In Vitro Cell.Dev.Biol.—Animal
DOI 10.1007/s11626-012-9505-0

Plant Contributed Papers

P-1000

Optimized Site-specific Recombination Systems for Improved Plant Biotechnology. MIN SHAO[1,2] and James Thomson[1]. [1]USDA-WRRC-ARS Crop Improvement and Utilization, Albany, CA 94710 and [2]UC Davis Department of Plant Sciences, Davis, CA 95616. Email: James.Thomson@ars.usda.gov

Site-specific recombinases are enzymes which can precisely manipulate DNA. Certain recombinases have the ability to integrate a target DNA sequence into the plant genome as well as remove unwanted DNA, such as selectable marker genes, during genetic engineering. As biotechnology is becoming more readily utilized to confer agronomically important traits in crops, optimization of site-specific recombination systems will facilitate precise genetic engineering and potentially speed current deregulation procedures. Our lab has developed and tested a number of novel recombinase systems for plant engineering. Here we present a comparison of activities among wildtype, mutagenesis-enhanced and codon-optimized versions of FLP, R, phiC31, Bxb1, ParA and U153 recombinase systems using a transient excision-based dual luciferase expression assay in onion. The activity levels from all recombinase versions were normalized against the Cre recombinase. Our results indicate that the wildtype version of FLP demonstrates only a 2 % activity rating; however, the mutagenesis-enhanced version of FLP (FLPe) showed 37 % activity, and the codon-optimized version of FLP (FLPo) had an activity level of 104 %. For the R/RS recombination system, the wildtype version had 29 % activity, while the optimized version showed an efficiency of 52 % against Cre. The Bxb1 recombination system in its wildtype form had 19 % activity, while the monocot codon-optimized version demonstrated an activity level of 74 %. In phiC31, ParA and U153 systems, the excision activities of enhanced and optimized versions were all higher than their respective wildtype versions. Our results also suggest that sequences adjacent to the recombinase recognition sites can drastically affect recombination efficiency. Our research demonstrates an effective system to rapidly test recombinase activities, allowing direct comparison between systems studied. Our goal is to develop a suite of recombinase systems and applications for public utility providing more efficient molecular tools for genetic engineering in crops.

P-1001

Heat-shock Induced Marker-gene Excision by Cre/*lox* and Its Stable Inheritance in FLP/*FRT* Mediated Site-specific Gene-integration Lines of Transgenic Rice. SOUMEN NANDY[1] and Vibha Srivastava[1,2]. [1]Department of Crop, Soil & Environmental Sciences and [2]Department of Horticulture, University of Arkansas, Fayetteville, AR 72701. Email: snandy@uark.edu

Transgene containment technologies have become a major focus in current transgenic research with the marker-free transgenic technology running in the fore-front. Though selectable marker genes are necessary for efficient production of transgenic events, their presence in the transgenic products creates public and regulatory concerns. Strategies are being developed to generate marker-free transgenic plants that would help allay the concern. Here, a 'clean' transformation system, based on two independent recombination systems, FLP/*FRT* and Cre/*lox,* is reported. These recombination systems are versatile tools for precise genomic manipulations such as DNA excision and integration. By using FLP/*FRT*, site-specific integration lines were developed consisting of gene-of-interest and selection marker genes. The placement of 4 *lox* sites within the integration locus was an important part of the strategy, which primed the locus for excision of marker genes and other undesirable genes within the locus by heat-inducible Cre activity, generating a 'clean transgene locus'. Young plants of four integration lines were subjected to heat-treatment that resulted in production of the 'clean' locus. Each line transmitted the 'clean' locus to next generation, establishing 'clean' transgenic lines. The efficiency of the transmittance of 'clean' locus varied from 100 % to 40 %. This work demonstrates the integration of two applications of recombination systems, marker excision and precise transgene integration, for efficient production of 'clean' transgenic plants.

P-1002

Recombinase-mediated Technologies for Production of Plants and Microbes with Enhanced Traits. MERIDITH COOK and James Thomson. USDA-ARS-WRRC Crop Improvement and Utilization Research Unit, Albany, CA 94710. Email: Meridith.Cook@ARS.USDA.GOV

Published online: 28 April 2012

Biotechnology offers many opportunities for crop improvement. Recombinase-mediated cassette exchange (RMCE) is a strategy that can be used to genetically modify plants in a precise manner, and allows production of selectable marker-free plants. RMCE involves the use of site-specific recombinases to both integrate and excise sequences of interest. The initial step in RMCE is production of "founder" lines, which contain the recombinase recognition sites flanking a selectable marker, referred to as the "TAG" region. Transformation of the founder lines with an incoming "exchange" vector results in two recombination reactions: first, integration of the entire exchange vector occurs, and then excision of the region between the excision-specific recognition sites takes place. The RMCE strategy used in this study was first tested in the eukaryotic model system *Saccharomyces cerevisiae* (brewers' yeast). Yeast founder lines were produced and subsequently transformed with an exchange vector. Integration of the exchange vector was followed by the predicted excision event, resulting in the "swapping" of the selectable marker for the gene of interest. Both events were verified using PCR and phenotypic assays. This technology is currently being utilized to engineer yeast for improved biofuel production. Furthermore, these yeast experiments demonstrate that the RMCE strategy is functional and give support for further testing in plants. The plant system used to test RMCE was the oilseed crop *Camelina sativa*. Founder *C. sativa* lines containing recombinase recognition sites were produced using the herbicide resistance gene *bar* or the antibiotic resistance gene *hptII* and were confirmed using PCR and Southern blot analyses. An exchange vector is currently being investigated that will allow site-specific integration of a gene(s) of interest followed by excision of the unneeded markers. The RMCE strategy presented here has widespread applications in both plant and microbial systems for precise engineering of enhanced traits of interest.

P-1003

Improving End Use Functionality of Grain Sorghum. TEJINDER KUMAR MALL[1], Ismail Dweikat[1], Shirley Sato[2], Zhengxiang Ge[2], Natalya Nersian[2], Han Chen[2], Tom Elthon[1], Brian Loerger[3], Mike Tilley[3], Scott Bean[3], and Tom Clemente[1,2,4]. [1]Department of Agronomy & Horticulture, University of Nebraska-Lincoln; [2]Center for Biotechnology, University of Nebraska-Lincoln; [3]USDA/ARS, Kansas State University; and [4]Center for Plant Science Innovation, University of Nebraska-Lincoln. Email: tclemente1@unl.edu tejinderkumar@rediffmail.com

Sorghum serves as a staple food and feed source throughout the world. However, the grain has a few drawbacks in relation to functionality and digestibility. Sorghum prolamins, termed kafirins, are categorized as α, β, and γ kafirins. These are co-translationally translocated to the endoplasmic reticulum where they are assembled into discrete protein bodies. These protein bodies tend to be poorly digestible with low functionality in food and feed applications. To address these issues we initiated genetic engineering approaches to deliberately alter seed storage protein characteristics. To this end we first introduced wheat high-molecular-weight glutenin subunit (HMW-GS), which are known to have impact on end-use functionality of flour. In separate complementary set of sorghum transformations we introduced genetic cassettes designed to specifically down-regulate the sorghum alpha and gamma kafirins. These genetic cassettes were transformed into sorghum via Agrobacterium-mediated genetic transformation system. The successful introduction of genetic cassettes into sorghum was confirmed with southern blots and expression of related gene was confirmed at RNA and protein level. All the transgenic events showed improvement in protein digestibility of the uncooked ground grain. Transgenic events downregulated for alpha kafirin showed presence of distorted protein bodies in the immature seed. Mature seeds showed changed amino acid profile in various transgenic events. Selected transgenic sorghum events are currently being bulked-up to allow for additional down-stream functionality testing of the derived flour. The long-term goal is to stack the HMW-GS trait with the modulated kafirins events, as a means to address both end-use functionality and digestibility.

P-1004

Gene Silencing in Multiple *Arabidopsis* Genes by Terminator-less Transgene Constructs. M. A. AKBUDAK[1], S. J. Nicholson[1,4], J. Thomas[1] and V. Srivastava[1,2,3]. [1]Department of Crop, Soil & Environmental Sciences; [2]Cell & Molecular Biology Program; [3]Department of Horticulture, University of Arkansas, Fayetteville, AR 72701; and [4]Present address: USDA-ARS, 1301 N. Western Rd, Stillwater, OK 74075. Email: aydin@uark.edu

Transgene-mediated gene silencing is a prominent biotechnology and research tool. There are several RNAi-mediated techniques available for silencing genes in plants. The basis of all these techniques is to stimulate the generation of double stranded RNA precursors in the cell, which are recognized by the surveillance system in the cell and marked for degradation by the dicer family RNases into siRNAs. Improperly terminated, unpolyadenylated RNA are potent precursors of double stranded RNA and therefore considered as silencing triggers in plants. Such transcripts can easily be synthesized from transgene constructs lacking transcription-terminator signals (terminator-less constructs). The present study determined the efficiency of terminator-less constructs on 4 different genes in Arabidopsis, VAR2, AP1, BRI1 and CO. Expression of terminator-less VAR2, AP1, BRI1 and CO constructs resulted in dramatic

decline in transcript level up to 90 % compared to wild type. This suppression was accompanied with mutant phenotype in selected transgenic lines. Thus, terminator-less constructs are efficient tools for inducing silencing of genes in plants.

P-1005

Wound-induction of GmERF Promoters in Soybean. CARLOS M. HERNANDEZ-GARCIA[1], Cheri Nemes[1], Michelle L. Jones[1], Paul J. Rushton[2], and John J. Finer[1]. [1]Department of Horticulture and Crop Science, OARDC/ The Ohio State University, 1680 Madison Ave., Wooster, OH 44691 and. [2]Department of Biology and Microbiology, South Dakota State University, Brookings, SD 57007. Email: hernandez-garcia.1@buckeyemail.osu.edu

Promoters are the main regulators of gene expression at the transcriptional level. In soybean only a few promoters have been well studied, and wound-inducible promoters have received little to no attention. As plants show similar responses to mechanical wounding, pathogen invasion and damage from chewing insects, studies of wound-inducible promoters could provide insights to the mechanisms of regulation of stress-responsive genes. Here we studied induction of ten *GmERF* (*Glycine max Ethylene Response Factor*) genes and their promoters. Although the *ERF* gene family is one of the best characterized stress-responsive gene families, reports describing ERF promoters are surprisingly scarce. In this study, transcripts of *GmERF* genes accumulated to high levels in soybean seedlings wounded or treated with either methyl jasmonate or ethylene. Four GmERF promoters were subsequently isolated, fused with *GFP*, and reintroduced into soybean for expression analysis. In transgenic plants, the studied GmERF promoters directed low basal GFP expression in roots, pods, the epidermis, and vascular tissues. However, these promoters were highly inducible by wounding in cotyledons, hypocotyls and leaves. Wound-inducible expression was not detected in wounded roots, indicating that wound induction of these GmERF promoters is organ-dependant. Further deletion analysis for the GmERF3 promoter suggests complex regulation of expression. Candidate promoter regions likely responsible for induction and enhancement of gene expression were also identified using transient expression and expression in soybean hairy roots. This study increases our understanding of ERF promoter functionality and expands the toolbox of soybean wound-inducible promoters for potential use in both basic and applied research.

P-1006

Inhibition of Human Low Density Lipoprotein (LDL) Oxidation by Ginger (*Zingiber officinale*) Extracts: An *In Vitro* Study. K. D. P. P. GUNATHILAKE and H. P. Vasantha Rupasinghe. Department of Environmental Sciences, Nova Scotia Agricultural College, PO Box 550, Truro, NS, B2N 5E3, CANADA. Email: gunathilakep@nsac.ca

Ginger is a medicinal plant widely used in various herbal medicines all over the world for wide array of ailments. Currently, there is a renewed interest in ginger because of its cardio-protective properties. Oxidative modification of LDL is thought to play a key role in the pathogenesis of atherosclerosis and identifying natural bioactives which can inhibit LDL oxidation is physiopathologically important for developing functional foods for preventing atherosclerosis. In this study, we have examined the effect of ginger extracts prepared using ultrasonic-assisted extraction at 30°C for 20 min and some secondary metabolites of ginger on copper-induced oxidation of human LDL *in vitro*. Total phenolic contents and antioxidant capacity of the extracts were determined by Folin-Ciocalteau and FRAP assay, respectively. Results showed that phenolic content and the antioxidant capacity in water extract were lower than that of organic solvent extracts. The percent inhibition of LDL oxidation by ethanol, methanol, ethyl acetate, hexane and water extracts of ginger were 63.91 %, 66.24 %, 58.09 %, 54.95 %, and 42.74 %, respectively.. Phenolic bioactives of ginger; 6- gingerols, 8- gingerols, 10-gingerols and 6-shogaol, are seems to be strong inhibitors of copper induced-LDL oxidation compared with farnesene, an abundant isoprenoid of ginger. This study revealed that ginger extract inhibited copper-catalyzed LDL oxidation *in vitro* at varying levels subjected to the extraction conditions, suggesting that ginger as a potential ingredient to be used in functional foods designed for reduction of the risk of cardiovascular disease.

P-1007

MS Mineral Nutrients Limit Growth and Development of Micropropagated Red Raspberries. SUKALYA POOTHONG[1] and Barbara M. Reed[2]. [1]Department of Horticulture, 4017 ALS, Oregon State University, Corvallis, OR 97331 and [2]USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333–2521. Email: sukalya_p@hotmail.com; Barbara.Reed@ars.usda.gov

In vitro propagation is important for rapid multiplication of a wide range of nursery crops, including red raspberry. The genetic variation of the many red raspberry cultivars makes it difficult to successfully apply one growth medium for all. Although most cultivars will grow on Murashige and Skoog (1962) medium (MS), some display stunting, hyperhydricity, discoloration, callus, leaf spots, or necrosis. The poor growth symptoms are likely caused by suboptimum

concentrations of important mineral salts. This study investigated the effect of five groups of MS mineral salts in a 5-dimensional experimental design. Five red raspberry cultivars were tested with 46 treatments selected from the design sphere of five concentrations each of NH_4NO_3, KNO_3, meso elements ($CaCl_2$, KH_2PO_4 and $MgSO_4$), minor elements (Zn-Mn-Cu-Co-Mo-B-I), and iron. Shoot cultures were grown for three cycles of 3-wk transfers before data was taken. Plant quality, multiplication, shoot length and 5 other parameters were evaluated. Results varied by cultivar for some characteristics, but all cultivars had improved growth or appearance with some treatments compared to MS. Mesos were significantly the most limiting factor associated with changes in plant quality, multiplication and shoot length in all cultivars. Increasing iron and minors significantly decreased growth and multiplication in all cultivars except 'Willamette'. Nitrogen effects varied with the cultivar. Increasing NH_4NO_3 and KNO_3 decreased shoot number in 'Canby', 'Indian Summer' and 'Nootka' but only KNO_3 affected the multiplication in 'Trailblazer' and 'Willamette'. Future experiments will address optimizing mesos and nitrogen ratios to improve media for development of red raspberry micropropagation.

P-1008

An Improved Temporary Immersion Bioreactor Design for Plant Tissue Culture Propagation. SYDNEY SHAW, Matt Curtis, Sergio Florez, Jeff Larsen, and Wayne R. Curtis. The Pennsylvania State University, Department of Chemical Engineering, University Park, PA. Email: sydneyshaw@psu.edu, wrc2@psu.edu

Temporary immersion bioreactors (TIB) have been used extensively for plant propagation. Nearly all designs have utilized pneumatics (air pressure) to move the culture media either between chambers or between compartments to provide intermittent immersion of the plant tissue. We report here a design that overcomes two disadvantages of typical designs: (1) large volumes of gas use, and (2) a rigid culture vessel. By using a media reservoir that is raised and lowered, the movement of liquid is completely decoupled from gas flow rates. Since the culture vessels are not pressurized, the compartment containing the plant tissue can be constructed from a simple plastic bag. The ability to minimize gas flows provides efficient use of gas mixtures such as elevated oxygen or CO2 to enhance plant tissue culture growth. Liquid flows are observed to induce small but significant suction pressures on the order of several inches of water, which introduces the potential for contamination. This can be overcome by a simple inexpensive manifold design for gas delivery to multiple reactors at very low flow rates. The media reservoir manipulation is accomplished with an inexpensive stepper motor and pulley configuration that includes the ability to provide remote monitoring. Progress on developing this reactor system using seedless watermelon tissue will be presented. Two goals of this work are to achieve superior growth to static agar culture (as demonstrated with a rigid flask TIB operated pneumatically), and photoautotrophic growth in the absence of sugar in the culture media.

P-1009

Agrobacterium-mediated Transformation and Regeneration of Pumpkin Ash (*Fraxinus profunda*) Hypocotyls. MICAH E. STEVENS[1] and Paula M. Pijut[2]. [1]Purdue University, Dept. of Forestry and Natural Resources, Hardwood Tree Improvement and Regeneration Center (HTIRC), 715 West State St., West Lafayette, IN 47907 and [2]USDA Forest Service, Northern Research Station, HTIRC, 715 West State St., West Lafayette, IN 47907. Email: steven31@purdue.edu or ppijut@purdue.edu

Fraxinus profunda is both ecologically and economically important and faces extirpation from attack by the non-native emerald ash borer (EAB). The disjointed native range of pumpkin ash (PA) confined mainly to wetlands, floodplains, or river bottoms of the eastern United States further exacerbates this threat, and PA has been listed as a threatened or endangered species in several states. The EAB larvae feed centripetally under the bark effectively girdling and eventually killing an infested tree. There are no reports of innate resistance in any North American *Fraxinus* species resulting in the loss of millions of trees. Ash containing genes that impart resistance to the EAB would be of great economic and ecological importance to landowners, forest and conservation managers, as well as to the lumber industry. The objective of this research was to develop a protocol for genetic transformation and regeneration of PA. Hypocotyls isolated from mature seeds were cultured for 3–5 d on Murashige and Skoog (MS) medium with 22.2 μM 6-benzyladenine (BA), 4.5 μM thidiazuron (TDZ), 50 mg L^{-1} adenine hemisulfate (AS), and 10 % coconut water (CW). Hypocotyls were then exposed to *Agrobacterium* strain EHA105 containing the pq35GR vector, with both a β-glucuronidase (GUS) and neomycin phosphotransferase (*nptII*) gene. Hypocotyls were transformed in a bacterial suspension with 100 μM acetosyringone, 90 s sonication, and 10 min vacuum-infiltration. After 2–3 d co-culture, hypocotyls were rinsed to remove excess *Agrobacterium* prior to culture on shoot regeneration medium. Adventitious shoots regenerated on MS medium with 13.3 or 22.2 μM BA, 4.5 μM TDZ, 50 mg L^{-1} AS, 10 % CW, 400 mg L^{-1} timentin, and 20 mg L^{-1} kanamycin. Results of a replicated

factorial experiment indicated that 400 mg L^{-1} timentin was optimal for control of *Agrobacterium* growth, while 20 mg L^{-1} kanamycin was optimal for shoot regeneration and selection of transformed tissue. The presence of GUS and *nptII* were confirmed by polymerase chain reaction. This research provides the framework for genetic transformation of PA with a gene specific for EAB resistance.

P-1010

Development of P1 Gene Constructs to Confer Broad, Effective and Durable Resistance against Rice Yellow Mottle Virus. OLALEKAN BANWO[1,3], Martina Paape[2] and Stephan Winter[2]. [1]Department of Crop Protection, Ahmadu Bello University, Zaria, NIGERIA; [2]DSMZ Plant Virus Department, c/o Julius Kühn Research Institute, Braunschweig, GERMANY; and [3]current address: Arkansas Biosciences Institute, College of Agriculture and Technology, Arkansas State University, Jonesboro, AR. Email: obanwo@astate.edu or banleks@yahoo.co.uk

Rice yellow mottle virus (RYMV) is endemic to Africa. It is the major pathogen of rice and a very serious problem for African rice growers. Infection of susceptible lowland cultivars with RYMV leads to total yield loss. To date, there are only few cultivars with a natural resistance controlled by the recessive rymv 1 locus. The rymv 1 encodes the eukaryotic translation initiation factor eIF (iso) 4G. Comparison of susceptible and resistant varieties appears either as substitution or short deletion of amino acids in the resistant alleles rymv 1–2;1–3;1–4. Since several RYMV isolates were able to break this natural resistance, genetic engineering approaches were followed to introduce resistance against RYMV. However, since transgenic resistance is also not absolute and sustainable, novel strategies are needed for RYMV resistance development. This project used inverted repeat mediated RNA silencing, a powerful and unique method to confer effective and durable resistance in plants against RNA viruses. The four different hpRYMV P1 constructs designed and tested exhibited silencing of P1. The silencing was complete and specific to P1 sequence and the hpRYMV is currently being used for producing transgenic rice plants to test their capability to induce resistance at high frequency.

P-1011

Micro-RNA Regulation for Boron Toxicity Tolerance in Barley. TURGAY UNVER[1], Emine Gulden Erkilic[1], Vahap Eldem[2], Serdal Sakcali[3], Arif Ipek[1], Serkan Uranbey[1], and Sebahattin Ozcan[4]. [1]Cankiri Karatekin University, Faculty of Science, Department of Biology, Cankiri, TURKEY; [2]Istanbul University, Faculty of Science, Department of Biology, Istanbul, TURKEY; [3]Fatih University, Faculty of Arts and Science, Department of Biology, Istanbul, TURKEY; and [4]Ankara University, Faculty of Agriculture, Department of Field Crops, Ankara, TURKEY. Email: turgayunver@gmail.com

Micro-RNAs (miRNA) are small, endogeneuos and regulatory RNAs playing important roles in plant development and stress responses. Boron toxicity is one of the important abiotic stress factors for plant growth and development. Barley (*Hordeum vulgare*) is a mainly produced crop plant for human nutrition and animal feeding. To investigate the regulatory roles of miRNAs for boron toxicity tolerance in barley a comperative approach is applied for naturally boron tolerant model barley (Sahara). On the other hand limited number of barley miRNAs have been identified to date. Excess amount of boron treated barley leaf and root samples were used for Illumina Solexa deep sequencing (miRNA identification) and degradome (miRNA-target gene discovery) sequencing. Comparative expression analyzes showed that many of the miRNAs are differentially expressed upon excess boron treatment in tissue specific manner. Target gene expressions were measured as correlated with miRNA expression upon toxic level of boron treatment. GO and KEGG analyzes for target genes have shown few pathways take roles in boron toxicity tolerans in root and leaf tissues. Quantitative RT PCR (qRT-PCR) measurements validate our miRNA and target gene expression results in boron treated barley tissues. Additionally, the genes known as playing roles in boron uptake, export and tolerans mechanisms were searched to find out their specific regulatory miRNAs via bioinformatic analyzes.

P-1012

Biotechnological Strategies for Conservation and RAPD Analysis of an Endangered Forest Tree *Wrightia tinctoria* - Important in Toy Making Industry. RAMASWAMY NANNA and Madhusudhan Kairamkonda. Kakatiya University, Warangal- 506009, INDIA. Email: swamynr.dr@gmail.com

Many artisans in Chennapatna, Etikoppaka and Kondapally (India) depend upon wood of *Wrightia tinctoria* for their lively-hood and it is used by the lacware handicraft industry. Due to lack of natural regeneration and over-exploitation, the species has become an endangered. In view of the demand from artisans for conservation of this forest tree, an attempt has been made in the present investigation by using *in vitro micro propagation*. To develop the disease-free and true-to-type of plants through mericlone technology, the shoot tip explants were cultured on MS medium supplemented with

different concentrations of BAP/Kn/TDZ as a sole growth regulator. High frequency number of multiple shoots formation was observed at 0.8 mg/L BAP with highest percentage (98 %) of response compared to all other concentrations of BAP/Kn and TDZ used. Nodal segments were cultured on MS medium supplemented with different concentrations (0.2-5-5.0 mg/L) of BAP/Kn/TDZ individually and also in combination with 0.5 mg/L IBA/IAA/NAA. BAP had shown superiority in inducing more number of multiple shoots. The plantlet regeneration through direct somatic embryogenesis from cotyledon explants was also been established in *W.tinctoria*. For *in vitro* rooting, the micro-shoots were transferred on to ½ strength MS, MSO and MS medium fortified with different concentrations of IAA/IBA/NAA. RAPD analysis has also been carried out in 8 accessions of W. *tinctoria* collected from different ecoclimatic zones by using a total of 50 RAPD primers. Among all these primers, only eight primers are generated reproducible, informative and easily scorable RAPD profiles. A UPGMA dendrogram was also constructed based on the matrix of genetic distance. The *in vitro* rooted plantlets were hardened on four different types of soil mixtures. Acclimatization of *in vitro* plantlets has been established in *W. tictoria* for the first time. The development of protocol for *in vitro* micropropagation in an endangered tree species *W. tinctoria* is a break- through.

P-1013

Micropropagation of Native Plants for the Desert Rehabilitation Program in Kuwait. C. SUDHERSAN[1], A. M. Al-Dousari[2], J. Ashkanani[1], and S. Al-Melhem[1]. [1]Biotechnology Department, Food Resources and Marine Sciences Division and [2] Costal and Air Pollution Department, Environment and Urban Development Division, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, KUWAIT. Email: schellan@kisr.edu.kw

Native plants are the key components of the desert ecosystem. Desert rehabilitation program requires a large number of perennial native plant species. Production in large quantities of selected native perennial plant species in limited time is difficult and expensive in the arid regions. Therefore, *in vitro* micropropagation is an alternative process in large scale native plant production for the desert rehabilitation program. This study was undertaken to develop *in vitro* micropropagation techniques for the large scale production of Kuwait's perennial native plant species *Nitraria retusa*, *Lycium shawii* and *Ochradenus buccatus*. Stem nodal segments of the above mentioned plant species were established in *in vitro* cultures using MS culture media containing low concentrations of cytokinin (BA or Kinetin at 0.1-1.0 mg/l). Sterile nodal explants collected from the *in vitro* grown plantlets of all the three species were inoculated in different concentrations of cytokinin (BA at 0–10 mg/l) and auxin alone (2,4-D at 0–10 mg/l) or in combination with low concentration of a cytokinin (2,4-D at 0–10 mg/l with K 0.1-1 mg/l). After 30 d cultures were transferred to hormone free MS culture media for plant growth or somatic embryo germination. Somatic embryogenic callus development occurred only in the presence of 2, 4-D in the culture media. Shoot development occurred in the control treatment or with 0.1-1.0 mg/l BA or Kinetin. Somatic embryo maturation and germination occurred only in the growth hormone free MS culture medium. Somatic embryo multiplication occurred continuously when the subculture carried out frequently once in 15 d. Large number of somatic embryos was produced in limited time. However, only a small number of somatic embryos germinated into complete plantlet. Synthetic seeds of these plant species were produced by sodium alginate encapsulation technique. However, germination of synthetic seeds occurred in *in vitro* cultures and failed in soil media. A large number of plants were produced through *in vitro* stem nodal multiplication of plantlets obtained from somatic embryogenesis method and axillary shoot induction method coupled with photoautotrophic culture system. This study helped in large scale plant production for the desert rehabilitation program in Kuwait.

P-1014

Tubulin Manipulations Alter Wood Properties and Drought Tolerance Characteristics in *Populus*. RASHANT SWAMY[1], Shawn Mansfield[2], Jeng-Der Chung[3], Christopher Frost[1], Scott Harding[1], and C.-J. Tsai[1,4]. [1]Warnell School of Forestry and Natural Resources, University of Georgia, GA; [2]Department of Wood Science, University of British Columbia, CANADA; [3]Taiwan Forestry Research Institute, TAIWAN; and [4]Department of Genetics, University of Georgia, Athens, GA. Email: psswamy@uga.edu

In plants, alpha-(TUA) and beta-(TUB) tubulins are encoded by small multi-gene families. The *TUA* and *TUB* gene families have undergone comparatively unequal expansions in *Populus*. The TUA proteins of *Populus* also exhibit C terminal residues not found in other sequenced plant species. Transcript levels of a small subset of tubulin genes, including *TUA1*, are comparatively high in the developing xylem of poplar stems. *TUA1* encodes a protein with a C terminal tyrosine residue which in animals is implicated in a post-translational modification (PTM) thought to regulate tubulin function. The objectives of this study are to i) investigate the effects of ectopic expression of xylem-abundant tubulins along with their PTM mimics on growth and development and ii) to analyze the effects of transgenic manipulation on wood quality traits. We produced transgenic *Populus* plants that overexpress PTM

mimics of *TUA1* in combination with two different *TUB* genes. A beneficial effect of PTM mimics for obtaining viable tubulin transformants was discovered during production of those plants. QPCR analysis revealed that transgene transcript levels comprised a small percentage compared to endogene levels in xylem, but exceeded endogene levels in leaves. The transgenic trees were comparable to wild-type trees in terms of their growth and biomass. However, mature leaves from transgenic trees exhibited lower length-to-width aspect ratios. Under acute drought conditions, photosynthesis and transpiration rates decreased more in wild type plants than in the transgenics. Total wood lignin content was similar in transgenic and wild-type plants but lignin S/G ratio and wood density were lower in the transgenic lines. The data indicate that ectopic expression of xylem abundant tubulins can have pleotropic effects on overall plant development e.g. altered leaf expansion, stomatal behavior and wood quality traits.

P-1015

A Novel JAZ1 Protein from Peach Negatively Regulates Flower Opening and Is Not Degraded by the Ubiquitin/26S Proteasome Pathway. S. SHERIF[1,3], I. El-Sharkawy[3], J. Mathur[2], G. Paliyath[1], and S. Jayasankar[3]. Departments of [1]Plant Agriculture and [2]Molecular & Cellular Biology, University of Guelph, Guelph ON; and [3]Vineland Research Station, Department of Plant Agriculture, University of Guelph, Guelph ON, CANADA. Email: ssherif@uoguelph.ca

Flower opening is indispensable for fruit set in stone fruits where most plum, cherry and some apricot varieties are self-incompatible and require cross-pollination for fruit set. In contrast, the vast majority of peach varieties are self-fruitful and they can carry either showy chasmogamous (CH) or preanthesis cleistogamous (CL) flowers. The application of methyl jasmonate (MeJA) could fasten petal elongation and flower opening of CL varieties. Two peach accessions, namely V85331and VABM29, were used as representatives for CL and CH flowers, respectively, in order to investigate the role of JA pathway components on regulating flower opening. JA-biosynthesis genes; *e.g. Pp-LOX3*, *Pp-OPR3* and *Pp-AOC* showed slight changes in transcripts level before and after anthesis in both types of flowers. Nevertheless, the genes encode for JAZs, the key repressors of JA signaling, showed significant differences in gene expression between VABM29 and V85331. Transcripts of *Pp-JAZ1*, in particular, were more abundant in CL flowers after anthesis indicating a potential role of this gene in flower closure. To test this hypothesis, we cloned this *Pp-JAZ1* gene and overexpressed it in tobacco. Transgenic tobacco expressing *Pp-JAZ1* exhibited CL flowers. The exogenous application of MeJA could partially restore the anthesis of CL flowers, suggesting a suppressive role of Pp-JAZ1 into JA-mediated flower opening. JA-insensitivity in transgenic plants is attributed to enhanced stability of Pp-JAZ1. This became evident after Pp-JAZ1-GFP chimeric protein expressed in peach leaves remained intact after 60 min of MeJA application, while its *Arabidopsis* orthologue (At-JAZ1) was degraded completely within 30 min. Further analysis using yeast two-hybrid assay indicated that Pp-JAZ1 is not interacting with Pp-COI1, the JA receptor and the part of SCF^{COI1} ubiquitin ligase. The comparison between Pp-JAZ1 and At-JAZ1 identified amino acid substitution in Jas domain that might be essential for Pp-JAZ1 interaction with Pp-COI1 and subsequently its degradation by the ubiquitin/26S proteasome pathway.

P-1016

Tissue Culture and Induced Mutation of Giant Miscanthus. DINUM PERERA[1], Brian S. Baldwin[1], and Nancy A. Reichert[2]. [1]Dept. of Plant & Soil Sciences, Mississippi State University, Mississippi State, MS 39762 and [2]Dept. of Biological Sciences, Mississippi State University, Mississippi State, MS 39762. Email: hnp21@msstate.edu

Giant miscanthus (*Miscanthus* x*giganteus*; Mxg) is a bioenergy crop with the potential to produce liquid fuel from cellulosic biomass. Since Mxg is seed sterile, it can only be propagated vegetatively, often through rhizomes, making classical breeding techniques impossible for crop improvement. The purpose of this research was to optimize a tissue culture protocol for Mxg and to utilize the developed protocol for mutation induction. Immature inflorescence explants grown in a medium of 13.6 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.44 μM 6-benzylaminopurine (BAP) resulted in highest shoot regeneration rate. A medium containing only 2,4-D (9 μM) primarily produced direct shoots. Tissue cultured plants have been evaluated for somaclonal variations. Calli were subjected to mutagenesis using various concentrations (0.01 %, 0.1 %, 0.5 %, 1 %, 3 %) of ethyl methanesulfonate (EMS) for 45 min. Effective mutagen concentration was optimized using a dose response curve and the potential mutants were regenerated. Potential mutants will be screened and evaluated in the field for mutations and will be further analyzed for genetic variability in the lab. Optimization of culture conditions, including PGR combination and concentration resulted in efficient *in vitro* proliferation of Mxg. The optimized protocol was helpful in generating potential Mxg mutants.

P-1017

Overexpressing CBP and AVP1 Confers Drought Tolerance in Rice (*Oryza sativa*). S. Y. LEE[1,2], R. Gaxiola[3], G. Yang[4], D. Robertson[1], and R. Qu[2]. [1]Department of Plant Biology,

North Carolina State University, Campus Box 7620, Raleigh, NC 27695; [2]Department of Crop Science, North Carolina State University, Campus Box 7287, Raleigh, NC 27695; [3]Department of Cellular & Molecular Biosciences, Arizona State University, Tempe, AZ 85287; and [4]Department of Natural Resources and Environmental Design, North Carolina A&T State University, Greensboro, NC 27411. Email: slee10@ncsu.edu

Drought and salinity are the leading causes for crop losses around the globe. Calcium and other nutrient elements play important roles in plant response to drought and salinity. It was previously shown that overexpressing CBP, the calcium binding domain of the maize calreticulin gene, and AVP1, the *Arabidopsis* gene encoding H^+-pyrophosphatase each confers drought tolerance in transgenic *Arabidopsis* plants. We introduced CBP and AVP1, respectively, into rice, a model cereal crop plant, and crossed CBP rice transgenic plants with AVP1 rice transgenic plants to see what effects these two genes have on plant element homeostasis and abiotic stress response. We used inductively coupled plasma (ICP)-emission spectrometer to measure the amount of elements in transgenic rice plants and found that CBP x AVP1 transgenic rice plants had increased total calcium and phosphate content compared to that of control plants. CBP rice transgenic plants, AVP1 rice transgenic plants and CBP x AVP1 rice transgenic plants all exhibited better drought tolerance compared to control rice plants. CBP x AVP1 transgenic rice plants showed higher leaf chlorophyll content and relative water content, and less wilting after intermittently withholding water for a period of two wk. We did not, however, observe synergistic effects of CBP and AVP1 on transgenic rice plants. We are currently testing our transgenic rice plants for salt tolerance and the results will be presented at the meeting.