

## Plant Contributed Papers

### P-1000

Isolation and Evaluation of New *Agrobacterium* Strains for Transformation of Soybean and Sunflower. K. BENZLE<sup>1</sup>, D. Marty<sup>2</sup>, L. McHale<sup>3</sup>, B. Goodner<sup>4</sup>, K. Finer<sup>5</sup>, C. Taylor<sup>2</sup>, and J. Finer<sup>1</sup>. The Ohio State University, <sup>1</sup>1680 Madison Ave., Wooster, OH 44691, <sup>2</sup>210 Selby Hall, Wooster, OH, and <sup>3</sup>312B Kottman Hall 2021, Coffey Rd., Columbus, OH 44720; <sup>4</sup>Hiram College, 113 Colton Hall, Hiram, OH 44234; and <sup>5</sup>Kent State University, 6000 Frank Ave NW, Canton OH 44720. Email: benzle.2@osu.edu

Although most laboratories routinely use *Agrobacterium* for generation of transgenic plants, only a few *Agrobacterium* strains are widely available and none of those strains were isolated, selected and disarmed based on efficiency of transformation. For this research, new *Agrobacterium* strains were isolated from crown galls of various plants in Ohio and rhizospheric soil collected from soybean fields throughout the Midwest US. Wild type strains were isolated by plating gall and soil extracts on 1A selective medium and screening the isolates for the presence of *virG* using PCR. Additional known wild type strains and disarmed strains were also used. For evaluation of transformation efficiency in sunflower and soybean, a pCambia-derived construct containing a Gmubi-regulated *gfp* gene, was first introduced into each of these strains. Plant transformation efficiency was evaluated by quantifying GFP expression in hypocotyl and cotyledonary tissues of sunflower and soybean seedlings, and in proliferative embryogenic tissue of soybean. In sunflower, seedling cotyledonary tissue was almost unresponsive to all strains but the hypocotyl tissues were very responsive with the highest transformation rates obtained using EHA105. Sunflower hypocotyl tissues showed high tissue specificity with EHA105 as 90% of the transformed cells were located in the vascular tissues. In soybean seedling tissues, tissue-specific transformation was not observed with any strain as transformed cells were evenly distributed throughout target tissue. With soybean hypocotyl, cotyledon and embryogenic tissues, a single strain from a soybean field gave 5-10x higher transformation rates than EHA105, while a strain from a gall gave 3-5x higher rates. Surprisingly,

transformation efficiencies for all strains were similar across tissue types for soybean.

### P-1001

Optimizing *In Vitro* Mineral Nutrition and Plant Density Increases Greenhouse Growth Rate of Turmeric. R. EL-HAWAZ, D. Park, W. Bridges, and J. W. Adelberg. Clemson University, SC 29634. Email: rabiae@clemson.edu; nonaopener@yahoo.com

Micropropagated plants gain most of their value in the greenhouse, yet there is no medium optimized for growth of the microplant *ex vitro*. Increasing the number of plants in a vessel increases the mineral concentrations needed to maintain optimal *in vitro* growth. This work will show *in vitro* medium designed to provide mineral nutrition to large numbers of *in vitro* plants to maximize subsequent *ex vitro* growth. By selecting treatment combinations using D-optimal criteria and a response surface model, MS liquid medium was modified with 5 mM NH<sub>4</sub>, 6.25 to 10.25 mM P, 2 to 10 mM Ca, and 18 to 100 mM KNO<sub>3</sub> for two *Curcuma longa* L. genotypes (35-1 and 22-5) with 2 to 10 buds in a vessel with 40 mL of medium. MS medium was also tested with these genotypes at the same plant densities. The residual minerals in spent media were analyzed after the 4<sup>th</sup>, 35-day cycle under treatment conditions. Plants were transferred to the greenhouse with effort made to assure near uniform plant density and mist patterns. The first 3-weeks of *ex vitro* growth was measured by the gain in fresh mass/mass in. High-density with increased minerals *in vitro* increased production to 30 and 23 new plants/vessel for clones 35-1 and 22-5, respectively. In the greenhouse, high density MS medium had 1.2x growth rate, while in the mineral optimization model, KNO<sub>3</sub> was the major factor and interacted with 10 buds/vessel and 2 mM Ca to raise the growth rate to 2.3x, with 42.6 mM KNO<sub>3</sub>. Plants from MS medium had low P concentrations when compared agronomic standards for turmeric. In treatment conditions, P was raised into the optimal range where P concentration in dry mass was interrelated with genotype, Ca, KNO<sub>3</sub>

and plant density. More *in vitro* plants in a fixed volume of media lowers the mineral contents and the *ex vitro* growth rate for large numbers of plants can be maximized with a multifactor approach.

#### P-1002

RNAi Mediated Silencing of Endogenous Wheat Genes eIF4E2 and eIF4G Induces Resistance to Potyviruses Wheat Streak Mosaic Virus and Triticum Mosaic Virus. J. RUPP<sup>1</sup>, L. Cruz<sup>1</sup>, J. Fellers<sup>2</sup>, and H. Trick<sup>1</sup>. <sup>1</sup>Kansas State University, 4024 Throckmorton, Manhattan, KS 66506 and <sup>2</sup>USDA-ARS-HWWGRU, Manhattan, KS. 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 eIF4E2 and eIF4G to interrupt this process to induce resistance to these wheat viruses. RNAi expression vectors were independently created from the sequences of the wheat genes eIF4E2 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. Putative transformed plants were analyzed through PCR for the presence of the appropriate RNAi gene. Transgenic T<sub>1</sub> seeds were collected and each line was tested for transgene expression via RT-PCR. To determine viral resistance, the progeny were also mechanically inoculated with the WSMV. Viral presence was established by ELISA. Transgenic lines were advanced to homozygosity. Although transgene silencing and deletion was detected with a few events in the T<sub>2</sub> generation, a consistent stable resistance response was demonstrated in three transgenic lines of eIF4E2 construct and four transgenic lines of eIF4G. T<sub>3</sub> progeny were independently subjected to inoculation with both WSMV and TriMV and were resistant. T<sub>4</sub> progeny were co-infected with both viruses and were also demonstrated to be resistant to both potyviruses. The transgene expression in the T<sub>5</sub> generation of either construct continued to exhibit high levels of viral resistance. 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-1003

Development of Switchgrass (*Panicum virgatum* L.) Transformable Cell Suspension Culture and a Screening System for Rapid Assessment of Cell Wall Genes for Improved Biomass for Biofuels. J. WILLIS<sup>1</sup>, L. Kline<sup>2</sup>, S. Allen<sup>1</sup>, P. Bhattacharya<sup>2</sup>, A. Collins<sup>1</sup>, J. Grant<sup>1</sup>, G. Montgomery<sup>1</sup>, N. Labbe<sup>2</sup>, and C. N. Stewart<sup>1</sup>. <sup>1</sup>University of Tennessee, Department of Plant Sciences, Knoxville, TN 37996 and <sup>2</sup>Center for Renewable Carbon (CRC), The University of Tennessee, TN. Email: jdwillis@utk.edu

Transformation and chemical characterization of plant cell wall traits for switchgrass is arduous and time consuming. We have developed transformable switchgrass cell culture lines with corresponding chemical fingerprinting to rapidly screen cell walls with modified genes. Transgenic down-regulated lignin switchgrass plants (COMT and MYB) were used as donors for inflorescence meristem tissue to induce callus. COMT and MYB callus were added to a liquid culture system to produce aggregate and non-aggregate cells to be evaluated by spectral and chemical analysis for cell wall properties. Callus generated from wildtype plants were used to develop a liquid culture system. Wildtype aggregate and non-aggregate cell cultures were transformed using *Agrobacterium tumefaciens* harboring the pANIC vector carrying the COMT and MYB genes and cell wall traits were analyzed. Transgenic liquid cultures from transformed plants and transformed cultures from wildtype plants were analyzed by chemical (PyGC/MS) and spectral (FTIR/Fluorolog) techniques to generate a prediction model for detecting cell wall changes. Development of this simple cell switchgrass culture is our first steps for developing a multiplex automatic genome engineering (MAGE) system for plants.

#### P-1004

A Leading Intron of a Soybean *elongation Factor 1A* Gene Interacts with Soybean Promoter Elements to Regulate Gene Expression. N. ZHANG, L. McHale, and J. Finer. Department of Horticulture and Crop Science, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691. Email: zhang.2241@osu.edu

Introns, especially the first intron in the 5'UTR, can increase gene expression by intron-mediated enhancement (IME). However, little is known about intron-enhancement mechanisms since only a few plant introns have been intensively studied. To further gain insight into IME in plants, we studied the interaction of intron components with the regulatory element sequences from promoters of a group of highly-expressed soybean genes called “GmScream”. To gauge promoter strength and specificity, synthetic promoters consisting

of an element tetramer and a core promoter were constructed and used to regulate a *gfp* gene. Gene expression was analyzed using both transient expression in lima bean cotyledons and stable expression in soybean hairy roots. Element tetramers, placed upstream of a GmScream core promoter (GmSM8 core), showed very high activity, only if the native leading intron was included, indicating that interesting interactions may exist among intron-containing sequences, promoter elements and the core promoter. Partial deletions of the leading intron were performed to see if the intron contained regulatory elements that interacted with the synthetic promoter containing an element tetramer. A 222 bp intronic sequence significantly contributed to very high GFP expression. Additional intron variants were generated to further understand the interaction between the intronic sequences and specific promoter element sequences. Intron variants with a monomeric or trimeric repeat of this 222 bp intronic sequence, gave almost two-fold higher expression compared to the original intron, which further demonstrated that the 222 bp intronic sequence was essential for the intron-element interaction enhancement. The 222 bp intronic sequences may contain splicing enhancers that interacted with the promoter elements to increase gene expression during gene transcription and the intron splicing process.

#### P-1005

Insulator Mediated Transgene Performance in Sugarcane. Y. ZHAO<sup>1</sup>, J. Kim<sup>1</sup>, C. Fan<sup>2</sup>, W. Yu<sup>2</sup>, S. Dong<sup>2</sup>, and F. Altpeter<sup>1</sup>. <sup>1</sup>University of Florida, 1692 McCarty Drive, McCarty Hall D, RM 3062, Gainesville, FL 32611 and <sup>2</sup>Syngenta Biotechnology Inc., Research Triangle Park, NC. Email: yangzhao779@ufl.edu

Prediction of transgene performance is difficult in sugarcane due to the random insertion into the large and highly polyploid sugarcane genome and associated position effects. Insulators have been identified which establish genomic barriers and block the activity of enhancers. These two properties make it desirable to include insulators in vector construction to provide predictable expression of transgenes, support the direct comparison of different promoters and/or facilitate the tissue specific expression of stacked transgenes. EXOB from bacteriophage  $\lambda$  and TBS from petunia were recently reported as effective insulators in model plants. Our objective is to explore the influence of these insulators on transgene expression in sugarcane. Constitutive *nptII* expression cassettes without insulators or flanked by insulators were bombarded into sugarcane leaf whorl explants. Plants were regenerated following selection with geneticin via direct somatic embryogenesis. Taqman<sup>®</sup> qPCR, PCR with multiple primer combinations

and Southern blot were performed for determination of transgene copy number and integration pattern. NPTII ELISA was conducted from replicated protein extracts of the mid section of the top visible dewlap leaf from single copy lines for quantification of transgene expression. The 11 single-copy lines with complete expression cassettes and without insulators displayed an average expression level of 9.1 ng/10 $\mu$ g soluble protein, ranging from 0.0 to 21.5 ng/10 $\mu$ g soluble protein. The 7 single-copy lines with insulators displayed an average expression level of 17.0 ng/10 $\mu$ g soluble protein, ranging from 14.5 to 20.5 ng/10 $\mu$ g soluble protein. The line to line variation of transgene expression appeared to be reduced in transgenic lines with insulators. The average transgene expression level of lines with insulators was almost two times higher than that of lines without insulators. Data from additional single copy lines with the different constructs are currently generated and will also be presented.

#### P-1006

TALEN Mediated Targeted Mutagenesis in Sugarcane. J. JUNG and F. Altpeter. University of Florida, 3085 McCarty Hall, Gainesville, FL 32611. Email: jehyeong@UFL.EDU

Transcription activator-like effector nuclease (TALEN) is a recently developed tool enabling precise genome modifications, such as targeted mutagenesis, gene replacement, or insertion. Sugarcane is a prime feedstock for bioethanol production, and utilizing both sucrose and cell wall bound sugars for fermentation will enhance the biofuel yield. Down-regulation of lignin biosynthetic genes has been a successful strategy to improve bioethanol production from lignocellulosic biomass by diminishing biomass recalcitrance derived from lignin. In this study, one of the lignin biosynthetic genes, Caffeic acid O-methyltransferase (COMT) was targeted for the TALEN induced mutagenesis to modify lignin biosynthesis in sugarcane. Two alternative TALEN scaffolds were compared to optimize mutation frequency. TALEN recognition sites were selected using the TALEN<sup>TM</sup> Hit software, and corresponding TALEN arms were custom synthesized with different scaffolds. Constitutive TALEN expression cassettes were constructed and co-introduced into callus with a selectable *nptII* gene by Agrobacterium or biolistic gene transfer. Putative events were selected on geneticin containing culture media. Targeted mutations were identified by restriction digestion of the PCR amplicon of the targeted mutation site. Events were confirmed by sequencing of the targeted mutation site. This is the first report of successful targeted mutagenesis in sugarcane. Restriction digest and sequencing of the targeted mutation site in the sugarcane COMT gene revealed the presence of insertions and deletions at the target site. Thus, TALEN can be utilized as a precise genetic engineering tool

for sugarcane improvement. Currently more than 100 transgenic lines are regenerating for each TALEN construct. The effect of two alternative TALEN scaffolds on mutation frequency will be presented after evaluating all transgenic lines. Future work will include the analysis of lignin content and composition.

#### P-1007

TIR1-like Auxin-receptors Are Involved on the Regulation of Plum Fruit Development. I. EL-SHARKAWY, S. Sherif, and J. Subramanian. University of Guelph, Department of Plant Agriculture, 4890 Victoria Ave., N., PO Box 7000, Vineland Station, ON L0R 2E0, CANADA. Email: ielshark@uoguelph.ca

Ethylene is the main regulator of ripening in climacteric fruits. Recent evidences showed that auxin also plays an important role during fruit ripening. However, the nature of the interaction between the two hormones remained unclear. To understand the cross-talk between ethylene and auxin, we compared two plum cultivars with widely varying fruit ontogeny. We show that exogenous auxin is capable of accelerating fruit development and ripening in early and late cultivars Early-Golden (EG) and V9, respectively, but in a different manner due to diversity in auxin sensitivity. To investigate the molecular basis of auxin action in fruit development, plum genes encoding three novel proteins that belong to *F*-box TIR1/AFB family of auxin-receptors were isolated. Their expression during fruit development did not elucidate the differences between the two cultivars in terms of auxin-sensitivity. Genome sequence analysis revealed that though plum genome has one allele for *Ps/TIR1* and *Ps/AFB2*, two different alleles were identified for *Ps/AFB5*. Genetic analysis shows that the differences in auxin-sensitivity between plum cultivars may be associated with *Ps/AFB5*, which belongs to a class of auxin-receptors that have been shown to negatively regulate auxin-responsiveness. We demonstrate that the *Ps/afb5* allele present in EG is inactive because of a SNP occurred within the *F*-box domain, leading to the loss of a proline residue. The reduced auxin levels and sensitivity in V9 is associated with *Ps/AFB5/afb5* heterozygosity. Protein localization, Y2H and BiFC assays of WT and modified versions of *Ps/TIR1*-like proteins highlighted the contribution of various TIR1/AFBs domains and the importance of the *F*-box/proline residue substituted in the *Ps/afb5* allele. Moreover, ectopic expression of *Ps/TIR1* in tomato resulted in a pleiotropic phenotype consistent with an altered auxin-response. Our results indicate the prominence of auxin in plum fruit development and the potential for differential auxin-sensitivity among cultivars, leading to changes in the seasonality (early/late) in stone-fruits.

#### P-1008

Artificial MicroRNA as an Alternative Method to Improve the Resistance to Soybean Cyst Nematode. B. TIAN, J. Li, T. Oakley, T. Todd, and H. Trick. Kansas State University, Department of Plant Pathology, Manhattan, KS 66506. Email: btian@k-state.edu

Soybean cyst nematode (SCN), *Heterodera glycines*, is one of the most important pests to limit the soybean production worldwide. Current SCN management relies mainly on host-plant resistant cultivars derived from PI88788 which has relatively narrow genetic background. Previous studies in our lab showed that resistance against SCN was improved by transgenic composite plants expressing dsRNA targeting several SCN genes. With the discovery of endogenous microRNAs as a mode of gene regulation in plants, artificial microRNA (amiRNA) methods have become an alternative for gene silencing having the advantage that they could lead to more specific silencing than traditional RNAi vectors. The amiRNA can also limit off-target silencing of related sequences as they contain only very short (19-24nt) complementary sequence to the target gene. To explore application of amiRNAs for improving resistance to SCN, we have constructed amiRNA vectors targeting 3 SCN genes (J15, J20, and J23). Using a rapid hairy root system established in our lab, we have transformed composite soybean with the three amiRNA vectors individually as well as stacking multiple targets in one vector. The bioassay for amiRNA vector silencing GFP in soybean confirmed that the designed amiRNA vector suppressed soybean endogenous target gene efficiently. Our preliminary bioassay results also showed significant reduction of SCN populations. For the amiR-J15, the number of eggs was significantly reduced by 53%, although the reduction of cysts (21%) was not significant. The number of cysts and eggs on amiR-J23 hairy roots were both significantly reduced by 34% and 45%, respectively. The evaluation of SCN resistance of the stable transgenic soybean and other stacking amiRNA vectors is in the process. Our current results indicated that the amiRNA methods had the potential ability to improve soybean resistance to SCN as it should limit undesired phenotypes associated with the transformation which is important for commercial crops.

#### P-1009

Metabolic Engineering of Orange Fruit for the Development of Novel Blood and Cara Cara Like Citrus Varieties. K. DASGUPTA<sup>1,2</sup>, R. Thilmony<sup>2</sup>, and J. Thomson<sup>2</sup>. <sup>1</sup>University



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The Blood orange and the Cara cara orange are special cultivars of navel orange that are distinguished by their color derived from the expression of anthocyanins and lycopenes in the ripening fruit. They are superior to other navel orange varieties in flavor, taste, health benefits and are often in high demand when they are in season. Despite increasing consumer interest, production of these citrus varieties remains unreliable due largely to a dependency on cold for full color formation. We propose to generate Blood and cara cara cultivars via genetic engineering by targeting anthocyanin and lycopene metabolic pathway using fruit specific promoters from citrus and tomato. Constitutive promoters may be suitable for proof of concept experiments, but have potential disadvantages for use in crop breeding. Fruit specific promoters which enable precise manipulation of gene expression and metabolic pathways are fundamental for engineering fruit cultivars and improve fruit quality. We have identified 3 candidate citrus fruit-specific promoters using bioinformatics tools and citrus gene expression data. The candidate promoters should express in orange fruit, but should exhibit little or no expression in vegetative tissues. The promoters of the selected candidate genes have been identified using the available citrus genome sequences and 1.5-2kb upstream sequence has been PCR amplified and cloned. These candidate promoters have been fused to the GUSPlus reporter gene in a binary vector to test their activity in tomato and citrus fruits. The promoter strength and expression specificity is currently being tested in tomato fruit using a transient expression assay and stably in transformed Micro-Tom tomato plants. The required enzymes necessary for anthocyanin and lycopene production are well characterized. MybA transcriptional activation gene has been shown to up-regulate the expression of anthocyanin biosynthetic genes in many species, including citrus and lycopene production requires a minimal metabolic pathway of three genes for most plants. With the existing citrus candidate promoters and lycopene/anthocyanin genes in our hand, the proposed research will be useful for generating oranges that accumulate anthocyanin or lycopene or for modifying other citrus fruit quality traits

#### P-1010

Genetic Instability of Long-term Micropropagated Mature Pistachio. H. AKDEMİR<sup>1</sup>, V. Suzer<sup>1</sup>, E. Tilkat<sup>2</sup>, A. Onay<sup>3</sup>, and Y. Ozden Çiftçi<sup>1</sup>. <sup>1</sup>Gebze Institute of Technology Faculty of Science, Department of Molecular Biology and Genetics,

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Determination of genetic stability of micropropagated plants provides information about the applicability of the developed technique for mass propagation of mature pistachio trees. A non-destructive assay for hydroxyl radicals, using DMSO as a radical trap, was used to determine hydroxyl radical formation during tissue culture. The result showed that shoot tips excised from mature pistachio buds were subjected to oxidative stress especially in the beginning of culture period. We investigated genetic stability of long-term (5-7 years) micropropagated plantlets, via apical bud proliferation followed by organogenesis using RAPD, ISSR and AFLP markers to obtain possible effects of the oxidative stress or other tissue culture induced stresses. Each molecular marker system showed genetic polymorphism between donor plant and its clones. 15 RAPD primers produced 141 scorable fragments and PIC value of the primers was 0.226. Average genetic similarity values was determined as 0.84. 7 ISSR primers produced 62 scorable fragments and PIC value of the primers was 0.220. Average of genetic similarity values was determined as 0.82. In case of AFLP marker, totally 789 scorable bands were produced by 10 AFLP primer pairs and PIC value of the primers was 0.241. Genetic similarity value of the donor plant and its clones varied from 0.57 and 0.90 and the average genetic similarity values was 0.75. Our results showed that applied marker systems are useful to reveal out specific genomic alterations associated with tissue culture variation. In conclusion, this is the first study on occurrence of genetic instability, which is slightly informative (PIC

#### P-1011

Overexpression of a Soybean Salicylic Acid Methyltransferase Gene Confers Resistance to Soybean Cyst Nematode. J. LIN<sup>1</sup>, M. Mazarei<sup>1</sup>, N. Zhao<sup>2</sup>, M. Rudis<sup>1</sup>, V. Pantalone<sup>1</sup>, P. Arelli<sup>3</sup>, F. Chen<sup>1</sup>, and C. N. Stewart<sup>1</sup>. <sup>1</sup>Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996; <sup>2</sup>Biosciences Division, Oak Ridge National Laboratory, Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831; and <sup>3</sup>USDA-ARS-MSA, 605 Airways Blvd., Jackson, TN. Email: jlin11@utk.edu

A salicylic acid methyl transferase gene (*GmSAMT1*) was identified from soybean as a candidate soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN) defense-related gene in our previous analysis using GeneChip microarray experiments. Using in vitro enzyme assay, the *Escherichia coli*

expressed *GmSAMT1* was confirmed to function as salicylic acid methyl transferase catalyzing the conversion of salicylic acid to methyl salicylate. The transgenic hairy roots overexpressing *GmSAMT1* were tested for the soybean response to SCN race 2, race 3 and race 5 by analyzing the proportion of nematodes developed beyond J3 stage at 15 days post-inoculation. Different levels of increased resistance were found. Stably-transformed soybean were produced using *Agrobacterium tumefaciens* whereby *GmSAMT1* was overexpressed and T<sub>2</sub> plants were assayed against SCN race 2 and race 3, respectively. The elevated resistance was also detected through analyzing the nematode reproduction at 35 and 42 days post-inoculation. Taken together, these data suggest that the increase in expression of *GmSAMT1* contributed to the soybean resistance against SCN.

#### P-1012

Twenty Five Years of Innovations on Date Palm Micropropagation. S. CHELLAN, J. Sudharsan, and J. Ashkanani. Kuwait Institute for Scientific Research, P. O. Box 24885, Safat 13109, KUWAIT. Email: schellan@kisir.edu.kw

Date palm micropropagation is of high importance in date producing countries. Basic culture media and protocol developed during 1979 was adopted or modified and utilized worldwide. The true-to-type fruiting from the first set of plantation was confirmed in 1990. However, variations and abnormalities were reported from the latter micropropagated date palm plantations. The senior author of this study conducted research on date palm micropropagation continuously for the last 25 years. During this period, date palm micropropagation technology for commercialization was refined through innovative findings. Thousands of plantlets were produced from leaf primordial and shoot meristem explants and commercialized. Date palm *in vitro* plant production was influenced by several internal and external factors including *ex vitro* and *in vitro* conditions. Specific requirements during stages of micropropagation, such as the explant selection, sterile explant preparation, culture media, establishment of *in vitro* cultures, somatic embryogenesis or organogenesis, *in vitro* plant production, adventitious rooting, acclimatization and field evaluation were studied and refined. New approaches for increased plant production at low-cost without changing the high quality produce for commercial purpose were developed and adopted during this period.

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#### P-1013

Development and Comparison of Two Different PVS2-based Procedures for Cryopreservation of Sugarcane (*Saccharum* spp.) Germplasm. E. KAYA<sup>1</sup>, F. Souza<sup>2</sup>, L. Rodrigues<sup>3</sup>, A. Alves<sup>4</sup>, E. Ozudogru<sup>5</sup>, C. Maroon-Lango<sup>6</sup>, and D. Ellis<sup>7</sup>. <sup>1</sup>Gebze Institute of Technology, Gebze Institute of Technology, Istanbul Str., Kocaeli 41400, TURKEY; <sup>2</sup>Embrapa Mandioca e Fruticultura Empresa Brasileira de Pesquisa Agropecuária (Embrapa) Cruz das Almas/BA, BRAZIL; <sup>3</sup>Universidade Federal de Lavras, Lavras, MG, BRAZIL; <sup>4</sup>Embrapa Labex-USA, USDA-ARS, Fort Collins, CO; <sup>5</sup>CNR-IVALSA, Istituto per la Valorizzazione del Legno e Delle Specie Arboree, Florence, ITALY; <sup>6</sup>USDA-APHIS, Plant Germplasm Quarantine Program, Beltsville, MD; and <sup>7</sup>International Potato Center, Lima, PERU. Email: kayaer19@gmail.com

Sugarcane germplasm is conserved as *ex situ* collections of plants in several locations in the USA and in India. However, maintenance cost of large collections is very high and plants in natural conditions are exposed to pests and pathogens as well as to natural disasters. Because of that 61% of the clones of the USA collections have been lost between 1957 and 1977. *In vitro* collections have been developed for a large number of plant species, which allow the reduction of these problems. Advances in biotechnology provide new methods for conservation of plant genetic resources and evaluation. Cryopreservation, developed during the last 25 years, is an important and the valuable method for long-term conservation of biological materials. The main advantages of cryopreservation are the simplicity and the applicability to a wide range of genotypes. The encapsulation-vitrification technique is a combination of encapsulation-dehydration and vitrification procedures, where samples are encapsulated in alginate beads, then subjected to freezing in liquid nitrogen following application of vitrification solutions. The droplet freezing method where samples are frozen in a drop of cryoprotectant is proving to be an efficient method across many species. For instance it gave the highest regeneration rates in comparative studies with different vitrification methods for yam and banana. In the present study, *in vitro* shoot tips of three *Saccharum* spp. clones (Halaii, H83-6179, NG 57-024) were successfully cryopreserved via droplet-vitrification. Two different PVS2-based procedures, i.e., encapsulation-vitrification and droplet-vitrification, were compared for shoot tips of sugarcane line Halaii, as well. 45 minutes of PVS2 exposure yielded the highest regeneration for the lines Halaii (70.9%) and NG 57-024 (63.3%), while the best regeneration rate for line H 83-6179 (76.3%) was obtained with 30 minutes of PVS2 treatment.

**P-1014**

*In Vitro* Mutagenesis and Selection of *Fusarium sacchari*-tolerant Sugarcane Plants for Biological Control of *Eldana saccharina*. T. MAHLANZA<sup>1</sup>, S. Rutherford<sup>1</sup>, S. Snyman<sup>1</sup>, and P. Watt<sup>2</sup>. <sup>1</sup>South African Sugarcane Research Institute, 170 Flanders Drive, Mount Edgecombe, Durban 4300, SOUTH AFRICA and <sup>2</sup>University of KwaZulu Natal, Westville Campus, PBag X54001, Durban 4000, SOUTH AFRICA. Email: tendekai.mahlanza@sugar.org.za

*Eldana saccharina* is a destructive pest of the sugarcane crop in South Africa. *Fusarium sacchari* PNG40 (a fungal strain harmful to *E. saccharina*) has the potential to be an endophytic biological control agent of this stalk borer. However, the fungus causes *Fusarium* stalk rot in sugarcane, thus limiting its usefulness. Sugarcane plants tolerant to *F. sacchari* PNG40 were obtained by exposing embryogenic calli (cultivar N41) to the chemical mutagen ethylmethanesulfonate (EMS), followed by *in vitro* selection during somatic embryogenesis and plantlet regeneration on media containing *F. sacchari* culture filtrates (CF). The incorporation of 100 ppm CF in the culture media at the embryo maturation stage, at germination, or at both, resulted in callus necrosis and consequent plantlet yield reduction. Subsequent trimming of the roots of regenerated plants and their exposure to 1500 ppm CF served as a further selection treatment. More plants produced from EMS-treated calli (10.2 %) displayed improved root re-growth in the presence of CF pressure than those from non-treated calli (6 %). Greenhouse tests performed by inoculating putative-tolerant plants with *F. sacchari* PNG40, re-isolation of *Fusarium* spp. from asymptomatic plants and establishment of the identity of fungal isolates as PNG40 using molecular analysis, confirmed the tolerance of the plants. Random Amplified Polymorphic DNA (RAPD) analysis of the plants revealed genetic differences between some mutants and the parent cultivar. Currently, insect bioassay trials to test the effect of PNG40 on *E. saccharina* using the tolerant genotypes which permit endophytic colonisation by the fungus are ongoing and results on stalk damage, survival and weights of artificially inoculated larvae will be presented. The *in vitro* mutagenesis and selection approach employed in the present study has potential application in generating tolerance to other diseases of sugarcane.

**P-1015**

Direct Somatic Embryogenesis and Shoot Organogenesis from Apical and Lateral Meristems of Soft Taro (*Colocasia Esculenta* Var. *esculenta*). V. VERMA. College of Micronesia-FSM, Micronesia Plant Propagation Research Center, Kosrae

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Soft taro (*Colocasia esculenta* var. *esculenta*), a member of the family Araceae, is an ancient crop grown throughout the humid tropics for its edible corms, leaves and petioles as well as for its traditional uses. A study was undertaken to develop an economically feasible, efficient, rapid and reproducible regeneration protocol for taro. Taro plants were regenerated via somatic embryogenesis and organogenesis from lateral meristems and shoot meristem, respectively. Murashige and Skoog, 1962 (MS) medium was used throughout the study. A two-step protocol was used to regenerate somatic embryos. Initially, lateral meristems were cultured on medium augmented with 10  $\mu$ M 2,4-dichlorophenoxyacetic acid (2,4-D) and 2  $\mu$ M thidiazuron (TDZ) followed by a culture phase with 5  $\mu$ M TDZ. Embryos at various developmental stages (globular, heart and torpedo shaped) were observed. In organogenesis, cultures were initiated on medium augmented with 2  $\mu$ M TDZ and then transferred on medium with 5  $\mu$ M TDZ. For further growth and subsequent multiplication, shoots were transferred on medium augmented with 5  $\mu$ M indole-3-acetic acid (IAA) and 7.5  $\mu$ M 6-benzylaminopurine (BAP). The number of multiple shoots produced from each explant after two subcultures varied from 40 to 50. MS medium augmented with 2  $\mu$ M IAA was used for rooting. Mature plants were produced from both somatic embryos as well as from induced shoots. A 12-h photoperiod with a temperature of 28°C day and 24°C night, light intensity of 40  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, and 55±5% relative humidity were maintained for multiplication. Rooted plants were transferred to soil: vermiculate (1:1, v/v) mixture in pots and acclimatized with 100% survival rate. Fully acclimatized plants were planted in the field

**P-1016**

Improvement of Sunflower Transformation Through the Use of Cotyledon Explants from Mature Seeds. Z. ZHANG. OARDC/The Ohio State University, 1680, Madison Ave, Williams Hall, Wooster, OH 44691. Email: zhang.653@buckeyemail.osu.edu

Despite the high susceptibility of sunflower tissue to *Agrobacterium*, a reliable and efficient transformation protocol has not been available for routine gene characterization and manipulation in this plant. In order to use biotechnological tools for improvement of sunflower, sunflower transformation protocols must first be developed and optimized. A sunflower line RHA280 was identified as having high response to shoot induction, with an average of over 80 shoot primordia obtained from each of the 6 cotyledon explants, isolated from each seed. Sonication Assisted *Agrobacterium*-mediated Transformation

(SAAT) was evaluated to improve the transformation efficiency, and a construct containing a *gfp* reporter gene driven by a sunflower polyubiquitin gene promoter (HaUbi) was used to monitor transgenic cell proliferation and shoot development. Imbibition of dry cotyledons in liquid medium for 1 d seemed to enhance their susceptibility to SAAT, by softening the dry cotyledon, leading to better wounding. A selection scheme in which hygromycin was applied immediately at the end of co-culture, instead of waiting 5 d after co-culture, increased the frequency of transgenic shoot production. Additionally, use of an intermediate level of hygromycin (15 mg/L) applied directly after co-culture yielded over 20% explants with transgenic shoot primordia, and over one transgenic shoots per explant on average. Due to the problems with shoot elongation and shoot rooting from cotyledon explants, a micrografting approach was also developed for the recovery of transgenic shoots, with a success rate over 30%. Transgenic shoots were also obtained from cotyledon tissue of cotyledon node explants which contained embryo shoot apex and the proximal end of cotyledon. The shoot primordia could elongate more easily than those from cotyledon explants even though the frequency of adventitious shoots was much lower, and rooting of these shoots remained problematic.

#### P-1017

A Series of DNA Promoters and a Versatile Gene Expression Vector pSiM24 Useful for Transient Assays and Plant Made Products as Well as Stable Expression of Foreign Genes in Plants. I. MAITI and D. Sahoo. University of Kentucky, 1401 University Drive, KTRDC Bld-Room 224, Lexington, KY 40546. Email: imaiti@uky.edu

We have created a series of DNA promoters useful for expressing foreign genes in transgenic plants. Those promoter systems have been published in referred journals. We could make available those DNA promoters to scientific community in nation and abroad for evaluating in economically important various crop plants like soybean, tomato, maize, cotton, to name a few and other plants. We have also designed and constructed a small and highly efficient binary Ti vector pSiM24 for transformation of higher plant cells. We reduced the size of the backbone of earlier binary vector pKYLXM24 (GenBank Accession No. HM036220), a derivative of pKYLX71 (Schardle et al., 1987) from 12.8 Kb to 7.1 kb. The binary vector pSiM24 is composed of following genetic elements: left and right T-DNA borders (127 and 161-bp, respectively); modified full-length transcript promoter (M24) of *Mirabilis mosaic virus* with duplicated enhancer domains (638-bp); three multiple cloning sites; 3' rbcSE9 terminator; replication functions for *Escherichia coli* (ColE1, 678-bp) and *Agrobacterium tumefaciens* (pRK2-OriV, 620-bp) and replicase trfA gene (1504-bp); selectable marker genes kanamycin resistance (KanR, nptII) gene (1338-bp) and ampicillin

resistance (AmpR, 'bla') gene (1136-bp). The pSiM24 plasmid offers a wide selection of cloning sites, high copy numbers in *Escherichia coli* and high cloning capacity for easily manipulation of different genetic elements. It has been fully tested in transferring the transgene (GFP and GUS) in both transiently (Agro-infiltration, protoplast electroporation and biolistic), and stably in plant systems (Arabidopsis and tobacco) using both *Agrobacterium* and biolistic procedures, hence this would be useful for both nuclear transformation, and plant made products (PMP) applications. In addition, the pSiM24 plasmid can act as a platform for various applications like gene expression studies and different promoter expression analyses. The fully annotated sequence information of the pSiM24 is provided in NCBI data base (GenBank accession no. KF032933).

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#### P-1018

Optimizing Critical Factors Affecting *Agrobacterium* Mediated Genetic Transformation of Cassava (*Manihot Esculanta*) Cultivars. R. CHAUHAN, G. Beyene, and N. Taylor. Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132. Email: rchauhan@danforthcenter.org

Key steps in achieving successful transformation of cassava are to establish optimal conditions for T-DNA transfer while retaining viability and capacity for proliferation of transformed cells after *Agrobacterium* co-culture. Critical factors influencing transformability of cassava cultivars TME204 and Ebwanatereka were studied. Introduction of the antibiotic moxalactamin the final subculture cycle of friable embryogenic callus prior to *Agrobacterium* infection increased transformation efficiency over 50 fold. The average number of GFP dividing units recovered per ml settled cell volume obtained without moxalactam pretreatment were two as compared to over 100 from moxalactam treated tissue. *Agrobacterium* strain LBA4404 was found to be more effective than EHA105 and AGL-1. Diluting the LBA4404 suspensions to below OD<sub>600nm</sub> 0.5 increased the transformation rate. An average of 33 plants were obtained per ml SCV using an OD<sub>600nm</sub> of 0.05 compared to 15 plants OD<sub>600nm</sub> 0.5.

#### P-1019

Constitutive Expression of *Osa-miR528* Alters Plant Development and Enhances Plant Tolerance to Salinity Stress and Nitrogen Starvation in Transgenic Creeping Bentgrass. S. YUAN, Z. Li, D. Li, Q. Hu, and H. Luo. Clemson University, 105 Collings Street BRC, Clemson, SC 29634. Email: syuan@clemson.edu



One of the adaptive mechanisms that plants have evolved in stress response is mediated by microRNAs (miRNAs). Large-scale expression analyses reveal that a conserved monocot-specific miRNA, miR528 has the potential of mediating multiple stress responses. So far, however, the experiment support is lacking. To determine the role miR528 plays in plant response to abiotic stress, and the underlying molecular mechanisms, we analyzed its impacts on a perennial monocot crop species, creeping bentgrass (*Agrostis stolonifera* L.). Our stem-loop RT-PCR analysis demonstrates that the expression of miR528 was up-regulated under salt and drought stresses, but down-regulated under N deficiency in the wild type (WT) creeping bentgrass. Furthermore, we generated transgenic creeping bentgrass plants overexpressing a rice miR528 (*Osa-miR528*). Our data indicate that both plant development and stress response have been altered in transgenic plants. Morphologically, transgenic plants display shorter internodes, more tillers and upright growth than WT controls. Resistance to salt stress and N deficiency was enhanced in transgenics. Improved salt stress resistance was associated with increased water retention, cell membrane integrity, and chlorophyll content, while enhanced tolerance to N deficiency was associated with increased biomass, total nitrogen and chlorophyll content. Gene expression analysis identified four putative target genes, *AsASO*, *AsLACII*, *AsCSD2*, and *AsLPRI*, which function in oxidation-reduction. Additionally, the expression level of *AsNir* encoding for nitrite reductase is increased in transgenic plants compared to WT controls, which might contribute to enhanced N use efficiency. The data obtained indicate the potential of manipulating *miR528* in improving plant abiotic stress resistance.

#### P-1020

An Intragenic Approach for the Genetic Improvement of Citrus. M. DUTT<sup>1</sup> and J. Grosser<sup>2</sup>. <sup>1</sup>UF/IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850 and <sup>2</sup>University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850. Email: manjul@ufl.edu

To develop a genetically modified sweet orange (*Citrus sinensis* (L.) Osbeck) acceptable to the consumer, juice processors and packing houses, utilization of precision breeding strategies resulting in the development of intragenic citrus is essential. This intragenic plant would have genetic material derived from sweet orange itself or from closely related species capable of sexual hybridization such as other citrus species, trifoliate oranges and kumquats. Use of this gene pool,

which is similar to other genetic improvement methods such as conventional breeding and protoplast fusion, could lead to easier approval of a genetically modified product. We are exploring several strategies to create intragenic citrus. We have evaluated a single base pair mutant (A122V) of the *Citrus sinensis* acetolactate synthase gene as a herbicide resistance selectable marker gene. We have developed a transformation system to generate reporter gene expression free citrus by coupling a visual anthocyanin producing transcriptional factor gene with an embryo specific promoter to regenerate genetically modified plants that have the marker gene switched off. We are utilizing the cre-lox system to remove selectable marker genes from plants following transformation. In addition, we are also evaluating a large number of citrus derived promoters, genes and terminators to create an all citrus transformation vector, which is being incorporated into sweet oranges and mandarins by conventional *Agrobacterium* mediated and our unique protoplast transformation methods.

#### P-1021

NMR-based Metabolomics Profile Comparison to Distinguish Between Embryogenic and Non-Embryogenic Callus Tissue of Sugarcane at the Biochemical Level. K. CHOWDHURY, I. Mahmud, B. Shrestha, and A. Boroujerdi. Claflin University, 400 Magnolia St., Biology Department, Orangeburg, SC 29115. Email: kchowdhury@claflin.edu

Nuclear magnetic resonance (NMR)-based metabolomic profiles comparison of embryogenic and non-embryogenic calli of sugarcane was compared using principal component analysis (PCA) to determine possible relationship between certain metabolites and embryogenesis. Mahalanobis distance analysis showed significant score plot differences between the embryogenic and non-embryogenic callus groups. Significantly different spectral buckets and their corresponding metabolites have been identified using volcano and loading plot analyses where glucose, fructose, sucrose, maltose, and alanine were observed as increased concentration and melatonin, o-acetylcholine, malonate, lysine, 4-aminobutyrate, asparagine, aspartate, beta-alanine, citrate, glutamine, 2-hydroxyisobutyrate, valine, and isoleucine were observed as decreased concentration in embryogenic callus when compared with non-embryogenic callus. Possible role of these significantly differing metabolites in sugarcane somatic embryogenesis is discussed.