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Plant Contributed Papers

P-1000

Aflatoxin-free Transgenic Maize. DHIRAJ R. THAKARE, Peter J. Cotty, and Monica A. Schmidt. School of Plant Sciences, University of Arizona. Email: drthakare@email.arizona.edu

Aflatoxin is a toxic secondary compound produced by a fungal source and can be responsible for massive agricultural losses world-wide. It is estimated that 25% of the world's crops are contaminated with some sort of mycotoxin, aflatoxin being chief among them. World-wide there is a net loss of 16 million tons of maize due to aflatoxin contamination. Aflatoxin contamination in crops, and subsequently livestock, threatens greater agricultural development, food security and human health. The fungus*Aspergillus* produces aflatoxins that are toxic and carcinogenic to livestock animals and humans. When aflatoxin-contaminated food/feed is ingested it can result in hepatotoxicity, liver cancer, kwashiorkor and Reye's syndrome. Due to its high toxicity over 100 countries restrict the level of aflatoxin in food and feed. The US Department of Agriculture regulates the allowable level of aflatoxin in maize for livestock feed and human consumption. Maize destined for human food and dairy cattle feed has the tightest limit of 20 parts per billion (ppb). To put this number into perspective, 1 ppb is equivalent to a single drop of water in a 21,700 gallon (82,135 liter) swimming pool or from a time perspective, 1 sec in 31.7 yrs. The objective of this project is to generate aflatoxin-free transgenic maize by RNAi directed against an *Aspergillus* aflatoxin biosynthesis gene. We have produced 9 transgenic lines of maize (cultivar B73 hybrid) by *Agrobacterium*-mediated transformation and have confirmed by molecular means the insertion of both the bar selectable marker gene, conferring the added bioalaphos herbicide resistance trait, and the RNAi aflatoxin suppression cassette. To date, two lines have been bred to homozygosity. Results from preliminary on-plant cob*Aspergillus* infections, with subsequent toxin quantification, indicates this RNAi strategy is effective to reduce aflatoxin by at least 80% in developing transgenic maize kernels.

P-1001

Production of Transgenic Soybean Seeds Expressing Human Epidermal Growth Factor (hEGF) for a Therapeutic Formula to Prevent Neonatal Necrotizing Enterocolitis (NEC). YONGHUA HE[1], Monica A. Schmidt[1], Raphael Sun[2], Chris Erwin[2], Brad Warner[2], and Eliot M. Herman[1]. [1]The University of Arizona, School of Plant Sciences/BIO5 Institute, Tucson, AZ 85718; [2]Division of Pediatric Surgery, Washington University School of Medicine, St. Louis, MO 63110; and [2]Department of Surgery, St. Louis Children's Hospital, St. Louis, MO 63110. Email: yonghuahe@email.arizona.edu

Each year in the United States, more than 530,000 babies are born prior to full 37 weeks of gestation. One of the major problems associated with prematurity is the development of a condition known as necrotizing enterocolitis (NEC). In 50% of infants with NEC a large amount of damaged and dead intestine must be surgically removed, often resulting in death or lifelong health problems. A growing body of experimental and clinical evidence supports a conclusion that deficient quantities of epidermal growth factor (EGF) leads to the development of NEC. The EGF peptide appears to be fundamental for normal intestinal development and repair. Soy-milk containing engineered EGF may be a suitable therapy if given to premature infants to prevent NEC. Human mature EGF is 6 kDa protein with three intramolecular disulfide bonds. To produce EGF a synthetic codon-optimized gene was transferred to soybean by biolistic transformation. The resulting transgenic lines were regenerated into somatic embryos that were screened by PCR and the production of EGF assayed by ELISA. Crude extracts of somatic embryos producing EGF were assessed for bioactivity by induction of phosphorylation of the EGF receptor in HeLa cells that showed soy-produced EGF exhibited the same activity as authentic EGF. T_0 transgenic EGF seeds have showed positive ELISA for EGF demonstrating the feasibility to produce a therapeutic soy-milk to mitigate the development of NEC by premature infants. The efficacy trial of Soy-hEGF in a mouse model with short bowel syndrome will be discussed.

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P-1002

Regeneration of *Dioscorea rotundata* Through Somatic Embryogenesis. RAJESH MANOHARAN, Evans Nyaboga, Jaindra N. Tripathi, and Leena Tripathi. International Institute of Tropical Agriculture, Nairobi, KENYA. Email: R.Manoharan@cgiar.com

White yam (*Dioscorea rotundata* Poir) is one of the most important staple food crop widely cultivated in West Africa. The consumer demand of yam is very high in sub-Saharan region of Africa, but its production is declining in this region due to factors including viral and fungal diseases, pests such as nematodes and decline in soil fertility (Nyaboga *et al*. 2014, Frontiers in Plant Science 5:463). The genetic improvement of yam through conventional breeding is very difficult due to constraints such as the long growth cycle (8-10 mo), dioecious and poor flowering nature, polyploidy, vegetative propagation and heterozygous genetic background (Mignouna *et al*. 2008, In: Moore P, Ming R (eds) Plant genetics and genomics: crops and models, vol 1. Springer, pp 549–570). In view of these problems, genetic engineering may provide an alternative route for the improvement of yam cultivars. However, the availability of successful regeneration protocol is a pre-requiste for the transfer of desirable genes into *D. rotundata* to improve this crop. Among the different regeneration systems, somatic embryogenesis is widely considered to be an efficient system for developing transgenic plants. To our knowledge, there is no report describing somatic embryogenesis of *D. rotunda*. Hence the present study was aimed to develop plant regeneration through somatic embryogenesis amenable for *Agrobacterium*-mediated transformation. Different explants (immature leaf, internode, root segments, axillary bud) of *D. rotundata* cultivar TDr 2436was tested for their embryogenic potentials on medium supplemented with different auxins (2,4-D, NAA, Picloram) . Axillary bud explants cultured on MS medium supplemented with Picloram (12 mg/l) favored embryogenic callus production. Addition of organic nitrogenous additives like casein hydrolysate and proline improved the embryogenic callus induction. The embryos attained complete maturity and germinated into normal plants when cultured in MS medium supplemented with BAP (0.4 mg/l). The results from this study will be presented.

P-1003

Wheat Gene Expression Differences Induced by Six Races of *Puccinia triticina*. KERRI NEUGEBAUER[1], Myron Bruce[2], Harold Trick[1], and John Fellers[2]. [1]Kansas State University, Department of Plant Pathology, 4024 Throckmorton, Manhattan, KS 66506 and [2]USDA-ARS-GMPRC, 4006 Throckmorton, Manhattan, KS 66506. Email: kerrin@ksu.edu

Puccinia triticina, the casual agent of wheat leaf rust, is a devastating disease that can cause up to 40% yield loss. During fungal infection the host plant recognizes pathogen effectors, which trigger a host defense response. Changes in the pathogen effectors due to host selection pressure are responsible for the rapid development of new rust races and make durable resistance hard to obtain. The objectives of this study are to identify and characterize wheat genes that are utilized by races differently throughout infection and to understand functions of these genes using gene silencing. Six races of leaf rust were inoculated on a susceptible wheat variety and tissue was collected at six days post inoculation. RNA was sequenced and 63 wheat genes were identified that showed varying expression in response to the six races. 54 of these genes were evaluated in a time course study from zero days to six days post inoculation with the same six races. Real-time PCR was then used to analyze the timing of expression during early infection. The characterized genes have proposed functions involved in plant defense and stress, energy and metabolism, protein transport, replication, and RNA binding. Majority of the candidate genes showed three main expression patterns. However, race specific expression was found in three wheat genes that are affected by race shifts on Lr2A, Lr2C, and Lr17A. Sixteen potential susceptibility genes were also identified. Host susceptibility genes could be altered to provide durable resistance. RNAi was used to silence seven wheat genes to further understand their roles in leaf rust infection. T_0 and T_1 plants have been obtained and confirmed for the gene of interest. T_2 plants were inoculated and observed for changes in susceptibility.

P-1004

Basis for Engineering an Improved Processing Trait in Sugar Beets. JOSE C. TOVAR[1,2], Jianfeng (Jay) Xu[2,3], and Brett J. Savary[2,3]. [1]Molecular Biosciences Graduate Program, [2]Arkansas Biosciences Institute, and [3]College of Agriculture and Technology, Arkansas State University, Jonesboro, AK. Email: Jose.tovar@smail.astate.edu, bsavary@astate.edu (corresponding author)

We are investigating a novel biotechnical application of a thermally-tolerant pectin methylesterase (TT-PME) for improving energy efficiency in sugar beet processing. Sucrose diffusion from beet root slices occurs in 60-65°C water, optimal conditions for TT-PME activity. Separating water from wet pulp is necessary to stabilize it for storage and reducing weight for shipping. Drying pulp is a highly energy intensive process (consumes up to 30% of total energy in a processing

factory). Beet pulp's high water binding is due to its high content of pectin, a complex cell wall polysaccharide that entraps water. We hypothesize TT-PME action can reduce water binding in pulp by promoting calcium-mediated crosslinking, which will facilitate improved mechanical de-watering, resulting in lower energy cost and environmental footprint for drying pulp. To test this hypothesis, we developed an in vitro assay to quantify water binding in pulp. We found that TT-PME action reduced water binding in beet pulp by 27% with supplemented calcium. This evidence for functional benefit provides basis for our goal to develop transgenic beet roots that express TT-PME as an improved output trait. To support this goal, we developed peptide-based monospecific antibodies to detect TT-PME in transgenic plant tissues. Current experiments are directed to assess the function and impact of the pro-peptide domain in expressing active recombinant enzyme. For this, we are using the rapid and well-established *Nicotiana benthamiana* transient expression system. This presentation will summarize our findings on TT-PME's pulp water binding reduction, specific antibody production, and progress on *N. benthamiana* TT-PME expression.

P-1005

Metabolic Redesign of Tocopherol Biosynthetic Pathway: Potential Application for Human Health and Abiotic Stress Alleviation in Plants. DEEPAK KUMAR[1], Mohd. Aslam Yusuf[1,2], and Neera Bhalla Sarin[1]. [1]School of Life Sciences, Jawaharlal Nehru University, New Delhi, INDIA and [2]Department of Bioengineering, Integral University, Lucknow, INDIA. Email: deepakinjnu@gmail.com

Oxidative stress is one of the major manifestations of unfavourable environmental conditions faced by plants as also of several different diseases, including cancers, in humans. α-tocopherol, the biologically most active form of vitamin E, is a major antioxidant that bulwarks the cells. It constitutes a small fraction of the total tocopherol pool in most oilseed crops. We generated transgenic (TR) *Brassica juncea* plants with ~6 fold higher α-tocopherol levels by overexpressing γ-tocopherol methyl transferase. To better understand the roles of different tocopherol forms in plants we compared the performance of TR plants under conditions of abiotic stresses induced by salinity, heavy metal and drought. This resulted in an increase in total tocopherol levels in both the wild type (WT) and TR plants. Seed germination and leaf disc assay showed that TR *B. juncea* had enhanced tolerance to these stress and that induced by high temperature and methyl viologen. Damage caused by the induced stress was lower in TR plants compared to WT plants as assessed by their higher relative water content, lower MDA and electrolyte leakage. Lesser superoxide and H_2O_2 accumulation was observed in TR seedlings exposed to these stress. The levels of antioxidant enzymes and molecules were higher in TR plants when compared to WT plants under similar stress. Analysis of chlorophyll a fluorescence kinetics showed that there were differential effects of the stress on different sites of the photosynthetic machinery. These effects were found to be alleviated in TR plants. We further checked the efficacy of feeding α-tocopherol enriched seeds in securing the antioxidant defense in mice. We found significant increase in the content of various phase I and phase II enzymes with a corresponding decrease in peroxidative damage. Also, the transgenic seeds were found to have chemopreventive effects against DMBA-induced skin papillomagenesis in mice model. Our results highlight the potential of increased α-tocopherol in transgenic *B. juncea* in health of humans and agricultural crop plants.

P-1006

In-Planta Expression of Synthetic Lytic Peptides for the Potential Inhibition of Human Immunodeficiency Virus Replication. S. SAMUELS[1], M. Egnin[1] C. Bernard[1], T. Nashar[2], M. Banasik[2], M. Cho[3], and J. Jaynes[3]. [1]CAENS Department of Agriculture & Environmental Sciences, Plant Biotech & Genomics Research Lab; [2]CVMNAH Department of Pathobiology, Tuskegee University, Tuskegee AL 36088; and [3]Molecular Virology and Vaccine Research, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. Email: Ssamuels6952@mytu.tuskegee.edu

Recombinant plant systems offer economic alternatives to producing large amounts of pharmaceutical proteins as well as providing the most promising opportunity to supply low-cost drugs and vaccines for major diseases such as HIV. Treatments of infectious diseases in humans and animals have traditionally been targeted by chemically synthesized drugs, with the majority of the burden of cost falling on the individual in need of treatment. With the new revolution of producing therapeutic compounds, such as peptides in plant based systems, the cost of production is dramatically decreased. Synthetic antiviral peptides capable of inhibiting the progression of HIV have been developed at Tuskegee University. These synthetic lytic peptides genes were designed with an intron to facilitate cloning in bacteria and were mobilized in *Agrobacterium tumefaciens* strain EHA105 for transformation of sweetpotato cultivar PI318846-3. Seven transgenic plants were confirmed by Southern and RT-PCR analyses, leading to testing of the efficacy and toxicity of crude and purified sweetpotato extracts. Treatment of infected cells showed an 80% inhibition of DH_{12} and SF_{162}, two well characterized pseudovirus strains, in neutralization assays. Peptide treated DH_{12} and SF_{162} show significant reduction as compare to no peptide control. Further biosafety, nutritional, and efficacy tests will

determine agronomic performance, proximate quality and potency of transgenics respectively. Successful development of sweetpotato expressing this novel therapeutic compound can be both a powerful tool in treatment of the HIV epidemic, as well as a road map for future treatment of viral mediated diseases. Work supported by Tuskegee University GWCAES, NIFA-Evans-Allen, Project Export, and UC-Davis-TU CREATE Igert, Iowa State University GWCIP.

P-1007

Expression Pattern and Activity Analysis of A New Tissue Specific Promoter Srf3. N. YUAN and H. Luo. Clemson University, Department of Genetics and Biochemistry, 105 Collings St., 104 BRC, Clemson, SC, 29634. Email: ningy@clemson.edu

Promoter is a specific DNA sequence regulating the expression of downstream coding sequence or other noncoding expression cassettes. CaMV 35S (Odell, Nagy et al. 1985, Benfey and Chua 1990) and maize ubiquitin promoter (Cornejo, Luth et al. 1993) are two broadly used constitutive promoters in transgenic plants, but their strong and constitutive activities may cause many adversity consequences to transgenic plants such as growth suppression or 'transgene silencing' (Kooter, Matzke et al. 1999, Dietz-Pfeilstetter 2010). To avoid the disadvantages of constitutive promoters, we exploited a new tissue specific promoter that could be used to drive foreign gene expression in transgenic plants. We identified and cloned a promoter region of a leaf specific protein kinase gene Stress Responsive Factor 3 (*SRF3*) named Srf3 from *Arabidopsis thaliana* genomic DNA. To investigate the regulation pattern and evaluate the level of this promoter activity, we constructed a series of GUS reporter systems (β-glucuronidase), in which GUS gene is under the control of CaMV 35s, maize ubiquitin, Srf3a (-1536 to -12), Srf3b (-1033 to -12), or Srf3c (-395 to-12) promoters. Histochemical staining analysis of stable transgenic *Arabidopsis* plants harboring these constructs shows that all three Srf3 promoters have as strong activity as CaMV 35S and maize ubiquitin promoters in leaf tissue. The results of quantitative GUS activity assay indicate that unlike the two constitutive promoters, the activity of Srf3a is restricted to leaf tissue, and Srf3b and Srf3c are also leaf-predominant promoters. None of them are active in *Arabidopsis* seeds. Histochemical staining of transgenic tobacco harboring Srf3a/GUS construct show that Srf3a also exhibits a strong leaf-specific activity. Another binary vector was constructed, in which Srf3c promoter was used to drive an herbicide resistance gene *bar*. After treated with PPT, the transgenic *Arabidopsis* plants harboring Srf3c/*bar* survived, indicating that Srf3 could be used as a useful strong leaf specific promoter in agriculture for crop improvement.

P-1008

Comparative Transcriptome Profiling of Perennial Grass *Paspalum vaginatum* in Response to Drought and Salt Stresses. PEIPEI WU, Zhigang Li, Xiaoxia Xia, Christopher Saski, Rooksana Noorai, Galen Collier, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634. Email: peipeiw@g.clemson.edu

Drought and salt stresses are always a big issue in agriculture production and turfgrass industry. Research on molecular mechanisms underlying plant tolerance to drought and salt has made a remarkable progress in model species through gene function discovery and large-scale data analysis. However, few studies have been reported using non-model plant species with high tolerance to the both stresses. Seashore paspalum (*Paspalum vaginatum*), a warm-seasoned perennial grass, is native to tropical and coastal regions, and tolerant of many environmental stresses including drought and salt. This study intends to gain insights into the mechanism of its tolerance to drought and salt at transcriptional level and identify key regulators that could be further applied for improvement of plant stress tolerance. In this study, two cultivars Supreme (high tolerance to salt) and Parrish (low tolerance to salt) were used for transcriptome analysis. The RNAseq libraries of cDNA from drought and salt treated plants as well as untreated controls were constructed and sequenced, and differentially expressed genes were analyzed. Illumina sequencing of the pooled mRNA generated about 318 million paired-end reads, and a total of 74,442 unigenes were assembled in Supreme and Parrish. EdgeR identified 683 and 52 genes that are differentially expresssed (by 2-fold or more) in Supreme under drought and salt treatment, respectively. In Parrish, 1727 and 196 genes are either up-regulated or down-regulated (by 2-fold or more) under drought and salt treatment, respectively. Quantitative PCR will be performed to validate the RNA-seq results and genetic engineering will be conducted using potentially functional genes identified in the future.

P-1009

Application of Micropropagation Technology in Desert Rehabilitation - A Success Story. C. SUDHERSAN, S. Jibi, L. Al-Sabah, J. Ashkanani, and S. Al-Melhem. Biotechnology Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, P. O. Box 24885, Safat 13109, KUWAIT. schellan@kisr.edu.kw

Desertification and land degradation are increasing in Kuwait due to anthropological activities and natural calamities. Desert rehabilitation requires suitable plant species in large numbers. Two native shrubs such as: *Lycium shawii* and *Ochradenus*

buccatus that are surviving under the harsh climatic conditions of Kuwait were identified as potential candidates for the desert rehabilitation program. These native shrubs having the high survival potential in the desert soil are under threatened condition and need to be multiplied. Large quantity of seed production in these shrubs is limited in Kuwait due to the high temperature and low rainfall. Seed collection and large-scale plant production for the desert rehabilitation purpose is difficult, expensive and time consuming. Therefore, plant tissue culture micropropagation technology was selected as an alternative tool for large-scale plant production in these native shrubs. An in vitro protocol for the micropropagation of these two shrubs was first developed and used for the large-scale plant production. A large number of plants were produced through micropropagtion method, hardened them all and reintroduced into the selected desert sites where the natural rehabilitation trial failed. The micropropagated native shrubs planted in the desert experimental sites showed very high rate of survival and tolerance to the frequently occurring sand storms. After three years of planting these two tissue culture derived native shrubs in thousands, the experimental site soil quality has improved through sand deposit and organic material enrichment. A microclimate suitable for the establishment of other native plant species has been established. Through the wind born native seeds, a desert vegetation having more than 100 species of native plants has been established successfully. Results of the study confirmed the application of Micropropagation technology in desert rehabilitation program.

P-1010

Plant Production with the SE-Fluidics System. E-M. ULRIKA EGERTSDOTTER[1,3,4] and Cyrus K. Aidun[1,2]. [1]G. W. Woodruff School of Mechanical Engineering; [2]Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA; [3]SweTree Technologies AB, Uppsala Business Park, Virdings alle 11, Uppsala, SWEDEN; and [4]Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish Agricultural University, UMEÅ, SWEDEN. Email: Cyrus.Aidun@me.gatech.edu, Ulrika.Egertsdotter@me.gatech.edu

High cost of production due to tedious manual handling is the major obstacle preventing large-scale commercial plant production through somatic embryogenesis (SE). Previously, we have presented a ‘fluidics-base’ approach to automating the SE plant production process. We now present the first preliminary results from early test production of Norway spruce somatic embryo plants using the 2-Line Pilot System (model number U-P2) at SweTree Technology’s facilities in Uppsala, Sweden. We show high yields of mature embryos from the partially immersed bioreactors designed for rapid attachment and extraction into U-P2, and subsequent development of embryos and germinants developing into plants ready for the nursery greenhouses. Tests at the forestry nurseries show that the SE plants develop equally or better depending on cell line compared to the control seedlings.

P-1011

Novel Applications of Medicinal Plant In Vitro Systems: Natural Product Drug Discovery and Amoeba-based Emergent Computing. V. RICIGLIANO and D. Howarth. St. John’s University, Department of Biological Sciences, 8000 Utopia Pkwy, Jamaica, NY, 11439. Email: Vincent.Ricigliano@gmail.com

Plant cell cultures can be used to produce valuable secondary metabolites, obtain fundamental metabolic information, and provide germplasm for biological activity assays. We are developing in vitro culture systems for medicinal plants to study and harness the unique metabolic capabilities driving the production of biologically active compounds. First, we developed the methodologies to generate transgenic hairy root cultures by infection with *Agrobacterium rhizogenes* and stable integration of root-inducing DNA into the host plant genome. Second we implemented recombinant root cultures with diverse chemical profiles to test the contribution of candidate biosynthetic genes to secondary metabolism. Third, we have been developing novel test platforms to assay the pharmacological potential of these in vitro cultures, as well as their modulatory potential in the cellular computing substrate *Physarum polycephlaum*. Progress in all of these areas will be presented.

P-1012

Preparation, Analysis, and Bioassay of Functional Oligosaccharides Isolated from Rice Bran Fiber. BRETT J. SAVARY[1,2], Jianfeng (Jay) Xu[1,2], Ningning Zhang[1,3], Keat (Thomas) Teoh[1], Shiguang Yu[1], and Fabricio Medina-Bolivar[1,3]. [1]Arkansas Biosciences Institute, [2]College of Agriculture and Technology, and [3]College of Science and Mathematics, Arkansas State University, Jonesboro, AR. Email: bsavary@astate.edu
Whole-grain rice products can contribute diverse functional components to the diet to promote improved gastrointestinal health in humans. We are isolating feruloylated arabinoxylan-oligosaccharides (FAXOs) from rice bran fiber to evaluate in vitro their bioactivity in modulating innate immune responses in human colonocytes. A better understanding of the function, bioavailability, and interactions of rice bran components in colon functioning will determine their dietary utility and identify effective processing technologies for formulating

safe, high quality rice products containing bioactive components in a sustainable manner. Our current objectives are to 1) compare yields of FAXO fractions following dilute acid hydrolysis and enzyme treatments, 2) evaluate profiles by hydrophilic interaction liquid chromatography (HILIC) separation chemistry, and 3) determine ability of FAXOs to modulate tight-junction protein levels in T84 colonocytes. Results from these objectives will be presented.

P-1013

Improving Wheat Yield Under Heat Stress by Expressing Putative Thermostable Starch Synthase Genes. HAROLD N. TRICK[1], Bin Tian[1], Shyamal K. Talukder[2], Hyeonju Lee[1], and Alan K. Fritz[3]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506; [2]Forage Improvement Division, The Samuels Robert Noble Foundation, Ardmore, OK 73401; and [3]Department of Agronomy, Kansas State University, Manhattan, KS 66506. Email: hnt@ksu.edu

As global climate shifts worldwide, high temperature becomes one of the major environmental stresses for wheat production. Heat stress during the grain filling stage reduces the kernal weight of wheat seeds by decreasing starch production. The soluble starch synthase (SSS), a key heat-labile enzyme, plays an important role in regulating the conversion of sucrose to starch in the wheat endosperm. Expression of putative thermostable SSS in genetically engineered wheat could increase the productivity under heat stress. In this study, we demonstrated that the grain weight of transgenic wheat was significantly improved under high temperature conditions by expressing a rice (*Oryza sativa*) SSS with the regulatory control of either the maize ubiquitin or wheat high molecular weight glutenin promoter. Under optimum growing conditions all agronomic traits evaluated (seed size, seed number, tiller number, and physiological maturity) had no significant variations. However, the concentration of soluble starch in transgenic lines was significantly higher compared to the controls and the transgenic lines demonstrated up to a 34% increase in thousand seed weight compared to non-transgenic controls under heat stress conditions ranging from 31/24°C (d/n) to 34/28°C (d/n) during the grain filling period. In addition, two other SSS genes from grape (*Vitis vinifera*) and black cottonwood (*Populus trichocarpa*) were selected according to a thermostability prediction algorithm, codon optimized for wheat, and expressed in transgenic wheat. Significant increases in seed weight were also observed in these transgenic lines under heat stress. Further analysis of transgenic wheat plants is in the process in hope to provide a novel strategy for improving heat tolerance for cool season crop plants.

P-1014

Transgrafting Enables Virus Resistance in Non-transgenic Sweet Cherry. GUO-QING SONG and Aaron E. Walworth. Michigan State University, Plant Biotechnology Resource and Outreach Center, Department of Horticulture, East Lansing, MI 48824. Email: songg@msu.edu

Transgrafting using transgenic rootstocks and non-transgenic scions has potential for the commercialization of transgenic plants for non-transgenic products. In our recent studies, we transformed a hairpin RNA (hpRNA) vector to sweet cherry rootstocks in order to silence *Prunus necrotic ringspot virus* (PNRSV), which is a major pollen-disseminated Ilarvirus. All transgenic rootstocks showed high resistance to the PNRSV. Subsequently, we performed grafting studies to investigate whether PNRSV-resistant transgenic rootstocks developed through small interfering RNA-mediated gene silencing can enhance virus resistance of non-transgenic scions. For the first time in woody plants, we developed profiles of transported (rootstock-to-scion) small interfering RNAs (siRNAs) that were detected in a non-transgenic scion of sweet cherry grafted on a transgenic rootstock expressing the hairpin RNA vector. More importantly, inoculation of non-transgenic scions with PNRSV revealed that the transferred siRNAs enhanced PNRSV resistance in the scions grafted on the transgenic rootstocks. Low amounts of transferred hpRNA siRNAs in scions, compared to those detected in PNRSV-infected but symptomless cherry plants using non-transgenic rootstocks, implied little concern of these siRNAs for food safety. These findings provide the basis for 'using transgenic rootstocks to produce non-transgenic products of scions in rootstock-scion grafted plants', while minimizing concerns about food and environmental safety.

P-1015

Host Reactions of Developing Sweetpotato Storage Roots Under Root-knot Nematode Challenge. GREGORY C. BERNARD[1], M. Egnin[1], S. Samuels[1], C. Bonsi[3], D. Mortley[3], W. Witola[2], C. Land[4], and K. Lawrence[4]. [1]Plant Cellular and Molecular Genetics Lab, College of Agricultural, Nutritional and Environmental Sciences, Tuskegee University, Tuskegee, AL 36088; [2]Molecular Parasitology and Immunology Lab, Tuskegee University, College of Agricultural, Nutritional and Environmental Sciences, Tuskegee AL, 36088; [3]George Washington Carver Experimental Station, Tuskegee University, College of Agricultural, Nutritional and Environmental Sciences, Tuskegee, AL, 36088; and [4]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849. Email: gbernard4673@mytu.tuskegee.edu

Five cultivars of sweetpotato [*Ipomoea batatas* (L.) Lam] were subjected to phenotypic and molecular screening analyses in efforts to distinguish levels of resistance to the root-knot nematode, *Meloidogyne incognita*. Eight cm long cuttings were obtained from healthy, vigorous sweetpotato cultivars Nugget, Georgia Jet, TUO2, Whatley Loretan, and DMO1 and propagated in 500-cm^3 containers containing a sterilized mix of 4:1 sandy/field soil for a period of 14 d prior to inoculation with 10,000 *M. incognita* eggs per pot. Plants were cultivated under greenhouse conditions, and root samples were harvested 84 d post-infection. Significant differences ($p < .05$) were shown between cultivars in fresh root weights, necrosis and galling index values, *inplanta* egg counts and nematode soil counts. Nugget showed the highest degree of resistance in comparison to other treated cultivars as indicated by minor necrosis and galling and lowest egg counts. Necrosis and galling scores were highest in susceptible cultivars Georgia Jet and DMO1 where extensive root cracking was shown in Georgia Jet. TUO2 and Whatley Loretan were designated as intermediately resistant. Differences in host responses to root-knot nematode challenge are often conferred by numerous genes. Preliminary genetic expression analyses showed specific differential expression patterns between genotypes at different time points throughout the study. Our findings have identified new intermediately resistant cultivars and will provide information on the discreet transcriptional events in sweetpotato genotypes during root-knot nematode infection, with efforts to identify molecular markers of resistance for successful root-knot resistant sweetpotato breeding programs. Work supported by Tuskegee University, GWCAES, NIFA-EVANS-ALLEN-T-CAP and UC DAVIS-TU CREATE-IGERT

P-1016

Using STTMs to Explore Plant MicroRNAs in Regulation of Soybean Cyst Nematodes Infection. BIN TIAN[1], Timothy C. Todd[1], Guiliang Tang[2], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 and [2]Department of Biological Sciences, Michigan Technological University, Houghton, MI 49931. Email: btian52@hotmail.com

Soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most important pests to limit the soybean production worldwide. The lack of understanding of nematode virulence and soybean defense mechanisms has limited the ability to develop novel and effective management strategies. MicroRNAs (miRNAs) are a class of small, non-coding RNAs that are known to play central roles in growth and development, genome integrity, and responses to biotic and abiotic stresses in plants. Recently, it was reported that many soybean miRNAs were responsible for interacting with microbes such as *Pseudomonas syringae*, *Bradyrhizobium japonicum*, and *Phytophthora sojae*. With Short Tandem Target Mimics (STTMs), it is able to easily target specific miRNAs of interest for the degradation without affecting other miRNAs. This robust technology allows us to block all members in a miRNA family so that it is much effective to explore and understand miRNA functions in plants. STTMs have been successfully demonstrated in the model plant Arabidopsis and a few economically important crops such as rice, and soybean. Here using a rapid soybean hairy root system established in our lab and combining this cutting-edge STTM technology, we investigated functions of several conserved soybean miRNAs, and their effects on SCN infection. This technology will provide a novel insight and knowledge about the role of soybean miRNAs in regulating the defense response to SCN infection. We will report progress on miRNA functions during plant-microbe interactions using the soybean hairy root system.

P-1017

RNAi Mediated Silencing of Endogenous Wheat Genes eIF4(iso)E2 and eIF4G Induces Resistance to Potyviruses *Wheat Streak Mosaic Virus* and Triticum Mosaic Virus. JESSICA RUPP[1], Luisa Cruz[1], John Fellers[2], and Harold Trick[1]. [1]Kansas State University, Dept. of Plant Pathology, 4024 Throckmorton, Manhattan, KS 66506 and [2] USDA-ARS. Email: jrupp@ksu.edu

Wheat streak mosaic virus (WSMV) and Triticum mosaic virus (TriMV) are two viruses affecting wheat in the Great Plains of the United States. The current disease management strategy incorporates the deployment of resistant varieties, mite vector control and various cultural practices; however, it is not fully effective. Both of these viruses belong to the family *Potyviridae* and use host eukaryotic initiation factors in order to facilitate replication of their genomes. We evaluated the use of RNAi to silence eIF4(iso)E2 and eIF4G to interrupt this process in order induce resistance to these wheat viruses. RNAi expression vectors were independently created from the sequences of the wheat genes eIF4E(iso)2 and eIF4G. Immature embryos of the wheat cultivar 'Bobwhite' were independently co-transformed by biolistic particle delivery system with RNAi expression vectors and pAHC20, which contains the *bar* gene for glufosinate selection. All progeny have undergone PCR and RT-PCR analysis. To determine viral resistance, the progeny were mechanically inoculated with the viruses. A consistent stable resistance response was demonstrated in three transgenic lines of eIF4(iso)E2 construct and four transgenic lines of eIF4G, each derived by single seed descent. T_6 progeny were co-infected with WSMV and

TriMV continue to be resistant. Traditional crosses have been performed with the winter wheat 'Overley.' Effectiveness of the RNAi construct has been evaluated using Real-time PCR. Results show up to 18-fold reduction in viral titer in the transgenic lines, the F1 cross and the BC1F1 in compared to control plants. This research provides evidence that a single transgene can provide resistance to multiple viruses and has great potential benefits to both breeders and producers.

P-1018

Precision Breeding Technology for the Genetic Improvement of Grapevine. D. J. GRAY[1], Z. T. Li[1], T. N. L. Grant[1], D. A. Dean[1], and S. A. Dhekney[2]. [1]Mid-Florida Research & Education Center, University of Florida/IFAS, Apopka, FL 32703 and [2]Department of Plant Sciences, University of Wyoming, Sheridan, WY 82801. Email: djg@ufl.edu

Precision breeding (PB), in which only defined genetic elements from sexually-compatible parents are utilized, is a logical extension of conventional breeding (CB) inasmuch as it is fully consistent with the plant lifecycle. PB builds upon decades of research aimed at bypassing the disruption of sexual reproduction (meiosis) by allowing gene insertion to be accomplished via the significantly more stable and predictable mitotic cell division pathway. For an increasing number of crop plants, it is now possible to transfer only pre-tested genes and other genetic elements that express known traits among sexually-compatible relatives. The PB approach has particular promise for grapevine (*Vitis* spp.), which is unique among all crop plants due to its special sensory attributes. A relatively small number (>40) of elite cultivars and their landraces account for the majority (66%) of world wine production. They require substantial chemical disease control and sanitation in most regions. They urgently require better genetic resistance to ease production losses. Elite cultivars of grapevine cannot be improved by CB because of major genetic obstacles, including inbreeding depression/self-incompatibility and a long lifecycle. However, crucial cell culture and gene insertion systems are in place and completion of the genome sequence of *V. vinifera* 'Pinot Noir' in 2007 finally enabled PB of grapevine. A strange concern expressed against PB has centered on the fact that insertion sites for such genetic elements are random and may cause "unintended consequences"; however such concerns overlook the basic botanical fact that plant sexual reproduction randomly induces far greater variability through recombination, crossing over and transposition. Importantly, due to their benign nature, even when compared to those derived via CB, PB plants likely will require no governmental regulation beyond that of CB, so that they easily will be released to growers for testing. It remains to be determined whether or not PB-derived versions of elite cultivars retain their highly-prized sensory attributes.

P-1019

Elimination of Non-germline Events Via Selection Early in the Soybean Transgenic Event Production Process. SIVA CHENNAREDDY[1], Dayakar Pareddy[2], Pon Samuel[2], Toby Cicak[1], Geny Anthony[1], Brandon Bishop[1], Nolan Shumway[1], and Rodrigo Sarria[2]. [1]Dow AgroSciences, West Lafayette, IN and [2]Dow AgroSciences, Indianapolis, IN. Email: schennareddy@dow.com

In soybean, the transformation methods involving somatic embryogenesis and organogenesis have both been commonly used for transgenic production. In the case of organogenesis-based transformation using organized meristematic target tissues such as cotyledonary node, embryonic axis or split-seed explants, germline and non-germline (chimeric) transformation events are produced. In these systems, between 40-70 percent of the events are expected to be non-germline events. Historically, primary transformants (T0 events) were maintained until maturity with T1 progeny screened for heritability using selection agents or through molecular analysis methods. Maintaining tissues and plants to T1 stage results in unnecessary cost. We have developed a simple and efficient method for earlier elimination of non-germline events using a selection agent at the rooting stage. Briefly, following *Agrobacterium*-mediated transformation of split-seed explants with a construct containing PAT (selectable marker gene) and YFP (fluorescent reporter gene); the regenerated shoots were cultured on 'Rooting Medium' containing glufosinate ammonium. The non-germline events and 'escapes' either produced no roots or produced unhealthy, brown roots. These events did not survive selection or were easily distinguished visually and eliminated. With this method, up to 90% of non- germline events were eliminated in the transgenic production process, thereby saving significant resources in the greenhouse and field.

P-1020

Improved Soybean Transformation for Efficient and High Throughput Transgenic Production. DAYAKAR PAREDDY[1], Siva Chennareddy[2], Tatyana Minnicks[1], Olga Karpova[2], Tejinder Mall[2], Geny Anthony[2], David Griffin[1], Brandon Bishop[2], Nolan Shumway[2], Lauren Clark[1], Pon Samuel[1], Kelley Smith[1], and Rodrigo Sarria[1]. [1]Dow AgroSciences, Indianapolis, IN and [2]Dow AgroSciences, West Lafayette, IN. Email: drpareddy@dow.comAlthough introduction of genetically

modified soybean dates back to 1996, genetic transformation of soybean is still inefficient. We developed an improved, organogenesis-based soybean transformation resulting in average transformation frequencies up to 20% using Maverick and Dow AgroSciences elite lines. This method involves *Agrobacterium*-mediated transformation of the split-seed explants with partial embryonic axis prepared from imbibed seed. Two selectable marker genes, i.e., PAT and DGT28 conferring tolerance to glufosinate and glyphosate respectively, were successfully employed for transgenic production. About 45% of the T1 progeny, similar to that of the cotyledonary node method, was found to be heritable based on herbicide screening with Liberty® and molecular analysis. This system is about 3-8-fold more efficient than the other methods, i.e., cotyledonary node and half-seed method, and serves as an efficient means for high throughput transgenic method for basic research studies and commercial development of transgenic soybean products. In addition, such high throughput *Agrobacterium*-mediated transformation platform as well as direct transformation methods provides an opportunity for development and deployment of precision insertion and gene editing technologies such as EXZACT™.

P-1021

Vectors to Modify Plant Genome Using CRISPR-Cas9-sgRNA Genome Editing System, Application to Reduce Harmful Products in Tobacco, and in Developing Broad Spectrum Virus Resistance in Crop Plants Like Brassica. INDU B. MAITI[1], Dipak Kumar Sahoo[1], Ankita Shreshta[2], Sunita Patro[2], and Nrisingha Dey[2]. [1]KTRDC, College of Agriculture, Food & Environment, University of Kentucky, Lexington, KY 40546 and [2]Department of Gene Function and Regulation, Institute of Life Sciences, Department of Biotechnology, Government of India, Nalco Square, Chandrasekherpur, Bhubaneswar, Odisha, INDIA. Email: imaiti@uky.edu

We have created a series of DNA promoters useful for expressing foreign genes in transgenic plants. We have also created an user friendly small and highly efficient binary Ti vector pSiM24 for transformation of higher plant cells(Sahoo et al., 2014. PLoS ONE 9(6): e98988. doi:10.1371/journal.pone.0098988). The pSiM24 plasmid can act as a platform for various applications like gene expression studies and different promoter expressional analyses. The sequence information of the pSiM24 and relevant genetic elements is provided in NCBI data base (GenBank accession no. KF032933). We have modified this plasmid to express Cas9 gene and specific sgRNAs to edit target gene in plant genome. The single RNA-guided DNA recognition of CRISPR-Cas9-sgRNA method is a simple and powerful tool for selected genome engineering. These technologies allowed scientists to develop new strategies to develop agricultural products and gene therapy to control diseases, and abiotic and biotic stress related effects. We are interested to explore this CRISPR-Cas9-sgRNA-mediated genome editing technology, an effective nontransgenic approach, in dismantling gene structure and subsequent expression of nicotine N-demethylase (NND) to minimize the conversion of nicotine to nornicotine in high conversion tobacco lines to reduce tobacco harmful carcinogen tobacco specific nitrosoamines (TSNAs). Developing genetic resistance in host is the most sustainable and effective way to control plant pathogen invaders from damaging crop plants in agricultural practice. We sought to develop broad-spectrum potyvirus resistance brassica plants through genome editing. We are interested to explore this CRISPR-Cas9-sgRNA-mediated genome editing technology in dismantling host gene structure. We will target eukaryotic translational initiation factors (eIF4E genes) responsible in virus-host interaction. Knock down of eIF4E genes in host will confer broad-spectrum resistance against plant viruses including potyviruses in tobacco.

P-1022

Application of CRISPR/Cas9 Technology for Characterization of Developmental Genes in Tomato. J. VAN ECK[1], C. Brooks[2], V. Nekrasov[3], and Z. Lippman[2]. [1]Boyce Thompson Institute for Plant Science, Ithaca, NY 14853; [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; and [3]Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK. Email: jv27@cornell.edu

Tomato (*Solanum lycopersicum*) has quickly become a tractable model plant for genetic, biological, and functional genomics studies. Its utilization as a model has increased because of the numerous resources developed that include a high quality genome sequence, mapping populations, a mutant collection, and a highly efficient transformation system. These resources also provided the perfect platform for testing the feasibility of genome editing technology in tomato. We chose to use the CRISPR/Cas9 editing technology for studies of plant developmental genes. For our proof of concept experiment, we targeted a gene (*ARGONAUTE7; SlAGO7*) that when function was lost would result in an easily visible phenotype (needle-like leaves; wiry) evident as early as the plant regeneration stage from *Agrobacterium*-infected cotyledons. The CRISPR construct contained two single guide RNAs (sgRNAs) to cause large deletions to ensure disruption. Twenty-nine independent transgenic lines were recovered and surprisingly 14 plants had the expected phenotype, which indicated the successful disruption of *SlAGO7* function. PCR analysis and DNA sequencing revealed the CRISPR lines contained homozygous, biallelic or chimeric small insertions and deletions (indels), and that the indels were present at various locations near both sgRNA targets. Following this proof

of concept experiment, we designed 34 CRISPR constructs to target selected genes that were shown through RNAseq analysis to possibly play a role in flowering, stem maturation, and inflorescence branching. The CRISPR/Cas9 technology has been a powerful tool for our reverse genetics approach to elucidate the roles of these genes. Results will be presented.

P-1023

Iterative Modification of Transgene Locus Towards Multigene Stacking into a Single Genomic Site. VIBHA SRIVASTAVA, Soumen Nandy, and Shan Zhao. University of Arkansas, Dept. of Crop, Soil and Environ. Sci., Fayetteville, AR 72701. Email: vibhas@uark.edu

A method was developed for repeatedly directing the integration of genes into the selected sites in plant genomes. Transgenic plants developed by this method contain a stack of genes in a single genomic site without the presence of selection marker genes (SMG). Linking genes in this manner will simplify breeding and minimize gene expression variation through precise gene integration. The method utilizes site-specific recombination systems for driving efficient gene integration, and nucleases for SMG excision. The combination of the two steps enables unlimited rounds of transformation, and the resulting transgenic plants carry marker-free insertions . In the proof-of-concept study, 3 genes were inserted into the rice genome by Cre-*lox* at efficiencies ranging from 30 – 50%, and the SMG excised by I-*Sce*I at 30% efficiency. I-*Sce*I mediated SMG excision could be induced by transient or inducible I-*Sce*I gene expression, and most of the marker-free lines contained only a short deletions at the excision site. This data suggests that this gene stacking method works at an efficiency close to that of the standard transformation methods, and should therefore be easier to implement in other crop species.