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Plant Contributed Papers

P-1000

Effects of Plant Growth Regulators on In Vitro Tuber Initiation and Dormancy at Different Growth Phases in White Yam (*Dioscorea rotundata*). M. O. BALOGUN[1], S. Y. Ng[2], I. Fawole[3], H. Kikuno[4], N. Q. Ng[2], and H. Shiwachi[5]. [1]Department of Crop Protection and Environmental Biology, University of Ibadan, NIGERIA; [2]FAO Regional Office for Asia and the Pacific, 39 Phra Atit Road, Bangkok 10200, THAILAND; [3]Bells University of Science and Technology, Otta, NIGERIA; [4]International Institute of Tropical Agriculture, Ibadan, NIGERIA; and [5]Department of International Agricultural Development, Tokyo University of Agriculture, JAPAN. Email: kemtoy2003@yahoo.com

Yam tuber production and dormancy are linked. They are also affected by plant growth regulators (PGRs). However, exogenous control of tuber dormancy is still inadequate. We investigated in vitro, microtuber production and dormancy at some PGR regimes applied at selected growth phases spanning the life cycle of yam. Three concentrations each of uniconazole-p (UP), gibberellic, jasmonic (JA) and naphthalene acetic (NAA) acids were applied at culturing, primary nodal complex (PNC) formation and to harvested microtubers in a split-plot design in two genotypes of white yam. Duration to 50% microtuber initiation (MI) and sprouting (SPR) were recorded. NAA applied at culturing initiated microtubers 28 d earlier than the control, but delayed it by 56 d when applied at PNC formation. GA (3 μM) applied at culturing also initiated microtubers 28 d earlier than the control in TDr 93-23. JA (3 μM) applied at PNC formation delayed microtuber initiation by 56 d in TDr 608 while 0.3 μM applied at culturing induced earlier tuber initiation than the control in TDr93-23. The effects of JA and NAA on sprouting of microtubers were not significant. All regimes of UP inhibited microtuber production, except 1.7 μM UP applied at PNC formation with MI of 126 d in TDr 608. Microtubers produced from cultures treated with 1.7 μM UP at PNC formation sprouted 90 d earlier than the control in TDr 608, while those produced when cultures were treated with 0.15 μM GA at culturing or PNC formation did not sprout for 376 d post culturing. These results suggest an important role for GA and its inhibitor UP applied at different growth phases in in vitro microtuber initiation and dormancy.

P-1001

FLPe-mediated Marker Gene Excision from Site-specific Gene Integration Locus in Rice. M. AYDIN AKBUDAK[1,2] and Vibha Srivastava[1,2,3]. [1]Department of Crop, Soil & Environmental Sciences, [2]Cell & Molecular Biology Program, and [3]Department of Horticulture, University of Arkansas, Fayetteville, AR 72701. Email: aydin@uark.edu

Site Specific Recombination (SSR) systems carry out precise recombination between target DNA sequence resulting in DNA integration or excision depending on the orientation and position of the target recombination sites. This principle has been applied to drive integration of transgenes into the engineered genomic sites and removal of marker genes in plant cells. These two major applications have been individually developed using different SSR systems such as Cre–*lox*, FLP–*FRT*, and R–*RS*. In the present work, a molecular strategy for combining the two applications was tested. This strategy was designed to accomplish Cre–*lox*-mediated gene integration followed by FLP–*FRT*-mediated marker excision to generate a marker-free site-specific integration locus. Two site-specific integration loci containing FRT sites to remove marker gene

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were developed to study the efficiency of FLP-FRT mediated marker excision. First, wild-type FLP (FLPwt) was introduced either transiently or constitutively to initiate *FRT* x *FRT* recombination. FLPwt-mediated marker excision on two integration lines resulted only in poor to undetectable excision of marker genes. Next, the enhanced (thermo-stable) version of FLP protein, FLPe, was introduced in the two lines. FLPe catalyzed efficient excision of marker genes that was detected by PCR and Southern analysis. The excision efficiency as calculated by real time PCR was found to be ~100% in majority of the cases. Thus, FLPe–*FRT* system is suitable for developing a robust marker-removal technology for plants.

P-1002

Overexpression of a Cyanobacterial Flavodoxin in Transgenic Creeping Bentgrass (*Agrostis stolonifera* L) Leads to Enhanced Drought Tolerance. ZHIGANG LI, Archana Rangabashyam, Qian Hu, Man Zhou, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: hluo@clemson.edu

Flavodoxin (Fld) plays an important role in photosynthetic microorganisms as an electron carrier flavoprotein. It can be induced in response to undesirable environmental stress and has been demonstrated to be able to efficiently replace the stress-sensitive plant chloroplast ferredoxin (Fd) in most electron transfer processes. When introduced into tobacco, transgenic plants displayed enhanced tolerance to various sources of abiotic stress. We have studied the feasibility of using cyanobacterial Fld in perennial grasses for improving plant performance under environmental adversities. We generated transgenic creeping bentgrass plants, in which the overexpressed Fld was directed to chloroplast by a pea rbcS transit peptide. Twelve of the eighteen independent transgenic (TG) plants exhibited dramatically improved drought tolerance compared to non-transgenic wild type (WT) plants. The enhanced drought tolerance was associated with the higher relative water content and lower electrolyte leakage. Under water deficient conditions, the biomass increments in the TG plants were higher than in WT controls, and the TG plants had higher chlorophyll contents than WT controls under both normal and drought stress conditions. Moreover, transgenic creeping bentgrass plants also exhibited enhanced performance under other abiotic stresses including heat and salinity. Our results demonstrated that overexpression of cyanobacterial Fld gene in transgenic turfgrass significantly impacted plant response to adverse environmental conditions, leading to enhanced performance under water, salinity and temperature stresses. This points to the promising potential of using similar strategy for genetic engineering of food crops and other environmentally and economically important perennial grasses, enhancing agriculture production.

P-1003

Flower and Fruit Developmental Defects Caused by a Spontaneous DNA Deletion in a *SEPALLATA3*-like Gene in *Fragaria vesca*. S. H. HOLT [1,2], Y. Dan[1,2], V. Shulaev[3], and R. E. Veilleux[1]. [1]Department of Horticulture, Virginia Polytechnic Institute & State University, Blacksburg, VA; [2]The Institute for Sustainable and Renewable Resources, The Institute for Advanced Learning & Research, Danville, VA; and [3]Department of Biological Sciences, University of North Texas, Denton, TX. Email: holtsh@vt.edu

Floral morphology and development are critical in the plant life cycle. Genetic control of floral patterning depends upon gene interactions, orchestrated by the MADS box transcription factors (TFs). These TFs are divided into five classes (ABCDE) of genes corresponding with the ABCDE model of floral morphology. These genes have overlapping function across the floral whorls and are necessary for proper organ development. Understanding how each TF impacts floral morphology has been greatly aided by the use of knockout mutants, either caused by random genetic mutation or intentional T-DNA insertional mutagenesis. *Fragaria vesca* L. is a rapidly developing model for Rosaceae and other fruit crops. This diploid strawberry possesses simple flowers and fleshy fruits that provide an excellent opportunity for studying MADS box TFs. Phenotypic screening of a T_1 pCAMBIA insertional mutant population revealed a floral morphology mutation in *F. vesca* named *Green Petal* (GP). The phenotypic effects of the GP mutation include sepaloid petal morphology, pollen abortion, indehiscent anthers, spontaneous conversion of anthers to sepals, presence of trichomes on pistils, green carpels and ectopic fruit formation, thus affecting all of the three inner whorls. Southern analysis indicated that two T-DNA insertions were present in the GP mutant line. The flanking regions of the T-DNA insertions were sequenced by HiTAIL PCR. T_1 Segregation analysis through T-DNA Zygosity PCR showed that neither T-DNA insertion associated with the GP phenotype. Based on the GP phenotype, we selected 30 MADS box TFs from strawberry as potential candidate genes responsible for this phenotype. RT-PCR of floral bud cDNA revealed a 37 bp deletion of the C-terminal domain in a putative *SEPALLATA3* E-class MADS TF. Segregation analysis confirmed association of the deletion with the GP phenotype. The wide range of expression of the mutation suggests that this gene's

function may affect many downstream processes, offering new insight into flower development.

P-1004

RNA Interference Suppresses Lignin Biosynthetic Genes Caffeic Acid 3-O-methyltransferase (COMT) and/or 4-Coumarate-CoA Ligase (4CL) in Sugarcane. J. H. JUNG, Y. Xiong, J. Y. Kim, W. Fouad, W. Vermerris, M. Gallo, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program and Genetics Institute, University of Florida - IFAS, Gainesville, FL. Email: Altpeter@ufl.edu

A large amount of lignocellulosic biomass such as leaf litter residues and bagasse are generated during the sugarcane harvest or after the sugar refining process, respectively. Therefore, lignocellulosic biomass from leaf and processing residues will likely become a valuable feedstock for future biofuel production. However, lignin is recognized as the major limitation to efficient conversion of lignocellulosic biomass to biofuel. Therefore, altering lignin composition or reducing lignin content via RNAi suppression of lignin biosynthetic genes is a promising strategy to increase the efficiency of biofuel production from lignocellulosic sugarcane residues. In the lignin pathway, 4-coumarate-CoA ligase (4CL) and caffeic acid 3-*O*-methyltransferase (COMT) are key enzymes that catalyze the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates or the methylation of 5-hydroxyconiferaldehyde to sinapaldehyde, respectively. In this study, *COMT* and *4CL* genes were isolated from the commercially important sugarcane cultivar CP 88-1762 by a combination of cDNA library screening and PCR based approaches. More than 100 transgenic lines harboring *COMTi* or *4CLi* or both *COMTi* and *4CLi* constructs were generated via biolistic gene transfer. Quantitative real-time PCR identified transgenic lines ranging from no suppression to almost complete suppression of the target genes. Accumulation of siRNAs was confirmed in 23 transgenic lines by Northern blot analysis of low molecular weight RNA. These transgenic lines were vegetatively propagated and are currently grown to maturity in a replicated and randomized design. The analysis of the Klason lignin content will be carried out in the Spring of 2011 and presented at the conference. These results demonstrate that RNAi is effective in suppression of individual or co-suppression of multiple endogenous genes of the complex sugarcane genome. Further these results allow a determination of the relative importance of the targeted alleles or gene families for lignin biosynthesis and biofuel applications in sugarcane.

P-1005

Rooting for Artemisinin in *Artemisia annua* Shoots. K. T. NGUYEN and P. J. Weathers. Worcester Polytechnic Institute, 100 Institute Road, Department of Biology/Biotechnology, Worcester, MA 01609. Email: ktnguyen@wpi.edu

Artemisinin, synthesized in glandular trichomes of *Artemisia annua*, is the most effective treatment for malaria, but since the plant produces so little of the drug, supply is inadequate to cost effectively treat all infected patients. Although the biosynthetic pathway is reasonably elucidated, little is known on how the pathway is regulated. *A. annua* shoots produce less artemisinin than rooted shoots. No artemisinin or its pathways metabolites are produced in the plant's roots. Furthermore, rooted shoots respond to some signals, e.g. DMSO, but shoots do not, suggesting the roots are altering some aspect of the pathway. We measured trichome populations, and gene transcripts and metabolites in the artemisinin pathway in shoots as they form roots. Shoots were inoculated into either shooting or rooting medium for 16 d and plants were harvested periodically for analysis. Metabolites were analyzed via GC-MS and the first 3 genes in the pathway (*ADS, CYP, DBR2*) were measured using qPCR. As roots began to form, the end products of several branches of the pathway increased, but two of their immediate precursors remained high in the shoots. Consistent with these results, there was no difference in the mRNA transcript levels of the 3 genes. Although trichome number did not change, they were larger after roots formed. It is possible that roots do not regulate the transcription of the genes in the pathway, but instead, regulate the last non-enzymatic and photo-oxidative step in the pathway. Roots also seem to affect trichome size. When rooted shoots were transferred to either shooting or rooting medium, there was no significant difference in artemisinic metabolites in the shoots from either medium even after 20 d. This suggested that once roots develop, the artemisinin pathway is committed and the two phytohormones present within shooting medium (NAA and BAP) are unable to inhibit the pathway. Future work will explore how NAA and BAP impact trichomes and the artemisinin biosynthetic pathway.

P-1006

RNAi Mediated Viral Resistance in Transgenic Wheat. JESSICA L. RUPP[1], John P. Fellers[2], and Harold N. Trick[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 and [2]USDA-ARS, Center for Grain and Animal Health Research Manhattan, KS 66506. Email: jrupp@ksu.edu

Wheat streak mosaic virus (WSMV) and *Triticum mosaic virus* (TriMV), are two viruses of the wheat mosaic complex affecting *Triticum aestivum* (L.) in the Great Plains of the United States. Although not fully effective, the current disease management strategy incorporates the deployment of resistant varieties, mite vector control and various cultural practices. As an alternative strategy, the use of interference RNA to generate resistance to these wheat viruses was evaluated. RNAi expression vectors were independently created from the sequences of the coat proteins (CP), the HC-Pro, 6K2-NIa and a portion of the VPG of both WSMV and TriMV. Immature embryos of the spring 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. After tissue culture, putative transformed plants were analyzed through PCR for the presence of the appropriate RNAi gene. Transgenic T_1 seeds were collected and each line was tested for transgene expression via RT-PCR. To determine viral resistance, progeny were mechanically inoculated twice (day 1 and day 14) with the corresponding virus, and leaf samples were screened fourteen days after last inoculation by ELISA. Resistance was observed in the transgenic lines however the results did not segregate in a Mendelian fashion; there was evidence of transgene silencing. Analysis of T_2 and T_3 generation followed similar results. Regardless of these phenomena, consistent resistance response in two lines of WSMV CP-RNAi construct and one TriMV CP-RNAi transgenic line was found. T_3 generations continue to exhibit a high level of resistance among these CP-RNAi constructs. Twenty-two crosses have been made with the commercially important winter wheat cultivar 'Overly.' In addition to the CP-RNAi events, plants expressing 6k2-NIa-RNAi transgene have been grown to the T_2 generation and are currently undergoing bioassays and analysis. HC-Pro and VPG RNAi constructs for both viruses are also in the tissue culture process.

P-1007

Overexpression of a Rice M*icroRNA319* Gene Enhances Drought and Salt Tolerance in Transgenic Creeping Bentgrass (*Agrostis stolonifera* L.). MAN ZHOU, Dayong Li, Zhigang Li, Qian Hu, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: hluo@clemson.edu

miroRNAs (miRNAs) are short single-stranded molecules arising from primary miRNA transcripts (pri-miRNAs) encoded by miRNA genes. They regulate gene expression by negatively controlling both the stability and translation of target messenger RNAs. In plants, miRNAs mostly regulate other transcription factors and have critical functions in plant growth, development and stress responses. The miR319 family is one of the first characterized and conserved miRNA families in plants and it has been demonstrated to target *TEOSINTE BRANCHED/CYCLOIDEA/PCF (TCP)* genes encoding plant-specific transcription factors known to be largely involved in leaf development. In rice, the *miR319* comprises two members, *OsmiR319a* and *OsmiR319b* with the same mature sequence. We have investigated the role miR319 plays in plant development and plant response to abiotic stress in perennial grass species. Transgenic creeping bentgrass plants overexpressing the full-length cDNAs of *OsmiR319a* were produced. All transgenics exhibited dramatic morphological changes, including significantly decreased tiller numbers, wider leaves, and coarser stems. The five putative *miR319* target genes in turfgrass, *AsPCF5, AsPCF6, AsPCF7, AsPCF8* and *AsTCP14* were all down-regulated in transgenic plants. Overexpression of *miR319* also led to dramatically enhanced salt and drought tolerance in transgenics, which is associated with the down-regulation of at least one of the target genes, *AsPCF5*, indicating its direct involvement in plant response to salt stress. Transgenic analysis further elucidated the biochemical and physiological basis of the enhanced stress tolerance in transgenic plants. Data obtained in this research reveal the importance of *miR319* in plant resistance to environmental adversities, and identify its direct target specifically involved in plant stress response. This will lead to development of novel molecular strategies to genetically engineer crop species for enhanced performance under unfavorable environmental conditions, contributing to agriculture production.

P-1008

RNAi-mediated Metabolic Engineering of Cottonseed for Improving Its Nutritional Value. KEERTI S. RATHORE[1], S. Sundaram[1], G. Sunilkumar[1], L. M. Campbell[1], L. K. Tollack[1], Lorraine Puckhaber[2], Robert D. Stipanovic[2], S. R. Palle[1], and S. Krishnan[1]. [1]Institute for Plant Genomics & Biotechnology, Texas A&M University, College Station, TX 77843-2123 and [2]USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX 77845. Email: rathore@tamu.edu

Cotton, grown mainly for its fiber, is a major crop in the U.S. and several other, mostly poor countries. Cottonseed, a byproduct, remains an abundant but greatly underutilized source of protein because of the presence of gossypol, a

toxic terpenoid that otherwise serves a beneficial function in protecting the plant from biotic stresses. Annual, worldwide production of 44 million metric tons (MMT) of cottonseed contains ~10 MMT of protein, enough to meet the basic protein requirements of 500 million people. We employed RNA interference (RNAi) to inhibit the expression of the δ-cadinene synthase (dCS) gene in a seed-specific manner, thereby disrupting a key step in the biosynthesis of gossypol. Compared to an average gossypol value of 10 μg/mg in the wild-type cottonseeds, seeds from RNAi lines showed values as low as 0.2 μg/mg. Importantly, the levels of gossypol and related terpenoids that are derived from the same pathway were not diminished in the foliage and floral parts of mature plants and thus remain available for plant defense against insects and diseases. The stability of the engineered trait has been confirmed by evaluation of several lines over five generations in the greenhouse and also in field studies. Further, we have obtained molecular and biochemical evidence showing that the germinating, RNAi seedlings are capable of launching terpenoid-based defense response when challenged with pathogens. Thus, the "silenced-state" of the dCS gene that existed in the seed, does not leave a lasting, residual effect that can interfere with the normal functioning of the cotton seedling during and following its germination. Thus, the use of modern molecular tools has allowed us to successfully address the problem that had evaded earlier attempts by plant breeders.

P-1009

A Novel Platform Technology to Control Metabolic Pathways. F. TURANO, J. Thoguru, S. Cheepineeti, and K. Turano. Plant Sensory Systems, 1450 S. Rolling Rd., Baltimore, MD 21227. Email: fturano@plant-ss.com

The traditional methods to modify metabolic pathways involve the inhibition or over-expression of rate-limiting genes. Oftentimes the result is non-optimal and creates an imbalance in pathway intermediates, which have a detrimental effect on the organism. The Metabolic Regulator (MR) technology is a novel technology that avoids this limitation and effectively controls metabolic pathways. The MR technology introduces a gene whose product limits the availability of small signaling molecules. As a proof of concept the MR technology was used to increase the oil content in oilseeds in response to the need to increase the production of oil produced per acre of land. Oilseed crops are typically bred to maximize either seed oil content or seed protein content. A trade-off exists between the two products in the seed because they compete for the same carbon pool. The MR technology was utilized to decrease the pool of a signaling molecule that typically inhibits the carbon flow into oil biosynthesis. The MR technology was transferred into *Brassica napus* L. The transgenic plants produced seeds that had a 5.6% average increase in total oil relative to wildtype or empty vector control plants. A second utilization of the MR technology involved the expression of the same peptide in a different tissue and the result was a significant increase in lignin. The MR technology is extremely versatile, having the ability to target different pathways, including the uptake, assimilation and distribution of nitrogen, sulfur, phosphate, or minerals to improve plant growth, development, and yield.

P-1010

Brassinosteroids Increase Protein Accumulation in Skeletal Muscle Cells. D. ESPOSITO[1], T. Rathinasabapathy[1], A. Poulev[1], S. Komarnytsky[1], and I. Raskin[1]. Biotech Center, SEBS, Rutgers University, 59 Dudley Rd, New Brunswick, NJ 08901. Email: esposito@aesop.rutgers.edu

Brassinosteroids are plant-derived polyhydroxylated derivatives of 5α-cholestane, structurally similar to cholesterol-derived animal steroid hormones and insect ecdysteroids. We recently showed that a natural brassinosteroid (22S,23S)-homobrassinolide (HB) [**1**] stimulates protein synthesis, inhibits protein breakdown, and increases muscle mass in rats, but has very low androgenic activity both in vitro and in vivo. In this study we synthesized a set of brassinosteroid analogues including (22S,23S)-homocastasterone [**2**], (22S,23S)-3α-fluoro-homobrasinolide [**3**], (22S,23S)-3α-fluoro-homocastasterone [**4**], (22S,23S)-7v-aza-homobrassinolide [**5**], (22S,23S)- 6-aza-homobrassinolide [**6**], and studied their anabolic efficacy in L6 rat skeletal muscle cells in comparison to other synthetic and naturally occurring brassinosteroids (22R,23R)- homobrassinolide [**7**], (22S,23S)-epibrassinolide [**8**], and (22R,23R)-epibrassinolide [**9**]. Presence of the 6-keto group in the B ring and stereochemistry of 22α,23α-vicinal hydroxyl groups in the side chain were critical for the anabolic activity, possibly due to higher cytotoxicity of the 22β, 23β-hydroxylated brassinosteroids. All anabolic brassinosteroids tested in this study selectively activated PI3K/Akt signaling pathway as evident by increased Akt phosphorylation in vitro. The pharmacogenomic properties of HB were characterized in healthy rats orally administered with 60 mg/kg HB for 24 d and indicated that HB potently stimulated two sets of genes involved in muscle cell growth and carbohydrate metabolism. In summary, plant brassinosteroids and their synthetic derivatives may offer a novel therapeutic strategy for promoting growth, repair, and maintenance of skeletal muscles.

P-1011

Characterization of Novel Opium Poppy Micro-RNAs: A New Insight for Alkaloid Biosynthesis. TURGAY UNVER[1], Arif Ipek[1], Iskender Parmaksiz[2], Serkan Uranbey[1], and Sebahattin Ozcan[3]. [1]Cankiri Karatekin University, Faculty of Science, Department of Biology, Cankiri, TURKEY; [2]Gaziosmanpasa University, Faculty of Arts and Science, Department of Biology, Tokat, TURKEY; and [3]Ankara University, Faculty of Agriculture, Department of Field Crops, Ankara, TURKEY. Email: turgayunver@gmail.com

We have identified and cloned opium poppy micro-RNAs by using small RNA cloning and deep sequencing strategies. As a result 12 novel species specific poppy miRNAs have been sequenced and characterized. Of the 12250 miRNA sequences 281 conserved miRNA families were identified. The miRNA expression patterns of the different tissues including stem, root, leaf and young capsule have been compared via miRNA microarray chip and qRT Real Time-PCR analyses. A modified 5' RACE was applied to discover candidate miRNA target genes. Some of the miRNAs have been found to be targeting and regulating genes involved in alkaloid biosynthesis. Therefore, our results represent new insights for metabolic engineering in plants.

P-1012

ASGR-BABY BOOM-like (*ASGR-BBML*), a Candidate Apomixis Gene for Parthenogenesis Identified in Pennisetum and Cenchrus Species. J. A. CONNER[1], H. Huo[1], W. Hanna[2], M. Mookkan[1], and P. Ozias-Akins[1]. [1]Department of Horticulture and [2]Department of Crop and Soil Sciences, University of Georgia-Tifton Campus, Tifton, GA 31793. Email: jconner@uga.edu

Apomixis is a developmental process that leads to the clonal propagation of the maternal plant through seed. Apomictic Pennisetum and Cenchrus species reproduce via apospory. For apospory, unreduced embryo sacs are derived from nucellar cells of the ovary followed by parthenogenesis. In *P. squamulatum* and *C. ciliaris*, apospory is controlled by the Apospory-Specific Genomic Region (ASGR). Given the large physical size of the ASGR (>50 Mbp), a homology-based candidate gene discovery strategy was employed to identify candidate apomixis genes. The tightly-linked *ASGR-BBML* gene has high protein similarity to *BABY BOOM (BBM)* genes. *BBM-like* genes, found in many plant species, are thought to help regulate embryogenesis. Within Pennisetum and Cenchrus species, *ASGR-BBML* gene is conserved and transcribed in twelve apomictics while absent in eight sexual species. Expression of *ASGR-BBML* in *P. squamulatum* ovaries begins 1-2 d prior to fertilization and proceeds to at least 10 d after pollination. Insitu hybridization demonstrates *ASGR-BBML* expression at the globular stage and beyond of embryo development. Expression of *ASGR-BBML* precedes the expression of LEC, a gene required for early embryogenesis. This suggests that ASGR-BBML is expressed prior to the globular stage of embryo development but remains undetectable by the insitu hybridization methods used. A correlation between the level of *ASGR-BBML* gene transcription and the number of cells in two day old unfertilized developing embryos has been identified in apomictic F_1 transgenic lines containing an *ASGR-BBML* RNAi construct. The generation of transgenic lines showing complete knock-down of the *ASGR-BBML* gene and transgenic lines with conditional overexpression of *ASGR-BBML* is now being pursued.

P-1013

Overexpression of Osmotin and Chitinase in *Medicago sativa* by *Agrobacterium* Mediated Transformation for Improving Stress Tolerance. S. V. SAHI, J. Kancharla, and B. Sinilal. Department of Biology, Western Kentucky University, Bowling Green, KY 42101-1080. Email: shiv.sahi@wku.edu

Osmotin and chitinase were introduced into *Medicago sativa* for improving its tolerance against drought, salinity and fungal pathogens. Osmotin from *Nicotiana tabacum* and chitinase from *Phaseolus vulgaris*, cloned under two individual 35S promoters in the binary vector pBTEX was used for agrobacterium mediated transformation. As part of the standardization of a regeneration protocol, the regeneration capabilities of different accessions of *M. sativa* were verified and *M. sativa* ssp. *sativa*, African/ NSL 4142/ USA was identified as the best. Among the different explants used, hypocotyl region of the seedlings showed higher rate of regeneration and hence used for transformation. Plantlets developed from the transformed tissue were screened by PCR and the transformants were further confirmed by southern hybridization.

P-1014

A Simple In Vitro Protocol for Multiple Plantlet Regeneration Pathways and Control of Physiological Disorders in Date Palm. C. SUDHERSAN, Y. Al-Shayji, S. Jibimanuel,

and J. Ashkanani. Biotechnology Department, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109, KUWAIT. Email: schellan@kisr.edu.kw

Tissue culture technology has been successfully applied in date palm mass clonal propagation worldwide. Organogenesis and somatic embryogenesis are the common methods of regeneration practiced for this purpose. Complicated culture media with different combinations of growth hormones and organic additives were reported in the literature for date palm micropropagation and control of physiological disorders. In the present study we established a simple and efficient protocol to regenerate plantlets through eight different regeneration methods: 1. Direct somatic embryogenesis, 2. Indirect somatic embryogenesis, 3. Direct shoot induction, 4. Indirect shoot induction, 5. Axillary shoot induction, 6. Meristemoid induction, 7. Direct somatic embryogenesis from shoot buds and 8. Direct plant regeneration from meristem. Commonly occurring physiological disorders like tissue browning, hyperhydricity of somatic embryos and dwarfism which affect the commercial plant production were also controlled in this method. This protocol was successfully applied over 25 female and 5 male date palm cultivars in our laboratory for commercial scale plant production. Perhaps the present work is the first report on multiple regeneration and control of physiological disorders through a simple 3 stage culture media and protocol for the commercial plant production of different date palm cultivars. Field evaluation on the plantlets produced by this method confirmed the clonal nature which is important for commercial benefits.

P-1015

In Vitro Regeneration of Plantlets Through Callus Cultures in *Trichosanthes dioica* Roxb. cv. nimia. RASHMI KOMAL. Tissue Culture Laboratory, Department of Botany, Patna University, Patna-06, Bihar, INDIA. Email: rashmi0911@gmail.com

Trichosanthes dioica Roxb. commonly known as pointed gourd, is said to be the native of South East Asia and mostly cultivated in the Northern and Eastern states of India also well known for its economic and medicinal properties. Among the various cultivars extensively being grown along the river beds of Bihar (India) cultivar nimia is preferred as it can be successfully grown in uplands as well as reverine belts. Secondly, fruits of cv. nimia have better shelf life as compared to the other cultivars of pointed gourd. Cv. nimia carrying its name after a village Nimmi of Shekhpura district in Bihar is an underexploited summer vegetable crop. Though there are earlier reports on in vitro propagation of this crop still plantlet regeneration from the callus has neither been achieved nor attempted in this cultivar. Hence, the present study is an attempt to induce callus and regenerate plantlets from the callus of this cv. nimia. An efficient protocol for plantlet regeneration from the callus derived from shoot tip and axenic node was developed for cv. nimia. The explants were inoculated on semisolid Murashige and Skoogs's medium supplemented with different concentrations and combinations of (BAP and 2,4-D) alone and in combination. The cultures were incubated in the culture room maintained at 26°C $^{\pm}$ 2°C with a relative humidity of about 55-65% and 16 h photoperiod, 2000-2500 lux light intensity from fluorescent tubular lamps. The cultures for callus induction were kept in the dark for a week prior to exposing them to 16 h photo period. After callus formation these calluses were transferred to a cytokinin rich media for shoot initiation. The best callus formation was observed in MS + (2 mg/l BAP + 4 mg/l 2,4-D) after 45 d of culture. Proliferation of about 13 shoots was obtained from the callus on MS + 1.5 mg/l BAP along with 0.3 mg/l NAA after 20 d of culture. The elongated shoots rooted within seven to eight days in 1/2 MS + 1.0 mg/l IBA. IBA proved to be better than IAA for efficient rooting. The in vitro raised plantlets were acclimatized by transferring them in an inorganic MS salt solution, before planting in potted soil. The plantlets successfully established in earthen pots filled with soil, sand and farmyard mixture in a ratio of 1:1:1, showing 75% survival rate.