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Plant Contributed Papers

P-1000

Dissection of the Gmubi Promoter Intron Reveals Possible Regulatory Elements That Affect Promoter Activity and Transgene Expression. T. GRANT, C. de la Torre, and J. Finer. The Ohio State University, OARDC, 1680 Madison Avenue, Wooster, OH. Email: grant.343@osu.edu

The soybean ubiquitin promoter, Gmubi, is an intron-containing polyubiquitin promoter that expresses at high levels in many different soybean tissues. Typically recognized for their function in messenger RNA processing and stability, introns may also contain enhancer elements that can affect gene expression. Removal of the intron from the Gmubi promoter resulted in reduced levels of gene expression, which suggest an important role for the Gmubi intron in promoter activity. Various intron derivatives were evaluated using transient expression of GFP in lima bean cotyledons. Tetrameric repeats of small intron fragments were placed upstream of a minimal CaMV35S core promoter, which was used to regulate the green fluorescent protein. Tetramers were also cloned within a modified, native intron, which was then placed downstream of Gmubi and CaMV35S core promoters. Intron derivative constructions were introduced into lima bean cotyledons via particle bombardment and GFP expression was evaluated at peak expression times. Intron fragments, used as tetramers upstream of minimal promoters, regulated GFP expression at least two times higher than levels obtained with the CaMV35S core promoter, with some fragment tetramers yielding up to eighty times higher expression. Tetrameric intronic fragments, cloned within an intron, also demonstrated increased GFP expression up to four times higher than Gmubi alone and up to forty times CaMV35S alone. These intron fragments likely contain regulatory elements that could provide increased expression. The use of intron regulatory elements provides an additional tool for increasing transgene expression.

P-1001

GmCPI1, a Soybean Cysteine Protease Inhibitor Is Involved in Plant Development and Response to Biotic Stress. ZHIGANG LI, April Warner, Qian Hu, Ning Yuan, Cong Li, Benjamin Matthews, Halina Knap, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: hluo@clemson.edu

Proteases as secreted extracellular proteolytic enzymes in many insects and pathogenic microorganisms play important roles in pathogenesis, and are essential for the maintenance and survival of their hosts. Cysteine protease inhibitors are among the effective antidigestive compounds produced in plants to fight against herbivory or pathogenic infection. We have cloned a soybean cysteine protease inhibitor gene GmCPI1 from a nematode-resistant genotype. Transgenic Arabidopsis plants overexpressing GmCPI1 exhibited dramatically enhanced resistance against pests. Transient essay using soybean root transformation demonstrated that compared to wild-type control plants, transgenic soybean roots overexpressing GmCPI1 had a 60 % decrease in nematode infection. In addition, overexpression of GmCPI1 led to dramatically enhanced plant growth associated with modified global gene expression profiles. These data point to the great potential of using similar strategy to improve other food plants & economically important crops for enhanced pest and disease resistance as well as seed and biomass yield, contributing to agricultural production.

P-1002

Isolated Microspore Culture in Cereals by Mediating Stresses and Nursing. RAKESH KUMAR SINHA and François Eudes. Agriculture and Agri-Food Canada, Lethbridge, T1J 4B1, AB, CANADA. Email: rakesh.sinha@agr.gc.ca, Francois.Eudes@agr.gc.ca

Isolated microspore culture is a double haploid production platform instrumental in breeding programs. Development of microspore into embryo and green plant is dependent on a series of factors. An estimated 50 % of the isolated microspore undergoes programme cell death within 24 hours of culture, and few microspores succeed to form scutellar stage embryo in wheat and triticale. Studies were conducted to reduce the frequency of microspore cell death during the early stage of culture, to nurse their embryogenic development and enhance the production of green plants while minimising albinism. Various groups of antioxidants, including reactive oxygen species scavenger dimethyl tyrosine group, and Phytosulfokine-alpha (PSK-α) were evaluated in triticale and spring wheat genotypes. We report the number of embryo like structure and green plants were enhanced when induction medium was supplemented with proline (10 mM) or Glutathione (2 mM). The use of dimethyl tyrosine labelled organelle targeting peptides, allowing mitochondrial and chloroplast targeted delivery, greatly enhanced frequency of microspore going through embryo like formation and plant production. Complementary to these treatments, we report a dose effect of the nursing PSK-α on the number of embryos and the rate of green to albino plant formations, which resulted in an efficient doubled haploid production platform in wheat and triticale.

P-1003

Improving the Process of Somatic Embryogenesis Using Transient Expression of Transcription Factors and the Implementation of a Novel Bioreactor Design. S. FLOREZ[1], M. Curtis[1], S. Maximova[2], M. Guiltinan[2] and W. Curtis[1]. [1]Department of Chemical Engineering, Pennsylvania State University, PA and [2]Department of Horticulture, Pennsylavania State University, PA. Email: Slf5256@psu.edu

The process of somatic embryogenesis (SE) allows for the propagation of superior plants from non-embryonic tissue. Traits such as disease resistance can be maintained in all the plants generated through SE resulting in a greater economic value. While the process of SE has been commercialized for many economical important crops, the mechanism behind this process is not completely understood. Using *cacao* (chocolate tree) as a model, we are implementing whole genome transcriptomics to identify the key genes responsible for the reprograming of a somatic cell into an embryo. Further understanding of this regulation will be used to control SE with the goal of making it a more efficient, economical feasible process. Potential transcription factors responsible for this regulation have been isolated and characterized. Preliminary results testing the function of these genes in *cacao* have led to improvements in embryo production. Overexpression of the transcription factor BABY BOOM resulted in twice as many embryos produced per explant after one hundred days post culture initiation when compared to the control. To fully develop this "enhanced" SE process and show the feasibility of implementing at a commercial scale, we are developing a novel low cost temporary immersion bioreactor. This system will allow genes to be transiently expressed via *Agrobacterium*-mediated transformation. We are also trying to improve the process by exploring novel *Agrobacterium* techniques including the use of auxotrophic strains and suicide genes to control overgrowth to increase transformation efficiency. The employment of a bioreactor system to complement a transient gene expression strategy to improve SE can be a very useful platform for the propagation of many commercial crops.

P-1004

Insights into the Role of α-Tocopherol in Plants: Multiple Abiotic Stress Tolerance Via ROS Scavenging. DEEPAK KUMAR[1,2], Mohd. Aslam Yusuf[1], Preeti Singh[1], Meryam Sardar[2], and Neera Bhalla Sarin[1]. [1]School of Life Sciences, Jawaharlal Nehru University, New Delhi, INDIA and [2]Department of Biosciences, Jamia Millia Islamia, New Delhi, INDIA. Email: deepakinjnu@gmail.com

Alpha (α)-tocopherol, the biologically most active form of vitamin E, is a major antioxidant that bulwarks the cells against oxidative damage. It constitutes a small fraction of the total tocopherol pool in most oilseed crops. We generated transgenic (TR) *Brassica juncea* plants with ~6 fold higher α-tocopherol levels compared to the wild type (WT) plants by overexpressing γ-tocopherol methyl transferase. This enzyme catalyzes a rate limiting step in the α-tocopherol biosynthetic pathway. To better understand the roles of different tocopherol forms in plants we compared the performance of TR plants under conditions of abiotic stresses induced by NaCl (salinity), $CdCl_2$ (heavy metal) and mannitol (drought). This resulted in an increase in total tocopherol levels in both the WT and TR plants. Seed germination, shoot growth, and leaf disc senescence showed that TR *B. juncea* had enhanced tolerance to these stress and that induced by high temperature and methyl viologen. Damage caused by the induced stress was lower in TR plants compared to WT plants as assessed by their higher relative water content, lower MDA and H_2O_2 accumulation and lower electrolyte leakage. Lesser superoxide and H_2O_2 accumulation was observed in TR seedlings exposed to these stress. Enhanced levels of different antioxidant enzymes and molecules were present in TR plants when compared to WT plants under similar stress. Analysis of chlorophyll a fluorescence rise kinetics showed that there were differential effects of the applied stress on different sites of the photosynthetic machinery. These effects were found to be alleviated in TR plants. Thus, biofortification by metabolic engineering not only offers sustainable alternative to vitamin E supplementation for improvement of human health but also plays an important role in the alleviation of various environmental stress conditions in plants.

P-1005

Increased Mesos Components ($CaCl_2$, $MgSO_4$ and KH_2PO_4) Improve Growth and Quality 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

Genetic variation in red raspberries makes it difficult to successfully apply a standard *in vitro* growth medium. Many red raspberry cultivars grown on Murashige and Skoog medium (MS) display stunting, hyperhydricity, discoloration, callus, leaf spots or necrosis, and these are likely caused by non-optimum concentrations of important minerals. Earlier studies modeling MS mineral components concluded that the mesos ($CaCl_2$, $MgSO_4$ and KH_2PO_4) components significantly affected the quality and growth of red raspberries. This study investigated the effect of the individual mesos components in a 3-dimensional experimental design based on response surface methodology. Five red raspberry cultivars were tested with 29 treatments computer selected from all possible combinations of five concentrations of each. Shoot cultures were grown for three of 3-week transfers before data were taken. Plants were evaluated for quality, multiplication, shoot length and plant mineral content. Results varied by cultivar for some characteristics, but modeling indicated that all cultivars require significantly more of all three meso components for improved plant quality. High concentrations of KH_2PO_4 and $CaCl_2$ and medium to high $MgSO_4$ significantly increased shoot length and shoot number in most cultivars. Increasing the meso components improved leaf size and leaf color in some cultivars. High $CaCl_2$ increased hyperhydricity in 'Canby' 'Indian Summer' and 'Willamette'. Based on this study, the optimal mesos composition for improving overall quality of five cultivars was 2.5x the MS concentration of mesos or $3xCaCl_2$:$3xKH_2PO_4$:$2.5xMgSO_4$. Additional experiments to improve red raspberry micropropagation will address optimizing nitrogen ratios and minor elements.

P-1006

Introgression of a Novel Aldose Reductase from *Xerophyta viscose* (*ALDRXV4*) Confers Multiple Stress Tolerance to Evolutionary Diverse Organisms. PREETI SINGH[1], Deepak Kumar[1,2], Mohd. Aslam Yusuf [1]and Neera Bhalla Sarin[1]. [1]Jawaharlal Nehru University,New Delhi, INDIA and [2]Jamia Milia Islamia, New Delhi, INDIA. Email: preetimku@gmail.com

The enzyme aldose reductase plays an important role in the osmo-protection mechanism and detoxification of reactive aldehyde compounds. The aim of this study was to monitor whether over-expression of the aldose reductase homologue *ALDRXV4* from the resurrection plant *Xerophyta viscosa* could provide protection to prokaryotic (*E.coli*) and eukaryotic (tobacco and blackgram) cells from methylglyoxal (MG) toxicity during abiotic stressess.When over expressed in bacteria, recombinant *ALDRXV4* conferred multiple stress tolerance. Furthermore, *ALDRXV4* was cloned in a plant expressing vector under constitutive promoter and transformed into model plant tobacco and subsequently used for the transformation of a highly recalcitrant pulse crop, *Vigna mungo* (blackgram). Compared with wild type plants, transgenics showed improved photosynthetic efficiency, less electrolyte leakage and higher relative water content under drought and salinity stress. The increased synthesis of aldose reductase in the transgenic plants correlated with an elevated level of sorbitol and reduced methylglyoxal (MG) accumulation under stress conditions, consistent with its suggested role in osmoprotection and detoxification. They also showed less membrane damage, favorable ionic balance, and higher chlorophyll content under stress. The transgenic lines showed normal growth, morphology and seed production as compared to the WT plants without any yield penalty under stress conditions. The overall results demonstrate the profound effect of *ALDRXV4* in bestowing multiple abiotic stress tolerance at cellular and whole plant level via ROS detoxification. To the best of our knowledge this is the only report of engineering multiple abiotic stress tolerance in blackgram.

P-1007

Ovule-embryo Culture and Plant Regeneration of *Thermopsis turcica,* Critically Endangered Turkish Endemic. D. TEKDAL and S. Cetiner. Biological Sciences and Bioengineering Program, Sabancı University, Istanbul, 34956, TURKEY. Email: dilektekdal@sabanciuniv.edu

The possibility of micropropagation for the endangered *Thermopsis turcica* investigated through an fruit ovule-embryo culture. This technique prevents embryo degradation at early development stage, shortens breeding time, and efficient propagation of many plants from a single embryo, without the need for breaking seed dormancy. Although a number of efficient propagation protocols of *T. turcica* has been reported for multiple shoot induction and plantlet regeneration from the seeds of *T. turcica*, to date no publication exists on any protocol on fruit embryo culture of *T. turcica*. The main constraint to the transfer of desired traits into cultivated from wild relatives *T. turcica* are the habitat conditions, and the presence of uncertain seed predators consuming *T. turcica* seeds for larval development. This paper reports on the preliminary optimisation of protocols for rescuing *Thermopsis turcica* fruit embryos. The protocol for embryo culture used in this research consisted of

cracking the fruit, pitting open to remove the seed, excising the ovule-embryo from the seed, placing it on the medium modified from established *Fabaceae* embryo rescue tissue culture media, extracting embryos from ovular integuments, and culturing. During culturing, cotyledons began to arise and grow plantlets. These results show that embryo culture technique can be an alternative ex-situ conservation technique for the protection of threatened rare plant species *Thermopsis turcica*.

P-1008

Engineering Hydroxyproline-*O*-Glycosylated Peptide Motifs in Hairy Roots for an Enhanced Bioproduction Platform. NINGNING ZHANG, Fabricio Medina-Bolivar, Brett Savary, and Jianfeng Xu. Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401. Email: ningning.zhang@smail.astate.edu, jxu@astate.edu

Hydroxyproline-*O*-glycosylation involves post-translational hydroxylation of proline to hydroxyproline (Hyp) and subsequent glycosylation, a modification unique to plants. Evidence has showed that Hyp-*O*-glycosylated peptide motifs, e.g. tandem repeats of "Ser-Pro", engineered in plant cells could function as a "carrier" to significantly excrete tagged proteins into culture media and stabilize the proteins. However, the precise process of Hyp-*O*-glycosylation in plants has yet been elucidated. Hairy root cultures are among the most attractive plant-based production systems for recombinant proteins as they combine merits of both suspension cell culture and whole-plants cultivation. The purpose of this research is in two aspects: 1) to test if the Hyp-*O*-glycosylation technology can be applied to an alternative production system-hairy root; 2) to study the Hyp-*O*-glycosylation process with hairy root culture system. Two major types of HRGP motifs, an extensin consisting of tandem repeats of "Ser-Hyp-Hyp-Hyp-Hyp" motif, and an arabinogalactan protein (AGP) consisting of tandem repeats of "Ser-Hyp" motif, were respectively engineered in tobacco hairy roots as fusion with a reporter protein, enhanced green fluorescence protein (EGFP). As in plant cell cultures, significantly enhanced secretion of the target protein (EGFP) carried by the Hyp-*O*-glycosylated motifs (by more than 10 folds) was detected in hairy root cultures. While fully glycosylated extensin motif was identified in both the culture media and roots, interestingly, two types of glycoforms of the AGP motif were observed; they were completely segregated with the partially glycosylated form (presumably with single galactose) retained inside the roots while the fully glycosylated form (with arabinogalactan polysaccharides) was recovered in the culture media. This research demonstrated high potential of engineering Hyp-*O*-glycosylated motifs in hairy roots for an enhanced production platform, and that hairy root culture may provide a unique platform to study the Hyp-*O*-glycosylation process.

P-1009

Toward Chromosome Length Contigs: Single-molecule, Electronic, Solid-state Sequencing of Long DNA Fragments. JOHN OLIVER, Debra Dederich, Peter Goldstein, William Heaton, Dona Hevroni, Maryam Jouzi, Hsu-Yi Lee, Mark Nadel, and John Thompson. NABsys Inc., 60 Clifford Street, Providence, RI 02903. Email: oliver@nabsys.com

NABsys is developing a single-molecule solid-state electronic sequencing platform that obtains sequence information over very long length scales (100 kb+). Specifically, the platform locates, with sub-diffraction-limit resolution, the positions of probes that have bound to long DNA fragments. Long-range information is preserved in these reads so structural rearrangements and duplications are easily identified and correctly assembled. The maps can be assembled into contigs whose lengths approach the lengths of chromosomes. Collections of these maps may be used to *de novo* sequence genomes of any length and complexity. Single maps are also useful in their ability to provide the large-scale information that is missing from short-read DNA sequencing platforms. The sample preparation, single-molecule detectors, and algorithms used to implement the Nabsys technology will be described. Experimental results for the assembly of maps from single-molecule reads will be presented. Simulations for the assembly of the Maize genome using the experimentally determined error model demonstrating the ability of the technology to generate extremely long contigs will also be presented.

P-1010

Micropropagation Integrated with Fingerling Culture Method for Cost Effective Clonal Plant Production. C. SUDHERSAN and J. Ashkanani. Biotechnology Department, Kuwait Institute for Scientific Research, KUWAIT. Email: schellan@kisr.edu.kw

The major advantage of *in vitro* micropropagation is the rapid production of superior quality, vigorous, healthy and uniform planting material on year-round basis. Micropropagation technology is more expensive in many cases than the conventional methods of propagation due to the requirement of technical know-how, skilled technicians and costly equipment and chemicals. The success and survival of commercial scale clonal plant production via micropropagation technology depends on the low cost plant production. The low cost micropropagation technology is the adoption of new simple methods, avoidance of costly equipments at certain levels, reduction in manpower for subculture and high cost culture media. The low cost options should lower the unit production cost without lowering the quality of planting material. The present study was undertaken to develop a low cost clonal plant production method without lowering the quality of the planting material. Normal axillary shoot multiplication method using MS culture medium

consisting culture initiation, multiplication, growth and elongation, *in vitro* rooting and acclimatization stages was used as control and micropropagation integrated with fingerling culture method was used for an experiment to compare the unit cost of the propagule. The plant species tested were *Lycium shawii*, *Adenium obesum*, *Ficus robusta* and *Moringa pterigosperma.* Shoot tip cultures were initiated from the above mentioned plant species and multiplied by axillary shoot multiplication method, and produced rooted plants continuously for a year. In the experimental method 1000 rooted plantlets were produced by the same axillary shoot multiplication method and planted in photoautotrophic culture medium and maintained inside the growth room. After 30 days terminal shoots of a finger length (3 inches) were removed and planted in new photoautotrophic culture medium. The mother plants were maintained in the photoautotrophic culture media inside the growth room or inside the greenhouse for continuous axillary shoot production. Axillary shoot tips of 3 inches length were continuously harvested and planted in the photoautotrophic culture medium. Plantlets were multiplied in large numbers through this method which was more strong, healthy, uniform and high quality. The total expenses for one year production and the total number of plants produced by two methods were calculated and used for finding out the unit cost of production. The unit cost of production by the new method was 20 times lower than the control method. Hence this method could be used for many plant species in commercial plant tissue culture production industries for a profitable business.

P-1011

An Improved Microinjection Method for *Agrobacterium tumefaciens* Mediated Genetic Transformation in Tomato. N. JAYABALAN, S. Vinoth, and P. Gurusaravanan. Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-24, Tamilnadu, INDIA. Email: jayabalan5419@gmail.com

Globally, cultivation and consumption of tomato have been increased gradually for the past two decades and it is referred as luxury crop. Even though it is cultivated in almost all the parts of the country, often it is exposed to various adverse environmental stresses such as biotic and abiotic stress that limits its yield and quality. The factors influencing the efficient transformations of tomato are still need to be optimized for its higher efficiency .So there is in need of reproducible and genotype independent protocols to obtain a number of positive plants through transformation technology. A simple and efficient protocol for *Agrobacterium* mediated genetic transformation of tomato was developed using combination of non-tissue culture and micropropagation systems. Initially, Embryonic Shoot Apical Meristem (ESAM) region of one day old germinated tomato seeds were microinjected for 1–5 times with *Agrobacterium* inoculums (OD_{600}=0.2-1.0).The germinated seeds were co cultivated in the MS medium fortified with (0–200 mM) acetosyringone and minimal concentrations of (0–20 mg L^{-1}) kanamycin and the antibiotic concentration was doubled during the second round of selection..In another set of experiment, an improved and stable regeneration system was adapted for the explants from the selection medium. Four day old Double Cotyledonary Node (DCN) explants were excised from the microinjected seedlings and cultured on to the MS medium supplemented with 1.5 mg L^{-1} TDZ, 1.5 mg L^{-1} IBA, 30 mg L^{-1} kanamycin and 0–1.5 mg L^{-1}Ads. Maximum 9 out of 13 micropropagated shoots were shown positive to GUS assay. The transgenic plants were analysed and confirmed by using PCR, RT PCR and southern blot analysis. By this technique, the transformation efficiency was increased from 46.28 % to 65.90 %. This optimized protocol is simple, reproducible and less genotype dependent. The protocol clearly indicates that the level of escapes can be reduced and chimerics can be eliminated.

P-1012

Enhanced Production of Edible Food Colorants in Growtek Bioreactor by a Callus Line of *Amaranthus tricolor* L. SATYAHARI DEY and Mousumi Biswas. Plant Biotechnology Laboratory, Biotechnology Department, Indian Institute of Technology Kharagpur, INDIA-721302. Email: satyahari01@yahoo.com

The red amaranth, *Amaranthus tricolor* L, produces edible betalain pigments having identical chemistry to those of sugar beet. The pigments in *A. tricolor* are, however, produced by the aerial parts of the plant. Betalains are safe edible pigments and have enormous importance as a natural colorant in food and healthcare industries. There has been no report on *in vitro* production of these metabolites from *A. tricolor.* The first ever callus line of this plant producing red-violet amaranthin and related compounds has just been reported by our laboratory. This article is also the first one on its suspension culture in a bioreactor. In an optimized medium (B5; 3 % sucrose; 2.26 μM of 2,4-D and 8.88 μM of benzyladenine; 21 days) the callus yielded 340 μg g^{-1} fresh weight colorant that was reduced to 130 μg g^{-1} (38.2 %) in shake flask but regained with enhanced yield of 590 μg g-1 (1.74 folds) in suspension culture in Growtek bioreactor (Indian patent No. 183604). The cell lysis in shake flask was among important causes of lower yield, and the floating perforated tissue support in Growtek was observed to be the main reason for superior performance, preventing cell lysis.

P-1013

RNAi-mediated Resistance to Cucumber Mosaic Virus (CMV) in Transgenic Tomato. VALENTINE OTANG NTUI, Kong Kynet, Ikuo Nakamura, and Masahiro Mii. Laboratory of Plant Cell Technology, Graduate School of Horticulture, Chiba University, 648 Matsudo, Matsudo, Chiba 271–8510, JAPAN. Email: ntuival@yahoo.com

Cucumber mosaic virus (CMV) is a tripartite, positive sense RNA virus, which causes severe infections and yield losses to many plant species. Here, we generated two RNAi constructs; one, pEKH2IN2CMVai, contains inverted repeat of 1100 bp fragment of a defective CMV replicase gene derived from RNA2 of cucumber mosaic virus strain O (CMV-O), while the other, TRV-based VIGS vector (pTRV2CMVai), contains the same fragment of the replicase gene, but without inverted repeat. These constructs were used to produce transgenic tomato lines expressing CMV-derived double stranded (dsRNA). Transgenic lines derived from pEKH2IN2CMVai showed small interfering RNA (siRNA) accumulation before and after virus challenge, whereas those derived from pTRV2CMVai showed siRNA expression after virus challenge. When transgenic lines were challenged with CMV-O or CMV-Y, three categories of plants were discriminated: plants that showed complete resistance, these plants were free of symptoms; highly resistant plants, which had mild symptoms, but later recovered because new leaves that emerged were free of symptoms; and susceptible plants, which showed moderate to severe symptoms similar to wild-type plants. Comparison of infectivity caused by both strains of CMV indicates that infectivity of CMV-O was lower than that of CMV-Y in the highly resistant and susceptible plants. Plants derived from pEKH2IN2CMVai were more resistant to both CMV strains than those obtained from pTRV2CMVai. The presence of CMV-specific siRNA in the resistant phenotypes is indication of RNA silencing.

P-1014

Transcriptional Activity and Practical Use of Ubiquitin and PR-1 Gene Promoters from Grapevine (*Vitis* spp.) in Transgenic Plant Recovery. D. J. GRAY, Z. T. Li, K.-H. Kim, J. R. Jasinski, D. D. Silva, and M. R. Creech. Mid-Florida Research and Education Center, University of Florida/IFAS, 2725 Binion Road, Apopka, FL 32703–8504. Email: djg@ufl.edu

Native promoters are an essential genetic element in the development of cisgenic and intragenic plants via biotechnology. In order to overcome the shortage of constitutive native promoters for use in transformant selection, variant DNA fragments upstream of 7 ubiquitin (Ubi) genes and a PR-1 gene were amplified from various genotypes of grapevine (*Vitis spp.*). They were then fused to reporter genes including VvMybA1, GUS and EGFP/NPTII fusion genes, respectively, for functional test following transformation of grapevine somatic embryos (SE) and tobacco. A color histogram analysis method was utilized to determine transient anthocyanin production in non-pigmented SE explants to infer quantitatively transcriptional activity. Results revealed that 6 out of 15 Ubi promoters generated constitutive activities reaching up to 100 % of that of the d35S promoter. In particular, the high activity levels of VvUbi-6 and VvUbi-7 promoters were verified by transient GUS quantitative assay as well as stable anthocyanin expression in sepal and corolla tissue of transgenic tobacco. Activity variations among these Ubi promoters did not correlate with the presence and size of 5' UTR introns, but seemed to be related positively and negatively to the number of positive *cis*-acting elements and root-specific elements, respectively. In addition, several promoters derived from a PR1 gene produced a higher basal activity as compared to previously reported similar promoters, suggesting higher levels of inducible activity. Stable transformation experiments using constructs containing the EGFP/NPTII fusion gene indicated that the majority of tested promoters with a wide range of activity levels were capable of driving selectable marker gene expression in tobacco, thus resulting in recovery of transgenic shoots. Transformation experiments using grapevine SE explants are being conducted to determine the efficacy of these promoters to support transformant selection in the native host. The fidelity of the anthocyanin-based assay system for promoter analysis will be discussed.

P-1015

Hydroxyproline-*O*-Glycosylated Biopolymer Carriers for Competitive Plant Cell and Tissue Bioproduction Platforms. JIANFENG XU[1], Maureen Dolan[1], Ningning Zhang[1], and Marcia Kieliszewski[2]. [1]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72401 and [2]Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701. Email: jxu@astate.edu

In vitro plant cell and tissue culture has been demonstrated to be a safe and cost-effective bioproduction platform for therapeutic proteins. However, low protein production is a common bottleneck in driving the commercialization of this technology. This research seeks to address this bottleneck by leveraging a new strategy, termed "HypGlyco" technology, where increases in recombinant proteins yields as high as 1500-fold compared to controls has been achieved. This technology exploits the glycosylation "code" of plant hydroxyproline (Hyp)-rich glycoproteins (HRGPs) for *de novo* design of short biopolymer tags, such as tandem repeats of "Ser-Pro" motif, which are targeted for extensive Hyp-*O*-glycosylation with arabinogalactan polysaccharides in plant cells. Such designer biopolymer tags appear to function as a "carrier" in promoting efficient transport of the recombinant protein into the culture media as well as protecting the protein from proteolytic

degradation. While originally characterized in plant cell culture, the HypGlyco technology appears to be universally applicable to other plant-based expression systems including transient plant expression, whole-plants, hairy roots and green microalgae with significant enhancement in protein production demonstrated. The underlying mechanism(s) of the Hyp-*O*-glycosylation process for enhancing recombinant protein production expressed on the various plant bioproduction platforms is explored.

P-1016

Synthetic TAL Effectors for Targeted Gene Activation in Plants. C. NEAL STEWART, JR.[1], Wusheng Liu[1], Mary R. Rudis[1], Yanhui Peng[1], Mitra Mazarei[1], Reginald J. Millwood[1], Jian-Ping Yang[2], and Jonathan D. Chesnut[2]. [1]Department of Plant Sciences, The University of Tennessee, Knoxville, TN and [2]Synthetic Biology Research and Development, Life Technologies, CA. Email: nealstewart@utk.edu

Transcription activator-like effectors (TALEs), secreted by the pathogenic bacteria *Xanthomonas*, specifically activate expression of targeted genes by binding to the TATA-box regions of plant resistance (R) genes. Here we designed synthetic TALEs that bind to the flanking regions of the TATA-box motif on the CaMV 35S promoter for the purpose understanding the engineerable "hot-spots" for increasing transgene expression. We demonstrated that the *de novo*-engineered TALEs could increase reporter gene expression by up to 3 fold in stable transgenic tobacco harboring the fluorescent protein reporter gene *pporRFP* under the control of synthetic inducible, minimal, or full-length 35S promoters. Moreover, the synergistic effects of a combination of different synthetic TALEs could activate reporter gene expression by up to 5.3 fold. We also studied the orientation, activation domain, position, and dosage effects of synthetic TALEs on targeted gene activation. Furthermore, TALE activation of the *Arabidopsis* MYB transcription factor *AtPAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1)* in stable transgenic tobacco gave rise to a dark purple color on infiltrated leaves when driven by four copies of *cis*-regulatory elements of pathogenesis-related gene (*PR1*) with enhancer motifs B and A1 from the 35S promoter. These results provide novel insights into the potential applications of synthetic TALEs for targeted gene activation of transgenes in plants.

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Somatic Embryogenesis from Immature Embryos for the Transformation and Regeneration of Sorghum Lines (*Sorghum bicolor* L.). SHIREEN K. ASSEM[1], M. M. Zamzam[1], S. H. El-Mansy[1], and E. H. A. Hussein[2]. [1]Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Reseach Center (ARC), Giza, EGYPT and [2]Department of Genetics, Faculty of Agriculture, Cairo University, Giza, EGYPT. Email: shireen_assem@yahoo.com

Regeneration of *sorghum bicolor* L. through somatic embryogenesis from immature embryos is genotype dependent. We report here an in vitro culture system for *sorghum bicolor* L. in attempts to develop a successful regeneration protocol using different media combinations and different genotypes. Nineteen of local and commercially important elite sorghum germplasms were used in two groups. Immature embryos were cultured on thirteen of MS based callus induction media supplemented with different levels of vitamins, hormones and growth regulators. One B5 besed and one N6 based media were also used. Media based on MS salts resulted in the formation of higher frequency (up to 72 %) of embryogenic calli than media based on N6 or B5 salts. 1.5-2.0 mg/l 2,4-D and 0.7-1.0 mg/l L-proline greatly enhanced the formation of embryogenic calli. While addition of L-asparagine enhanced the formation of embryogenic calli only in one genotype. Not only the creamy to yellowish embryogenic calli were able to regenerate but also the white compact embryogenic calli formed shoots on regeneration media and regenerated into whole plants. All embryogenic calli formed on media containing 0.5 mg/l kinetin and 0.5 mg/l BAP developed mature somatic embryos and regenerated into normal mature plants. Immature embryos explants were used for the optimization of *Agrobacterium-mediated* transformation using the selectable marker *bar* and *uida* reporter genes. Optimization experiments were carried out on sixteen sorghum lines, with two *Agrobacterium* strains (EHA101and LBA4404) at four optical densities (0.4, 0.5, o.6 and 0.7) for bacterial infection. Transgenic embryogenic calli survived on bialaphos selection were obtained from eleven of the tested lines. Embryogenic calli from nine of these lines were able to produce putatively transgenic plantlets. Six independent transformation events from two lines were regenerated into fertile T_0 transgenic plants. These plants were resistant to local application of the herbicide "Basta". The integration of *uida* and *bar* genes was confirmed by southern blot analysis.