and 156 under-expressed transcripts. The tRNAs were generally under-expressed with the notable exception of GlyGCC, which was over-expressed greater than 5-fold on average. The results of this study confirm differential expression of RNA species in urinary exosomes from Cd- treated versus saline control rats. Further studies are needed to examine exosomal RNAs as a source of biomarkers for Cd-induced kidney injury and to determine their role in Cd-induced kidney injury.

A-3010

Antioxidant and Cardioprotective Properties of Peanut Hairy Root-derived Prenylated Stilbenoids in Cardiac Cells. ROKIB HASAN[1,2], Sankalpa Chakraborty[1,2], Viswanathan Rajagopalan[1,2,3,4], and Fabricio Medina-Bolivar[1,2,4]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401; [2]Molecular Biosciences Graduate Program, Arkansas State University, Jonesboro, AR 72401; [3]Department of Basic Sciences, New York Institute of Technology-College of Osteopathic Medicine, Jonesboro, AR 72401; and [4]Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401. Email: mdrokib.hasan@smail.astate.edu, vrajagop@nyit.edu, fmedinabolivar@astate.edu

Despite great therapeutic advances, cardiovascular diseases (CVD) remain a major health problem and the leading cause of mortality worldwide. To further improve the symptoms and survival of CVD patients, novel therapeutic strategies are needed. Stilbenoids are a non-flavonoid class of polyphenols that are important for their potential medicinal applications. Resveratrol, a polyphenolic compound found in a select group of plants, exhibits antioxidant properties and cardioprotective function. Prenylated stilbenoids, which include arachidin-1 and arachidin-3, are derivatives of resveratrol found in the medium of elicitor-treated peanut hairy root cultures, and are potentially more bioavailable than the resveratrol. However, the molecular mechanisms underlying the bioactivities of prenylated stilbenoids remain unclear. To study the potential cardioprotective effect of prenylated stilbenoids against oxidative stress, we used hydrogen peroxide-treated rat H9c2 (2-1) model. Our results suggest that extracts enriched in prenylated stilbenoids and purified arachidins are not toxic to the cells at low concentrations. Both the extract and arachidins protected against oxidative stress in the hydrogen peroxide-treated cells. We have also found that arachidins regulate the expression of genes related to the detoxification of reactive oxygen species. These studies enhance our understanding of the antioxidant properties of arachidins in vitro, and carry important translational implications for the application of prenylated stilbenoids in cardioprotection.

A-3011

Pro-inflammatory Signaling Is Induced in Cancer Cells by Electroporation with a Synthetic dsRNA Analog. LOREE C. HELLER, Amanda Sales Conniff, Guido Encalada, Shreena Patel, Manya Bhandary, and Farah Al-Takrouri. Department of Medical Engineering, University of South Florida, 12901 Bruce B. Downs Blvd., MDC 111, Tampa, FL 33612. Email: lheller@usf.edu

Germline-encoded pattern recognition receptors (PRRs) are found in all mammalian cells and function in the detection molecular patterns associated with pathogen invasion or cellular damage. PRR activation induces the production of type I interferons (IFN), pro-inflammatory cytokines and chemokines, and cell death. The PRR subset termed RNA sensors detect inappropriately located or modified intracellular RNA. Polyinosinic-polycytidylic acid or poly(I:C) is a synthetic analog of double-stranded RNA (dsRNA) which binds and activates sensors specific to dsRNA. Consequently, this molecule has been tested as a sole or combination cancer therapy in preclinical studies and clinical trials with limited success. The purpose of this study was to evaluate the effects of poly(I:C) transfection via electroporation (EP) on cell lines from cancer of epithelial origin, 4T1 mammary carcinoma, and cancer of mesenchymal origin, WEHI 164 fibrosarcoma. After poly(I:C) EP, significant cell death was detected by quantifying cell metabolism. Significant secretion of Type I IFN and several cytokines and chemokines indicated the initiation of pro-inflammatory signaling. After poly(I:C) transfection, the mRNAs of RNA sensors DExD/H-box helicase 58/retinoic acid-inducible gene 1 protein (Ddx58/RIG-I) and DEAH-box helicase 9 (Dhx9) were not regulated, but the mRNAs of toll-like receptor 3 (TLR3), interferon induced with helicase C domain 1/melanoma differentiation-associated protein 5 (IFIH1/MDA5), interferon-inducible protein 204 (Ifi204), DEAD (Asp-Glu-Ala-Asp) box polypeptide 60 (Ddx60), and Z-DNA binding protein 1 (Zbp1) were upregulated in both cell lines. RNA sensors signal via interferon regulatory factors (IRFs). IRF1 and IRF7 mRNAs

were upregulated in both cell types; IRF3 was present but not upregulated. These data demonstrate that the baseline pro-inflammatory effects of poly(I:C) can be amplified by electroporation, potentially impacting the therapeutic use of this molecule.

A-3012

Lower Molecular Weight Polycyclic Aromatic Hydrocarbons: Carcinogenic Potential in Lung. ALISON K. BAUER,[1] Sabine Plottner,[2] Melanie Wolff,[2] Heiko U. Kafferlein,[2] and Brad L. Upham.[3] [1]Department of Environmental and Occupational Health, Colorado School of Public Health, University of Colorado Anschutz, Aurora, CO 80045; [2]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance Institute of the Ruhr University Bochum (IPA), 44789 Bochum, GERMANY; and [3]Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI 48842. Email: alison.bauer@cuanschutz.edu, upham@msu.edu

Air pollution is classified as a human lung carcinogen with ~30% of lung cancers worldwide the result of non-smoking related air pollution. Polycyclic aromatic hydrocarbons (PAHs) are major components of outdoor (e.g., wildfire smoke) and indoor air pollution (e.g. secondhand smoke). . Thus far, most research has focused on the higher molecular weight (HMW), genotoxic PAHs for all health effects. The IARC recognizes benzo[*a*]pyrene (B[*a*]P) and other HMW PAHs as human carcinogens. However, given a lack of studies directly investigating lower molecular weight (LMW) PAHs, they are currently not classifiable as carcinogens by IARC (group 3; except naphthalene). Importantly, both indoor and outdoor air pollution have far greater amounts of these LMW PAHs in than HMW PAHs. Our previous studies implicate them in altering critical signaling pathways involved in lung cell functions. Our recent novel *in vitro* and *in vivo* data supports the hypothesis that LMWs combined with B[*a*]P increase carcinogenic relevant events in lung, including tumor promotion. We evaluated several *in vitro* endpoints in human BEAS-2B (bronchial epithelial) and A549 (alveolar type II cell, tumorigenic) cells including *anti*-BPDE-DNA adducts and gap junction activity. In addition, a novel *in vivo* 2-stage tumor promotion model was developed where an initiator (ip., 3-methylcholanthrene; MCA) followed by 4 weekly oropharyngeal aspirations of B[*a*]P, an LMW PAH mixture of 1-methylanthracene, fluoranthene, and phenanthrene (equimolar), or a combination of both. Tumor endpoints were evaluated. Dysregulation of gap junctional intercellular communication and increased *anti*-BPDE DNA adducts were observed *in vitro*. *In vivo* significant lung tumors (all adenomas/adenocarcinomas) were *only* present in the MCA/B[*a*]P/LMW PAH mixture group with incidence at 89%, supporting carcinogenic activities of these LWM PAHs. Our data provides evidence that these LMW PAHs have carcinogenic potential and support the need for additional studies to understand the mechanisms driving tumor development and how to intervene.

A-3013

Sex linked Aberrant Behavior and Hippocampal Gene Expression in *Egr1* Conditional Knockout Mice. C. SWILLEY and H. Xie. Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, 24061. Email: Codys20@vt.edu

Early growth response gene-1 (*Egr1*) is a critical transcription factor involved in epigenetically regulated biological processes, including neuronal plasticity and memory formation. Despite numerous studies on mice with *Egr1* complete knockout, little information has been gathered regarding the sex differences in behavior and gene expression resulted from *Egr1* conditional knockout. In this study, we obtained mice with *Egr1* removal in neuronal lineage cells using Nestin-Cre recombinase drivers. Age-matched controls were included to study the sex-linked influence of *Egr1* loss on growth and behavior. We have performed four behavioral assays and female mice have shown higher levels of anxiety and delayed responses to heat intolerance. All mice in the breeding paradigm have been tracked for growth from postnatal day P0 to 12 weeks, and the conditional knockout *Egr1* mice are smaller in stature. In addition, RNAseq will be explored to determine changes in hippocampal gene expression. Altogether, our study will determine sex linked changes in gene pathways and might shed new light on the molecular mechanisms associated with aberrant behaviors observed in *Egr1* conditional knockout mice.

A-3014

Biophysical Characterization of the HIV-1 Nucleocapsid Protein RNA Interaction Properties. ANISSA BELFETMI[1], Loussine Zargarian[1], Assia Mouhand[2], Marjorie Catala[2], Philippe Fossé[1], Carine Tisné[2], and Olivier Mauffret[1]. [1]Laboratoire de Biologie et de Pharmacologie Appliquée (LBPA), UMR 8113 CNRS, Institut D'Alembert, École Normale Supérieure Paris-Saclay, Université Paris-Saclay, 4, Avenue des Sciences, 91190 Gif sur Yvette, FRANCE and [2]Expression Génétique Microbienne, UMR 8261, CNRS, Institut de Biologie Physico-Chimique (IBPC), Université de Paris, 75005 Paris, FRANCE. Email: abelfetmi@crystal.harvard.edu

The HIV-1 nucleocapsid protein (NC) is a nucleic acid chaperone playing a crucial role in several steps of the virus life cycle. To delineate the biophysical properties and chaperon activity of this protein, we studied its binding to RNA molecules under different protein maturation state. Using nuclear magnetic resonance (NMR) spectroscopy, Förster Energy Transfer (FRET), and gel retardation assay (EMSA), we evaluated the destabilization and annealing of Trans-activation response element TAR RNA and complementary DNA cTAR. An important molecular process during the 1st strand transfer of the viral reverse transcription. At a low ratio of protein: nucleotide (NC:TAR), we observed the first destabilized base pairs on the apical stem loop, which suggested a nucleation site – Guanine 26. We also observed a hybridization of the TAR RNA-cTAR DNA even at low concentrations of NC. However, the conversion rate to the extended duplex seemed to be strongly dependent on a high ratio of NC:nucleotides. Furthermore, we investigated the properties

of NC under its three maturation forms, which appear successively during the viral replication cycle. Our internal dynamic studies showed that the additional C-terminal domains p1 and p6, present within the immature forms (NCp9 and NCp15) and not on the mature NCp7, influence the interaction properties to nucleic acids. We thus reconsider the properties of NC within the polyprotein precursor Gag, and the role of other domains (Matrix MA and Capsid CA) at the plasma membrane of the infected cell. Indeed, these domains tunes the properties of NC during the recognition and selection steps of the viral genomic RNA, to package it in the newly formed viral particles. To sum up, our study indicates that in addition to a conserved binding site of NC to nucleic acids, the protein can readjust its biophysical properties and play different roles during the HIV-1 viral life cycle. Since NC constitutes an attractive therapeutic target due to its conservation and importance in viral infectivity, deeper understanding of NC features is critical for the design of anti-NC inhibitors.

A-3015

High-throughput Genotyping by Sequencing with the AgriSeq™ Workflow. T. DUNBAR and A. Burrell. Thermo Fisher Scientific, 2130 Woodward St. Austin, TX 78701. Email: Tia.Dunbar@ThermoFisher.com

Genotyping by Sequencing (GBS) is a robust tool in plant and animal genomics that is used to identify SNPs for a wide range of applications in agrigenomics. Targeted GBS is an essential method not only for high-throughput and cost-effective marker-assisted breeding, but discovery of important variants. Such a workflow allows thousands of markers to be analyzed per day with minimal labor and laboratory inputs. Our AgriSeq™ targeted GBS workflow involves a customizable, multiplexed PCR for targeted SNPs and indels of interest. AgriSeq™ methods have been used to investigate a broad range of species and identify an array of traits including disease susceptibility and parentage. Up to 1536 uniquely barcoded samples can be prepared in just a matter of hours and processed on a single Ion Torrent™ sequencing run. Our workflow has been designed with flexibility and simplicity in mind, thus easily adaptable to individual labs using common laboratory equipment. In addition, our scaled-down library-prep reaction volumes and increased sample throughput cut traditional workflow costs for further ease-of-use. We validated the high-quality performance of our AgriSeq™ methods with multiple panels: 1536 barcoded samples were processed with the AgriSeq™ HTS Library Kit and sequenced on the Ion S5™ XL System. We used panels of different sizes that are tailored to different species—one plant and one animal—to illustrate workflow versatility. In addition, we exemplify reproducibility of results between and within library preparations through quality metrics such as call rate, coverage depth, uniformity, and genotype concordance. Here we provide an example report for the AgriSeq™ targeted GBS workflow and analysis to demonstrate its overall value as a high-throughput, low-cost library prep method. *For Research Use Only. Not for use in diagnostic procedures.*

ANIMAL VIRTUAL POSTER ABSTRACTS

A-3016

A Novel Approach to Bone Implant Infection Therapeutics Leveraging Proteins Derived from L. *acidophilus* and Modulating Anti-inflammatory Gene Expression. ANOUSKA SEAL. College of Medicine, University of Central Florida, Orlando, FL. Email: lilyseal205@gmail.com

Staphylococcus aureus bacteria is the world's leading cause of bone implant infection causing osteomyelitis. Osteomyelitis accounts for half of all chronic diseases in people over 50 and this number has doubled in 2020. The risk for infection after a bone implant is up to 50% (Seebach and Kubaztky). Osteomyelitis causes septic arthritis, skin cancer and may lead to death. Traditional antibiotics cannot cure this as S. aureus is due to antibiotic resistance. Osteomyelitis treatment is notoriously difficult as there are no non-invasive solutions as the current treatment is a bone debridement surgery with a strong regimen of non-traditional antibiotics, which is not cost or time effective, as the patient is required to follow them for up to 5 years. In this project the hypothesis being tested is about the proteins derived from L. acidophilus, will inhibit bacterial growth and inflammation when applied to S. aureus and macrophages. The experiment was designed to extract the proteins from the L. acidophilus by varying concentration concentrations of Ammonium sulfate precipitation. After gel electrophoresis was run, strips of the gels were then placed on a petri dish and agar with S. aureus was poured over each petri dish. While, all the concentrations had significant zones of inhibition, it was found that the 40% ammonium sulfate precipitated protein had the greatest zone of inhibition and inhibited the most S. aureus growth. To test for the anti-inflammatory genes, the proteins were added to the macrophage. After qPCR, we could see that all the anti-inflammatory markers tested for, TGF β, CD163, CD206 and IL-10 were significantly upregulated. This means that there was a greater presence of all anti-inflammatory genes once treated with proteins. The derived proteins were successful in both inhibiting the bacteria and reducing inflammation. This all-in-one solution combats osteomyelitis preventatively and drastically reduces costs for complicated surgeries and aids in the recovery process of patients, making it time and cost effective. A future protein spray therapeutics is envisioned.

A-3017

Evaluation of DJ-1 and PTEN as Prognostic Biomarkers in Cutaneous Melanoma. ZIYI SUN[1] and Hilal Arnouk[2]. [1]Midwestern University College of Osteopathic Medicine, Downers Grove IL and [2]College of Graduate Studies, Midwestern University, Downers Grove, IL. Email: ziyi.sun@midwestern.edu

Objective. The aim of this study is to evaluate the expression of two signaling molecules that are implicated in the progression of cutaneous melanoma: DJ-1, an oncogene, and PTEN, a tumor suppressor gene, in the normal skin and melanoma tissue samples, and to correlate their expression levels with various clinicopathological parameters of melanoma lesions. Thus, establishing DJ-1 and PTEN as prognostic biomarkers for

melanoma. Study Design. Immunohistochemistry (IHC) staining was performed on tissue microarray samples representing normal skin and melanoma biopsies of different clinical stages. High-resolution photomicrographs were evaluated with Aperio Imagescope using a positive-pixel-counting algorithm. A histo-score (H-score) was calculated based on staining intensity and the percentage of positive cells (%-staining). Mean differences in H-scores were compared with regard to their TNM parameters, separately and combined into clinical stages, according to the American Joint Committee on Cancer (AJCC) classification. Results. Both DJ-1 and PTEN were found to correlate with tumor thickness (T), which is a reliable indicator for survival rates. Specifically, DJ-1 and PTEN were both significantly downregulated in tumors with thickness over 2 mm (T3+T4) compared to tumors with thickness at or below 2 mm (T1+T2). Conclusions. DJ-1 and PTEN expression levels, as measured by immunohistochemistry, helped differentiate between tumors with thickness over 2 mm and tumors with thickness at or below 2 mm, making them potential prognostic indicators for the risk of melanoma progression and survival rates. Longitudinal studies evaluating risk stratification based on the expression of DJ-1 and PTEN are needed to establish the utility of these two biomarkers in the clinic as an adjunct for pathological examination.

A-3018

Per and Polyfluorinated Substances Dysregulate Gap Junctional Intercellular Communication. JAMIE E. LIEBOLD[1], Lizbeth Lockwood[1], Joo Hye Yeo[2], Jinu Lee[2], Alison K. Bauer[3], Erika Lisabeth[4], Richard Neubig[4], and Brad L. Upham[1]. [1]Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI 48824; [2]College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 21983, SOUTH KOREA; [3]Department of Environmental and Occupational Health, University of Colorado-Denver, Aurora, CO 80045; and [4]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824. Email: lieboldj@msu.edu

The selection of gap junctional intercellular communication (GJIC) as an endpoint is a significant step in developing a systems-based *in vitro* model to assess the toxic potential of environmental contaminants, as this biological phenomenon is crucial for integrating signaling mechanisms within cells with that of neighboring cells in a tissue and is an important early-stage event in abnormal cell proliferation. Thus, we are determining the effects of per and polyfluoroalkyl substances (PFAS) on GJIC, which are major environmental contaminants and known as "forever chemicals". We are using the well-established "scalpel load – dye transfer" assay to assess GJIC as a function of dose and time and comparing the dose response results with the lab's newly established high throughput screening (HTS) assay. Both assays used the F344 WB cell line, which is an excellent *in vitro* cell model of liver oval cells, a bipotent stem/progenitor cell that give rise to hepatocytes and hepatic biliary duct cells and self-renew. The new HTS is quite unique that depends on a subset of donor and subset of receptor cells where the donor cells are stably transfected with the iodide transporter gene and the acceptor cells with the yellow fluorescent protein (YFP) gene. The addition of iodide initiates the bioassay by entering the donor cells *via* the iodide transporter, and then transfers through gap junctions to the receptor cells, in which iodide quenches the YFP-fluorescence. Closed or partially closed gap junction channels prevents or partially prevents quenching in the receptor cells from iodide. My results will help validate the new HTS assay system and provide crucial data for assessing the toxicity of PFAS. Research supported: National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH) under Award Number R21ES031345. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH-NIEHS."

A-3019

Evaluation of DJ-1 and PTEN Expression in Oral Squamous Cell Carcinoma. REY DE LA TORRE and Hilal Arnouk. Midwestern University, College of Graduate Studies, Downers Grove, IL. Email: rey.delatorre@midwestern.edu, harnou@midwestern.edu

Objective. This study aims to evaluate the expression of DJ-1 and PTEN as prognostic biomarkers in oral squamous cell carcinoma (OSCC). DJ-1 is an oncogene and an inhibitor of PTEN, which is a tumor suppressor gene that is defective in several types of cancer. Study Design. Immunohistochemistry analysis was performed on tissue microarray (TMA) samples representing normal oral mucosa and OSCC biopsies of different clinical TNM stages and histopathological grades using anti-DJ-1 and anti-PTEN antibodies. Tissue images were analyzed with Aperio ImageScope (Leica Biosystems) utilizing a positive-pixel-counting algorithm to quantify the staining intensity. A histo-score (H-score) was calculated based on staining intensity and percentage of positive cells staining. Comparisons were made for the Mean H-scores of normal oral mucosa versus OSCC samples and across the T, N and M parameters, overall clinical stages, and the histopathological grade using a two-tailed T-test. Results. The study was able to find significantly increased DJ-1 expression in OSCC samples in comparison to the normal oral mucosa, and significantly increased DJ-1 expression in high histopathological grade OSCC in comparison to low histopathological grade OSCC. The study also found significantly increased PTEN expression in OSCC samples in comparison to normal oral mucosa, and significantly decreased PTEN expression in high histopathological grade OSCC in comparison to low histopathological grade OSCC. Conclusions. DJ-1 and PTEN expression levels can reliably differentiate between the normal oral mucosa and oral squamous carcinoma. Moreover, DJ-1 and PTEN expression significantly correlates with the histopathological grade/differentiation status of OSCC, adding to their potential utility as prognostic biomarkers for oral cancer. Follow-up studies to monitor of DJ-1 and PTEN levels throughout the progression of OSCC and relative survival rate data are needed to firmly establish these two biomarkers in the clinical setting to supplement current pathological assessment and prognosis.

A-3020
Using Nanoparticle-based Magnetic Hyperthermia to Hinder Planarian Regeneration. THOMAS C. COMMANDER and Marion Zeiner. Episcopal School of Jacksonville, FL. Email: Commanth01@esj.org

The purpose of this investigation is to evaluate the effectiveness of chitosan-coated magnetic iron oxide nanoparticle-based magnetic hyperthermia in hindering or halting stem cell-driven regeneration in planaria, flatworms with a large population of stem cells, and forms of the PTEN gene, a commonly mutated gene in human cancers, thus making planaria worthwhile subjects for research upon this novel form of thermal ablation and its effects on stem cells. It was hypothesized that the iron oxide nanoparticles, when subjected to an alternating current, would create enough friction to raise the temperature of the treated planarian to halt stem cell growth and that chitosan alone would promote stem cell regeneration. Iron oxide nanoparticles were made in a jacketed glass reactor. The nanoparticles were administered to half of the amputated planarian tails used in trial 1. Either an AC or a DC was then administered via solenoid coil to 2⁄3 of planarian tails. Temperature after treatment was measured and planarian mortality rates over a week were recorded. In trial two, 9 planarian tails were given various treatments and after a designated amount of time, they were photographed under a microscope and the lightness of the photographs was measured to quantify the regeneration. For the first trial, experimental groups that displayed a significant rise in temperature also saw much higher mortality rates than groups that did not display a significant increase in temperature. In the second trial, the tails treated with just chitosan showed much more cellular regrowth than control tails with a 39.85% difference in cell population. The tails treated with the chitosan-coated nanoparticles did not show as much cellular regrowth, but they did show stronger growth than control groups with a 9.56% difference in cell population. Cancer stem-like cells are the leading cause of cancer metastasis and are extremely drug-resistant and difficult to target. This method provides an opportunity to target stem cells and ultimately treat them locally, reducing collateral damage and making cancer more easily dealt with.

PLANT CONTRIBUTED PAPER ABSTRACTS

BIOTECHNOLOGY, GENOME EDITING AND GENETIC ENGINEERING CONTRIBUTED PAPER SESSION

Monday, June 6, 5:00 pm – 6:00 pm

P-1015
Molecular Physiological Responses of *Paulownia* and Turmeric Under Various Abiotic Stress Conditions. C. BASU[1], M. Chaires[1], K. Cooper[2], D. Gupta[3], N. Joshee[4], N. Katiyar[5], S. Pakala[6], N. Ramadoss[7], B. Bharadwaj[1], and K. Musaev[1]. [1]Dept. of Biology, California State University, Northridge, CA; [2]School of Animal and Comparative Biomedical Sciences, University of Arizona; [3]Department of Molecular and Cell Biology, University of California, Berkeley; [4]College of Agriculture, Family Sciences and Technology, Fort Valley State University, GA; [5]Jackson Laboratory, CT; [6]Vanderbilt University Medical Center, Nashville, TN; and [7]Department of Biology, San Diego State University, San Diego, CA. Email: chhandak.basu@csun.edu

Abiotic stresses, including heat, cold, drought, and salinity, are significant causes of global crop loss. Plants are sessile organisms and hence can not move away from environmental stresses. They must adapt to stress or succumb to these conditions. High throughput sequencing technologies, including NGS (Next Generation Sequencing) technologies, assist us in understanding the physiology of plant growth and development in stressful environments. The overall goal of our lab is to understand the molecular physiological mechanisms of stress tolerance in plants. We have studied various abiotic stresses in two plant species: (1) Paulownia elongata (salinity and heat stresses) and (2) turmeric (drought and salinity stresses). Plants were undergone through various abiotic stresses as described below, followed by RNAseq to compare the transcriptomes of stressed versus unstressed plants. A miseq instrument was used to sequence barcoded cDNAs from control and stressed plants. Paulownia plants were grown with 125 mM NaCl for ten days for the salt stress experiments. RNAseq analysis revealed differential expression of 645 genes due to salt stress, and some of the salt stress-related genes we found upregulated are given below: GDSL esterase/lipase, glucan-1,3-beta-glucosidase, and glyceraldehyde-3-phosphate dehydrogenase. For the heat stress, Paulownia and turmeric plants were grown at 40C for 24 hours. For Paulownia, we identified 4,435 genes that were differentially expressed when plants experienced heat stress, and we identified the following three genes, which were highly upregulated: tonoplast dicarboxylate transporter, heat shock protein, and triacylglycerol lipase. For drought stress, water was withheld from the turmeric plants grown for 14 days. We are in the process of conducting RNAseq with the turmeric plants undergoing heat and drought stress. Understanding how plants respond to abiotic stresses will help us develop stress-tolerant crops and enhance agricultural productivity in the future.

P-1016
DNA-Free Genome Editing in Hexaploid Sweetpotato Directed by Preassembled CRISPR-Cas9 Ribonucleoprotein Complexes. ADRIANNE BROWN, M. Egnin, F. Bukari, D. Mortley, C. Bonsi, O. Idehen, D. Alexander, and G. Bernard. Tuskegee University Plant Biotechnology and Genomics Research Laboratory, Department of Agriculture & Environmental Sciences, College of Agriculture Environment and Nutrition Sciences (CAENS). Tuskegee, AL, 36088. Email: abrown9633@tuskegee.edu, megnin@tuskegee.edu

The simplicity and specificity of CRISPR-Cas9 has become the primary method of editing asexually propagated polyploid plant species. One such plant, sweetpotato, endures genetic modification challenges due to multiple allelic polymorphisms or integrations. Though CRISPR-Cas9 and Cas13 have been utilized to improve sweetpotato using naked DNA or Agrobacterium tumefaciens delivery routes, removing foreign DNA through breeding is impossible. In addition, extended-expression of the constitutive promoter-driven Cas9 integration results in increased cleavage activities, often leading to continuous off-target editing.

Therefore, this study seeks to develop the first DNA-free genome editing protocol in hexaploid sweetpotato utilizing the preassembled Cas9 ribonucleoprotein (RNP). Several single guide RNAs were designed to target the eukaryotic translation initiation factor 4E superfamily. Freshly isolated sweetpotato protoplasts were edited with CRISPR-CAS9-RNP complex via polyethylene glycol (PEG) mediated transfection under various parameters. The most efficient editing factors in sweetpotato were obtained with 25% PEG mixed with a 3:1 ratio of purified preassembled Cas9 protein and in-vitro transcribed guide RNAs under a 25-minute incubation period. Different allelic InDels were obtained with an editing efficiency of 10-20%, demonstrating that PEG-mediated RNP transfection can be a viable editing system toward developing DNA-free integration tools for polyploid and vegetatively propagated crops. Work Supported by USDA-NIFA Grants: 2017-38821-26414-GE, and Tuskegee University CAENS-GWCAES-NIFA-EVANS-ALLEN Grants.

P-1017

Constitutive Expression of a miR169 Gene Alters Plant Development and Enhances Drought and Salt Tolerance in Transgenic Creeping Bentgrass. XIAOTONG CHEN, Jason Yeung, Andrew Fiorentino, Qian Hu, Morgan Kuess, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634. Email: xiaotoc@clemson.edu

Abiotic stresses, such as salinity, drought and heat, are important limiting factors for plant growth and development, significantly impacting crop production and agriculture economy. Plants have evolved various protection mechanisms coping with different environmental adversities. Manipulation of genes involved in plant stress regulation to genetically engineer enhanced performance in transgenics plays an increasingly important role in sustainable modern agriculture. MicroRNAs (miRNAs) are endogenous small non-coding RNAs identified in plants that engage in post-transcriptional target gene regulation, crucial for plant development and environmental adaptation. Here, we investigated the role of miR169g, a conserved plant miRNA that targets CCAAT transcription factors in regulating plant development and stress response and the underlying physiological and molecular mechanisms using transgenic analysis in an important perennial grass species, creeping bentgrass (Agrostis Stononifera). Our data indicate that miR169 overexpression in transgenics alters plant development and significantly improves tolerance to drought and salt stresses associated with modified physiological and molecular characteristics. The results obtained demonstrate the importance of miR169 as a key coordinator in plant development and stress responses, providing information for the development of novel biotechnology approaches to genetically engineer crops for enhanced agricultural production.

P-1018

T-DNA-Free Gene Editing through Transient Suppression of the POLQ Gene in Plants. HEQIANG HUO[1], Guiluan Wang[1], and Zhanao Deng[2]. [1]Department of Environmental Horticulture, Mid-Florida Research and Education Center, University of Florida, Apopka, FL 32703 and [2]Department of Environmental Horticulture, Gulf Coast Research and Education Center, University of Florida, Wimauma, FL 33598. Email: hhuo@ufl.edu

The current CRISPR/Cas genome editing in plants heavily relies on the delivery of CRISPR reagents into plant cells to create transgenic plants, which are subjected to strict regulations by different legislations. The current gene-editing approach to create transgene-free mutants in annual crops is not suitable for clonal plants and trees that are highly heterozygous and have long juvenile periods. Previous studies have demonstrated POLQ plays a critical role in microhomology-mediated end joining (MMEJ) during double-strand break (DSB) repair and in T-DNA integration. In this study, we exploited this previous discovery and demonstrated that transient silencing of the POLQ gene greatly increased the frequency of transgene-free mutants in tetraploid tobacco. We first screened the optimal concentration and duration of kanamycin selection to promote preferential growth of edited cells with the transiently expressed transgenes. With the optimized conditions, the frequency of T-DNA-free mutants reached as high as ~50% for two POLQ-suppressed genome editing vectors(antiPOLQ/pds3 and POLQ-RNAi/pds3), whereas only ~20% of mutants from the control vector were T-DNA-free. Expression analysis confirmed that suppression of POLQ gene resulted in lower integration of T-DNA, which were validated by dynamic detection of GFP fluorescence and PCR genotyping. Sequencing results showed that the larger deletions are more freququent in T-DNA-free mutants from antiPOLQ/pds3 and POLQ-RNAi/pds3 with a frequency of 83% or 50%, respectively, relative to the 25% in T-DNA-free mutants from the pds3 control. In summary, high frequencies of T-DNA-free gene edits could be easily achieved by transient suppressing the evolutionary conserved POLQ gene using a routine Agrobacterium-mediated transformation method. Our study reports the first case of achieving high T-DNA-free gene editing frequency through transient suppression of the POLQ gene in plants, which is of great impact on the application of CRIPSR genome editing in clonally propagated plant species.

PLANT INTERACTIVE POSTER ABSTRACTS

BIOTECHNOLOGY, GENOMICS, AND PLANT PHYSIOLOGY

Monday, June 6, 1:30 pm – 2:30 pm

P-3000

A Rapid and Simplified Transformation and Genome Editing Method for Maize Inbred B104 Using Agrobacterium Ternary Vector System and Immature Embryos. MINJEONG KANG[1], Keunsub Lee[1], Todd Finley[2], Hal Chappell[2], Veena Veena[2], and Kan Wang[1]. [1]Department of Agronomy, Iowa State University, Ames, IA and [2]Plant Transformation Facility, Donald Danforth Plant Science Center, Saint Louis, MO. Email: mjkang@iastate.edu

Maize genetic transformation is a critical technology for maize genomic studies as well as trait improvement. As a recalcitrant plant species, only a limited number of maize genotypes such as Hi Type II, A188 and B104 are amenable for genetic

transformation. B104 is an attractive public inbred for transformation because it shares a high genetic similarity with B73, an important public inbred serving as the reference genome. Although the transformation of maize inbred B104 has been developed, it is not broadly applicable in academic lab settings due to labor-intensive and time-consuming procedure (Frame et al., 2006; Raji et al., 2018). Here we described an improved B104 transformation and genome editing protocol using *Agrobacterium* ternary vector system and CRISPR/Cas9. The original B104 transformation protocol requires about 120 to 160 days to produce rooted transgenic plants from the starting day of transformation experiment. The improved protocol described here reduces the transformation process to about 50 days, which saves about 70 to 110 days. Using this protocol, the T0 plants can be obtained with an average of 6.6% transformation frequencies (the number of transgenic T0 plants per 100 infected embryos). Transgenic T0 events containing Cas9 cassette targeting *Glossy2* gene showed over 66% of indel frequency at the cleavage site. We expect that this simplified and improved B104 transformation protocol can be readily transferrable to many academic groups that desire to set up the maize genetic transformation and CRISPR/Cas-mediated genome editing for fundamental and applied research.

P-3001

CRISPR/Cas9 Mediated Cell Wall Engineering of Plant Cells for Enhanced Recombinant Protein Production. UDDHAB KARKI[1,2], Hong Fang[1,2], and Jianfeng Xu[1]. [1]Arkansas Bio-sciences Institute, and [2]Molecular Biosciences Program, Arkansas State University, Jonesboro, AR 72401. Email: Uddhab.karki@smail.astate.edu

Plant cell culture has been established as a cost-effective alternative production platform for therapeutic proteins in industrial scale due to its intrinsic safety, low cost and the capability of post-translational modification. Due to its central role in bio-production and fundamental research, tobacco BY-2 cell has been referred to as the "CHO-cell in molecular farming" and the "HeLa cell in the biology of higher plants". However, major challenges exist for the BY-2 cell bioproduction system, such as low protein production and secretion, presence of large vacuoles, and difficulty in cryopreservation. These problems can largely be attributed to the distinctive plant cell wall structure that is composed of a complex matrix of interconnected polysaccharides. The complete cell wall structure may not be crucial for *in vitro* cultured plant cells because optimized culture conditions are provided to the cells to support their rapid propagation. This project aims to explore top-down cellular engineering approaches to create novel cell wall-deficient or "animal cell-like" plant cell lines for enhanced therapeutic protein production. Major cellulose synthase A catalytic subunit (CESA) genes were targeted to inactivate using the CRISPR/Cas9 genome editing technology. The morphology, cell wall structural composition, cell growth, bioproduction properties and transcriptomics of the knockout BY-2 were characterized. A BY-2 cell line with the CESA3 gene knocked out showed significantly reduced cellulose content (40.2% reduction) and significantly changed cell wall sugar composition. This study establishes proof-of-concept for top-down plant cell wall engineering to create new plant cell lines for the production of recombinant proteins.

P-3002

Post-wildfire Soil Microbiome Analysis of eDNA from the Angeles National Forest. SAVANAH SENN[1,2], Gerald Presley[2,3], and Sharmodeep Bhattacharyya[2,4]. [1]Los Angeles Pierce College Department of Agriculture Sciences, 6201 Winnetka Ave., PMB 553, Woodland Hills, CA 91371; [2]Environmental Sciences Graduate Program, Oregon State University, Corvallis, OR 97331; [3]Department of Wood Science and Engineering, Oregon State University, Corvallis, OR 97331; and [4]Department of Statistics, Oregon State University, Corvallis, OR 97331. Email: stclais@piercecollege.edu

Wildfires are increasing in frequency and severity in Southern California due to climate change; furthermore, acres per wildfire have grown yearly since 1950 (CARB). This paper presents a soil microbiome study of different wildfire areas in the Angeles National Forest, Gold Creek Preserve. Soil samples were taken from plant rootzones of *Quercus agrifolia*, *Eriodictyon crassifolium*, *Dendromecon rigida*, and *Arctostaphylos glauca*. The *Q. agrifolia* had burned in different combinations of fires, and the thickleaf Yerba Santa and Tree Poppy, which require scarification to germinate, established large stands at the sampling site after the 2017 Creek Fire and 2016 Sand Fire, respectively. The manzanita trees resprouted from trees that had been established after the Station Fire and regenerated following the 2017 Creek Fire. These plants were of interest since they produce secondary metabolites, such as tannins, coumarins, protopine, and essential oils. Coumarins and terpenes influence the composition of microbial communities in the rootzone (Jacoby 2021); microbes regulate plant secondary metabolite production by enhancing gene expression or through horizontal gene transfer (Pang 2021). The NGS datasets from December 2020 and June 2021 were analyzed using phyloseq, Ranacapa, DESeq2, and EZBioCloud MTP. The main results showed significant differences in bacterial and fungal taxa associated with different fire areas in the Gold Creek Preserve. There were changes in fungal taxa associated with soil samples that were affected by the Creek Fire in combination with the 2009 Station Fire, 2016 Sand Fire, or the 2017 Creek Fire alone (p adj.< 1*10^-7). There was evidence of seasonal shifts in the alpha diversity of the bacterial communities, based on the ACE index. An unexpected result is that the plant species was not a significant factor in determining the alpha or beta diversity of the communities. These results emphasize the importance of protecting forests from repeated wildfire events.

P-3003

Advanced Plant Reporter Genes for Transient Expression. NATHAN VORODI, Natalie Thompson, David Samson, Rekha Kandaswamy, Vijay Sheri, Aliya F. Anwar, Michael Ream, Anna Filipkowski, Maia Clipsham, Sairam Rudrabhatla, and Wayne R. Curtis. Department of Chemical Engineering, Pennsylvania State University, State College PA. Email: nathan.vorodi@curtislab.org

Reporter genes are useful tools to distinguish cells expressing proteins that are easily visualized, and/or quantitatively assayed. mNeonGreen (mNG) is a relatively new fluorescent protein that is several-fold brighter than green fluorescent protein (GFP), and does not require oxygen for the catalytic formation of its chromophore. mCherry is developed to avoid background autofluorescence in tissue cultured duckweed. Mushroom luciferase and nano-luciferase provide orders of magnitude greater signal-to-noise ratio based on fluorescence, with mushroom luciferin substrate being readily synthesized in plants rather than supplied as an exogenous substrate. A limitation with using reporter genes for transient expression, is that expression can occur in both the Agrobacterium vector, as well as the target plant cells. This work describes efforts to create intron-containing reporters to avoid Agrobacterium expression that includes different intron types, locations, while correcting for cryptic splice sites created by plant codon optimization. Three separate mNG CDS locations were chosen for insertion of the potato (PIV2) intron based structure predictions and previous placements in GFP - with very different performance. Begomovirus viral vector payloads were tested with different size introns to accommodate genome size constraints (Arabidopsis, AGO and Castor bean, PDK). Five different strong constitutive promoters displayed orders of magnitude different levels of expression of mushroom luciferase-intron, including testing of 35s promoters with different truncations, with and without TMV 5'UTR. These transient reporters were developed for the highly compact Agrobacterium binary vector pLSU1 (~4.5Kb), which has the characteristic of high copy number and stability in the absence of selection pressure to enhance transient expression. This extensive effort of improving these new reporter genes was undertaken to provide tools for the plant biotechnology community with a particular focus on optimizing Agrobacterium-mediated transient gene expression.

P-3004

Establishment of an Axenic Whitefly Colony for *In Vitro* Virus Transmission Studies. WAYNE R. CURTIS[1,4], Natalie Thompson[1], David Krum[1], Yun-Ru Chen[1], Mariela Torres[1,2], Marena Trauger[1], Dalton Strike[1], Zach Weston[1], April Hile[1], and Jane Polston[3]. [1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802; [2]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802; [3]Department of Plant Pathology, University of Florida, Gainesville, FL 32611; and [4]Intercollege Program in Plant Biology, The Pennsylvania State University, University Park, PA 16802. Email: wrc2@psu.edu

An axenic co-culture of the phloem-feeding sweet potato whitefly (*Bemisia tabaci*) was established on tissue cultured plants. Establishment involved surface sterilization of eggs laid on photo-trophic meristem propagated sweet potato, followed by 'rescue' of the adults before contamination emerged from vascular feeding during egg pedicil attachment. The absence of microbial contamination in serially cultured cabbage for over a year suggests that the whitefly colony is free of culturable gut microbiome. Serial subculture on four cabbage seedlings in a GA7 tissue culture vessel can produce over 500 whiteflies from a dozen inoculated adults in roughly 6 weeks. Large differences in whitefly accumulation are observed for a dozen different plant species, but nearly all display full whitefly life-cycle development. This includes plant tissue culture initiated from surface-sterilized seed: *Brassicaceae* (cabbage, broccoli, radish), *Solanacea* (pepper, tomato), *Cucurbitaceae* (squash, cucumber), okra, greenbriar and others, as well as meristem-propagated sweet potato, and Asian yam (*Dioscorea*). Proliferation on two different Dioscorea species (cayenensis and rotundata) are dramatically different suggesting a native resistance. In addition to measurements of whitefly adult accumulation with time, ongoing studies include preference (in the same tissue culture vessel) as well quantitative viability assessments based on application of models of proliferation into planted screen cages. An acyl-sugar knockout line of *Nicotiana benthamiana* allows for healthy proliferation that does not occur on wild-type plants. Since *N. benthamiana* is a host to a broad range of begomovirus plant pathogens, this work sets the stage for the study of *in vitro* virus transmission. An autoclavable membrane feeding device has been developed to provide feeding (as demonstrated by confocal microscopic uptake of fluorescent dye). The *in vitro* tissue culture environment provides for sufficient biocontainment for USDA-permitted study of virus transmission.

P-3005

Genome-wide Analysis of Alpha-amylase Gene Family in Major Cereal Crops. SHABDA VERMA[1], Kanwardeep S. Rawale[2], Navraj Kaur Sarao[1], Johar Singh Saini[1], Gagandeep Singla[1], and Kulvinder S. Gill[2]. [1]Punjab Agricultural University, Ludhiana 141004, INDIA and [2]Washington State University, Pullman, WA. Email: shabdav@gmail.com

Starch breakdown involves a complex network of enzyme processes that are influenced by plant growth stages, hormones, and environmental factors. Alpha-amylase is one of the most important starch degrading enzymes in plants. The majority of information on gene evolution, copy number, conservation, and divergence in major cereal crops is based on protein isoforms, which is frequently erroneous. Genome-wide analysis of the alpha-amylase gene family was done with the goal of performing extensive analysis of the structural copies of the gene, which found at least 10 gene copies in each of the diploid cereal ancestors. Physical mapping of the genes in wheat revealed gene copies on five of the seven chromosomes, including tandem duplications, implying that the alpha-amylase gene family in cereals has evolved in a complicated way. Wheat showed 36-fold amplification of the alpha-amylase gene copy. In rice, barley, maize and sorghum, the gene amplification ranged from 7 to 12 fold. Apart from group-specific patterns in gene structure, further study indicated that gene size exhibited 14-fold difference across all crops. The amino acid sequences of distinct alpha-amylase groups indicated substantial conservation. Furthermore, domains, motifs, and functional sites analysis showed that functional sites 6 and 13 contain group-specific signatures. Furthermore, promoter analysis revealed that the duplicated alpha-amylase gene family had different gains or losses of motifs, indicating likely functional divergence.

MICROPROPAGATION/MEDICINAL PLANTS INTERACTIVE POSTER SESSION
Tuesday, June 7, 1:30 pm – 2:30 pm

P-3006

Mutation Rates in Micropropagated *Cannabis sativa* Detected Through Genotyping by Sequencing, Are SSRs Antiquated? KRISTIAN ADAMEK[1], Andrew Maxwell Phineas Jones[1], and Davoud Torkamaneh[2]. [1]Department of Plant Agriculture, University of Guelph, Guelph, N1G 2W1, ON, CANADA and [2]Département de Phytologie, Université Laval, Québec, G1V 0A6, QC, CANADA. Email: kadamek@uoguelph.ca, davoud.torkamaneh.1@ulaval.ca

Micropropagation is a powerful technology that has revolutionized horticulture due to rapid clonal propagation generating large amounts of disease-free planting material. While the goal is to produce genetically identical planting material, growers and researchers have noted an increased rate of mutations, referred to as somaclonal variation. In the late 20th century, researchers developed numerous markers to investigate tiny fragments of DNA, such as Restriction Fragment Length Polymorphism (RFLP), Random Amplification Polymorphic DNA (RAPD), and Simple Sequence Repeats (SSR). Historically and even today, people have used these markers to evaluate genetic fidelity and justify true-to-type statements despite the abysmal whole genomic coverage. Such statements imply plants are genetically identical to the stock plants however, this is not valid to claim with only a few markers. Nevertheless, in the 21st century, Genotyping-by-sequencing (GBS) was developed to further broaden Next-Generation Sequencing (NGS) uses with the sequencing and genotyping of thousands of markers compared to only hundreds with older genotyping platforms. GBS can sequence several million to billions of DNA bases throughout an individual genome or pooled population in a flexible, swift, and low-cost approach. In this study, we set out to compare the effectiveness of genotyping from GBS versus SSR using micropropagated cannabis. In the end, GBS discovered significant amounts of genetic diversity that SSR could not. As a result, this raises the question of using SSRs for genetic fidelity studies and previous research conclusions using them or similar markers. Thus, this study is to compare the power of GBS to SSR in evaluating mutation rates in micropropagated cannabis clones and encourages and supports NGS-based technologies as a standard for 21st century genotyping research.

P-3007

Direct Field-to-lab Introduction of Shoot Tips and Nodal Sections from Wild Nuttal's Scrub Oak (*Quercus dumosa*) for the Purpose of *Ex Situ* Conservation Via Micropropagation. J. REE and C. Powell. San Diego Zoo Wildlife Alliance, 2920 Zoo Dr, San Diego, CA 92101. Email: jree@sdzwa.org

Nuttall's scrub oak (*Quercus dumosa*) is critical to the wellbeing of the chaparral ecosystem of Southern California and Baja California and is listed as endangered on the IUCN Red List of Threatened species. Unfortunately, urban development and frequent wildfires will continue to threaten the species for the foreseeable future. To help preserve the remaining diversity of the species *ex situ*, we developed tissue culture methods suitable for a wide range of genotypes. Shoot tips and internode segments harvested from wild plants were placed on a medium composed of Woody Plant Medium, 3% sucrose, 2 mg L^{-1} 6-benzylaminopurine, and solidified with 8g L^{-1} agar. Explants swiftly exudated phenolic compounds and turned necrotic within several days. Different types and strengths of basal salts, plant growth regulators, explant orientation, and several other factors failed to alleviate runaway necrosis, and supplementation with ascorbic acid, citric acid, polyvinylpyrrolidone, or activated charcoal only delayed necrosis. The first critical factor was the amount of sucrose: increasing the concentration from 3% to 4.5% dramatically reduced the rate of necrosis, allowing cultures to survive for several weeks. The addition of 20-100 uM sodium nitroprusside, an NO donor, proved to be a second critical factor, yielding improved explant survival and stimulating regrowth for the first time. Furthermore, this regrowth occurred in 19 of 20 harvested trees, but the proportion of explants showing regrowth was genotype-dependent. Current ongoing experiments focus on further optimizations for large numbers of wild genotypes to determine how effective this method is for *Q. dumosa.*

P-3008

Clonal Propagation of Avocado (*Persea americana*) from Adult Trees: Establishment *In Vitro.* J. TIN, and R. Folgado. Botanical Center, The Huntington Library, Art Museum, and Botanical Gardens, 1151 Oxford Road, San Marino, CA 91108. Email: jtin@huntington.org, rfolgado@huntington.org

Avocado (*Persea americana* Mill.) is a tropical fruit crop highly appreciated for its nutritional value. Although the demand is growing, the availability of commercial clonal trees is limited. An alternative to producing high-quality plants while reducing the cost and time is micropropagation. Generally, this method has four stages; initiation, multiplication, rooting, and acclimation. However, woody plants like avocado are known to be recalcitrant to micropropagation. Additionally, material taken from adult trees requires an extra establishment stage before multiplication. This work studied the effect of the media on the initiation and establishment stages of *in vitro* cultures. Bud woods from adult trees of three avocado cultivars ('Mexicola', 'Pinkerton', and 'Lamb Hass') were collected and sterilized. Then, 64 buds per cultivar were subjected to four conditions (n=192); 1) initiation and establishment in Woody Plant medium (WPM), 2) initiation in WPM and establishment in WPM with coconut water (CW), 3) initiation in WPM and establishment in persea medium (P3G) with CW, and 4) initiation in P3G and establishment in P3G with CW. Every 6 weeks, the quality of the explants (leaves, stem and base) and the number of shoots were recorded before refreshing the medium. The initiation medium did not affect the establishment of the cultures for all 3 cultivars. Instead, the media and the presence of CW determined if the shoots establish *in vitro* after 24 weeks; the effect of the conditions was genotype-dependant. Cultures from cv 'Mexicola' established in both WPM and P3G with CW. Cultures from cv 'Lamb Hass' declined in all media. On the other side, shoots from cv 'Pinkerton' were established in three conditions, but quality and quantity were significantly better when P3G with

CW was used. The *in vitro* establishment of avocado bud woods from adult avocado trees depends not only on the used medium but also on the cultivar's nutritional requirements.

P-3009

Isolation and Characterization of Two Novel *Chryseobacterium sp.* Genomotypes from the Rootzone of the Medicinal Plant *Datura inoxia* by Whole Genome Sequencing. SAVANAH SENN[1,2], Kelly Pangell[1], Maryam Saraylou[3], Adrianna Bowerman[1], Karu Smith[1], and Ryan O'Halloran[1]. [1]Los Angeles Pierce College Department of Agriculture Sciences, 6201 Winnetka Ave., PMB 553, Woodland Hills, CA 91371; [2]Oregon State University Environmental Sciences Graduate Program, Corvallis, OR 97331; and [3]Department of Soil Science and Engineering, Agricultural Science and Natural Resources University Khuzestan, Mollasani, IRAN. Email: stclais@piercecollege.edu

Datura sp. are important medicinal and entheogenic plants which were model organisms in classical biotechnology and tissue culture studies (Guha and Maheshwari 1964, Konoshima 1970). There has been new interest in *Datura sp.* and alkaloid biosynthetic pathways involving polyketide synthases (Rajewski 2019, Rajewski 2021, Velazquez-Marquez 2021, Senn 2022). Our study (Senn 2022) identified 6 isolates with high partial 16S sequence completion and low % similarity to the closest known species. Two of these isolates were WG14 and WG23 from the *Datura* rootzone, which were closely related to *Chryseobacterium rhizospherae* and *C. phosphatilyticum*, respectively. WGS data was then obtained from streak plates via Chelex DNA extraction. Cluster generation and DNBseq were performed by BGIA. For assembly and 16S ID we used TruBacID. The results gave definitive ID of each isolate as *Chryseobacterium sp. nov.* from 16S sequences. Subsystems analysis was performed using RAST. Microscopy of the two isolates revealed gram negative rods. Later attempts to subculture the isolates after cold storage were unsuccessful. The 2 isolates exhibited functions on the ascorbate pathway; ascorbate has antioxidant activity and was linked to gains in secondary metabolite production in common bean (Gaafar et al 2020). A high number of functions associated with biotin metabolism were observed, and with the degradation of lysine. Functions were associated with cyclic amino acid precursors. WG23 had sequence differences in 2 lipid-related processing genes when compared with the genome of WG14. The first was lipid 3 flippase, and the second was Acetyl-coenzyme A synthetase, which is closely related to chalcone synthase. For functional differences, 15 TEs and a CRISPR protein appeared in the WG 23 genome but not in WG14. Further analysis will discuss evolutionary relationships between the new types and other species, structural differences in the genes, and the role that transposons may play in the evolution of enzymes whose products may support plant growth, or enhance the virulence of the bacteria.

P-3010

Moringa oleifera leaf Extract as a Potential Plant Growth Stimulant to Enhance Salt Stress Tolerance in Wheat. TALAAT AHMED, Mustafa Elshiekh, and Muhammad Fasih Khalid. Environmental Science Center, Qatar University, Doha 2713, QATAR. Email: t.alfattah@qu.edu.qa

Moringa oleifera is well known as a miracle tree. It is considered as a vegetable in many countries. Moringa leaves are rich in iron, calcium, vitamin A and C, b-carotene, phenolic, and riboflavin. It has antioxidant properties and rich with many plant growth promoters like Zeatin and cytokinin. Zeatin stimulates cell division and elongation. Qatar's wheat production is very low. This low productivity is mainly due to the lack of well-adapted bread wheat varieties and suitable production technologies. High yielding varieties and production technologies should be developed considering the limited water resources and poor soil quality to pave the way for Qatar's sustainable wheat production. Several strategies have been applied to improve wheat growth under salt stress, including using exogenous phytoprotectants, seed priming, and application of plant growth promoters. The current study aimed to investigate the performance of three wheat genotypes under salt stress as a response to foliar application of Moringa leaf extract (MLE). Three wheat genotypes (Salt tolerant, moderate and sensitive) were sown in 48 pots; five seeds were sown in each pot filled with soil. Ten days after sowing, wheat seedlings were thinned, and three uniform seedlings per pot were left for future study. The salt stress treatment was applied in 15 days old plants with 150 mM NaCl in addition to control treatment (Distilled water). Each treatment was replicated four times. Salt stress treatments were applied to the plants at 10 days interval under shade-net house condition. Pots were divided into two groups. One group was foliar sprayed with Moringa leaf extract (MLE) and the other group was foliar sprayed with distilled water. Results revealed that salt stressed plants sprayed with MLE were significantly improved in growth characteristics such as flowering time, plant height, number of tillers, flag leaf area, spike length, number of grains per spike, and grain weight by alleviating the inhibitory effects of soil salinity stress. The three-wheat genotypes responded differently to salt stress and MLE foliar spray.

P-3011

In Vitro Production of Adventitious Root Biomass and Important N-alkylamides Using Bioreactor Cultivation of Medicinally Important Plant *Spilanthes paniculata* Wall. ex DC. KRISHNA KANT PACHAURI and Rakhi Chaturvedi. Department of Biosciences & Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam – 781039**,** INDIA. Email: rakhi_chaturvedi@iitg.ac.in, krishana@iitg.ac.in

In vitro tissue culture methods provide an excellent alternative to reduce the overharvesting of medicinally important natural flora. *Spilanthes* (Asteraceae) is a group of medicinally important plants, recently declared threatened in the red data book due to its overexploitation in last few years. The plant is popularly known to possess anthelmintic, antimalarial, insecticidal, anti-inflammatory, anaesthetic and immune-modulating properties. In the current study, the large scale biomass and metabolites production are explored through in vitro adventitious root culture. The adventitious root culture of *S. paniculata* was established at shake flask level by using modified Murashige and

Skoog (MS 1962) medium and further scaled-up in a stirred tank bioreactor (3 litre capacity), fitted with marine type impellers in the same medium composition. Aeration at 0.1 vvm (volume of air per volume of liquid per minute) was optimum for biomass growth. The growth kinetics studies showed that maximum biomass and metabolites accumulation occur on 15th days of inoculation. After that, in vitro biomass was harvested from the bioreactor, dried and grounded to a fine powder, and the extracts were prepared using organic solvent. The extracts were analysed and characterized for the presence of N-alkylamides through High performance liquid chromatography (HPLC) and Mass spectrometry (MS). The presence of spilanthol was detected and quantified by using HPLC and further confirmed through MS analysis. Apart from spilanthol, extracts showed the presence of two other major metabolites, identified as Ethane; N-ethylcyclohexa-1,5-diene-1-carboxamide and N-Isobutyl-(2E,4Z,8Z,10E/Z)-dodecatetraenamide. The mass spectrometric analysis of the collected HPLC eluted peaks confirms their molecular weight as 211 and 247, respectively. The results of the study showed the presence of important N-alkylamides in in vitro grown adventitious roots. The study also provides an alternate technique for commercial production of bioactive metabolites of *S. paniculata.*

GENETIC ENGINEERING AND METABOLIC ENGINEERING INTERACTIVE POSTER SESSION

Tuesday, June 7, 1:30 pm – 2:30 pm

P-3012

Increased Engineering and Editing Efficiency of *Sorghum bicolor* Using Morphogene-assisted Transformation. KIFLOM AREGAWI[1], Jianqiang Shen[1], Grady Pierroz[1], Manoj K. Sharma[1], Jeffery Dahlberg[2], Judith Owiti[1], and Peggy G. Lemaux[1]. [1]Department of Plant and Microbial Biology, University of California, Berkeley, CA and [2]University of California Ag & Natural Resources, Kearney Agricultural Research & Extension Center, Parlier, CA. Email: lemauxpg@berkeley.edu, karegawi@berkeley.edu

Sorghum bicolor (L.) Moench, the fifth most important cereal worldwide, is a multi-use crop for feed, food, forage and fuel. Identifying genes associated with its notable abiotic stress tolerances requires a detailed molecular understanding of those traits. Our in-depth sorghum transcriptome study revealed over 40% of its transcriptome was not annotated. One approach to determine gene function is transformation; however, current sorghum transformation methods are time-consuming and genotype-dependent. Our efforts to develop efficient tools to engineer, edit, annotate and characterize sorghum's genes involved morphogene-assisted transformation (MAT). The morphogenes used were Zea mays *Baby boom (Bbm)* and *Wuschel2 (Wus2). Bbm* is an AP2/ERF transcription factor that promotes cell proliferation during embryogenesis and *Wus2* maintains stem cells in the shoot meristem. This led to accelerated times to achieve transformation, nearly half the time required for classical non-MAT approaches. MAT also resulted in more genotypes amenable to transformation, including several not previously transformed or historically recalcitrant, and improved transformation efficiencies reaching 52.8% with certain constructs. Morphogenes were also included in a CRISPR/Cas9 editing construct that led to a knock-out editing efficiency of 16.7% and the first report in sorghum of a biallelic edit in the T_0 generation. Another transformation approach, termed altruistic MAT, involved introducing a gene of interest in a separate Agrobacterium from the one with morphogenes, which leads to plants with the gene of interest but without the morphogenes. A high-throughput technique was used to identify single-copy plants, as well as a novel method to determine transgene-independent integration. Together, these advances provide a straightforward path to determine gene function in numerous genotypes of this multi-use crop.

P-3013

Inducible Expression for Acceleration of Design-Build-Test-Learn Cycles in the Metabolic Engineering of Oilcane. MONI QIANDE[1], Dang Viet Cao[1,2], Hui Liu[2,3], John Shanklin[2,3], and Fredy Altpeter[1,2]. [1]University of Florida - IFAS, Gainesville, FL; [2]DOE Center for Advanced Bioenergy and Bioproducts Innovation; and [3]Brookhaven National Laboratory, Upton, NY. Email: mqiande@ufl.edu, altpeter@ufl.edu

Sugarcane is an ideal target crop to fuel the emerging bio-economy. It combines superior biomass production and photosynthetic efficiency with hyperaccumulation of sucrose in its stem, which offers great prospects for diversion to alternative products. We recently reported the generation of oilcane, a sugarcane which has been metabolically engineered for hyperaccumulation of triacylglycerol (TAG) in its vegetative biomass (Zale et al. 2016; Parajuli et al. 2020). Inducible promoters may allow lipid production at will, at a time when tissue culture or critical stages of plant development are already completed. Since sugarcane tolerates elevated temperatures between 40°C and 45°C for an extended period of time, we explored different heat shock promoters (HSP) and the combination of heat-inducible and constitutively expressed lipogenic factors to accelerate Design-Build-Test-Learn cycles. Data describing transgene expression and resulting TAG accumulation before and after activation of different lipogenic factors and their combinations will be reported.

P-3014

Application of Developmental Regulators to Improve *In-Planta* or *In Vitro* Transformation in Plants. HEQIANG HUO[1] , Zhaoyuan Lian[1], Chi Dinh Nguyen[1], Jianjun Chen[1], Sandra Wilson[2], and Peggy Ozias-Akins[3]. [1]Department of Environmental Horticulture, Mid-Florida Research and Education Center, University of Florida, Apopka, FL 32703; [2]Department of Environmental Horticulture, University of Florida, Gainesville, FL 32611; and [3]Department of Horticultural Science, University of Georgia, Tifton, GA 31793. Email: hhuo@ufl.edu

Plant genetic transformation is a crucial step for applying biotechnology such as genome editing to basic and applied plant science research. Its success primarily relies on the efficiency of gene delivery into plant cells and the ability to regenerate transgenic plants. In this study, we have examined the effect of several developmental regulators (DRs) , including *PLETHORA*

(PLT5), *WOUND INDUCED DEDIFFERENTIATION 1 (WIND1)*, *ENHANCED SHOOT REGENERATION (ESR1)*, *WUSHEL (WUS)*, and a fusion of *WUS* and *BABY-BOOM* (*WUS-P2A-BBM*), on *in planta* transformation success through injection of *Agrobacterium tumefaciens* in snapdragon (*Antirrhinum majus*). The results showed that *PLT5*, *WIND1* and *WUS* promoted *in-planta* transformation of snapdragon. An additional test of these three DRs on tomato *(Solanum lycopersicum)* further demonstrated that the highest *in-planta* transformation efficiency was observed for *PLT5*. *PLT5* significantly promoted calli formation and regeneration of transformed shoots at the wound positions of aerial stems, and the transgene was stably inherited to the next generation in snapdragons. Additionally, *PLT5* significantly improved the shoot regeneration and transformation of the two *Brassica* cabbage varieties (*Brassica rapa*), and promoted the formation of transgenic calli and somatic embryos in sweet pepper (*Capsicum annum*). Despite some morphological alternations, viable seeds were produced from the transgenic Bok Choy and Snapdragon plants. Our results have demonstrated that manipulation of *PLT5* could be an effective approach for improving *in planta* and *in vitro* transformation efficiency, and such a transformation system could be used to facilitate the application of genome editing or other plant biotechnology application in modern agriculture.

P-3016

Protein Engineering in *Chlamydomonas reinhardtii* for Improved Production of Recombinant Proteins. CORBIN ENGLAND and Jianfeng Xu. College of Science and Mathematics, Arkansas State University, Jonesboro AR. Email: corbin.england@smail.astate.edu

Plant cells are an attractive production platform for biotherapeutics and other recombinant proteins due to their ability to conduct posttranslational modifications (PTMs) without playing host to animal pathogens. The green microalgae *Chlamydomonas reinhardtii* shares these traits while also possessing a haploid genome, simple and inexpensive media requirements, easily scalable culturing conditions, and is classified by the US Food and Drug Administration as Generally Recognized as Safe. *C. reinhardtii* is also a model green algae with sequenced nuclear, mitochondrial, and plastid genomes and a large body of literature exists regarding the amplification of nuclear transgene expression. By investigating two PTMs in this species, including the glycophosphatidylinositol anchor (GPI anchor) common to eukaryotes and the hydroxyproline-O-glycosylation (Hyp-O-glycosylation) specific to higher plants and green algae, we intend to design and engineer new polypeptides with these two PTMs in *C. reinhardtii* for improved biomedical and industrial applications. Several GPI anchor signal peptides have been identified *in silico* in *C. reinhardtii*. Testing *in vivo* will determine whether these peptide sequences can be correctly processed, leading to the addition of the GPI glycolipid moiety to the target protein and displaying the protein on cell surface. Certain Hyp-O-glycosylation modules, such as the tandem repeats of the "Ser-Pro" motif, have been demonstrated to be functional in *C. reinhardtii* but their glycosylation patterns have not been closely examined. We expect their glycosylation patterns to be different from those of plants, which will be tested in this project. This abstract represents a doctoral research plan and is work-in-progress.

P-3017

Development of an *In Vitro* Regeneration and Transformation System for Hop (*Humulus lupulus*). CHRISTOPHER J. WILLIG[1], Michele S. Wiseman[2], John A. Henning[2,3], David H. Gent[2,3], and Steven H. Strauss[1]. [1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR; [2]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; and [3]USDA-ARS, Forage Seed and Cereal Research Unit, Corvallis, OR. Email: chris.willig@oregonstate.edu

Hops are a high-value specialty crop grown in the US grown primarily for brewing beer. The rapid growth of the craft beer industry has led to increased demand and overall production has steadily expanded over the past decade. Hop growers face several challenges such as disease pressure from powdery mildew leading to increased input costs. Hop germplasm improvement by conventional breeding is a long process with an 8 to 15-year period between initial cross and commercial release of a new cultivar. With the recent development of genomic resources, including a high-quality chromosome-level genome assembly in hop, there is an opportunity for the application of modern biotechnological tools to enable investigation into gene function; utilization of these tools will inform breeding programs and ultimately decrease the timeline for generating varieties with enhanced agronomic traits. Our work has sought to establish a repeatable *de novo* shoot organogenesis and *Agrobacterium*-mediated transformation protocol in selected varieties of hop. We have screened several cultivars of economic importance in the US for their regeneration potential upon induction by various applications of exogenous hormones in tissue culture. Cultivars were also screened for their ability to uptake T-DNA using constructs carrying fluorescent markers. The effect of various media amendments, environmental conditions, and physical manipulations were then evaluated on explants of the cultivars that displayed the best performance in initial screens. The cultivar 'Cascade' gives a 40 to 50% regeneration frequency on media containing 10.0 mg/L BAP and 0.25 mg/L IAA, while 'Fuggle' achieves near the same frequency with 2.0 mg/L TDZ and 0.25 mg/L IAA. We will report on the results of several experiments aimed at increasing efficacy of T-DNA delivery, regeneration frequency, and the recovery of transgenic events. To date, we have found that different genotypes have very distinctive hormone requirements for regeneration. We thank the USDA-AFRI for grant support for our work (USDA grant #2021-67013-34739).

PLANT POSTER ABSTRACTS

P-3018

Development of a Meristem-based Transformation System for Barley (*Hordeum vulgare* L.) Targeting Mature Embryo Explants. LUCAS GONTIJO SILVA MAIA[1,3], Edward Williams[1], Mike Petersen[1], Ray Collier[1], Phil Bregitzer[2], Ramamurthy Mahalingam[2], Marcus Vinje[2], Shawn Kaeppler[1,3], and Heidi Kaeppler[1,3]. [1]Wisconsin Crop Innovation Center (WCIC),

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The utility of plant transformation and gene editing for genetic manipulations in crops is well-documented. For many crop species, however, current transformation systems are limited by genotype-dependence and protocol inefficiencies. Barley (*Hordeum vulgare* L.) is among those crops that are considered challenging to transform and current transformation systems are highly genotype-dependent. Additional limitations include soma clonal variation in regenerants, tedium of target explant isolation, lack of explant storability, lengthy, complex and low efficiency of protocols. Development of a genotype-flexible, meristem-based transformation system for barley targeting easily isolated and storable target explants would overcome many limitations of current transformation methods. Research was conducted to develop methods to isolate and store barley meristem explant tissues and establish a meristem-based transformation system for targeting an important but recalcitrant variety, Gemcraft. Mature barley embryonic axis and embryonic shoot tip explants were extracted from sanitized mature seeds and dried to 12% moisture. Prior to infection, the explants were rehydrated and treated with Agrobacterium strain AGL1 containing a a binary vector for delivery of T-DNA carrying a visual marker gene and NPTII, HPTII or bar as selection genes. After four days on coculture medium, treated explants were placed on selection medium for two weeks, following which green shoots were isolated and placed on rooting medium containing the selection agent. Rooted shoots were moved to soil and grown to maturity in the greenhouse. Transgenic T0 plants were obtained in 8-10 weeks from transformation to greenhouse. Marker gene expression was tracked in multiple leaves of T0 plants, and T1 seed from different spikes were separated to monitor chimerism and effects on transgene segregation. Germline transformation was confirmed via screenable marker and molecular assays of T1 progeny.

P-3019
Construction of Wheat Transformation System Using *GRF-GIF* Chimera Gene Cloned from Korean Wheat cv. Keumgang and 'Speed breeding' Conditions. GEON HEE LEE, Sang Yong Park, Tae Kyeum Kim, and Jae Yoon Kim. Department of Plant Resources, Kongju National University, Yesan, Chungnam, REPUBLIC OF KOREA. Email: jaeyoonkim@kongju.ac.kr

Agrobacterium-mediated transformation is a good way to integrate transgenes into the host genome of plant as a single copy event. However, there are still obstacles to achieve the Korean wheat transformation events using *Agrobacterium* in Korea, and even studies using particle bombardment had been low probability. Sequentially embryogenesis is one of most important factors in wheat transformation. Therefore, research to improve the *Agrobacterium*-mediated transformation efficiency of the Korean wheat varieties is essential. In the previous study, we reported the successful possibility of immature embryogenesis culture method applied 'Speed Breeding' in Korea. In this experiment, wheat transformation was applied to immature embryos collected in 'Speed breeding' and the efficiency of wheat transformation was confirmed by measuring the transgene integration rate. In addition, the recently reported GRF-GIF chimera gene was cloned from cv. Keumgang, predominant variety in Korea, and constructed, and its ability to improve regeneration and shorten the tissue culture period was confirmed. As a result, the transgene integration was confirmed by PCR screening for calli and one regenerated callus that had been selected for 5 weeks by G418 antibiotics. Immunostrip test results using putative transformants, stably transgene-integration efficiency was 0.15%. Finally, PCR screenings using the putative transgenic plants were confirmed that was integrated transgene. This study can be useful information in the development of the wheat transformation system in Korea. [Acknowledgements] This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ016528)" Rural Development Administration, Republic of Korea.

P-3020
Effects of Organellar Targeting of the aadA1a Selectable Marker Protein on Transformation Frequency of Meristem-based Soybean Transformation. S. MASSMAN[1], R. Collier[2], E. Williams[2], M. Petersen[2], R. Harnish[2], B. Martinell[2], and H. Kaeppler[1,2] [1]University of Wisconsin Madison, 161 Bascom Hall, 500 Lincoln Drive, Madison, WI 53706 and [2]Wisconsin Crop Innovation Center, University of WI-Madison, 8520 University Green, Middleton, WI 53562 Email: massman2@wisc.edu

Current soybean transformation protocols are limited by genotype dependence, low efficiencies, and/or intellectual property or regulatory restrictions. Previous research has demonstrated that the delivery of the selection marker protein into chloroplasts results in an increase in germline transformation efficiency in soybean. To examine in more detail how targeting of selectable marker proteins to subcellular compartments might impact plant transformation efficiency we designed, built, and tested selection marker expression vectors which directed the aadA1a protein, imparting to plants spectinomycin resistance, into either the cytoplasm (nontargeted), endoplasmic reticulum (ER), chloroplasts (CPT), or simultaneously, via a dual Targeting Peptide (dTP), into mitochondria and chloroplasts. Selection marker containing T-DNA were delivered from binary vectors, via *Agrobacterium*, into embryonic axis explants of soybean genotypes Williams 82 and IL3613 in replicated experiments with subsequent selection of transgenic shoots conducted on spectinomycin-containing medium. Mean transformation frequencies, recorded from 5 replications of Williams 82 treatments, were 8.1%, 0%, 12.4%, and 13.8% for cytoplasm, ER, CPT, and dTP targeting vectors, respectively. Similarly, mean transformation frequencies observed from 4 replications of IL3613 were 5.1%, 0%, 6.8%, and 7.8% for cytoplasm, ER, CPT, and dTP targeting vectors, respectively. Overall, the highest transformation frequencies were achieved with CPT and dTP mediated targeting of aadA1a, while the ER vector resulted in the lowest transformation frequencies across genotypes. These results indicate that we have developed an improved genotype-

flexible, meristem-based transformation system for soybean. This novel dTP-aadA1a selection marker now underpins the WCIC standard meristem-based soybean transformation system.

P-3021

Optimizing *Agrobacterium*-mediated Transformation and CRISPR-Cas9 Gene Editing in the *Tropical Japonica* Rice Variety Presidio. MARCO MOLINA-RISCO[1], Michael Thomson[1], Mayra Faion-Molina[1], Oneida Ibarra[1,2], Backki Kim[1,3], and Endang M. Septiningsih[1]. [1]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX; [2]Avance Biosciences Inc., Houston, TX; and [3]Plant Genomics and Breeding Institute, Seoul National University, Seoul, KOREA. Email: marco.molinarisco@ag.tamu.edu

Bottlenecks in plant transformation and regeneration have slowed progress in applying CRISPR/Cas-based genome editing for crop improvement. Rice (*Oryza sativa* L.) has highly efficient *temperate japonica* transformation protocols, along with reasonably efficient *indica* protocols using immature embryos. However, rapid and efficient protocols are not available for transformation and regeneration in *tropical japonica* varieties, even though they represent the majority of rice production in the U.S. and South America. The current study has optimized a protocol using callus induction from mature seeds with both *Agrobacterium*-mediated and biolistic transformation of the high-yielding U.S. *tropical japonica* cultivar Presidio. Gene editing efficiency was tested by evaluating knock-out mutations in the *phytoene desaturase* (*PDS*) and *young seedling albino* (*YSA*) genes, which provide a visible phenotype at the seedling stage for successful knockouts. Using the optimized protocol, transformation of 648 explants with particle bombardment and 532 explants with *Agrobacterium* led to a 33% regeneration efficiency. The *YSA* targets had ambiguous phenotypes, but 60% of regenerated plants for *PDS* showed an albino phenotype. Sanger sequencing of edited progeny showed a number of insertions, deletions, and substitutions at the gRNA target sites. These results pave the way for more efficient gene editing of *tropical japonica* rice varieties.

P-3022

A Dual Recombination System for Transgene Containment and Elimination in Perennial Grasses. XIAOTONG CHEN[1], Charles Henry[1], Annalise Enger[1], Qian Hu[1], Zhenyuan Pan[2], Xiaoyuan Gao[2], Lynda McMaster-Schuyler[2], Peiyu Zeng[2], and Hong Luo[1]. [1]Department of Genetic and Biochemistry, Clemson University, Clemson, SC 29634 and [2]Department of Natural Sciences and Math, State University of New York, Cobleskill, NY 12043. Email: xiaotoc@clemson.edu ZengP@cobleskill.edu

Biotechnology for crop improvement provides excellent opportunities to efficiently enhance agricultural production. However, transgenic technology application, especially in perennial grasses, raises the question of transgene escape into the nature and its unforeseen environmental consequence. We have developed an integrated strategy that combines a dual site-specific recombination system and total sterility induction mechanisms for transgene containment and removal in switchgrass and turfgrass. This should generate transgenic products self-contained for desirable transgene, but free of undesirable foreign DNAs, upon hybridization of two parental transgenic lines harboring different chimeric gene constructs. In the first line, a FLO/LFY RNAi expression cassette is separated from an upstream promoter by the Cre recombinase target site *loxP*-flanked *phiC31* recombinase, hygromycin resistance (*hyg*) and *Cas9* endonuclease genes. Another line contains an active herbicide resistance gene *bar*, recombinase gene *Cre* and *FLO/LFY* homolog gene guide RNA (sgRNA), and an inactive stress-regulating gene, *AVP1*. When the two transgenic lines are cross-pollinated, the phiC31 recombinase in the hybrids would excise the phiC31 target site *att*-flanked *bar*, activating Cre target site *loxP*-flanked Cre and *FLO/LFY* sgRNA, and consequently removal of itself and the sgRNA. This will activate *AVP1* leading to enhanced plant stress tolerance. Cre will also excise the *loxP*-flanked *phiC31*, *hyg* and *Cas9*, activating *FLO/LFY* RNAi, leading to total sterility. Additionally, Cas9/sgRNA-mediated gene editing will be active in the hybrids, ensuring *FLO/LFY* lockout for total sterility. Similar strategy can also be adapted to other crop species.

P-3023

Transcriptome and Metabolite Mechanisms Related to Pre-harvest Sprout (PHS) of Common Korean Wheat (*triticum aestivum*). SANG YONG PARK[1], Geon Hee Lee[1], Woo Joo Jung[2], and Jae Yoon Kim[1]. [1]Department of Plant Resources, Kongju National University, Yesan, Chungnam, REPUBLIC OF KOREA and [2]Institute of Life Science and Natural Resources, Korea University, Seongbuk-Gu, Seoul, 02841, REPUBLIC OF KOREA. Email: jaeyoonkim@kongju.ac.kr

Pre-harvest sprouting (PHS) is a representative moisture disaster in wheat, and is one of the factors that greatly damage the quantity and process ability of wheat. Transcriptome analysis was performed using samples obtained through experiments derived from PHS on 'Keumgang', 'Jeonju 377ho' and 'Woori'. The RNA-seq was constructed using the Truseq standard mRNA library prep kit (Illumina, CA, USA). In addition, the 100-base paired-end procedure was implemented by Illumina Novaseq 6000 sequencing system (Illumina, CA, USA). After clean-up was performed on raw data, the analysis of Eukaryotic Orthological Groups (KOG) and Kyoto Encyclopedia of Genomes (KEGG) analysis and Gene Ontology was conducted on established transcripts. Starch and sucrose metabolism, carbohydrate metabolism, energy supply in seeds, and germination mechanisms were identified. In GO analysis, related DEGs were mainly found. In addition, Ingenuity pathway analysis (IPA) experiment was conducted to confirm the expression in the protein. Data from proteomics and RNA-seq experiments were compared and analyzed with bioinformatics data and confirmed through heatmap and clustering. For the expression test of germination and dormancy-related DEGs and pathway-related genes, additional validation was performed by the differential expression of genes through qRT PCR. It is necessary to identify genes and mechanisms related to seed germination and dormancy through continuous research and to identify previously unknown related mechanisms or other functions. This study to understand the mechanisms and genetic factors between seed germination and

dormancy can increase the value of wheat in future agriculture. In addition, an understanding of genes and protein mechanisms is expected to help clear and precise wheat breeding program. [Acknowledgements] This work was carried out with the support of "The RND program for forest science technology (Project No. 2021400C10-2225-CA02)" Korea Forest Service, Republic of Korea.

P-3024

Plant Host Defense Peptides: Potential Tools for Disease Control. CHRISTIE STEPHEN and Dmytro P. Yevtushenko. University of Lethbridge, Department of Biological Sciences, 4401 University Drive, W, Lethbridge, AB, T1K 3M4, CANADA. Email: christie.stephen@uleth.ca

During the coming decades we need to find ways to increase crop productivity on existing farmlands to maintain global food security. Disease accounts for over one-third of crop losses worldwide so it is vital that strategies are developed to protect crops from major phytopathogens in an environmentally sustainable manner. This may be achieved by developing crops with broad spectrum, enduring disease resistance through the ectopic expression of host defense peptides (HDPs). HDPs are small membrane-active peptides which form a first-line of defense against pathogens; they are inherent to the immune systems of all living organisms. The aim of our research is to test four HDPs of plant origin for antimicrobial activity against important potato pathogens and assess their cytotoxicity toward plant and mammalian cells, *in vitro*. This will allow us to determine which HDPs are suitable for expression in potato plants. The peptides that have been selected are: Shepherin 2, from the roots of *Capsella bursa-pastoris* (Shepard's purse); Cr-ACP1, from *Cycas revoluta* (Sago palm); Skh-AMP1, from the medicinal plant *Satureja khuzestanica*; and Cn-AMP1, from green coconut water of *Cocos nucifera*. The antimicrobial activities of the HDPs are being evaluated against *Alternaria solani, Fusarium sambucinum, F. culmorum, and Pectobacterium carotovorum*. These peptides are being tested singly as well as in combination with each other in order to reveal any antagonistic, additive or synergistic interactions. A combination of Cn-AMP1 and Skh-AMP1 peptides has been found to be particularly promising. Individually, these two HDPs did not inhibit the germination of *F. sambucinum* conidia by much: the highest percentages of inhibited conidia for Cn-AMP1 and Skh-AMP1 were 55% and 11%, respectively. However, when tested together, Cn-AMP1 and Skh-AMP1 inhibited over 95% of fungal conidia even at very low peptide concentration (0.5 μM each), indicating a strong synergistic interaction between these two peptides. The results of this research will pave the way for development of plants with potent disease resistance.

P-3025

Transforming *P. hallii,* a Model for Perennial Bioenergy Grasses. KANKSHITA SWAMINATHAN[1], Anthony Trieu[1], Xiaoyu Weng[2], Rebekah Wood[1], and Thomas Juenger[2]. [1]HudsonAlpha Institute for Biotechnology, Huntsville, AL and [2]The University of Texas at Austin, Austin, TX. Email: kswaminathan@hudsonalpha.org

Switchgrass (*Panicum virgatum L.*) is native to the tallgrass prairie of North America. The high yield potential and the ability to grow well in marginal lands makes switchgrass a promising bioenergy feedstock. Switchgrass is self-incompatible, has a long-life cycle, and varied in ploidy, with tetraploid and octoploid being the most common. This makes genetic and gene function analysis a challenge. *Panicum hallii* is a perennial, diploid, self-compatible relative of switchgrass, with a short stature and shorter generation time. These features enable easier replicated experimentation in laboratory settings. Similar to switchgrass, *P. hallii* also shows substantial genetic variation in many traits important to biomass production. These include variations in flowering time, growth rate, disease susceptibility, and drought tolerance. This combination of genomic and physiological attributes makes *P. hallii* an ideal genetic model for switchgrass as well as other large C4 perennial grasses. Here, we describe the development of genetic transformation in *P. hallii* using FIL2 genotype (*P. hallii* var. filipes), a representative of lowland ecotype of *P. hallii*. This research was supported by the US Department of Energy, Office of Science, Office of Biological and Environmental Research aunder award numbers DE-SC0021126

P-3026

Engineering Novel Designer Biologics in Plant Cells for Oral Treatment of Inflammatory Bowel Disease. JIANFENG XU[1], Wenzheng Guo[1], Jonathan TrejoMartinez[2], and Uddhab Karki[2,3]. [1]Arkansas Biosciences Institute, [2]Department of Biological Sciences, and [3]Molecuar Biosciences Program, Arkansas State University, Jonesboro, AR 72401. Email: jxu@astate.edu

Plant cell culture has proved to be a safe and cost-effective bioproduction platform for therapeutic proteins. A unique feature of the plant cells is that they could serve not only as the "bio-factory," but also the oral delivery vehicle for recombinant biologics. Recent advances have demonstrated that plant cell walls, made primarily of cellulose microfibrils, can act as an excellent natural capsule for oral delivery of biologic drugs. This project aims to leverage two unique posttranslational modifications – "glycosyl-phosphatidylinositol (GPI) anchor" and "plant-specific hydroxyproline (Hyp)-*O*-glycosylation" – to strategically design and engineer novel anti-TNFα biomolecules in plant cells to develop a new class of oral biologic drugs for the treatment of inflammatory bowel disease (IBD). The designer anti-TNFα biomolecules consist of three functional domains: a N-terminal single-chain fragment variable (scFv) of an anti-TNFα antibody, a proprietary Hyp-*O*-glycosylation module comprised of tandem repeats of the "Ser-Pro" motif, or $(SP)_n$ (n= 5 to 30), and a C-terminal GPI anchor. While the GPI anchor "displays" the expressed anti-TNFα biomolecules at the plant cell surface to presumably create a high local concentration of the biologics, the $(SP)_n$ glycomodule stabilizes the protein from degradation during both the bioproduction and oral delivery processes. Designer anti-TNFα biomolecules consisting of different sizes of the $(SP)_n$ glycomodule are investigated for their accumulations in tobacco BY-2 cells, biological activity, and stability in a simulated gastric fluid, which determines the $(SP)_{20}$ module as an optimal design for the biomolecules. The therapeutic effectiveness of the orally administrated designer anti-

TNFα biologic (optimal design) in mitigating the IBD symptom is assessed in a dextran sulfate sodium (DSS)-induced colitis mouse model. The research may develop a new platform to produce effective oral biologic drugs for the treatment of UC and other inflammatory diseases of colon.

P-3027

Chromosomal Aberrations and Micronuclei of Somaclonal Variation in Onion Tissue Culture (*Allium cepa*). ABDELRAHEM YOUSEF[1], Abdel-Rahem T. Abdel-Rahem[2], Kasem Z. Ahmed[2], and Sayed Osman[2]. [1]Field Corps Research Institute, Agriculture Research Centre, Giza, EGYPT and [2]Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Eg-61519, EGYPT. Email: a.a_yousef@hotmail.com

Somaclonal variation occurs among the population of plant resulted from *in vitro* culture. It is apparently caused by gene amplification, the alteration of a basic couple, transposing migration, methylation transform, chromosome instability, chromosome inversion, gene spot mutation, translocation, ploidy change, restructuring or deletion (Kumar and Marthur, 2004). Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. In the present work we examined the effect of Plant tissue culture and Somaclonal variation on mitotic abnormalities and the mitotic index (MI) of onion (*Allium cepa.* L), the preparations were made from the root tips and Callus of three onion varieties (Giza 20, Giza 6 and BeheriRed**)**. The mitotic index for total dividing cell was ranged between 2.88±0.2 to 5.70±0.9, MI of donor plants of all tested cultivars was higher than derived Callus. Abnormalities of micronuclei percentage ranged from 0.05% (donor plants) to 0.76% (Callus), and abnormalities percentage of derived Callus of all cultivars were higher than donor plants and were significant in many cases.

P-3028

Transcriptomic Analysis of *Dunaliella salina* Under Salt and UV Stresses. M. J. HEIN[1], C. Basu[1,2], and S. M. Perl[2]. [1]California State University Northridge, Department of Biology, 18111 Nordhoff St, Northridge, CA 91330 and [2]NASA Jet Propulsion Laboratory, Origins and Habitability Laboratory, 4800 Oak Grove Dr, Pasadena, CA 91109. Email: mackenzie.hein.560@my.csun.edu

Dunaliella salina is a single celled eukaryotic algae that is capable of surviving high salt concentrations and high-intensity UV radiation. The ability to alter intracellular glycerol concentration and elastic cellular structure allow the algae to survive osmotic stress from very high or low salt concentrations. The cells are also capable of producing high intracellular concentrations of beta carotene to protect themselves from light and UV stress. Our lab has established protocols for the *in vitro* liquid culture of *Dunaliella salina* cells in a medium of filtered, sterilized, nutrient-fortified seawater. The growth of the cultures is being monitored with the use of a hemocytometer, and recordings have been made of live, motile cells. Cultures vary from green to reddish brown in coloration depending on beta carotene levels, and often contain floating, clumped colonies of cells. Cells from reddish brown cultures appear colorless on a hemocytometer, and are still alive and motile. An attempt was made to grow the cells in a medium with sharply increased salt concentration (3.5 M) compared to the baseline media in order to compare cell growth in the presence and absence of hypersaline stress. However, the cells were unable to survive the sharp increase in salinity, and no growth was observed. In the future, when testing the effects of increased salt concentration on growth, salinity will be increased in small increments to better acclimate the cells, and to produce a 'kill curve' across a range of salinities. Raman spectroscopy will be used to compare the proteomes of cells exposed to increasing levels of salt and UV stress. GC-MS will be used to determine other differences in chemical composition of cells in response to stress. The transcriptomes of cells exposed to increasing levels of stressors will be compared using RNA-Seq. The long-term goals of this study are to compare the transcriptomes, proteomes, metabolomes, chlorophyll content, and photosynthetic processes of cells exposed to varying levels of salinity, UV radiation, and possibly Mars-like conditions.

P-3029

Genetic and Physiological Responses of Lentil Plants Under Flood Stress. AVETIS MISHEGYAN[1], Bhiolina Bharadwaj[1], Sanjeevi Nagalingam[2], Alex Guenther[2], Nirmal Joshee[3], Samantha H. Herman[3], and Chhandak Basu[1]. [1]Department of Biology, California State University of Northridge, Northridge CA; [2]Department of Earth System Science, University of California Irvine, Irvine CA; and [3]College of Agriculture, Family Sciences and Technology, Fort Valley State University, Fort Valley GA. Email: avetis.mishegyan.882@my.csun.edu

Lentil (*Lens culinaris*) is a crop plant which cannot endure prolonged flooding. Flooding causes destruction of crops and property in the billions. The purpose of this project was to investigate physiological and molecular responses of lentil plants under flood stress or anoxia. In accomplishing this task flooding was induced for 4 days on 6-8 week old lentils. Molecular studies involved analyzing differential expression of the following genes by qPCR: acetyl-CoA carboxylase carboxyltransferase **β**-subunit (accD), aldolase, chloroplast Cu/Zn superoxide dismutase (SD), PBA flash photosystem II D1 (PsbA), and petD-cytochrome b6/f complex subunit IV (PetD). The results of our qPCR experiments exhibited an upregulation of accD, aldolase, and petD genes; but the downregulation of PsbA and SD. Besides gene expression studies, physiological studies were also conducted. These tests incorporated catalase activity, chlorophyll content analysis, and sugar content estimation. Flooded lentils exhibited increased levels of production of soluble sugar. In addition, electron microscopy analysis revealed higher surface roughness and more wax deposition in flooded plants. However, waterlogged lentils exhibited lowered levels of catalase activity, chlorophyll content, and volatile emissions. Gas chromatography-mass spectrometry (GC-MS) analysis of the plant volatiles revealed that several wound compounds were emitted (2-hexenal, 3-hexenal, cis-3-hexenol, 3-pentanone, and cis-3-hexenyl acetate) and that the emissions were largely downregulated in the flood-stressed plants relative to the control plants. Our results will help us to understand molecular and

physiological responses of lentils under anoxia and help us develop stress-tolerant lentil plants.

P-3030

Transcriptomic and Physiological Effects of Heat Stress on Turmeric (Curcuma longa). KIRILL MUSAEV and Chhandak Basu. Department of Biology, California State University, Northridge, 18111 Nordhoff St., Northridge, CA, 91330. Email: kirill.musaev.911@my.csun.edu

Turmeric (Curcuma longa) is primarily known for containing curcumin – a bioactive compound linked to anti-inflammatory, antioxidant and anti-cancer activity in humans, and a number of other potentially beneficial effects. Although turmeric has been used as a spice in India and Asia for centuries, its production has recently seen a significant increase to ≈1.7 million tons worldwide and is projected to increase further; this can be explained by a renewed interest in dietary supplement products, which market curcumin as a healthy supplement. With the global market for curcumin totaling US$21.9M (2019), turmeric is an economically important crop. However, while ample research is available on the bioactivity of curcumin and its related compounds – curcuminoids, there are not many reports on well-designed scientific investigations into the curcumin compounds production under various environmental stressful conditions. In this experiment we explore the effects of heat stress on gene expression and physiological responses in turmeric plants, potentially providing insight into the molecular physiology of the plant. Soil-grown turmeric specimen of the experimental group will be heat stressed at 42°C for 24 hours, then each plant will be tested for chlorophyll content, photosynthesis efficiency, catalase activity and curcumin content, and have samples collected for downstream RNA sequencing using Illumina MiSeq Next-Generation Sequencing procedure. The corresponding data from the control group, grown in normal conditions and sampled at the same time as the experimental group, will determine which properties are affected by the heat stress, including differential gene expression as identified by RNA sequencing. Correlating the physiological measurements with the gene expression data may additionally elucidate the pathways involved in heat stress mitigation. Furthermore, since curcumin is known to exhibit antioxidative properties, we speculate that its content might rise after a period of heat stress, to be detected in our assay.

P-3031

Expanding the Toolbox of Maize Promoters. NATHANIEL SCHLEIF[1], Frank McFarland[1], Ray Collier[2], and Heidi F. Kaeppler[1,2]. [1]University of Wisconsin - Madison, Department of Agronomy, 1575 Linden Dr, Madison, WI 53705 and [2]Wisconsin Crop Innovation Center, 8520 University Green, Middleton, WI 53562. Email: nschleif2@wisc.edu

The scope of research questions that can be asked is limited by the tools available, and this is especially true in crop functional genomics research. For maize, a globally important crop, only a limited selection of promoters are available for modulating transgene expression. This is an issue, as process efficiency and genetic/epigenetic research is critically dependent on promoters behaving in an expected and specific manner. Currently, there are very few characterized promoters available which impart tissue/organ/timing-specific expression, which limits maize genetic research and enhancement efforts. To address the need for a wider array of characterized maize promoter choices, we developed protocols for the identification, cloning, and characterization of promoters in maize. We then used these methods to identify and characterize promoters which confer leaf, embryo, or root-specific gene expression across a variety of developmental points. Candidate promoters were identified through analysis of RNAseq data from the Maize Gene Expression Atlas with distance metrics to an idealized promoter profile. Additional analysis was performed to identify and remove genes with long-range activating regions with available ATAC-seq data in maize. Potential cis-regulatory regions within the promoters were also identified to be in synthetic promoters. All sequences were domesticated for use in Golden Gate cloning. Both unmodified and conjugate promoters sequences were used to drive expression of the GUS screenable marker gene. The constructs were transformed into maize inbred line LH244. MUG assays to quantify GUS protein levels are ongoing, but preliminary results indicated that 4 out of the 15 promoter sequences tested showed the desired tissue/organ-specific expression.

P-3032

The Plant Genetic Engineering Network. J. VAN ECK[1,2], H. Kaeppler[3,4], B. Gordon-Kamm[5], K. Lee[6], W. Parrott[7], N. Taylor[8], and V. Veena[8]. [1]Boyce Thompson Institute, Ithaca, NY; [2]Plant Breeding & Genetics Section, Cornell University, Ithaca, NY; [3]Department of Agronomy, University of Wisconsin-Madison, Madison, WI; [4]Wisconsin Crop Innovation Center, University of Wisconsin-Madison, Madison, WI; [5]Corteva Agriscience, Johnston, IA; [6]Department of Agronomy, Iowa State University, Ames, IA; [7]Department of Crop & Soil Sciences, University of Georgia, Athens, GA; and [8]Donald Danforth Plant Science Center, St. Louis, MO. Email: jv27@cornell.edu

Plant genetic engineering (transformation) and gene editing are critical tools for the advancement of plant functional genomics research, and genomics-based crop improvement. Current transformation systems are significantly limited by inefficient, complicated methods, and a lack of training and expertise in the art and science of transformation biology and techniques. There is a critical need for increasing plant transformation capacity worldwide. Improving capacity will require 1) research advances across transformation technologies; and 2) enhanced knowledge exchange and training in transformation biology and techniques. The Plant Genetic Engineering Network Research Coordination Network (PlantGENE) has been established to facilitate research and to ensure sharing of technology, knowledge, and expertise. The goal of PlantGENE is to ensure that existing knowledge and new information gained from research and development on transformation are rapidly and inclusively shared across the research community. To meet this goal, PlantGENE will 1) establish a global network of researchers from public and private organizations to collectively address the current challenges; 2) facilitate collaboration and training opportunities; 3) exploit

established relationships with international research institutes to enable transfer of knowledge, technologies, and methodologies to laboratories in underserved regions; 4) share proven and state-of-the-art methods, develop training modules, and offer virtual and in-person workshops designed and led by experts in plant transformation; and 5) engage with plant biotechnology organizations such as the Society for In Vitro Biology (SIVB) and the International Association of Plant Biotechnologists (IAPB).

P-3033

Synteny Analysis: Genes Linked for Powdery Mildew Among Landraces of *Pisum sativum* and *Lathyrus sativus*. R. BISHNOI[1], S. Marker[2], and P. K. P. Meena[1]. [1]Department of Genetics and Plant Breeding, Agriculture University, Kota, Rajasthan, INDIA and [2]Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, INDIA. Email: vishnoi.ruchi25@gmail.com

Pea (*Pisum sativum*) and its closely related legume, *Lathyrus* (*Lathyrus sativus*) are drastically affected by powdery mildew (*Erysiphe pisi*), which is the most prevalent disease in India, especially in the eastern part of Uttar Pradesh. The landraces have wider adaptability and higher tolerance capability for biotic and abiotic stresses. Therefore, landraces of pea and *lathyrus* were collected from local farmers of Uttar Pradesh, after extensive survey, characterization, purification and documentation. Distinctiveness, Uniformity and Stability analysis were done during *rabi* 2019-20 and 2020-2021 according to the guidelines provided in Protection of Plant Variety and Farmers Right Act (PPV&FRA), 2001. They were found distinct from public domain varieties, uniform in their expression and were having stable performance during two consecutive seasons. Moreover, pea genotypes *viz*. PATK-278, PRAV-230 and PARA-501, were mildly tolerant to powdery mildew with 28%, 25% and 22% disease severity, respectively, whereas *lathyrus* genotypes *viz*. LKKK-227 and LMKK-232, were found to be resistant (8% disease severity). Advanced analysis of their genome and genes linked for powdery mildew can be done through high-throughput genotyping. Synteny analysis helps in identification and isolation of homologous genes and its order for powdery mildew resistance and/or sensitivity in both the species. It may be helpful in future breeding program for disease resistance and wide hybridization. Hence, synteny analysis and its significance in pea and *lathyrus* will be discussed for its full exploitation in powdery mildew resistance.

P-3034

Genome Editing in Three Species of the Bioenergy Grass *Miscanthus*. NANCY A. REICHERT[1,2], Anthony Trieu[2,3], Mohammad Belaffif[2,3], Shilpa Manjunatha[2,3], Pradeepa Hirannaiah[2,3], Rebekah Wood[3], Yokshitha Bathula[3], Rebecca Billingsley[1,2], Anjali Arpan[1,2], Erik Sacks[2,4], Stephen P. Moose[2,4], Tom E. Clemente[2,5], and Kankshita Swaminathan[2,3]. [1]Mississippi State University, Department of Biological Sciences, Mississippi State, MS; [2]University of Illinois, Center for Advanced Bioenergy and Bioproducts Innovation, Urbana-Champaign, IL; [3]HudsonAlpha Institute for Biotechnology, Huntsville, AL; [4]University of Illinois, Department of Crop Sciences, Urbana-Champaign, IL; and [5]University of Nebraska, Department of Agronomy and Horticulture, Lincoln, NE. Email: nar1@msstate.edu, kswaminathan@hudsonalpha.org

Miscanthus is a C4 member of the Poaceae (grass) family. These perennial, self-incompatible plants can be grown in many USDA hardiness zones with regrowth typically initiated from rhizomes in the spring. The majority of species contain a basic chromosome set of 19, and display a range in their number of basic chromosome sets (2x-6x). There is increased interest in various miscanthus species for lignocellulosic biomass for bioenergy applications. Three species of interest include *M. sacchariflorus* (2x and 4x), *M. sinensis* (2x) and *M.* × *giganteus* (3x; arose from cross between 4x *M. sacchariflorus* and 2x *M. sinensis*). *M. sinensis* may have arisen from a plant containing ancient *Sorghum*-like DNA that had doubled along with a post-doubling chromosomal fusion event; so, although diploid, *M. sinensis* is an allotetraploid. Transformation procedures (*Agrobacterium tumefaciens* and particle bombardment) to facilitate genome editing via CRISPR/Cas9 were developed in our labs. The lemon-white 1 gene (*lw1*), first identified in *Zea mays* seeds and seedlings, was targeted. Due to the importance of designing gRNA sequences that could target homeologous genes, both miscanthus and sorghum orthologs of *lw1* were aligned to identify conserved regions. Using various commercial software, three gRNA were designed and all were introduced into miscanthus embryogenic calli along with the neomycin phosphotransferase II (*nptII*) selectable marker gene. Selection was on media containing paromomycin (100-200 mg L^{-1}). Transgenic regenerants displayed various leaf colors ranging from pale green to green/white stripes to pure white. Confirmed deletions, based on Sanger sequencing, ranged from 1-26 bases and one large deletion (433 bases) spanned two gRNA target sites. This is the first report of successful genome editing in miscanthus, and was confirmed in three species – *M. sacchariflorus*, *M. sinensis* and *M.* × *giganteus*. This also demonstrated the successful targeting of a new gene that can be used as a visual marker to indicate editing has taken place.

P-3035

Development of Tissue Culture-free Genetic Transformation and Gene-editing Platform in Crops. ARJUN OJHA KSHETRY, Vikas Devkar, Luis Herrera-Estrella, and Gunvant B. Patil. Institute of Genomics for Crop Abiotic Stress Tolerance (IGCAST), Department of Plant & Soil Science, Texas Tech University, Lubbock, TX. Email: arjun.ojha@ttu.edu, gunvant.patil@ttu.edu

Gene editing technologies, especially CRISPR/Cas have revolutionized crop engineering research. However, delivery of gene editing reagents is largely depending on genetic transformation and *in vitro* regeneration (tissue culture) of plants. Moreover, plant regeneration and genetic transformation are highly genotype-dependent. Therefore, the lack of efficient genotype-independent plant transformation methods in several recalcitrant crops including cotton, soybean, sorghum, common bean, etc. has been a major limitation in applying gene-editing technology in crop improvement. To overcome these challenges,

we are creating a synthetic cascade to express developmental regulator genes involved in stem cell activity, rapid tissue differentiation, and the regeneration process. Importantly, these developmental regulators will be applied *in vivo,* and we envision the development of a robust regeneration and gene-editing methodology without a need for tissue culture. Currently, we are testing a series of developmental regulators in the model plant *Nicotiana benthamiana* (tobacco), and once successful, it will be applied to major recalcitrant crop species.

P-3036

Phenotypic Assessment of SnRK1C Mutants in Rice (*Oryza sativa var. japonica*) cv. Kitaake. M. C. FARIA CHAVES, C. Maurya, N. Soumen, S. Zhao, and V. Srivastava. Department of Crop, Soil and Environmental Sciences, University of Arkansas, 125 Plant Science Building W Maple St., Fayetteville, AR, 72701. Email: mcfariac@uark.edu

For normal growth and development and to cope with stress, plants rely on several signaling mechanisms involving the sucrose non-fermenting related protein kinase 1 (*SnRK1*). This gene affects many aspects such as regulation of autophagy, gene expression, sugar signals and coordination of transcriptional networks. However, the role of SnRK1 in rice development and yield is not well understood. Rice contains three functional paralogs of SnRK1, and this study focused on the function of SnRK1C (LOC_Os05g45420) by evaluating the morphological characteristics of its mutants developed by CRISPR/Cas9 mediated targeted mutagenesis. Earlier, these mutants were found to contain homozygous -1 to -3 bp mutations leading to early stop codon in the SnRK1C reading frame. The T1 seeds harboring homozygous mutations were subjected to phenotypic assessment along with the wild type (WT) *Oryza sativa var. japonica* cv. Kitaake. Plants were grown in randomized block design in a controlled environment in the greenhouse. Our results show that the mutant lines had lower shoot biomass and a lower number of seeds per panicle when compared with the WT, while no difference in the weight of roots and weight of 100 seeds was observed. These results show the importance of SnRK1C in the vegetative growth and fertility of rice. In the ongoing study, these validated SnRK1C mutant lines will be used for transcriptomic analysis to understand the underlying signaling mechanisms.

P-3037

CRISPR RNA-guided Integrase System for *Agrobacterium* Genome Editing. EPHRAIM ALIU[1,2,3], Keunsub Lee[1,2], and Kan Wang[1,2]. [1]Department of Agronomy, Iowa State University, Ames, IA 50011; [2]Crop Bioengineering Center, Iowa State University, Ames, IA 50011; and [3]Interdepartmental Plant Biology Major, Iowa State University, Ames, IA 50011. Email: ealiu@iastate.edu, kanwang@iastate.edu

Agrobacterium-mediated plant genetic transformation is critical for modern crop improvement to enhance fundamental biological research. For this reason, attempts have been made over the years to engineer *Agrobacterium*. Traditional technologies for gene knock-in or knockout in *Agrobacterium* require homologous recombination (HR) or transposon insertional mutagenesis. These methods are generally limited by low efficiency, reliance on recombination, laborious, and time-consuming. To address these shortcomings, we implement the INTEGRATE (**IN**sertion of **T**ransposable **E**lements by **G**uide **R**NA–**A**ssisted **T**arg**E**ting) system in *Agrobacterium tumefaciens* using a Tn7-like transposon system previously developed and optimized in *Escherichia coli* (Vo *et al.,* 2021, *Nature Biotech.*). We optimized the expression of INTEGRATE system for *Agrobacterium* and generated single and simultaneous multi-gene insertional mutants with high efficiency. Moreover, Sanger sequencing analysis confirmed highly specific genome-wide DNA insertion across all unique target sites. Furthermore, in conjunction with the Cre-*loxP* recombination system, we show that the INTEGRATE system can be utilized to achieve precise large fragment DNA deletion over 20 kilobases. This work thus provides new genetic engineering tools for *Agrobacterium* species to accelerate gene functional analysis.

P-3038

Transformation of Teosinte (*Zea mays* ssp. *parviglumis*) Via Biolistic Bombardment of Seedling Derived Callus Tissues. Jacob D. Zobrist, Susana Martin-Ortigosa, Keunsub Lee, MERCY K. AZANU, Qing Ji, and Kan Wang. Department of Agronomy, Iowa State University, Ames, IA. Email: mkazanu@iastate.edu

Teosinte (*Zea mays* ssp. *parviglumis*) is considered as the progenitor of domesticated maize (*Zea mays* ssp. *mays*), with ample genetic diversity that can be useful for maize breeding. The ability to access and introgress these genes into a crop improvement regimen would require a robust and timely regeneration protocol for teosinte. This also requires the right in vitro culture system that aids successful regeneration. Here, we provide a detailed protocol for teosinte regeneration via tissue culture, with an outline from seed sterilization till T0 plants. Emphasis on media composition and its effect regeneration is well discussed. We also report the first protocol for genetic transformation of teosinte (*Zea parviglumis)* using biolistic bombardment of seedling-derived callus tissues. We achieved a 4% transformation frequency, using a reporter plasmid pKL2155, which carries a mutant acetolactate synthase gene (HRA, for resistance of herbicide imazapyr) and a red fluorescent protein marker gene (*tdTomato*).This protocol provides not only a major enabling technology for studying domestication, gene function and creation of an ideotype maize plant from its wild progenitor, but also opens the door to endless access to the teosinte genome, which may be key towards a soon hunger-free world.

P-3039

Physiological and Transcriptomic Analysis of Turmeric Plants (*Curcuma longa)* Under Drought Stress. BHIOLINA BHARADWAJ and Chhandak Basu. Department of Biology, California State University, Northridge, CA. Email: bhiolina.bharadwaj.979@my.csun.edu

Turmeric (*Curcuma longa)* has been used as a medicinal plant for nearly 4000 years. In Southern Asia, it has been used not only as spices but also as a food preservative, beauty care product and a coloring material. In Indian culture, turmeric is used for religious ceremonies as well. However, it's concerning that, in many of the

parts of Southern Asia, turmeric yield is getting affected due to different abiotic stresses. Drought is one of the most concerning abiotic stresses in areas around India. But there are not many reports about how drought stress can affect turmeric plants on a molecular level.In our study we are investigating physiological and biochemical changes of turmeric plants under drought stress. Understanding differential expression of genes in physiological stressful conditions will be crucial to comprehend the entire mechanism responsible for drought stress tolerance in turmeric plants. To mimic drought stress, turmeric plants were not watered for 20 days. Physiological and molecular studies were conducted following the drought stress. Physiological studies involved photosynthetic rate, chlorophyll content, moisture content and catalase activity estimation. While for molecular studies, RNA was extracted from drought stressed and controlled plants and then Illumina Truseq kit was used to prepare barcoded cDNA libraries. Illumina Miseq sequencing system will be used to sequence the barcoded cDNAs from controlled and drought-stressed plants. Omicsbox software will be used to quantify differential gene expression among control and drought-stressed turmeric plants. Differential expression of genes will be confirmed by qPCR. Furthermore, with identified upregulated and downregulated genes and physiological studies, we can hypothesize a distinct pathway which will be likely responsible for drought stress tolerance in turmeric plants.

P-3040

Towards Genetic Engineering of Sunflower Lineage to Produce Biodiesel. F. SHAURYA, B. Bharadwaj, and C. Basu. California State University, Department of Biology, Northridge, CA. Email: shaurya.lnu.768@my.csun.edu

Sunflowers (,*Helianthus annuus* L) are widely grown crops all around the world. It's popularly known for its high oil content, which makes it an ideal crop for producing "Biodiesel". It yields well under various conditions which differentiates it from other oil crops as it's easy to grow regardless of farm sizes. Although, global warming is enormously becoming a major concern around the world. However, we developed effective ways to reduce the green house gas emission by using renewable sources of energy, such as biodiesel. Biodiesel is a domestically produced, clean-burning, renewable substitute for petroleum diesel. Therefore, the aim of our project is to develop genetically engineered sunflower plants for producing biodiesel. In this project, we plan to express germacrene synthase gene to increase production of the sesquiterpene compounds which happen to enhance over q uality of biodiesel in sunflower. Germacrene synthase is hypothesized to increase the abundance of sesquiterpene in the crop. Sesquiterpene has similar chemical properties as biodiesel. Our hypothesis is to use the sesquiterpene biosynthesis pathway to overexpress germacrene synthase which would further lead to `overproduction of biodiesel in sunflower. For this purpose we will use agrobacterium-mediated transformation methods to make our transgenic sunflower plants which are expected to produce biodiesel precursors in excess. We successfully established a tissue culture-based sunflower regeneration system in our lab. We used sterile cotyledons to initiate callus formation. The tissue culture media was a Murashige and Skoog media supplemented with NAA and BAP. We are in the process of infecting the calli with the LBA4404 strain of *Agrobacterium tumefaciens* harboring the germacrene synthase gene. A successful production of transgenic sunflower plants could be used for production of enhanced quality of biodiesel.

P-3041

Conservation of African Sweetpotato Landraces – Lessons from the Conservation of Global Vegetatively Propagated Genetic Resources Collections. DAVID ELLIS[1], Jan Low[2], Maria Andrade[3], Marian Dorcas Quain[4], Ted Carey[5], Matthew L. S. Gboku[6], Noelle L. Anglin[1,7], Godwill Makunde[3], Jebeh Samba[6], Norma Manrique-Carpintero[1,8], Rosemary Gatimu[2], Vania Azevedo[1], Charlotte Lusty[9], and Luigi Guarino[9]. [1]International Potato Center, Lima, PERU; [2]International Potato Center, Nairobi, KENYA; [3]International Potato Center, Maputo, MOZAMBIQUE; [4]CSIR-Crops Research Institute International, Kumasi, GHANA; [5]Reputed Agriculture 4Development Foundation, Kumasi, GHANA; [6]Sierra Leone Agricultural Research Institute, Freetown, SIERRA LEONE; [7]ARS-USDA Small Grains and Potato Germplasm Unit, Aberdeen, ID; [8]Colombian Corporation for Agriculture Research-Agrosavia, Bogota, COLUMBIA; and [9]The Global Crop Diversity Trust, Bonn, GERMANY. Email: davedellis07@gmail.com

The African continent is a secondary site of diversity for sweetpotato yet relatively few of the diverse and genetically unique farmer maintained sweetpotato landraces are securely conserved *ex situ*. The objective of this project was to collect, document, genotype, and ensure the secure long-term conservation of sweetpotato landraces from Ghana, Kenya, Mozambique, and Sierra Leone under the International Treaty for Plant Genetic Resources for food and Agriculture (ITPGRFA) to return virus-free plantlets to farmers in the contributing countries. Collected landraces from the different countries were established *in vitro* and shipped to the in trust sweetpotato collection at the CIP-genebank in Peru. The target was to ship 50 landraces per country in March 2020. However, shipment of the *in vitro* landraces was delayed by a year due to the COVID pandemic shut down of international shipments and sadly a significant amount of material was lost *in vitro*. Despite this, three shipments have been made resulting in 120 landraces undergoing phytosanitary cleaning in Peru, with a fourth and final shipment of 32 landraces scheduled for mid-2022. Genetic analysis (DArTseq) confirmed the unique nature of the material which clustered by country collected and as new pool when compared to the global sweetpotato collection in the CIP-Peru genebank. Although the project had letters of commitment from all countries to transfer the material under the ITPGRFA with the Standard Material Transfer Agreement (SMTA), to date, only Ghana and Sierra Leone have signed the SMTA due to within country challenges to obtain a signed SMTA. Lessons learned are that international collection projects of vegetatively-propagated materials should plan multiple shipments and contingency plans for maintaining material for a minimum of two years after collection. Additionally, a signed SMTA should be a prerequisite prior to shipping any materials. In the end, the project has been a success with novel sweetpotato genetic

resources now securely conserved and available under the ITPGRFA for use in breeding, research, and training.

P-3042

Global Plant Cryopreservation Initiative. DAVID ELLIS[1], Vania Azevedo[1], Marleen Engers[2], Badara Gueye[3], Charlotte Lusty[2], Nicolas Roux[4], and Peter Wenzel[5]. [1]International Potato Center, Lima, PERU; [2]Clobal Crop Diversity Trust, Bonn, GERMANY; [3]International Institute of Tropical Agriculture, Ibadan, NIGERIA; [4]Alliance-Bioversity International, Montpelier, FRANCE; and [5]Alliance-International Center for Tropical Agriculture, Cali, COLUMBIA. Email: davedellis07@gmail.com

The *Feasibility Study for a Safety Back-Up Cryopreservation Facility*, assessed the need for a cryopreservation facility for safety duplicates of germplasm collections that cannot be conserved as seed long-term in the Svalbard Global Seed Vault. These include vegetatively propagated crops and crops with recalcitrant or short-lived seed. There is a dire need to reliably protect these invaluable genetic resources, particularly in developing world, because collections are at risk of loss due to biotic and abiotic and many lack long-term resources for their secure maintenance. The study concluded: 1) cryopreservation is the best long-term conservation option for these collections; 2) cryopreservation has huge benefits for long-term safety back-up; 3) there is a critical need to accelerate the cryopreservation of clonal and recalcitrant seed collections; 4) the CGIAR is ideally situated to develop a proposal and seek donor sponsorship to meet these needs. To address this challenge, the CGIAR and Global Crop Diversity Trust, have proposed a collective effort, The Global Plant Cryopreservation Initiative (GPCI) to help countries worldwide securely safeguard vegetatively propagated crops long-term in cryopreservation. Phase One of the GPCI targets germplasm collections of five of humanity's ten most important crops (potato, cassava, sweetpotato, yam, banana) as well as five crops that are vital for the developing world (cacao, coffee, taro, coconut and ulluco). The initiative will build on the success of cryopreservation in potato and banana, where more than 80% of the global in-trust collections have been safeguarded in cryobanks under strict quality guidelines. It will establish regional "Center of Cryopreservation Excellence" hubs, starting with one each in Latin America, Africa, and the EU. These hubs will work with partners globally to protect germplasm collections under the terms of the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA) and thus strengthen the capacity for sustainable long-term cryobanking in the developing world.

P-3049

Development of an Efficient Method for Protoplast Isolation, Transfection, and Gene Editing form Soybean Roots. C. L. NWOKO, A. Ojha, V. Devkar, and Gunvant B. Patil. Institute of Genomics for Crop Abiotic Stress Tolerance (IGCAST), Department of Plant & Soil Science, Texas Tech University, Lubbock, TX 79401. Email: chnwoko@ttu.edu

Protoplasts are plant cells with degraded cell wall that behave like animal cells *in vitro*. Protoplast is a versatile system in modern plant biology that provides a platform for rapid analysis of diverse signaling pathways, studying functions of cellular machineries and functional genomics screening. Protoplast allows the direct delivery of DNA, RNA or protein into the plant cell and provides a high-throughput system to validate gene-editing reagents. However, this system is less exploited in several legumes crops (including soybean), and it is because of lower protoplast yields, transfection efficiencies and lack of working protocol for plant regeneration from protoplast Moreover, protoplast isolation in several plant is mainly focused on leaf mesophyll tissues. Although, root tissues provide several advantages, root protoplast isolation, transfection and gene-editing have not been established in soybean. To overcome these bottlenecks, we are developing a new robust method for high quality protoplast isolation and transfection from soybean roots (including transgenic hairy roots). With our newly developed the highest yield, 1.3 x 10^6 and 7.3 x 10^5) of protoplasts were obtained from soybean roots and hairy roots respectively. More importantly, we also describe a method for gene-editing in soybean protoplasts isolated from root tissues.

P-3050

Assessing Climate Resilience of Different Cowpea Cultivars Through Phenotypic and Molecular Analyses. INOCENT P. RITTE, M. Egnin, O. Idehen, D. Mortley, G. C. Bernard, and C. Bonsi. Plant Biotechnology and Genomics Research Lab, College of Agriculture, Environment and Nutritional Sciences, Tuskegee University, AL 36088. Email: iritte8222@tuskegee.edu, megnin@tuskegee.edu

Plants respond to environmental stresses through changes in gene expression, which make them more adapted to unfavorable conditions such as drought. The molecular mechanism of drought-tolerance in plants is not well understood. Knowledge of how genes are expressed in response to drought-stress may contribute to understanding of involved mechanism. This study aimed to investigate expression patterns of known stress and developmental genes in seven cowpea cultivars subjected to drought-stress at seedling stage. Three target genes; *9-cis-Epoxycarotenoid Dioxygenase-1* (*NCED1), Salt/drought-stress response/antifungal domain protein* (*SalT)*, and *Light-harvesting protein* (*Bio-PSII)* and reference gene, *Regulatory subunit of phosphatase 2A-protein (VuPp2A)* for comparing were evaluated by quantitative reverse transcription PCR (qRT-PCR). Cultivars were grown in greenhouse box experiment using Randomized Complete Design with three replicates. Plants were well-watered until first trifoliate leaf fully expanded. Drought-stress was imposed for 33d, and controls were well-watered. Morpho-physiological data were collected on stem diameter, chlorophyll-content, plant greenness, and recovery. Leaf tissues were sampled at three experimental time-points (d7, 14 and 28) for molecular analyses. All three genes were differentially expressed across cultivars in control and drought conditions. At d7, *Bio-PSII* and *NCED1* were up-regulated in control and down-regulated under drought conditions except *SalT* was up-regulated under drought in TVu11987, Aloomba and TVu2428. Significant up-regulation of all genes was observed at d14 in drought-stress condition and repressed during d28. Our results indicate, TVu11987, Lobia-I-

Sefade, K929 and Aloomba are more tolerant to drought along with confirmed drought-tolerant California-blackeye-#5. These results may contribute to identification of useful genes for enhancing cowpea drought-tolerance breeding. Research supported by USDA-NIFA Grants: 2017-38821-26414-GE and USDA-NIFA-Evans-Allen Grant No. ALX-FVC18 to Tuskegee University.

P-3051

Production-scale Transformation of Poplar Hybrid Clone INRA 717-1B4, *P. tremula* x *P. alba* by Particle Bombardment for Enhanced Photosynthesis Traits. MICHELLE TJAHJADI, Alex Crites, Dominick Tucker, Kenneth Donsky, Gary Orr, Karli Rasmussen, Jessica Du, Li-Wei Chiu, and Matt Heckert. Living Carbon, Hayward, CA. Email: michelle@livingcarbon.com

Increasing tree biomass and carbon capture efficiency are fundamental to an atmospheric CO2 drawdown and sequestration strategy at Living Carbon. Enhancing tree's natural ability to sequester carbon using a photorespiration shunt pathway to metabolize glycolate-2-phosphate with simultaneous downregulation of plastidal glycolate glycerate translocator 1 (*PLGG1)* was identified as a target for improving both carbon flux and fixation. To support this work, we show how Poplar hybrid clone INRA 717-1B4 *(P. tremula* x *P. alba)* can be transformed by particle bombardment at scale: Over a six-week period, three researchers bombarded 9000+ explants and generated a total of 125 independent, Geneticin resistant events with a transformation efficiency of ~1.5%. From stem-derived callus to putative transformed rooted plants, in soil it took on average 6 months. A systematic approach to expedite trait development from proof-of-concept through production is described.

PLANT VIRTUAL POSTER ABSTRACTS

P-3043

Bioinspired Superhydrophobic and Liquid-infused Slippery Coatings for Decreased Fouling, Thrombosis, and Infection of Medical Devices. E. OZKAN, A. Mondal, M. Douglass, S. P. Hopkins, M. Garren, R. Devine, R. Pandey, J. Manuel, P. Singha, J. Warnock, and H. Handa. School of Chemical, Materials and Biomedical Engineering, College of Engineering, University of Georgia, Athens, GA, 30602. Email: eo83417@uga.edu

Bacteria-associated infections and thrombus formation are two major complications plaguing the application of blood-contacting medical devices. To prevent these complications, we developed ultra-low fouling coatings that simultaneously mimic multiple functions of antimicrobial agents and bioinspired superhydrophobic (SH) or liquid-infused surfaces (LIS) to prevent complications over the life span of a device. Firstly, SH paints consisting of perfluorosilane-coated hydrophobic zinc oxide (ZnO) and copper (Cu) nanoparticles (NPs), and polydimethylsiloxane in tetrahydrofuran were prepared and subsequently applied onto widely used medical-grade silicone rubber (SR) tubes. Then, the SH tubes were converted to the LIS tubes upon lubrication with low-viscosity silicone oil. The morphological features, chemical compositions, and wettability of the tubes were investigated by SEM, EDX, and contact goniometry. Furthermore, the antimicrobial, antifouling, and cytotoxic properties of the tubes were thoroughly examined against clinically relevant *Escherichia coli (E. coli)*, platelets, and fibroblast cells. The introduction of hydrophobic ZnO and Cu NPs constructed micro/nanoscale structures on the SR surface, leading to superhydrophibicity (WCA = 159.16 ± 2.23°). After the lubricant infusion, the hierarchical microscale structures and void gaps were occupied by silicone oil, leading to a flat liquid film on the LIS surface with a WCA of 105.29 ± 1.06°. The LIS tubes exhibited superior resistance to clot formation and platelet adhesion than uncoated and SH tubes. The SH and LIS tubes significantly reduced bacterial adhesion and biofilm formation of *E. coli* relative to control tubes (> 5 log and > 3 log reduction, respectively). Also, 24 h cytotoxicity evaluation showed no cytotoxicity. This study demonstrates that novel LIS coating with antifouling and antimicrobial properties has huge potential to greatly increase the longevity of intravascular devices by reducing complications due to infections and thrombosis while improving patient care and quality of life.

P-3044

Influence of Plant Growth Regulators in Initiation of In Vitro Cell Cultures of *Tinospora cordifolia* for Biosynthesis of Silver Nanoparticles. VARTIKA SRIVASTAVA, Harshajit Baruah, and Rakhi Chaturvedi. Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, INDIA. Email: rakhi_chaturvedi@ iitg.ac.in.

Tinospora cordifolia, multipurpose woody liana has recently gained global recognition for its immense health benefits in immunizing the body against diabetes, cancer, SARS-CoV-2, dengue and other ailments. Along with, the plant participates in restoration and rejuvenation of the body as well. For fulfilment of requirement of the plant for food, drugs and as biomaterial, strategic methods for the germplasm conservation should also be employed. Plant cell culture is highly applicable approach which conserves the germplasm and provides superior plants for various applications, throughout the year. In the present study, in vitro cell culture was developed using leaf explants of *T. cordifolia.* Among tested media treatments, MS basal medium with growth regulators, BAP and NAA, gave highest callus proliferation rate in semi-solid and liquid culture medium. *T. cordifolia* of Menispermaceae family predominantly contains alkaloids, phenolics and terpenoid content. Likely, chemical analyses revealed higher alkaloid yield in in vitro cultures than the mother plant. Further, for exploring enhanced therapeutic and environmental benefits of in vitro cultures of *T. cordifolia*, they were transformed to nano-level. This report primarily proposed the methodology for biosynthesis of silver nanoparticles using in vitro cell cultures. The fabricated nanoparticles were characterized using UV-Vis spectroscopy, FTIR, SEM-EDX and FETEM. Results affirmed average particle size ranged from 10 nm to 24 nm at wavelength of ~450 nm, while the SAED pattern revealed the crystalline nature of the in vitro synthesized nanoparticle. This study is an initiative for tailoring cell culture mediated nanostructures and evaluating their future applications.

P-3045

Effect of BA on Shoot Organogenesis in Mature Leaves of *Cleome gynandra.* AUBRE JOYNER, Gloria Payne, and Margaret Young. Department of Natural Sciences, Elizabeth City State University, Elizabeth City, NC. Email: mmyoung2@ecsu.edu

Cleome gynandra is classified as a potential model C4 plant. C4 plants avoid photorespiration by separating their light dependent and light independent photosynthetic reactions (Krantz anatomy). This makes them more adapted to higher temperatures. In this experiment, we looked at three different concentrations of BA (1, 2 and 3 mg/l) on the effects of shoot organogenesis in mature leaf explants of *C. gynandra.* Mature leaves from 3 months old plants were sterilized using 5% bleach and placed on media containing BA. Data was taken every 2 weeks for 8 weeks and the experiment was repeated three times. Meristemoids were seen in 90% of treated explants. The best direct shoot formation (40.8 ± 19.3%) was observed on media containing 1 mg/L BA; whilst the media with 3 mg/ml BA had 14.4 ± 19.7% shoot formation. From previous experiments, in vitro grown leaves had a 95% direct shoot organogenesis rate with 2 mg/L BA. We are now testing three different concentrations of NAA (0.0, 0.1 and 0.2 mg/L) on root formation of these shoots.

P-3046

Tissues-wide Distribution of Azadirachtin Content in Neem (*Azadirachta Indica* A. Juss.), a Miraculous Medicinal Tree Species**.** RAJENDRA ADAK[1] and Rakhi Chaturvedi[1,2]**.** [1]School of Agro and Rural Technology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, INDIA, and [2]Department of Biosciences & Bioengineering, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, INDIA**.** Email: rakhi_chaturvedi@iitg.ac.in

India is globally recognized for ayurveda-based medicinal knowledge and practices, since prehistoric times. Folk healers are using plant extract for household remedies against various ailments. *Azadirachta indica* A. Juss. (family Meliaceae), commonly known as neem, is a miraculous medicinal tree, is well-known for its broad-spectrum therapeutic applications. Recent study shows that azadirachtin is a highly acknowledged secondary metabolite available in neem plants. Quantitative variability of azadirachtin content in neem plants depends upon different agro-climatic regions. Herein, a rapid selective and highly reproducible method has been developed for quantitative estimation of azadirachtin in reverse phase high performance liquid chromatography (HPLC). Comparative analysis of azadirachtin content has been performed among different tissues of neem plants, such as leaf, bark, stem, flower, root, and seed. Samples were harvested from 3-years-old neem plants from the experimental garden at IIT Guwahati. The samples were dried in an oven at 30±2°C temperature. Optimum separation and purification of azadirachtin was achieved by high-performance liquid chromatography (HPLC). The amount of azadirachtin content in increasing order was observed in leaves (1.37±0.05 mg/gm), bark (2.36±0.04 mg/gm), stem (3.13±0.07 mg/gm), flower (4.69±0.13 mg/gm), root (5.6±0.06 mg/gm) and seed (7.11±0.0.01 mg/gm). With this route, the highest azadirachtin content was obtained from seed and the least azadirachtin content from leaves. In conclusion, this study has screened tissue-specific variation of azadirachtin contents in neem plants to find out the unique source of targeted metabolite for extraction and commercialization.

P-3047

Determination of Rutin in *Musa* Leaves by High Performance Liquid Chromatography - A Step Towards Sustainability. IMNANARO LONGKUMER and Rakhi Chaturvedi. Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Assam, INDIA. Email: rakhi_chaturvedi@iitg.ac.in

The *Musa* sp. commonly known as banana, belongs to the family Musaceae, and is one of the most widely distributed and consumed fruit in tropical and subtropical countries. Nutritionally, it is one of the world's leading food crops with high content of minerals, vitamins, carbohydrates, flavonoids, and phenolic compounds. Banana leaves have a wide range of applications because of their large size, flexibility, and waterproof nature. They are used for cooking, wrapping and serving food and also as decorative elements in traditional ceremonies. However, apart from the traditional uses, the leaves of the banana are an undervalued commodity with a limited commercial value, and can be considered as an agricultural industry by-product and waste. Because banana leaves are widely available in large quantities, they can be used as a source of raw materials for the green technology industry. Rutin, a flavonoid, is naturally present in banana leaves and has several commercial properties. Rutin has several important pharmacological properties, which are beneficial to health and has powerful antioxidant properties. However, rutin cannot be used efficiently because of the high price. To reduce the cost of producing rutin, it is important to find a rich source, which is widely available and inexpensive. Therefore, the present study aims to report a methodology for extraction, and quantification of rutin from three different varieties of *Musa* sp., namely Malbhog (Musa paradisiaca, AAB), Chini champa (Musa paradisiaca, AAB) and Bhimkol (Musa balbisiana, BB), by using High Performance Liquid Chromatography (HPLC). Of the three varieties studied, Bhimkol variety showed the highest content of rutin with a crude methanolic extract of 387 mg, containing 11% of rutin, from 1 g of dried banana leaf powder. The results indicated that banana leaves, a food industry by-product and agricultural waste, has the potential for use as an inexpensive and new source of rutin.

PLANT SILENT ABSTRACTS

P-3048

Another Successful Target in the Suborder Sternorrhyncha (Hemiptera): Green Oligonucleotide Insecticides for Aphid Control. Y. V. PUZANOVA[1], I. A. Novikov[1], N. A. Marochkin[1], E. Eken[1], A. K. Sharmagiy[2], and V. V. Oberemok[1,2]. [1]Department of Molecular Genetics and Biotechnologies, V. I. Vernadsky Crimean Federal University, Simferopol, CRIMEA and [2]Laboratory of Entomology and Phytopathology, Nikita Botanical Garden, Yalta, CRIMEA. Email: liza.puzanova1996@gmail.com, emreeken1212@gmail.com

Aphids are serious pests of many field and forest crops. Among them, *Macrosiphoniella sanborni* Gillette, a common pest of chrysanthemums, causes significant damage to the world's floriculture. In addition to their direct negative effect on young shoots and buds, *M. sanborni* promote the spread of plant RNA viruses and provoke the development of sooty mold. These factors negatively affect the profitability of flower production. One of the issues facing floriculture is the urgent need to reduce the use of and dependence on contact chemical insecticides to lessen the ecotoxicological load on agrocenoses and reduce the danger to human health. Results from our earlier studies have demonstrated the high efficiency of green oligonucleotide insecticides (DNA insecticides) on armored scales, soft scales, mealybugs, and psyllids. This publication will describe for the first time an experiment on the aphid, *M. sanborni*. The DNA insecticide Macsan-11 5'-TGTGTTCGTTA-3' was developed based on the 5.8S rRNA sequence using the DNAinsector algorithm (dnainsector.com). The experiment was carried out on isolated *Chrysanthemum morifolium* plants under laboratory conditions at a temperature of 23°C. The density of the insects on the chrysanthemum plants at the beginning of the experiment was 3.44 individuals/cm^2. Seven days after the plants received a single treatment with a solution of Macsan-11 at a concentration of 100 ng/μL (mg per m^2 of leaves), aphid mortality reached 67.28±2.54%; for the control insects, the mortality rate for the same period was 5.6±0.13% ($p < 0.01$). The non-target oligonucleotide 5'-ACTGACTGACT-3' had a moderate nonspecific effect of 17.1 ± 2.07% on the insects. Thus, we have created an effective DNA insecticide for *M. sanborni* control that allows us to reduce the number of aphids on the target plant quickly. We plan to increase the efficiency of the Macsan-11 insecticide in further experiments using a double treatment at a daily interval. The research was funded by the grant of the Russian Science Foundation (project no. 22-16-20052).

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