In Vitro Cellular & Developmental Biology - Animal
https://doi.org/10.1007/s11626-022-00670-1

Plant Contributed Papers

P-1000

Field Evaluation of Metabolically Engineered Energycane for Hyperaccumulation of Triacylglycerol. VIET DANG CAO[1,2], Baskaran Kannan[1,2], Shelby Korynta[1,2], Hui Liu[1,3], Steve Long[1,4], John Shanklin[1,3], and Fredy Altpeter[1,2]. [1]DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL; [2]University of Florida, Gainesville, FL; [3]Brookhaven National Laboratory, Upton, NY; and [4]University of Illinois, Urbana, IL. Email: caodangviet@ufl.edu, altpeter@ufl.edu

Metabolic engineering to achieve hyper-accumulation of lipids in the vegetative tissues of high biomass crops is a promising strategy to improve lipid yields for biofuel production. Energycane is an ideal feedstock for this approach due to its superior biomass production and persistence. In this study, our objective is the evaluation of metabolically engineered energycane plants for the accumulation of triacylglycerol (TAG,) total fatty acid (TFA) and biomass under field conditions. A multigene expression construct for lipogenic factors and selectable marker gene was generated by modular Golden Gate assembly. Following biolistic gene transfer and selection of antibiotic resistant callus, transgenic plantlets were regenerated. The presence and expression of transgenes in the regenerated plants were confirmed by PCR and qRT-PCR analysis, respectively. Transgenic plants were vegetatively propagated in the greenhouse and were transplanted to a field site at the University of Florida Plant Science and Education Center near Citra, Florida state in randomized and replicated plots under USDA-APHIS permit in March 2021. The TAG and TFA contents for different leaf positions, stem sections, root, and juice from crushed cane were determined at the end of the growing season by a combination of TLC and GC/MS analysis. Biomass fresh and dry weight were also determined. The results indicated that TAG and TFA hyperaccumulated in all analyzed tissues under field conditions and differences in their accumulation in different tissues will be presented. The findings from this study will be useful in developing a high biomass feedstock for production of lipids.

P-1001

Evolution of Insect-resistant and Herbicide-tolerant Transgenic Cotton Lines Harboring Hybrid SN19 Gene. H. DEMIRCI[1], G. Akdoğan[1], S. F. Özcan[2], S. Uranbey[1], C. Sancak[1], and S. Özcan[1]. [1]Department of Field Crops, Faculty of Agriculture, Ankara University, Ankara, TURKEY and [2]Central Research Institute for Field Crops, Ministry of Agriculture and Forestry, Ankara, TURKEY. Email: hahmet@agri.ankara.edu.tr

Cotton is among the most valuable crops in the world with a considerable effect on the economy due to its fiber manufacturing quality. *Bt* cotton plant is a major GMO crop containing genes encoding *cry* endotoxin group for insect-resistant. The aim of this study was to transfer the hybrid SN19 (cry1Ba- domain I–III and cry1Ia-domain II) and GUS reporter genes under the control of 35S promoter to cotton. First, embryonic axis, embryonic hypocotyl, and plumule explants were cultured in vitro for optimization of shoot regeneration. Reproducible *in vitro* plant regeneration was obtained from the embryonic axis. For gene transformation, embryo axis explants from mature seeds were inoculated with *A. tumefaciens* strain GV2260 or EHA105 carrying the binary plasmid p35S-GUS-INT. After histochemical GUS, PCR and RT-PCR analysis, the transformation efficiency was recorded with varying GUS expression levels. In the second stage, to obtain insect-resistant and herbicide-tolerant plants the SN19 and bar genes were cloned into the T-DNA region of the pTF101.1 binary transformation plasmid and transferred successfully into cotton using embriyo axis explants. Putative transgenic cotton plants were primarily selected with phosphinothricin (PPT) thereafter both hybrid SN19 and bar genes confirmed by PCR analysis. Leaf bioassay with Lepidopteran *Spodoptera exigua* larvae showed 100% resistance.

P-1002

Optimizing CRISPR/Cas9-mediated Genome Editing in *Vitis*. PAPAIAH SARDARU[1], C. Jackson[1], A. Junior[1], B. Khatabi[1], X. Dai[2], Y. Zhao[2], and S. A. Dhekney[1]. [1]Department of Agriculture, Food and Resource Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853 and [2]Section of Cell and Developmental Biology, University of California San Diego, San Diego, CA 92903. Email: psardaru@umes.edu

Genome editing is a novel approach to plant genetic improvement that enables modification of specific traits while avoiding significant genome disruption, and is attractive for a perennial species such as grapevine. Recent advances in availability of the grapevine genomic information combined with optimized gene insertion systems now makes it possible to add or modify traits in elite grape cultivars without disruption of existing desirable characteristics. In the current study, a *Vitis* phytoene desaturase (PDS) 1 gene was targeted using CRISPR/Cas9 to demonstrate the concept of grapevine genome editing. Two guide RNAs were designed to target a large region of the PDS1 sequence and produce deletions for disrupting gene function. Embryogenic cultures were initiated from *Vitis vinifera* 'Thompson Seedless' and used as explants for *Agrobacterium*-mediated transformation. The efficiency of genome editing was evaluated based on the development of albino phenotypes and identification of deletions in callus and regenerated plant lines. Targeted mutations of the PDS1 gene resulted in large deletions and development of albino phenotypes, which could be identified following embryo germination. Of the 60 plant lines recovered, 10 exhibited an abnormal phenotype with an editing efficiency of 16.6%. A majority of the edited lines exhibited uniform bleached appearance in all organs while mosaic patterns were observed in a few lines. Deletions in the PDS1 gene was confirmed by PCR and sequencing results. Edited plant lines exhibited slow growth in the culture medium, compared to non-edited control plants and could not survive following transfer to soil and acclimation under conditions of high humidity. The study demonstrated the utility of the CRISPR/Cas9 system for efficient editing of the *Vitis* genome. Optimizing genome editing for grapevine will enable rapid improvement of elite table and wine grape cultivars for disease resistance and quality traits without changing existing desirable characteristics.

P-1003

Comparison of Constitutive Promoters for Spatial Regulation of Transgenes in Different Vegetative Tissues of Sugarcane. RAJESH YARRA[1,2], Evelyn Zuniga-Soto[1,2], and Fredy Altpeter[1,2]. [1]Agronomy Department, IFAS, University of Florida, Gainesville, FL and [2]DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL. Email: rajeshyarra@ufl.edu, altpeter@ufl.edu

Sugarcane is the source of 80% of the sugar and 26% of the bioethanol produced globally. However, its highly polyploid genome (2n = 100 – 120) is the most complex of any domesticated agricultural species (2n = 100–120) and impedes crop improvement by traditional breeding. With the help of crop biotechnology approaches sugarcane has become a prime feedstock to fuel the emerging bioeconomy. Recent examples include metabolic engineering to convert sugarcane into a crop that hyperaccumulates lipids for production of biodiesel and lubricants. Such approaches require coordinated expression of multiple transgenes and detailed knowledge of the level of transgene expression that can be achieved with different regulatory elements. Our objective is the detailed characterization of the spatial expression differences of several constitutive promoters to inform subsequent metabolic engineering approaches. The following ubiquitin promoters were compared including their 1st intron: *Zea mays* (ZmUbi1); *Brachypodium distachyon* (pBdUbi10); *Sorghum bicolor* (pSbUbi1) *Panicum virgatum* (pPvUbi1 & pPvUbi2) with *Cauliflower mosaic virus* 35S promoter (p35S); p35S with *Zea mays* HSP70 intron, *Oryza sativa* actin promoter including 1st intron (pOsActin); *Brachypodium distachyon* elongation factor 1α promoter (pBdEF1α) and Acetolactate synthase (*ALS*) promoter (pALS with & without *Zea mays* HSP10 intron). Promoters were subcloned via Golden Gate assembly 5' of the *gusA* gene & same terminator. *Npt*II was used as a selectable marker. Both transgene expression cassettes were flanked by insulators to minimize the effect of transgene position and introduced into sugarcane via biolistic transfer after removal of the vector backbone. Ten to twelve transgenic lines were regenerated for each construct and confirmed by PCR and histochemical GUS staining in leaves. Results detailing quantitative GUS expression analysis via MUG assay in leaf, stem and root tissues differing in maturity in low copy events will be presented. This study will inform promoter selection for multi-gene engineering of sugarcane.

P-1004

Wound Healing Property of a Gel Containing a Flavonoid-rich Extract of *Bryophyllum pinnatum* (Crassulaceae). S. M. ZUCOLOTTO[1,3], E. R. D. de Araújo[3], J. B. F. de Lima[3], J. M. Fernandes[3], J. B. Xavier-Santos[3], J. Schlam[1]; M. F. Fernandes-Pedrosa[3], A. A. da Silva Júnior[3], G. B. C. Guerra[3], and D. Esposito[1,2]. [1]Plants for Human Health Institute, NC State University, North Carolina Research Campus, Kannapolis, North Carolina; [2]Department of Animal Science, NC State University, Raleigh, NC; and [3]Federal University of Rio Grande do Norte, Health Science Center, Natal, BRAZIL. Email: szucolo@ncsu.edu

Bryophyllum pinnatum is a medicinal plant known as "coirama" or "saião". Popularly, extract from its leaves is applied on the skin to treat wounds. In the market there is no medicine or cosmetic containing this extract as an active ingredient. It shows easy cultivation and raw material during the year. The aim of this study was to evaluate the phytochemistry profile, phenolic and flavonoid content and dermal wound repair in vitro and in vivo assays and the anti-inflammatory properties of a formulation in gel containing a *Bryophyllum pinnatum* leaf extract. UFLC-DAD analysis showed a chromatographic profile rich in flavonoids glycosides (major flavonoid=quercetin3-O-α-L-arabinopyranosyl-(1→2)-O-α-L-rhamnopyranoside; content =33.12 ± 0.056). The phenol and flavonoid content at a concentration of 4 mg/ml was 80.86 mg/ml 38.96 mg/ml, respectively. Skin fibroblast (HDFa) migration assay was conducted using silicone based stoppers to create a central cell-free detection zone in each well of a 96-well plate. Extract accelerated fibroblast cell migration at concentration of 50 ug/ml after 24 and 48 h of incubation when compared with the vehicle. Wound healing in vivo model was conducted during14 days (punch of 12 mm). Gel + extract at 5% and the positive control (fibrinase), administered topically, accelerated the wound healing process in the 7º day ($p < 0.001$) and 14º day ($p < 0.001$) when compared with the placebo gel. Immunohistochemical analysis of the skin biopsies of animals treated with gel + extract at 5% and fibrinase at days 7º and 14º showed an upregulation of VEGF expression when compared with placebo gel; and decrease the level of IL-1β ($p < 0.01$) and TNF-α ($p < 0.05$). Regarding the anti-inflammatory property, extract suppressed the generation of ROS in LPS-stimulated mouse macrophage (RAW 267.4 cells) at concentrations of 50 and 125 ug/ml and the transcriptional levels of inflammatory regulatory genes including IL-1β, COX-2, iNOS, IL-4 and IL-6 were downregulated. Taken together, results showed the potential of this extract as a wound healing agent to develop new products.

P-1005

Highly Conserved sgRNA Target Sequences Support Cas9-mediated Mutagenesis of *LIGULELESS1* in Both Sorghum and Sugarcane. ELEANOR J. BRANT[1,2], Ayman Eid[1,2,3], Mehmet Cengiz Baloglu[1,4], Aalap Parikh[1], and Fredy Altpeter[1,2]. [1]Agronomy Department, University of Florida, IFAS, Gainesville, FL; [2]DOE Center for Advanced Bioenergy and Bioproducts Innovation, Gainesville, FL; [3]Current address: Department of Plant Science and Landscape Architecture, University of Maryland, Baltimore, MD; and [4]Current address: Department of Genetics and Bioengineering, Kastamonu University, Kastamonu, TURKEY. Email: eleanor.brant@ufl.edu, altpeter@ufl.edu

Sugarcane is a prime feedstock for commercial production of biofuel and table sugar. Its highly polyploid genome is the most complex of any domesticated agricultural species (2n = 100–120). This complicates crop improvement by both traditional breeding and genome editing. Sorghum has a diploid genome and its exons have a high level of sequence conservation to those of sugarcane. Establishing protocols to achieve robust, specific, and efficient multiallelic editing remains challenging in most C4 grasses despite the flexibility and efficiency of RNA guided nucleases like CRISPR/Cas9. Therefore, our objectives were to; (1) introduce CRISPR/Cas9-mediated mutations into *LIGULELESS1* (*LG1*) to create a rapidly identifiable phenotype by changing the leaf inclination angle, and (2) evaluate if highly conserved sgRNA target sequences for *LG1* support Cas9-mediated mutagenesis in both sorghum and sugarcane. Genome editing reagents were co-delivered into immature embryos of sorghum (var. Tx430; single *LG1* copy) and sugarcane calli (var. CP88-1762; 32 *LG1* copies) alongside the *npt*II selectable marker via biolistic gene transfer. The same guide RNAs were used in both crops. Transgenic lines were regenerated following selection, and edits were confirmed via Sanger or NGS sequencing. In sorghum, a single nucleotide, monoallelic insertion at the *lg1* target site conferred an upright leaf phenotype in tissue culture that persisted after transfer to soil. T1 progeny of a sorghum event carrying the insertion were analyzed and biallelic *lg1* knockouts resulted in a complete lack of ligules and more severe reduction in leaf inclination angle than monoallelic lines. Transgene free, edited events were also recovered following Mendelian segregation. In comparison, sugarcane lines exhibiting between 10-100% *lg1* knockout have been obtained and results from phenotypic analysis will be presented. This work highlights *lg1* knockout as a suitable strategy for creating a rapidly scorable phenotype and confirms the potential of sorghum as a model species for sugarcane gene editing.

P-1006

Knock-down of Vital Gene(s) of *Tuta absoluta* (Meyrick) (Lepidoptra: Gelechiidae) Using in Planta RNAi. M. H. HASHMİ[1], U. Demirel[1], and A. Bakhsh[2]. [1]Niğde Ömer Halisdemir University, Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, 51240 Niğde, TURKEY and University of the Punjab, Centre of Excellence in Molecular Biology, 53700, Lahore, PAKISTAN. Email: muneebshah9@gmail.com

RNA interference is a highly sequence-specific silencing process and can inhibit specific gene functions in targeted insect pests by utilizing dsRNA. To combat crop losses from damaging insect pests, dsRNA-based technologies proved to be an efficient and promising insect pest management strategy. Tomato is the seventh most important crop species after maize, rice, wheat, potatoes, soybeans, and cassava. Turkey is the fourth-largest producer of tomatoes and currently facing serious production losses due to pest infestation especially American tomato pinworm, *Tuta absoluta* (Meyrick) (*Lepidoptera: Gelechiidae),* which is a devastating insect pest of tomato. In this study, we reported a dsRNA-based approach against American Tomato Pinworm vital genes which comprises of development of transgenic tomato lines expressing Sec23dsRNA and Ache1dsRNA, which is reported for the first time and developed tomato cv. Rio Grande transgenic lines showed a significant level of resistance against *Tuta absoluta* (Meyrick). The gene *Acetylcholine esterase1* (*AChE1*) in insects particularly functions in neurotransmission, and *Sec23* protein present in insects is one of the components of the *coat protein complex II* (*COPII*) that promotes the formation of transport vesicles from the endoplasmic reticulum (ER). Insects fed on transgenic lines expressing dsRNA against *AChE1 and Sec23* mRNA resulted in the mortality of larvae, delayed pupation, abnormal growth causing the suppression of *Tuta absoluta* population. Hence, the reported dsRNA-based insect pest management strategy will serve as a breakthrough to be the cost-effective and eco-friendly approach.

P-1007

Antimicrobial, Cytotoxic, and Synergistic Activities of Five Linear Plant-derived Host Defense Peptides for Engineering Disease Resistance in Plants. NICK SCHIMPF and Dmytro P. Yevtushenko. Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, CANADA. Email: nick.schimpf@uleth.ca

Host defence peptides (HDPs) are more versatile successors to antibiotics, capable of reducing plant susceptibility to disease. Though variation exists, HDPs are generally small, cationic peptides with antimicrobial and/or immunomodulatory activity. The deleterious nature of chemical pesticides against non-target organisms and the environment are well known, whereas the selective nature of many HDPs prevents toxicity to mammalian and plant cells. Additionally, HDPs are commonly active against a broad range of pathogens, allowing for extensive application. The aim of this study is to evaluate linear plant-derived HDPs Sm-985, BnPRP1, P4650, Ib-AMP1 analog, and Shepherin 1 *in vitro* and to identify candidate HDPs suitable for expression in plants. Fungal conidia were incubated with various concentrations and combinations of peptides for 24 h to assess antifungal activity. Spores were considered inhibited if the emerging mycelium was less than twice its length. Similarly, peptide antibacterial activity was investigated. Cytotoxicity was assessed by incubation of mesophyll protoplasts with peptides and subsequent staining for cell viability. Peptide toxicity to mammalian cells was tested likewise by incubation with human embryonic kidney cells. In order of decreasing antifungal activity, the three most effective peptides were Sm-985, Ib-AMP 1Q, and Shepherin 1. Interaction ratios showed synergy between P4650, which alone had no antifungal activity, and Sm-985, Ib-AMP 1Q, and Shepherin 1, suggesting that P4650 had antimicrobial adjuvant activity. Among all individual HDPs tested, only P4650 was toxic to plant protoplasts, with ≥96% inhibition at 100 μM. The most promising peptide combinations will be ectopically expressed in potato plants to enhance host disease resistance. Transgenic plants will be challenged with potato pathogens and evaluated for disease susceptibility by the severity of symptoms. The results from this study will identify novel HDPs for engineering disease resistance in crops and contribute to improving food security.

P-1008

Efficient, Multi-allelic Editing for the Genetic Improvement of Bahiagrass (*Paspalum notatum* Flüggé). DAVID MAY[1], Jennifer Gilby[1], Sara Sanchez[1], and Fredy Altpeter[2]. [1]Agronomy Department, University of Florida - IFAS, Gainesville, FL and [2]Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville, FL. Email: dbm03@ufl.edu, altpeter@ufl.edu

Polyploidy, apomixis and self-incompatibility complicate the genetic improvement of bahiagrass (*Paspalum notatum* Flüggé), an important forage and turf grass in the Southeast United States. Gene editing techniques such as CRISPR/Cas9 would bypass chromosome doubling and repeated crosses necessary for conventional breeding of this species and could result in production of improved cultivars in a single generation. A pair of guide RNAs targeting magnesium-protoporphyrin IX chelatase (MgCh) along with Cas9 and the NPTII selectable marker were delivered to embryogenic callus cultures of the apomictic, autotetraploid bahiagrass cultivar ‘Argentine’ using particle bombardment. Chlorophyll-depleted lines, which were confirmed for multi-allelic edits with Sanger and next generation sequencing, were obtained across two independent experiments at efficiencies of 23 and 50% of total transgenic events. This approach has supported further endeavors to genetically improve turf and forage quality in bahiagrass including confirmed targeted mutagenesis of genes involved in functional stay-green, lipid accumulation and lignin composition. In addition, the transmission of edits to apomictic progeny was analyzed, and the feasibility of production of transgene-free edited apomictic lines *via* heat-inducible Cre/lox site-specific recombination was explored. Overall, these efforts have resulted in an efficient, reproducible CRISPR/Cas9 gene editing protocol for *Paspalum notatum*, a development with beneficial implications for future breeding efforts in this species.

P-1009

Targeted Mutagenesis of Vacuolar H^+ Translocating Pyrophosphatase (*V-PPase*) Promoter Limits Sucrose Formation and Disturbs Cytosolic pH During Germination in Rice. D. DHARWADKER[1], P. J. I. Gann[2,3], C. Maurya[3], S. Nandy[3], S. Zhao[3], and V. Srivastava[2,3]. [1]Department of Chemistry and Biochemistry, [2]Cell and Molecular Biology Program, and [3]Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR. Email: ddharwad@uark.edu

Germination and seedling growth in rice relies on the breakdown of starch to generate sucrose in the endosperm. This process is inhibited by inorganic pyrophosphate (PPi). An enzyme, Vacuolar H+ translocating pyrophosphatase (V-PPase), hydrolyzes PPi into inorganic phosphate (Pi) as it translocates H+ from the cytoplasm into the vacuole. Although the enzymatic activity of V-PPase is known, its function in germination and seedling growth is poorly understood. To explore the role of V-PPase in these stages, it was downregulated by heat and mutagenesis using CRISPR/Cas9. Downregulation by heat provides understanding on its mechanism in maintaining cytoplasmic pH while mutagenesis elucidates its contribution in germination and growth. In mutagenesis by CRISPR/Cas9, the resulting lines harbored a mutation in one of the four predicted promoter elements, an ATC deletion in GATA. Interestingly, when six selected lines with homozygous ATC deletions were subjected to gene expression analysis, *V-PPase* was found to be downregulated. Additionally, these lines showed lower germination rates and slower seedling growth. Understanding that V-PPase downregulation can inhibit sucrose formation due to the potential build-up of PPi, the lines were tested for sucrose content to explain their poor germination and growth. All lines had a lower sucrose content, but external supply reverted their growth to normal. In downregulation by heat, calli expressing the pH sensitive reporter, Green Fluorescent Protein (GFP), demonstrated a decrease in cytoplasmic pH. Altogether, *V-PPase* impacts cytoplasmic pH, a regulator of enzyme activities and the breakdown of starch to form sucrose, an important substrate for seedling growth.

P-1010

Deletion in the GATA Promoter Element of Vacuolar H+ Translocating Pyrophosphatase (*V-PPase*) by CRISPR/Cas9 Reduces Chalkiness in Rice. PETER JAMES ICALIA GANN[1,2], Dominic Dharwadker[3], Chandan Mayura[2], Soumen Nandy[2], Shan Zao[3], and Vibha Srivastava[1,2]. [1]Cell and Molecular Biology Program, [2]Department of Crop, Soil and Environmental Sciences, and [3]Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR. Email: pjicalia@uark.edu

Chalkiness is a concern in rice breeding as it drives down the market value of the grain. It is an outcome of improper starch packing that it is induced by heat stress and varies depending on the genotype. In one genotype, chalkiness has been linked to the vacuolar H+ translocating pyrophosphatase (V-PPase), an enzyme that hydrolyzes inorganic pyrophosphate (PPi) as it translocates H+ to the vacuole from the cytoplasm. Natural polymorphisms in the promoter of *V-PPase* were associated earlier with chalkiness. However, previously described promoters are not present in many genotypes and its mechanism in chalkiness remains unclear. Here, we elucidate the role of *V-PPase* in chalk formation during grain-filling by (i) comparing its expression across genotypes; (ii) mutagenesis in predicted promoter elements through CRISPR/Cas9 to investigate its impact on chalk size, sucrose content, and granule structure; and (iii) heat perturbation to explore its contribution in regulating cytoplasmic pH. Genotypes with higher chalk were found to have upregulated *V-PPase* at 10 days after flowering (10DAF). When a high chalk genotype was mutagenized through targeted deletion in the consensus sequence, AGATC, of a GATA promoter element, *V-PPase* was remarkably downregulated. Coincidingly, chalk size was reduced by approximately 10% under normal temperature and heat stress, granules became slightly more edgy indicating proper starch packing, and sucrose content decreased which suggests its potentially higher utilization for starch biosynthesis. For the heat perturbation in plants with pH sensitive reporters, *V-PPase* was downregulated and cytoplasmic pH dropped by ~0.21, a condition that could be optimum for enzyme activities essential in grain-filling. Overall, our findings herein illuminate an approach for reducing chalkiness and provide insights into the underlying mechanisms.

P-1011

Somatic Embryogenesis in Cell Suspension Cultures of Sweetpotato (*Ipomoea batatas*). K. DA[1,2], H. Leng[1], W. Liu[2], and G. C. Yencho[2]. [1]Plant Transformation Lab and

[2]Department of Horticulture, North Carolina State University, Raleigh, NC 27607. Email: kda@ncsu.edu

Sweetpotato (*Ipomoea batatas*) is one of the most important crops in the world. Apart from providing a valuable source of food, animal feed and industrial raw materials, sweetpotato offers a rich source of vitamins and antioxidants to human health. Great achievements have been made on yield and starch content breeding in sweetpotatoes by using conventional breeding methods. However, due to their highly complex genetic backgrounds, it is difficult to continuously improve the desirable characteristics of established elite lines and varieties by conventional breeding alone. Emerging plant genome editing techniques provide trait specific breeding for enhanced plant stress tolerance and disease resistance. Highly efficient regeneration and transformation system is a prerequisite for plant genome editing. Here, we report a protocol that focuses on efficient somatic embryogenesis from cell suspension culture in sweetpotato. Embryogenic callus was induced from in vitro leaf explants of sweetpotato on MS medium containing 1.5mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). Greenish-white, friable embryogenic calli were used to establish suspension cultures. A shaking speed of 100 rpm and 0.5 ml packed cell volume per 15 ml medium were found to be optimal for maintaining suspension cultures. Globular, heart-shaped and torpedo-shaped embryos were developed in two months after suspension cells were transferred to solid MS medium containing 0.5 mg/L 2,4-D. Maturation of cotyledonary-stage somatic embryos was achieved on MS medium containing 0.05 mg/L 2,4-D and 0.5mg/L abscisic acid. Embryos were converted into plants and survived in soil.

P-1012

Agrobacterium-mediated Genetic Transformation of Rose Embryogenic Cell Suspension Cultures. K. DA[1,2], H. Leng[1,2], D. Harmon[1,2], A. Nelson[1,2], N. Maren[1,2], W. Liu[2], and T. Ranney[2]. [1]Plant Transformation Lab and [2]Department of Horticulture, North Carolina State University, Raleigh, NC 27607. Email: kda@ncsu.edu

Rose (*Rosa*) is the most valuable ornamental crop in the world. Representing diverse markets including cut flowers, potted plants, landscape shrubs, perfume, and food/beverage additives, roses enhance the environment and human health and well-being. Conventional breeding has made great advancements in plant form, disease resistance, remontancy, and other commercial traits of roses. However, genetic improvement of rose through conventional breeding is challenging due to polyploidy, high heterozygosity, and low fertility of existing breeding lines. Genome editing permits precision trait engineering for enhanced stress tolerance, disease resistance, and improved ornamental features. Highly efficient regeneration and transformation systems are a prerequisite for rose genome editing. Here, we report an efficient method for *Agrobacterium*-mediated genetic transformation of embryogenic cell suspension cultures of rose. Competent callus from embryogenic cell suspension cultures derived from leaf callus were transformed with *A. tumefaciens* strain GV3101 harboring the binary vector containing a phosphinothricin N-acetyltransferase (bar) gene and a green fluorescent protein (GFP) reporter gene. A mean number of 28 putative transgenic cell lines were produced from three independent transformation of 0.5 gram of embryogenic competent callus with GFP visual selection. PCR analyses of the regenerated plants showed that 90% of them were transgenic. The development of this transformation method provides a highly efficient tool for rose transformation, trait engineering, and functional genomics studies.

P-1013

Identification and Validation of an Embryogenic Tissue Culture Response Gene from Maize Inbred Line A188 That Induces Embryogenic Callus Formation and Somatic Embryogenesis in Recalcitrant Maize Inbred B73. FRANK MCFARLAND[1,2], Ray Collier[2], Kaitlyn Vondracek[2], Dalia Macias Martinez[2], and Heidi Kaeppler[1,2]. [1]Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706 and [2]Wisconsin Crop Innovation Center, University of Wisconsin, 8520 University Green, Middleton, WI 53562. Email: fmcfarland@wisc.edu

Embryogenic cultures capable of regenerating into plants are critical for many crop transformation and gene editing systems utilized for functional genomics research and genome-based crop improvement efforts. Those applications are significantly limited, however, by genotype-dependent culture response in most crop species. Deciphering the genetic mechanisms which control embryogenic, regenerable culture response can aid in the development of genotype-flexible crop transformation/editing systems. Research was conducted by our group to determine the genetic factors underlying embryogenic, regenerable culture response in the globally important crop, maize. RNA-seq was utilized to characterize the transcriptome of immature embryo explants of the embryogenic maize inbred A188 in culture. We conducted genetic fine mapping and candidate gene identification based on molecular and phenotypic analysis of inbred backcross lines derived from the cross of A188 with the recalcitrant line B73. Fine mapping analysis led to the identification of a 3 Mb region on maize chromosome 3 significantly associated with embryogenic, regenerable culture response in maize. Comparative sequence and expression analysis between A188 and B73 sequences within the mapped region aided in identification of a small subset of candidate genes for investigation. One of the candidate genes tested was shown to induce somatic embryogenesis and embryogenic callus formation in the highly recalcitrant maize line B73. Delivery of constructs encoding constitutive expression of the candidate gene into immature embryos resulted in formation of somatic embryos and embryogenic callus. Plantlets were regenerated within six weeks, and transgenic events confirmed via visual and molecular assays. A transformation protocol based on the embryogenesis-inducing gene is being developed. In comparison to several existing methods that use genes which induce culture response, this method will not rely upon tissue-specific promoters nor the removal of the inducer gene to facilitate plant regeneration.

P-1014

Reproduction Control Tools for *Eucalyptus*: Knockout of Flowering and Meiosis Genes Using CRISPR/Cas9 Mitigates Concerns About Gene Dispersal While Maintaining Normal Vegetative Development. STEVEN H. STRAUSS, Michael F. Nagle, Surbhi Nahata, Bahiya Zahl, Alexa Niño de Rivera, Xavier V. Tacker, Estefania Elorriaga, Cathleen Ma, Greg Goralogia, Amy Klocko, Ellis Kline, and Michael Gordon. Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331. Email: Steve.Strauss@OregonState.Edu

Eucalyptus spp. are widely grown for wood, fiber, biofuels and ornamentals, and genetic transformation and gene editing have the potential to add or modify useful traits. However, eucalypts can become invasive exotics in some geographies, and movement of transgenes may present social and regulatory obstacles. To provide tools for management of dispersal, we used CRISPR/Cas9 to create loss-of-function mutations in three genes essential for diverse phases of male and/or female reproduction. Using both normal and early-flowering (*FT*-overexpressing) trees, we made biallelic knockouts of eucalypt homologs of *TAPETAL DEVELOPMENT AND FUNCTION 1 (TDF1), SYNAPTIC 1 (SYN1,* also known as *REC8)*, and *HECATE 3 (HEC3).* In the early flowering trees, the floral phenotypes resulting from each knockout appear to broadly mirror those seen in Arabidopsis and/or rice: *TDF1* knockouts produced flowers absent of any pollen, *SYN1* knockouts led to abnormally shaped and inviable pollen grains but otherwise normal flowers with fully developed stamens and nectar, and *HEC3* knockouts produced flowers with shortened stigmas and without pollen. In the non-flowering (no *FT* overexpression) trees, randomized greenhouse trials did not reveal morphologically distinguishable effects of gene knockouts on plant form or growth rate. Statistical tests also revealed an absence of significant effects of gene knockouts on vegetative traits studied, including leaf mass, relative stem volume, and relative chlorophyll density of leaves. Our results suggest that all these genes are useful for conferring male-sterility, and *SYN1*—because of its mechanism of action—is also likely to be useful for providing bisexual sterility. It may also be capable of producing sterile flowers that can provide at least some ecological functions in support of flower-dependent animals, such as nectar and partially developed pollen grains. Field trials of these knockout trees are needed to verify these greenhouse phenotypes and ecological service functions.