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ANIMAL POSTER ABSTRACTS

CANCER BIOLOGY

A-3004

High Content Imaging Characterization of the Cell Cycle. JONATHAN LOW¹, Shuguang Huang², Wayne Blosser¹, Michele Dowless¹, and Louis Stancato¹. Departments of Cancer Growth and Translational Genetics¹, and Statistics and Information Science², Eli Lilly and Company, Indianapolis, IN 46265. Email: lowja@lilly.com

Understanding the regulation and progression of the cell cycle is critical to the comprehension and treatment of cancer. Our current understanding of the cell cycle is based upon observations that, due to technological limitations, examined mixed populations of cells and were unable to analyze cellular subpopulations or determine how variability between cells affected the total population. High content imaging (HCI) quantifies treatment effects on each cell and on the population of cells as a whole using a variety of parameters. Although one parameter alone cannot define every phase of the cell cycle, the quantification of additional parameters further delineates cell cycle phases. The key to understanding and defining cellular subpopulations is multiparametric analysis using several relevant attributes that refine the definition of each population. As more attributes are added to the analysis additional subpopulations are often detected and previous subpopulations become more clearly defined. We have characterized the cell cycle of transformed mammalian cells using HCI in conjunction with multiparametric data analysis. The combination of these tools generates a phenotype for each phase of the cell cycle and shows that this phenotype is reproducible across mammalian cell lines in vitro. We illustrate that phase-specific arrest caused by common cell cycle inhibitors results in phenotypes identical to those observed for each phase of the cell cycle and that these results are detected both in vitro and in vivo. Multiparametric HCI cell cycle analysis quickly characterizes where in the cell cycle experimental molecules affect the target cells, and more importantly distinguishes the differential effects of these molecules on individual cells and cellular subpopulations across the total studied population. The flexibility inherent to this system also means that multiparametric analysis can be used in a variety of screening settings in which one or more phenotypic markers are affected by an experimental treatment.

CELLULAR MODELS

A-3000

Model of Insulin Resistance in Liver Cells. SANDHYA NAIR and Susan R Stapleton. Departments of Chemistry and Biological Sciences, Western Michigan University, Kalamazoo, MI 49008. Email: S3adiyod@wmich.edu

Insulin resistance, a hallmark of type 2 diabetes, is characterized by the inability of a cell or tissue to respond to physiological levels of insulin resulting in problems with glucose transport and metabolism. Several cellular models have been utilized to determine the mechanism of induction of insulin resistance but questions remain unanswered. There is evidence that high glucose induced insulin resistant may be mediated by products of the hexosamine biosynthetic pathway (HBP). The major end product of HBP, UDP-GlcNAc, is the substrate for O-GlcNAc transferase, an enzyme that catalyzes the O-linked transfer of GlcNAc to Ser/Thr residues of numerous proteins. This modification may play a role in induction of insulin resistance and thus needs to be evaluated in different cell types to fully understand its implication. Therefore we developed an insulin resistant model in primary hepatocytes by treating the cells with a precursor of a HBP product, glucosamine, at various concentrations over differing lengths of time. Insulin resistance was considered established when signal proteins such as AKT and the MAPK family were no longer phosphorylated in the presence of insulin. Increased glycosylation of proteins was also observed. Treatment of these cells with selenium, an insulin-mimetic, restored the phosphorylation of the signal proteins. Supported in part by a WMU FRACASF and Monroe Brown award and NSF DBI-0139204.

A-3001

Serum- and Glucocorticoid-inducible Kinase-1 Regulation of Human Cystic Fibrosis Transmembrane Conductance Regulator. J. D. SATO¹, M. C. Chapline^{1,2}, R. Thibodeau^{1,2}, R. A. Frizzell^{1,3}, and B. A. Stanton^{1,2}. ¹Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672; ²Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03755; and ³Dept. of Cell Biology and Physiology, University of Pittsburgh Medical School, Pittsburgh, PA 15261. Email: dsato@mdibl.org

Serum- and glucocorticoid-inducible kinase-1 (SGK1) has been shown by heterologous expression in *Xenopus* oocytes to regulate several transport proteins including epithelial sodium channels (ENaC), sodium-hydrogen exchanger isoform 3 (NHE3) and the human cystic fibrosis transmembrane conductance regulator (CFTR). This serine/threonine kinase shares a high degree of similarity in its catalytic region with the kinases protein kinase A (PKA), protein kinase C (PKC), protein kinase B (PKB)/Akt, phosphatidylinositol-dependent kinase-1 (PDK1), and p70 S6 kinase. Because SGK increases the plasma membrane expression of other ion channels, we tested the hypothesis that SGK1 stimulates CFTR-mediated Cl currents by increasing the number of CFTR Cl channels in the plasma membrane. CFTR Cl currents were measured in *Xenopus* oocytes by the two-electrode voltage clamp technique, and CFTR in the plasma membrane was determined by

laser scanning confocal microscopy. Wild-type SGK1 stimulated CFTR Cl currents by 42% and increased the amount of CFTR in the plasma membrane by 35%. A kinase-dead SGK mutant (K127N) had a dominant-negative effect on CFTR, reducing CFTR Cl currents by 38%. In addition, deletion of the C-terminal PDZ-interacting motif (SGK1- Δ SFL) increased CFTR Cl currents by 108%. Thus, SGK1- Δ SFL was 2.7-times as effective than wt-SGK1 in stimulating CFTR Cl currents. Neither wt-SGK nor the K127N mutant had any effect on Cl currents in oocytes when expressed alone in the absence of CFTR. We conclude that SGK1 stimulates CFTR Cl currents in *Xenopus* oocytes by increasing the number of channels in the plasma membrane. In addition, the effect of SGK may be mediated by protein-protein interactions involving the PDZ interacting motif. The finding that SGK1 regulates CFTR function in *Xenopus* oocytes may open up new approaches to discover therapies for cystic fibrosis if these results can be replicated in human airway epithelial cell lines or other relevant experimental models. This research was supported by grant RO1-DK45881 from NIDDK to BAS, a Cystic Fibrosis Foundation research development program grant to BAS, grant R01-DK68196 from NIDDK to RAF, NIEHS Center grant P30-ES03828, and by INBRE grant P20-RR016463 from NCRR. RT was supported by NSF Research Experience for Undergraduates grant NSF DBI-0453391.

A-3002

Role of the p38 MAPK Pathway in Oxidative Stress Induced Expression of Heme Oxygenase. SHRUTI SEVAK and Susan R. Stapleton. Departments of Chemistry and Biological Sciences, Western Michigan University, Kalamazoo, MI 49008. Email: s3sevak@wmich.edu

Reactive oxygen species (ROS) are highly unstable molecules capable of reacting with cellular material and can lead to cancer, and cellular death. The presence of ROS initiates a cellular stress response mediated by protein cascades in a process called 'signal transduction' by the activation of such proteins as the mitogen-activated protein kinases (MAPKs), namely extracellular signal-regulated kinases 1 and 2 (ERK1/2), the c-Jun N-terminal kinases (JNKs), and p38 kinase. Previous studies in our lab have shown that cadmium, a highly toxic heavy metal, can initiate the generation of ROS to cause oxidative stress and can induce the expression of the antioxidant protein heme oxygenase in the H4IIE rat liver cell line. However, determination of the exact signaling mechanism by which cadmium mediates induction of the heme oxygenase 1 (HO-1) gene is yet to be defined. Since it is known that cadmium augments production of ROS, and that p38 MAPK is a signal transduction pathway that responds to oxidative stress, we hypothesized that the p38 MAPK pathway mediates the induction of the HO-1 gene by cadmium. Our results show increased phosphorylation of the p38 MAPK protein when exposed to cadmium, and a significant decrease in cadmium-mediated induction of the heme oxygenase gene upon inhibition of the p38 MAPK. Therefore, p38 MAPK is involved in mediating cadmium-induced expression of heme oxygenase in the H4IIE rat liver cell line. Supported in part by a WMU Undergraduate Research and Creative Activities Award.

INVERTEBRATE CELLS

A-3003

New Cell Lines from Freshly Hatched Larvae, Embryos and Hemocytes of the Tarnished Plant Bug *Lygus lineolaris*. GUIDO F. CAPUTO, A. B. Broadbent, and S. Goodacre. Natural Resources Canada, Great Lakes Forestry Centre, 1219 Queen St. E. Sault Ste. Marie, ON P6A 2E5, CANADA and Agriculture and Agri-Food

Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford St., London, ON, N5V 4T3, CANADA. Email: gcaputo@nrnc.gc.ca

The tarnished plant bug, *Lygus lineolaris* is a major threat to both the agri-food sector and to coniferous nursery stocks in Canada, the United States and Mexico. It is an extremely polyphagous insect feeding on the developing reproductive organs resulting in shriveled seeds and seeds without embryos. Even though *Lygus lineolaris* has been reared in laboratories, there are no literature reports of a cell line from this insidious pest of seed crops. In this report we describe the establishment of twenty four continuous cell lines of *Lygus lineolaris*. The cell lines designated NRcan-LL-1030 consist of 22 neonate lines, with 12 freely suspended and 10 attached, 10 embryonic lines, with 8 freely suspended and 2 attached, and 8 hemocyte lines with 4 freely suspended and 4 attached. They were initiated in three different media namely Grace's Medium, Ex-Cell 401 and Mitsuhashi and Maramorosch Insect Medium. Concentration of FBS was started at 10% and gradually reduced to 3%. These cells are heterogeneous in nature with cells growing predominantly in large "soccer ball" like aggregates. These aggregates, visible with the naked eye, resist vigorous pipetting and even enzymatic treatment for dispersal. Other morphologies present include small and large individual round cells, epithelial and fibroblast-like attached cells and vacuolated cells. Initial cell growth was very slow with initial passages requiring six months intervals. This interval was reduced to one month by the 10th passage and to seven days by the 13th. The freely suspended cells grow well in 250 ml shaker flasks. All cultures can be cryopreserved and revived successfully.

PLANT POSTER ABSTRACTS BIOTECHNOLOGY

P-3000

Transgene Expression Threshold in Rice Cells. A. B. More, M. A. AKBUDAK, and V. Srivastava. Department of Crop, Soil, & Environmental Sciences, University of Arkansas, Fayetteville, AR 72701. Email: vibhas@uark.edu

High expression of transgenes is desired for molecular farming. However, transgenes are often subjected to gene silencing pathways in plant cells. Gene silencing may be triggered by the production of aberrant (hairpin) RNA molecule from a complex integration locus in plant genome or by the over-expression of transgene. Excessively transcribed RNA are subject to gene silencing even if they are produced from a single-copy locus. Therefore, we sought to determine how highly a transgene can be expressed before its transcript is subjected to gene silencing. In a previous study, we demonstrated that precise single-copy locus generated by Cre/lox-mediated site-specific integration (SSI) in rice is stably expressed at predictable levels through subsequent generations, and that its expression invariably doubled in homozygous progenies. To further explore the stability of SSI locus and determine the expression-threshold of rice genomic sites, we generated transformation vectors containing 1-3 copies of 35S-GUS and 35S-GFP transgenes. These vectors were used to develop SSI locus containing 1-3 copies of each transgene in two different varieties of rice, Nipponbare and Taipei 309. SSI lines will be analyzed by PCR and Southern blotting to ascertain the presence of precise integration structures consisting of 1-3 copies of each transgene. The precise SSI lines will be subjected to quantitative GUS and GFP assay to determine if gene expression indeed increased with the increase in gene dosage. Molecular and expression data of SSI lines will be presented.

P-3002

Transgenic Resistance to Potato Virus Y by Gene Silencing Depends on the Degree of Homology Between the Transgene and the Challenge Virus. V. GABA, A. Rosner, S. Singer, E. Kukurt, D. Liebmann, L. Maslenin, and A. Gal-On. Department of Plant Pathology and Weed Science, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, ISRAEL. Email: vpgaba@volcani.agri.gov.il

Tobacco plants (*Nicotiana tabacum* L.) were transformed with a construct based on pCAMBIA 2301 containing a "hairpin" inverted repeat of 598 nucleotides derived from the Potato Virus Y (PVY) replicase (NIB gene) of the N strain (Robaglia et al., J. Gen. Virol. 70, 935, 1989). Such constructs confer virus resistance by a post translational gene silencing mechanism. Homozygous (T3) plants were challenged with a range of PVY strains and resistance was measured by symptom expression, ELISA titer, and back-inoculation of controls with extracts from resistant plants. The nucleotide homology of PVY strains to the transgene was: WP (99.5%) PVY-NTN (96.3%), PVY-H (95.6%), PVY-O (88.9%), strain 52 (88.3%), and local field isolates from tomato (86.8%), and pepper (86.3%). A transgenic tobacco line was immune to the five PVY strains with which the transgene had the greatest homology (WP, NTN, H, O, 52). Infection with the PVY isolates from tomato and pepper, which had the lowest degree of homology with the transgene, caused delayed symptom appearance in the transgenic tobacco compared with control non-transgenic plants. Small RNA (20-25nt) related to the "hairpin" virus-derived transgene could not be detected in un-inoculated resistant plants. It was notable that a difference of 1.5% in homology at the nucleotide level (between PVY strain "52" and the tomato isolate) allowed viral infection. The homology between the virus strains and the transgene was calculated in units of fully homologous 22mer fragments (as such segments are produced by Dicer endonuclease). There is a notable difference in homology between strain 52 (to which the line is immune), producing 73 homologous 22mer fragments, and the PVY tomato and pepper strains, which would produce only 32 and 21 possible 22mer fragments respectively. This study allows us to design a transgene containing sequences which would give broad resistance against many viral strains.

P-3003

Metal-binding in Plants Overexpressing a Metallothionein Gene. B. JOYCE, M. Ayalew, B. Nelson, J. Abercrombie, E. Essington, B. Mullin, M. Z. M. Cheng, and C. N. Stewart, Jr. The University of Tennessee, Knoxville TN 37996. Email: bjoyce3@utk.edu

Metallothioneins were discovered as highly expressed proteins in the infection zone of *Alnus glutinosa* root nodules in symbiosis with the nitrogen-fixing actinomycete *Frankia* spp. Whereas the biological role of metallothioneins is not clear, they have been shown to have a high capacity for binding multiple metal atoms, including Co^{2+} , Al^{3+} , Cu^{2+} , Cd^{2+} and Hg^{2+} . Because these metals impact plant and animal health, we are investigating a phytoremediation strategy that overexpresses metallothionein genes in transgenic plants. A metallothionein cDNA (AgNt84) under the control of a CaMV35S promoter has been transformed into *Arabidopsis thaliana* and tobacco. The plants appear normal, indicating that over-expression did not interfere with normal cell metabolism. Northern blots revealed the presence of RNA, but no AgNt84 protein could be found in western blots. Immunostaining of pellets from cell wall protein extraction showed AgNt84 bound inside the cell wall. Transgenic tobacco lines stained in dithizone showed metal-binding properties in the presence of cadmium.

P-3004

A Spectrum of Possibilities: Engineering Altered Nutrition and Color in Soybean Seed. B. JOYCE, S. Burns, D. Tucker, P. LaFayette, and W. Parrott. The Center for Applied Genetic Technologies, The University of Georgia, Athens, GA 30602. Email: bjoyce3@utk.edu

Consumers associate quality of food with color and flavor. Without certain carotenoids egg yolks, butter, and even shrimp are white instead of their expected color. Hence, carotenoids must be incorporated into animal diets to obtain products that meet consumer expectations and meet animal nutritional requirements. Such carotenoids can be one of the most expensive feed ingredients, accounting for 15-25% of total food costs. Soybean meal is a chief source of animal feed in the US, but is devoid of carotenoids. If soybean feed already contained carotenoids, farming industries would have a more economical choice for animal feed. The goal of the project was to genetically engineer soybean to first produce carotene, and then the derived compounds, canthaxanthin and astaxanthin. Soybean embryos were shot in a gene gun with an engineered plasmid containing the phytoene synthase (*crtB*) gene for beta-carotene production from *Erwinia uredoformans* placed behind the cotyledon-specific soybean lectin promoter. Another plasmid which contained a cassette for *crtW*, a ketolase from *Nostoc punctiforme* in addition to *crtB*, was also used to attempt to create cell lines able to produce canthaxanthin. No expression of *crtW* was obtained, perhaps due to the presence of cryptic splice sites in *crtW*. Embryogenic cell lines and plants producing orange seed were recovered. Some cell lines also produced plants with orange leaves, suggesting that the lectin promoter is not completely seed-specific.

P-3005

A Highly Efficient Ovule Culture Method for Obtaining Hybrid Plants from Crosses between *Nicotiana tabacum* and *Nicotiana obtusifolia*. W. (Q.-W.) Huang, C. (H.) Xie, T. Bass, H. Qiu, and B.-C. Li. Kentucky Tobacco Research and Development Center, University of Kentucky, Lexington, KY 40546-0236. Email: bli2@uky.edu

Interspecific hybridization between *Nicotiana tabacum* ($2n=4x=48$, pollen donor) and *Nicotiana obtusifolia* ($2n=2x=24$) has been strategized as an important parent combination in an effort to produce varieties for applications of plant-made pharmaceuticals. However, hybrid plants from such crosses are very hard to obtain because the hybrid embryo and endosperm tend to degenerate at its early developmental stage. In order to develop an efficient method for obtaining hybrid plants from crosses between *N. tabacum* and *N. obtusifolia*, a factorial experiment was conducted to investigate effects of days after pollination (DAP, 4, 5 and 6) and effects of ovule culture media [OCM, a shoot induction medium (SIM) and a germination medium (GM), both with 8% sucrose] on recovery of hybrid plants. Our results indicated that DAP/OCM interaction was significant. The 6DAP/SIM combination was most effective: 22% of the ovules from this combination were responsive to shoot organogenesis and an average of 10.6 shoots could be produced per responsive ovule four weeks after being transferred to SIM containing 3% sucrose. All shoots recovered rooted and grew well when transferred to soil. The 5DAP/SIM combination responded the second best: 10% of the ovules produced shoots. The remaining combinations responded poorly. All three DAP/GM combinations, 4DAP/GM, 5DAP/GM and 6DAP/GM, yielded 2.8-7.5% germination rates, 11.6% of those that germinated were able to root and 12.5% of those rooted survived after being transfer to soil. Phenotypes of plants recovered from all three DAP/SIM combinations were

intermediate to both parents. The chromosome numbers were also determined using a root-tip staining technique for those plantlets obtained from 6DAP/SIM combination. Among the 34 plantlets examined, 32 had expected 36 chromosomes and the remaining two 48 chromosomes. In summary, we have developed a highly efficient method for obtaining hybrid plants from crosses between *N. tabacum* and *N. obtusifolia* by culturing hybrid ovules on a shoot induction medium, and as many as 233 hybrid plants could be recovered per 100 ovules.

P-3006

Suppression of Soybean Oleosin Results in Malformation and Aggregation of Oil Bodies into Organelle Complexes. M. A. Schmidt and E. M. Herman. USDA/ARS, Donald Danforth Plant Science Center, St. Louis, MO 63132. Email: mschmidt@danforthcenter.org

Soybeans are produced for their oil and protein content. Soybean seed oil is sequestered during seed maturation into discrete ER-derived organelles bounded by a half-unit phospholipid membrane into which is embedded oleosin proteins encasing the organelle in protein. Oleosins are found associated with seed and pollen oil bodies, while fruit oil bodies possess only the phospholipids membrane without any additional proteins. The existence of oil bodies without proteins appears to indicate that oleosins have a role in events after the formation of the organelle. The association of oleosins only with oil bodies contained in cells that undergo developmentally regulated desiccation and hydration has led to the proposal that oleosins prevent oil aggregation in the drying cell. Using RNAi technology, an essentially complete suppression of the immunologically detectable oleosin has been obtained. Specifically, an RNAi construct was transformed into soybean through somatic embryogenesis and biolistics, and it contained an oleosin sequence-specific hairpin loop driving by a seed-specific promoter. The resulting seeds have been assayed structurally with light and electron microscopy/cryofixation and proteomics. Two-dimensional gel analysis of the oleosin suppressed soybeans show few changes in the seed proteome. In contrast, light microscopy and high pressure cryofixed EM material of maturing soybean seeds show that the oil bodies are formed and remain in large interconnected complexes of oil bodies widely varying in size and aggregated with the ER. On desiccation, the oil bodies aggregated into massive oil bodies resulting in cellular disruption and a low germination rate. These results indicate that oleosins have a role in mediating the correct formation of soybean oil bodies.

P-3007

Engineering Aluminum Tolerance in Alfalfa. LANG SHEN, Peter LaFayette, Joseph Bouton, and Wayne Parrott. Department of Crop & Soil Sciences, University of Georgia, Athens, GA 30602. Email: shenlang@uga.edu

Alfalfa is the most important forage legume in the world. It is also the fourth most widely planted crop species in the USA. However, aluminum toxicity in acid soils poses a serious limitation to production of alfalfa and other crops. Application of lime can reduce soil acidity, but it is too expensive for poor farmers or low-value crops. Traditional breeding methods have not been able to breed aluminum resistance into alfalfa. Increased synthesis and exudation of organic acid is a natural mechanism for aluminum-tolerance in a wide variety of plant species. Accordingly, *Agrobacterium*-mediated transformation is being used to enhance aluminum tolerance in alfalfa. First, a citrate synthase gene from *Pseudomonas aeruginosa*, driven by either a constitutive promoter (MtHP) or by one of two root-specific promoters (MtPT1 and ROC) is being over-expressed in alfalfa to increase the citrate

content in cells, using kanamycin as the selectable marker. Another gene (DcPA1), encoding a plasma membrane proton-ATPase from *Daucus carota* and which has been shown to increase the export of citrate into the rhizosphere, is also being over-expressed with the same promoters, using hygromycin as the selectable marker. Transgenic lines expressing CS or DcPA1 will be crossed to produce alfalfa lines having two traits simultaneously.

EMBRYOGENESIS/REGENERATION/ MICROPROPAGATION

P-3014

Induction of Somatic Embryogenesis and HPLC Analysis of Chromones in *Saposhnikovia divaricata* Schischkin - a Medicinal Herb. HUNG-CHI CHANG¹, Dinesh C. Agrawal², Chao-Lin Kuo³, Ji-Luan Wen¹, Chung-Chuan Chen³, Chia-Yung Lu², and Hsin-Sheng Tsay². ¹Institute of Chinese Pharmaceutical Science, China Medical University, Taichung 40402, TAIWAN; ²Graduate Institute of Biotechnology, Chaoyang University of Technology, Wufong, Taichung 41349, TAIWAN; and ³School of Chinese Medicine Resources, China Medical University, Taichung 40402, TAIWAN. Email: hstsay@cyut.edu.tw

Root of *Saposhnikovia divaricata* Schischkin (Umbelliferae) is an important Chinese and Japanese traditional medicine called "Fang-feng" and "Bofu", respectively. It has been used for treatment of analgesic symptoms. Some of ingredients from roots of *S. divaricata* have been isolated. Among these, chromones; cimifugin, prim-O-glucosyl-cimifugin and 4'-O- β -D-glucosyl-5-O-methylvisamminol contribute to the pharmacological efficacy, such as anti-hypertension and analgesia. We report a rapid and simple in vitro propagation system by induction of primary and secondary somatic embryogenesis (SE) in *Saposhnikovia divaricata*. Plants derived from somatic embryos developed multiple roots, which could be of advantage since roots of *S. divaricata* are used as crude drug. HPLC analysis of different parts of tissue culture plants showed comparable quantities of chromones in above ground parts, especially leaves indicating a need of pharmacological function of these parts to be investigated, if these could be used as crude drug as supplement to or substitute of roots.

PLANT TISSUE CULTURE

P-3008

Variability Between Vials of Cryopreserved Shoot Tips from Tissue Cultured Plants. D. D. ELLIS, G. Holman, E. Staats, B. Ambruzs, and M. Jenderek. National Center for Genetic Resources Preservation, Plant Genetic Resources Preservation Program, Fort Collins, CO 80521. Email: david.ellis@ars.usda.gov

At the USDA-ARS National Center for Genetic Resources Preservation cryopreservation is used as a method of long-term storage of valuable plant genetic resources from vegetatively-propagated crop plants. The main germplasm collections for vegetatively-propagated crops are maintained as field plantings, with some also maintained as tissue culture collections. While the field plantings serve as a great source of propagules for distribution of germplasm, they are acutely vulnerable to loss through both biotic and abiotic factors. Tissue culture collections are expensive to maintain and are vulnerable to equipment malfunctions as well as contamination. The loss of any of this germplasm can be catastrophic as even if representatives still exist in the wild or other collections, since importation to the United States to replace the germplasm may not be possible. Therefore cryopreservation of these valuable genetic resources offers a secure and safe replicate of the collection. Already, cryopreserved material has been used to

replenish *Malus* accessions lost in field plantings due to disease. To be of practical use, duplicate back-up collections need to be readily available and replacement of field materials should not deplete the duplicates stored in the cryobank. One question that arises when creating a cryopreservation bank to duplicate field material is how many shoot tips are needed for long-term back-up and how many vials should be pulled to ensure the regeneration of a healthy plant. We, therefore, have investigated vial-to-vial variability in viable *Ribes*, *Fragaria* and *Rubus* shoot tips stored in liquid nitrogen to provide baseline information in the design of protocols for a cryopreservation genebank. In *Ribes*, viability of cryopreserved shoot tips was very high, >80% and vial-to-vial variation in viability was extremely low. In contrast, vial-to-vial shoot tip viability was much more variable in both *Fragaria* and *Rubus*, although the variability was still within acceptable ranges for cryobank management. These studies clearly demonstrate that with ten shoot tips/vial and a minimum acceptable viability of 40%, single vials can safely be pulled to ensure success in producing a viable plant for replacement of field plantings, leaving remaining vials in cryopreservation for future needs.

P-3009

The Effects of Mineral Nutrients on Callus Growth and In Vitro Shoot Multiplication. R. P. NIEDZ and T. J. Evens. USDA-ARS-US Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: Randall.Niedz@ars.usda.gov

The effect of the ratio and quantity of NH_4^+ , NO_3^- , and K^+ were characterized on sweet orange callus growth cv. 'Hamlin' and in vitro shoot multiplication of *Gerbera* daisy cv. 'Pasadena.' NH_4^+ , NO_3^- , and K^+ were treated as independent factors with all other mineral nutrients held constant, thus the experiment was free of ion confounding and the underlying effects on in vitro growth of these nutrients could be determined. Additionally, the effects of the quantity and form of Fe on callus growth, and the quantity and ratio of Fe to EDTA on shoot multiplication were determined. Sweet orange callus growth (i.e., fresh and dry weights) was increased in excess of 200%. For *Gerbera* daisy shoot multiplication, the measured response variables included multiplying clump weight, height, width, shoot number, leaf number, and a gestalt measure. The most significant result was the substantial effect of Fe form and concentration on the production of deep green shoots. The implications of this approach in defining the appropriate types and concentrations of mineral nutrients for in vitro responses, including the importance of mineral nutrition, the limitations of traditional methods of defining mineral nutrient formulations will be discussed.

PLANT TRANSFORMATION

P-3010

Advancing Efficiency of Genetic Transformation in Dicots. A. BOYKO and I. Kovalchuk. Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, T1K 3M4, CANADA. Email: oleksander.boyko@uleth.ca

The efficiency of plant transgenesis is mainly determined by combination of two factors, effective tissue regeneration and high frequency of transformation events, which if successful yields a large number of quality transformants available for further selection. The current understanding of foreign DNA integration in plant genome suggests that this event is strongly dependent on host DNA repair machinery. Using the knowledge of plant DNA repair mechanisms we were able to upregulate the activity of homologous recombination and establish experimental conditions

allowing manipulation of frequency and perhaps the quality of integration events. We report that changing media composition enhanced regeneration frequency of tobacco plantlets and significantly improved the frequency of transformation (number of transgenic plants regenerated). Our technique permits increasing the transformation rates by 2.2-3.0 folds. Application of our novel approach for the marker-free transformation leads to 9% of total regenerated plants being transgenic or in more general terms, it allows obtaining one transgenic plant out of each medium size tobacco leaf (2x3cm) used for transformation.

P-3011

Promoter Activity of the LTR Region of a Maize Centromeric Repeat Element. Peter LaFayette¹, DOUGLAS HECKART¹, Bao Phan¹, Guernot Presting¹, R. Kelly Dawe², and Wayne Parrott². ¹University of Hawaii-Manoa, and ²University of Georgia, 111 Riverbend Rd. Athens, GA 30602-6810. Email: heckartd@uga.edu

Maize centromeres contain centromere-specific retrotransposons (CRMs). It is thought transcription of these might induce epigenetic changes, perhaps through an RNAi mechanism. In an effort to verify the source of promoter activity, the Long Terminal Repeat (LTR) of a CRM family was cloned and placed in front of the GUSPlus gene in pCAMBIA 1305.2. This construct was used to engineer rice. A 35S- GUSPlus construct and a promoterless GUSPlus construct were used as controls. Twenty-nine independent transgenic rice cell lines were obtained with the LTR-GUSPlus construct, 4 with the 35S-GUSPlus construct, and 4 with the promoterless GUSPlus construct. Cell lines transgenic for the LTR- GUSPlus construct expressed GUS at levels comparable to that of plants transgenic for 35S- GUSPlus. These results suggest that some LTRs of centromeric repeat elements have promoter activity, and may serve as a source of new promoters for use in plant transformation.

PLANT TISSUE CULTURE

P-3012

Engineering Soybean for Resistance to Root-knot Nematodes. B. L. MARTIN, G. Huang, R. S. Hussey, P. LaFayette, and W. A. Parrott. Center for Applied Genetic Technologies, The University of Georgia, Athens, GA, 30602. Email: benlm@uga.edu

Root-knot nematodes (RKN), *Meloidogyne* spp., account for a loss of over \$74 million in soybean yield per year worldwide. They establish complex feeding sites within plant roots by altering gene expression in root cells, causing enlargement and hypertrophy, inevitably resulting in visible galls. The recent identification of RKN genes involved in plant parasitism has opened the possibility for RKN control by disrupting these genes using an RNA interference (RNAi) approach. Here, 271 bp of an RKN parasitism gene, 16D10 (GenBank DQ087264), was cloned into pHANNIBAL in its sense and antisense orientation and used for soybean transformation. Five individual transgenic lines were recovered and confirmed for presence of the transgene by PCR and Southern analysis. These lines were inoculated with *M. incognita* and 25% of the 83 T_0 plants showed high resistance. One T_1 line has been through an inoculation assay and showed high resistance in 36% of the 22 tested plants. Additional evaluations will be conducted once homozygous individuals are recovered from all lines. Other parasitism genes from RKN are also being assessed.

P-3013

Transgenic Tobacco BY-2 with cDNA of Human Interferon-gamma. M. T. SADDER. Plant Biotechnology Lab, Department of Horticulture and Crop Science, Faculty of Agriculture,

University of Jordan, Amman, 11942, JORDAN. Email: sadderm@hotmail.com

This study was conducted as one of a series of experiments to assess the implementation of bio-pharming human proteins in Jordan for their advantages over other cell systems. The first selected protein was the human interferon-gamma for its importance as bio-pharmaceutical and its relatively small size. The human interferon-gamma was amplified from genomic DNA. The four exons were amplified and the PCR products were assembled in two steps; first with two exons together and then the four together. The assembled gene was sequenced and was 100% correct. The gene was cloned into a pCAMBIA binary vector, where selection marker for bacteria is kanamycin and for plants is hygromycin. The *Agrobacterium tumefaciens* strain C58C1 (rifampicin and gentamicin) was used for transformation. The construct was electroporated into the *Agrobacterium*, and screened with mini-prep and PCR for specific interferon-gamma fragment. The *Agrobacterium* harboring the binary vector was co-cultured with BY-2 tobacco cells and putative transformants were obtained. Carbenicillin was found to be more effective than Cefotaxime in eradicating bacterial contamination. DNA was extracted from those putative transformants with a modified CTAB method. PCR test showed that some lines were harboring the interferon-gamma gene.

SILENT ABSTRACT

P-3001

Induction of Somatic Embryogenesis in *Campanula cochlearifolia* Lam. RODICA BLINDU. Vegetal and Animal Citobiology Laboratory, Institute of Biology, Bucharest, CP 65-53, ROMANIA. Email: rodibl2001@yahoo.com

The aim of this study is to achieve an efficient micropropagation protocol through somatic embryogenesis in *Campanula cochlearifolia* Lam. The genus *Campanula* (Fam. Campanulaceae) is large, about 300 species of annuals, biennials and perennials that are widely distributed throughout the northern hemisphere. The largest numbers occur in Europe (Austria, Czechoslovakia, Germany, Poland, Switzerland, Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Italy, Romania, Slovenia, Yugoslavia-Montenegro, France, and Spain) and Turkey. Species are found growing in a wide array of habitats from open meadows, woodland to the subalpine and alpine. Most of these species are easily cultivated and provide a long lasting display of flowers. Some are invasive (e.g., *Campanula rapunculoides*). The importance of these species is economical, being an ornamental plant species. *Campanula cochlearifolia* Lam is native to the mountains of Europe. Flower color is usually blue to blue-violet. The species is frequently in Romania (Rodnei, Ciucasului, Barsei, Bucegi, Iezerului, Fagaras Mountains), on rocky stones, detritus, alpine meadows, calcareous. The flowering time is between July-august. In vitro studies were done for some species. An efficient protocol has been developed to induce adventitious shoots in different types of explants of *Campanula carpatica* Jacq. It was observed that explants from cv. White Uniform were more organogenesis than those from cv. Blue Clip (Sridevy Sriskandarajah et al., 2001). In my experiment, were tried some media variants added with different hormones and concentrations. A high number of in vitro somatic embryos were obtained starting from leaves on the MS basal salts added with 2, 4, 5-T 2 mg/l. The established protocol can be used for the long term-conservation programs of endemics and rare plants species of this species.

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