

Plant Contributed Papers

P-1001

Transformation of Tomato with Antimalarial Genes with an Aim to Produce Edible Vaccines. KAMAL CHOWDHURY and Mihail Kantor. Biology Department, Claflin University, Orangeburg, SC 29115. Email: kchowdhury@claflin.edu

Malaria, a disease caused by protozoan parasites of genus *Plasmodium*, is one of the world's biggest scourges. It affects 300–500 million people annually, of which an estimated 3 million lives are lost, among them over 1 million children (majority under 5 yr of age). By conventional wisdom, the immune mechanisms responsible for protection against malaria will require a multiple of 10–15 antigen targets for proper protection against various stages of malarial infection. By standard vaccination protocols, such a large number of targets cannot be delivered to humans. To circumvent these logistical difficulties to deliver the malaria vaccines to most of the susceptible patients at a fraction of a cost, antimalaria edible vaccines in transgenic tomato plants (where different transgenic plants expressing different antigenic type(s), immunizing individuals against multiple antigens and against each stage of the life cycle of this multistage parasites) would be an efficient, inexpensive, and safe way of vaccination. To accomplish this goal, as a first step, five varieties of tomato were tested and identified two most regeneration-responsive varieties. These two varieties were used to optimize efficient transformation protocol for transforming those varieties with selected malarial antigens representing different stages of the life cycle of the parasite *Plasmodium falciparum*. Currently, vaccines against the merozoite surface protein (MSP) antigens are developed using conventional method and are at different stages of clinical trials. The MSP genes (*MSP-1*, *MSP-2*, and *MSP-3*) of parasite *P. falciparum* were cloned to appropriate Ti-plasmid vectors separately and used to transform the two varieties of tomato. Transgenic tissues are being selected for regenerating transgenic plants. Transgenic fruits will be tested for the expression of the antigen.

P-1002

In Planta Expression and Molecular Characterization of the Candidate HIV-1 Mucosal Vaccine CTB-MPR_{649–684}.

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Plants are potentially the most economical platforms for the large-scale production of recombinant proteins. Thus, plant-based expression of subunit HIV-1 vaccines provides an opportunity for their global use against the AIDS pandemic. CTB-MPR_{649–684} is a translational fusion protein comprised of cholera toxin B subunit and residues 649–684 of the HIV-1 gp41 membrane proximal region. The fusion protein has been previously shown in animal models to induce MPR-specific Abs, which successfully blocked a pathway of HIV-1 mucosal transmission in a human tight epithelial model. Here, we report on the molecular characterization of the CTB-MPR_{649–684} proteins expressed in transgenic *Nicotiana benthamiana* plants. It was shown that virtually all of the CTB-MPR_{649–684} proteins expressed in the selected line assembled into pentameric, GM1-ganglioside-binding complexes. Detailed biochemical analyses revealed that the protein is N-glycosylated, predominantly with high mannose type glycans (more than 75%), as predicted from a consensus Asn-X-Ser/Thr N-glycosylation sequon on the CTB domain and the KDEL ER retention signal attached at the C terminus of the fusion protein. Despite this modification, the plant-expressed protein was shown to retain the critical antigenicity of the MPR_{649–684} moiety and nanomolar affinity to GM1 ganglioside. Furthermore, the protein induced mucosal and serum anti-MPR_{649–684} Abs in mice after mucosal prime-systemic boost immunization. These results indicate that plant-based expression can be a viable alternative for the production of this subunit HIV-1 vaccine candidate.

P-1003

Production of Cervical Cancer-related HPV 16E7 as a Pharmaceutical Protein in Rice Seeds. AMIT MEHRA and Murphy J Brad. University of Arkansas, Department of Horticulture, 316 PTSC Building, Fayetteville, AK 72701. Email: amehra@uark.edu

Cervical cancer is still one of the main causes of cancer-related deaths in women of developing countries, and in developed countries, 5–6 million women per year are diagnosed with lesions caused by high-risk human papillomavirus (HR-HPV). The E6 and E7 oncoproteins of HR-HPV16 are considered to be involved in causing and maintaining the transformed state of tumor cells. A prophylactic vaccine (Gardasil) has recently been developed. However, there is long latency period between infection by HPV and cancer, thus a need exists to develop a therapeutic vaccine that can block the progression of preexisting lesions and eliminate tumorous cells. Plant expression systems have the potential for safe, economical, and large-scale production of recombinant proteins for pharmaceutical, industrial, and agricultural applications. Here we report the production of HPV 16E7 in rice seeds. Two different types of transgenic rice plants were produced with respect to subcellular targeting of recombinant E7. In one set, E7 was directed to the ER by utilizing the ER signal peptide of the rice seed storage protein, glutelin 1 (Glt 1). In another set, E7 was targeted to protein storage bodies by utilizing the CTPP of rice lectin. *Agrobacterium*-mediated transformation was used on callus of the rice cultivar Nipponbare. The Glt 1 promoter was used to control the expression of E7, and the expression cassette included nucleotide sequences for a 6X-His tag and FXa protease as a purification scheme. A large number of independent transformants were regenerated and characterized by PCR. The expression of E7 was confirmed by Western blot analysis and ELISA. The recombinant E7 was purified with a His-Trap column, the 6X-His tag cleaved by FXa protease, and the E7 separated from FXa on a benzamidine column. Western blotting showed that the CTPP signal on the carboxyl end of E7, targeting the protein to the protein storage bodies of rice seeds, was not cleaved efficiently.

P-1004

Plant-derived Intimin Vaccine to Prevent Colonization of Enterohemorrhagic *Escherichia coli*. EMEL TOPAL, Maria Lucrecia Alvarez, and Hugh S. Mason. Center for Infectious Diseases and Vaccinology (CIDV), The Biodesign Institute at Arizona State University, 1001 South McAllister Avenue, Tempe, AZ 85287. Email: topalemel@yahoo.com

Enterohemorrhagic *Escherichia coli* (EHEC) is one of the most common food-borne pathogens all over the world. *E. coli* serotype O157:H7 is the main EHEC serotype in these infections. The infection ranges from mild diarrhea to hemolytic uremic syndrome. Cattle are the direct or indirect source of the majority of human infections. The aim of this research is to understand the bacterial colonization mechanism in cattle and to create a vaccine that will remove

bacteria from its primary source to prevent human infection. Thus, we have established transgenic tomato using *Agrobacterium*-mediated transformation. We have selected elite lines which express intimin, a surface adhesin protein of *E. coli* O157:H7, from tissue culture plants. We used two expression plasmids, pNR49 and pNR50, which encode the carboxyl terminal 261 amino acids of intimin (int261). pNR49 contains a plant signal peptide (vspA) to direct ER targeting, whereas pNR50 has no signal peptide. As previously reported, ER targeting increased the expression level of intimin protein in tobacco cells, but resulted in highly glycosylated int261 with altered antigenicity. However, we did not detect glycosylation of tomato-expressed int261 by Western blot, and the expression levels of intimin in tomato plants that were transformed with pNR49 were 10 tissues higher than plants transformed with pNR50. Fruits from elite tomato plants were freeze-dried and the expression of intimin was determined by ELISA. The fruits expressing the highest level of intimin protein (~ 1 mg intimin per g of tomato powder) were selected to be used in future animal trials to test its immunogenicity in mice.

P-1005

Elimination of the Three Major Allergens in Transgenic Peanut (*Arachis hypogea* L). ANTHONY ANANGA, Hortense Dodo, and Koffi Konan. Food Biotechnology Laboratory, Department of Food and Animal Sciences, Alabama A&M University, 4900 Meridian St. Carver Complex S. P. O. Box 1628, Normal, AL 35762. Email: anthony.ananga@gmail.com

Peanut hypersensitivity is a common and serious food allergy with regards to the persistence of the affliction, the prevalence of peanut in food supply, and the severity of the response. Some individuals who are allergic to peanuts are susceptible to life-threatening reactions upon exposure to peanut allergens. Currently, there is no treatment to prevent these reactions other than avoidance of foods containing peanut proteins. The increasing use of peanut as an economic protein source in processed foods makes accidental ingestion inevitable. Genetic engineering of peanut plants through post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) represents a promising alternative to reduce the incidence of peanut allergy. An RNAi genetic construct containing a tandem fragment of the genes encoding the three major peanut allergens was used to produce transgenic peanut via *Agrobacterium*-mediated transformation. In this study, molecular and immunological analyses including SDS-PAGE, Southern and Western blot hybridizations, and ELISA were used to screen transgenic peanut seeds. The data revealed the reduction/elimination of Ara h 1, Ara h 2, and Ara h 3,

which occurred in 9%, 10%, and 16%, respectively in transgenic seeds. In addition, 3% seeds were found to be free of all three allergens. The IgE-binding capacity of the Ara h minus seeds was significantly reduced in at least nine transgenic seeds compared to that of wild-type control. This research has the potential to alleviate the phenomenon of peanut allergy.

P-1006

Seed Physiology of *Bletia purpurea* (Pine Pink; Orchidaceae)—Fluctuating Low Temperature and Dark Slow Development and Inhibit Germination. T. R. JOHNSON, M. E. Kane, and H. E. Perez. Environmental Horticulture Department, University of Florida, Gainesville, FL 32611. Email: timjohn@ufl.edu

Bletia purpurea is a threatened neotropical orchid restricted in distribution to south Florida, USA. Little is known about the seed physiology of orchids and the role temperature plays in controlling germination. The objective of this study was to determine the effect of (1) dark and day neutral light conditions and (2) simulated seasonal temperatures (22°/11° C [winter], 27°/15° C [spring], 33°/24° C [summer], 29°/19° C [fall], and 25° C [control]) on the germination and early development of *B. purpurea* seeds. Seeds cultured under day neutral 22°/11° C. conditions exhibited lower total germination (\pm SE) after 3 wk (37.3 \pm 7.5%) than other treatments (58.3–93.7%), but germination after 6 wk (92.5–98.8%) was not significantly different between treatments. After 3 wk culture, maximum protocorm development was limited to stage 4 (emergence of second leaf) when seeds received light at 25° C (2.5 \pm 0.5%), 29°/19° C (1.7 \pm 1.1%), and 33°/24° C (1.0 \pm 1.1%). Maximum development in other treatments was limited to stage 3. Maximum development of protocorms after 6 wk under 22°/11° C light or dark treatments was limited to stage 4. Stage 5 (elongation of second leaf) protocorms were observed under all other treatments. Development to stage 6 (emergence of third leaf) was only observed under 25° C lighted conditions (20.7 \pm 10.0%). These results indicate that early development of *B. purpurea* protocorms is facilitated by light and that low fluctuating temperatures inhibit both germination and development. Significantly greater germination of seeds cultured under 22°/11° C dark conditions than 22°/11° C lighted conditions indicate that light may inhibit germination at low seasonal temperatures.

P-1007

Asymbiotic Seed Germination and In Vitro Seedling Development of *Cyrtopodium punctatum*: a Propagation Protocol for an Endangered Florida Native Orchid. D.

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Cyrtopodium punctatum is an endangered epiphytic orchid restricted in the U.S. to southern Florida. Due to its ornamental value, the species was extensively collected from the wild in the past 100 yr. Today, only a few plants remain in protected areas. Currently, a conservation plan for the species is being designed. In this study, procedures for asymbiotic seed germination were developed. Five asymbiotic orchid seed germination media (*Phyto*Technology Orchid Seed Sowing Medium, Knudson C, Malmgren Modified Terrestrial Orchid Medium, Vacin & Went Modified Orchid Medium, and half-strength Murashige & Skoog) were examined for their effectiveness in promoting seed germination and protocorm development. The role of photoperiod on growth and development was also examined. Seeds were germinated under a 16/8 h, 12/12 h, or 8/16 h L/D photoperiod at 25 \pm 3° C and then allowed to develop in vitro for 10 wk. After 10 wk, developing seedlings were transferred to *Phyto*Tech Culture Boxes and returned to their assigned photoperiod treatments for continued seedling development for an additional 20 wk. Germination occurred regardless of photoperiod and media treatments; however, advanced stages of seedling development occurred only on *Phyto*Technology Orchid Seed Sowing Medium. The defined media and cultural requirements for asymbiotic seed germination and subsequent seedling development of *C. punctatum* will be used to produce plants for future reintroduction projects in southwest Florida.

P-1008

Synergistic Effect of Auxin and Cytokinin on In Vitro Androgenesis in *Azadirachta indica* A. Juss. RAKHI CHATURVEDI and Priyanka Srivastava. Department of Biotechnology, Indian Institute of Technology-Guwahati (IITG), Guwahati, 781039, INDIA. Email: rakhi_chaturvedi@yahoo.co.uk, rakhi_chaturvedi@iitg.ernet.in

In vitro androgenesis is the most prolific and popular approach of haploid production. The technique becomes a valuable tool, particularly in tree species, for producing double haploids in a short span of time, as the majority of the trees are outbreeding, highly heterozygous, and have long generation cycle. The perspective of raising haploid plants through in vitro androgenesis, thus, offers scores of foreseeable advantages like, shortening of breeding period, production of homozygous diploid lines in a single step

through chromosome doubling, and isolation of valuable recessive traits at the sporophytic level which, otherwise, remain accumulated and unexpressed in natural heterozygous diploid population. Because of immense medicinal and economic benefits, the present work was undertaken to raise in vitro haploids in neem. The neem has attained global importance as each and every part of the tree, particularly the leaves, bark, and seeds, has multiple uses. A number of significant factors are known to govern this process under in vitro conditions; combinations and concentrations of growth regulators present in the medium is one of them. Therefore, in the present work, the synergistic effect of auxin and cytokinin has been studied on in vitro androgenesis in neem (*Azadirachta indica* A. Juss.). A definite effect of growth regulators had been witnessed right from the initial stage of callus induction, multiplication to the stage of shoot differentiation, in anther culture of this tree species. Anther culture experiments were raised during the months of March–May, which is the flowering season of neem in India. The size of the bud was considered as a physical marker to select the appropriate developmental stage of microspores at culture. Thus, the anthers were excised from 2-mm size flower buds which carry microspores at early–late uninucleate stages. The stage of microspores were determined by 4,6-diamidino-2-phenylindole (DAPI), a fluorescent dye and acetocarmine squash preparations. After surface sterilizing the buds with 0.1% mercuric chloride solution followed by three rinses in sterilized distilled water, the anthers were excised and plated on Murashige and Skoog's (MS; 1962) medium with 9% sucrose and various combinations of growth regulators. A higher concentration of sucrose was used in the induction medium because it is known to suppress the divisions of diploid cells, thus facilitating proliferation from the haploid microspores. However, the concentration was restored to the normal 3% in the subsequent steps of callus multiplication and regeneration. MS basal medium alone did not show any response at any stage of culture during this study. When either auxin(s) or cytokinin(s) compounded with the medium, response was poor during the initial stages. However, the mixture of two auxins and one cytokinin proved to be beneficial for initial callus induction from the anthers; MS+2,4-D (1 μ M)+NAA (1 μ M)+BAP (5 μ M) served the purpose. For further multiplication and maintenance of callus, one auxin and one cytokinin were sufficient; MS+2,4-D (0.5 or 1 μ M)+Kn (4.5 or 10 μ M) were found to be most suitable. This interaction of auxin and cytokinin in the callus induction and callus multiplication medium also bore a profound effect on shoot organogenesis in the regeneration medium. MS medium supplemented with a cytokinin BAP at 5 μ M showed maximum shoot regeneration (75%). Shoots were elongated

well at a lower concentration of BAP (0.5 μ M) and rooted on an auxin-supplemented medium IBA at 0.5 μ M, thereby proving once again, the conventional roles associated with these two classes of growth regulators. Conclusively, a right mix of auxin and cytokinin is essential for the induction of cells during the early stages of culture. This synergism between the two helps and, thus, has been found mandatory to obtain organogenesis and development of haploid plants from anther cultures of neem.

P-1009

Standardization of Protocol for Efficient In Vitro Clonal Propagation of Rare Medicinal Plants e.g., *Elaeocarpus* and *Capparis*. L. N. SHUKLA, C. P. Shukla, B. K. Mishra, Manoj Kumar, Sushma Kumari, and T. Upadhyay. University Department of Botany, B. R. Ambedkar Bihar University, Muzaffarpur, Bihar, INDIA. Email: shukla_in@rediffmail.com

Elaeocarpus (Elaeocarpaceae) and *Capparis* (Capparidaceae) are important medicinal plants. Different parts of *Elaeocarpus* and *Capparis* are used for the treatment of different diseases. As both these species are endemic, restricted to smaller areas, and threatened too, in vitro clonal propagation appears an alternative for their conservation and reintroduction in nature in terms of their multiplication within restricted periods and conservation of genotype of both the species. In the present work, in vitro cloning of *Elaeocarpus* and *Capparis* has been carried out. Both MS basal medium and woody plant medium were supplemented with various concentration of BAP, KN, NAA, and IBA. BAP alone or along with KN or vice versa were used for axillary shoot bud initiation or tested. The nodal explants of both the species gave different results. However, in both the species, MS basal medium, supplemented with different concentration of BAP, gave better results. Similarly, BAP at various concentrations gave better results than KN. When both the growth promoting cytokinins were used together, significant differences were not found. Exudation of the phenolics and its oxidation was reduced by adding antioxidants like ascorbic acid and citric acid at an equal concentration. The in vitro raised axillary branches on subculturing produced multiple shoots and in some culture jars the number reached up to 40. Plantlets measuring about 8–10 cm with well-developed leaves were used for rooting experiments. These plantlets were cultured in half-strength MS medium supplemented with various concentrations of IBA and NAA. The number of roots varied in different culture conditions. *Abbreviation:* BAP—6 benzyl aminopurine, KN—kinetin, IBA—indole butyric acid, NAA—naphthalene acetic acid, MS—Murashige and Skoog.

P-1010

Germplasm Evaluation of *Andrographis paniculata* (Kalmegh) Through Chemoprofiling for In Vitro Mass Multiplication of Quality Germplasm from Satpura Plateau Region of Madhya Pradesh. S. K. TIWARI, Vijay Bahadur, Amit Pandey, Shweta Mishra, M. P. Goswami, and Pankaj Bhargava. Forest Genetics, Plant Propagation & Biotechnology Division State Forest Research Institute, Polipathar, Jabalpur (M. P.) INDIA. Email: Drsktiwari1963@rediffmail.com

Kalmegh (*Andrographis paniculata* Nees) commonly known as “king of bitter” belongs to family Acanthaceae, is an important annual medicinal herb widely distributed in Madhya Pradesh. The plant is used in Ayurvedic and Homeopathic systems of medicines. The whole plant part known as “Panchang” contains an important alkaloids viz. andrographolide and neoandrographolide, which are used in preparation of several drugs. The alkaloids percent in plant samples varies from place to place. To assess these variations in different agro-climate zones of Madhya Pradesh. A simple quick and accurate HPLC method using C-18 ODS-2 column for the determination of particular andrographolide is highlighted in this paper. The best germplasm in terms of highest alkaloid content of this species has been evaluated. The highest concentration of andrographolide was found 0.77% at Chhindwara region. By using axillary bud culture, large scale in vitro multiplication from this certified germplasm is in progress and on an average 20 to 25 fold multiplication has been achieved within 90 days.

P-1011

Micropropagation of *Boerhaavia diffusa*—a Valuable Medicinal Plant. P. REGHA¹, N. Vinod Kumar¹, M. S. Kavitha¹, M. Rajasekara Pandian², and E.G. Wesely¹. ¹Center for Biotechnology, Muthayammal College of Arts and Science, Rasipuram, 637408, Tamil Nadu, INDIA and ²Department of Zoology, Aaringar Anna Government Arts College, Namakkal, Tamil Nadu, INDIA. Email: pregha@yahoo.co.in

Boerhaavia diffusa is an herbaceous plant of the family Nyctaginaceae. The whole plant is known to have lot of medicinal properties (immunomodulator, hepatoprotective, and antidiabetic) and found to be one of the ten bestselling herbal medicines in developed countries. In vitro clonal propagation of *B. diffusa* was developed from mature field-grown plant using shoot tip and nodal explants. Multiple shoots were induced directly on MS medium fortified with various concentrations of BAP and kinetin. At the optimum

level of BAP (5 mg/l), the apical buds underwent a degree of dedifferentiation to develop a maximum of ten shoots per explant. The shoots regenerated in vitro had developed roots directly from the basal cut end without an intervening callus phase. However, rooting rates and root growth increased with increased concentrations of NAA and the reduction of the salt strength of the media. More than 90% of the rooted plantlets survived in the greenhouse and acclimatized to ex vitro conditions, exhibiting a normal development without any phenotypic aberrations. This standardized protocol will ensure the mass propagation of *B. diffusa* plants in limited time and space, thereby ruling out the dependence on natural stands for the plant material required for therapeutic purposes.

P-1012

Overexpression of the *Arabidopsis* Transcription Factor REVOLUTA Leads to Increased Soybean Seed Size and Yield. RUGANG LI^{1,2}, Bonnie Bancroft¹, Kristina Lum², Thu Nguyen², Jay De Rocher², and Daina Simmonds¹. ¹Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Ottawa, ON KIA 0C6, CANADA and ²Targeted Growth, Inc. 1441 North 34th Street, Seattle, WA, 98103. Email: rugang.li@targetedgrowth.com

Soybean is an important crop as food for human consumption, protein for animal feed, and oil for biofuel. Increasing demand, especially for use in animal feed in developing countries and for biodiesel globally, makes yield an even higher priority as a target trait for crop improvement. Targeted Growth initiated its soybean program in late 2004 focusing on increased soybean yield through effects on plant growth and/or seed size mediated by the overexpression of the REVOLUTA (REV) gene. REV is an HDZip transcription factor that functions in adaxial/abaxial cell fate determination and meristem growth. Phenotypes observed in *Arabidopsis* mutants and transgenic *Arabidopsis* overexpressing REV include increased seed size and thicker stems. Prior work in *Arabidopsis* and canola demonstrated that constitutive overexpression of REV results in detrimental pleiotropic effects. These effects were circumvented by overexpression of REV with appropriate promoters specifically during early embryo development with a resulting increase in seed yield and seed size. Here, we demonstrate that the application of this approach is also effective in a legume crop. Through our efficient transformation and plant regeneration protocols, we are able to produce transgenic T1 seeds in as little as 9 mo. Optimized plant growth conditions enable us to produce mature plants bearing sufficient T2 seed in no longer than 3.5 mo. REV transgene constructs

were delivered into two soybean lines from different maturity groups. T1 homo- and null-plants were identified by molecular analysis and T2 seeds expanded in greenhouses or fields (Canada, Chile, or Puerto Rico). The effect of REV on yield was evaluated in replicated field trials. Our data demonstrated that embryo-specific REV overexpression led to soybean yield increases of as high as 20%. Increased yield is due to both increased seed size and seed number. By developing novel yield technologies, Targeted Growth hopes to increase soybean yield and thereby help to meet the competing and growing demands for a limited crop resource.

P-1013

Field Testing Transgenic Grapevine for Disease Resistance. D. J. GRAY¹, Z. T. Li¹, S. A. Dhekney¹, D. L. Hopkins¹, and T. W. Zimmerman². ¹Mid-Florida Research and Education Center, University of Florida/IFAS, 2725 Binion Road, Apopka, FL 32703-8504 and ²University of the Virgin Islands, Agricultural Experiment Station, Biotechnology and Agroforestry, RR1 Box 10,000, Kingshill, St. Croix, VI 00850. Email: djg@ufl.edu; Website: <http://www.mrec.ifas.ufl.edu/grapes/genetics>

Efficient genetic transformation procedures established for an increasing number of grapevine (*Vitis* sp.) scion and rootstock cultivars facilitated in situ screening of genes for disease resistance. To date, two genes that encode hybrid lytic peptides (LIMA-A and LIMA-B) and one that encodes *V. vinifera* thaumatin-like protein (VVTL-1) control bacterial and fungal diseases in a greenhouse environment. As the next step in determining efficacy, USDA/APHIS-approved field sites were established in Florida and the US Virgin Islands to evaluate selected transgenic plants for resistance to bacterial Pierce's disease (PD) and a range of fungal diseases endemic to the region. In 2007, transgenic *V. vinifera* 'Merlot' and 'Thompson Seedless' and hybrid scion 'Seyval Blanc' and rootstock 'Freedom' were planted. In 2008, additional scion cultivars and rootstocks were added. In the future, transgenic rootstocks containing lytic peptide genes will be grafted to determine whether PD resistance is transmitted to nontransgenic scions. Non-transgenic control vines, along with blocks of native resistant varieties within the site and in border rows, serve as pollen trap plants to facilitate study of transgene flow.

P-1014

Overcoming Obstacles to Genetic Transformation in *Vitis*. S. A. DHEKNEY¹, Z. T. Li¹, T. W. Zimmerman², and D. J. Gray¹. ¹University of Florida/IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL

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Obstacles to genetic transformation of *Vitis*, including low embryogenic potential and transformation efficiency, were studied. Stamens and pistils from mature grapevines and leaves from micropropagation cultures were used to optimize parameters influencing somatic embryogenesis. Four developmental stages of stamens (stages 1–4) were identified for culture initiation. Embryogenic response was dependent on genotype, developmental stage of explants, and culture medium. Stages 2 and 3 stamens and pistils produced the best embryogenic response. Among media tested, the highest embryogenic responses were observed with MSI or PIV. Embryogenic cultures established in 22 *Vitis vinifera* varieties, *Vitis champinii*, *Vitis riparia*, *Vitis rotundifolia*, *Vitis rupestris*, and 8 *Vitis* hybrids were used in genetic transformation studies. Somatic embryos (SE) were subjected to various cocultivation treatments with *Agrobacterium* 'EHA 105' containing a EGFP/NPTII fusion gene, and transgenic SE were recovered via secondary embryogenesis. Among species and hybrids tested, SE cocultivated on filter paper exhibited increased transient (26–71%) and stable (37–400%) gene expression levels. Cotyledon excision of germinated SE resulted in a fourfold to sixfold increase in transgenic plant recovery. Transgenic plants were obtained in ten *V. vinifera* varieties, *V. champinii*, *V. riparia*, *V. rotundifolia*, *V. rupestris*, and five *Vitis* hybrids. Stable transgene integration in regenerated plants was confirmed by Southern blot hybridization.

P-1015

Camelina sativa Transformation by Floral Dip and Simple Large-scale Screening of Markerless Transformants. XUNJIA LIU, Sharon Leung, Jennifer Brost, Suzanne Rooke, and Thu Nguyen. ¹Targeted Growth Canada, 110 Gymnasium Place, Saskatoon, SK S7N 0W9, Canada and ²Targeted Growth, Inc. 1441 North 34th Street, Seattle, WA 98103. Email: xunjia.liu@targetedgrowth.com

Camelina (*Camelina sativa* (L.) Crtz. *Brassicaceae*), known as false flax or gold-of-pleasure, is a promising underexploited ancient oilseed crop with an unique fatty acid profile, low glucosinolate content and advantageous agronomic features. The ability to transform *Camelina* would allow the rapid introduction of new traits into this novel crop. Crop transformation methods typically rely on the use of tissue culture which requires specialized skills, is labor-intensive and slow, and generates transformants harboring undesirable mutations resulting from somaclonal variation. In addition, transformants are typically selected

based upon antibiotic or herbicide resistance conferred by cotransformed resistance marker genes. While such marker genes are useful in generating transgenic events intended for research purposes, they are problematic for transgenic events intended to be commercialized. Therefore, we have developed a *Camelina* transformation method based on a floral dip approach using markerless plasmid constructs and a simple large-scale screen for markerless transformants to bypass the issues described above. To establish an efficient floral dip transformation protocol, we transformed *Camelina* with four herbicide-marker constructs in a pilot experiment. A screen for herbicide-resistant transformants demonstrated that a transformation efficiency of 0.5–1.5% could be obtained. Forty percent of transgenic events had a single insertion which followed a 3:1 segregation pattern for herbicide resistance in the T2 generation. For T2 seeds of a given single-insert event positive for the selectable marker, one third of the T3 progeny were homozygous and two thirds segregated 3:1 for herbicide resistance, as expected for Mendelian inheritance. Typing transformants by PCR gave results consistent with the herbicide screen. We further transformed *Camelina* with four markerless plasmid constructs and screened for markerless transformants by a simple PCR method using regular filter paper disk spotted with cotyledons ground in TE buffer. We obtained a transformation efficiency similar to that of the herbicide marker transformation. In conclusion, we have developed and optimized the floral dip transformation for *Camelina sativa*. Furthermore, we have designed and validated a simple method for large-scale screening of transformants carrying marker-free T-DNAs.

P-1016

Comparative Analysis of Diploid and Polyploid Buffalograss Based on Transient Gene Expression and *in vitro* Regeneration. HIKMET BUDAK. Sabanci University, Orhanli 34956 Tuzla, Istanbul 34956, TURKEY. Email: budak@sabanciuniv.edu

Buchloe dactyloides is a warm season C4 type grass species. In this research, the objectives were to (1) evaluate the efficiency of gene delivery of in diploid, tetraploid, and hexaploid buffalograss accessions under two different combinations of bombardment pressures and sample plate distances using gold particles of different diameters and (2) develop an efficient *in vitro* regeneration system. The growth regulators with different types and concentrations supplemented with two different carbohydrate sources were also established for a reliable callus induction system using distinct explant sources. The growth regulators results indicated a high level of regeneration in hexaploid accession used compared to diploid accessions used in this

study. Successful gene delivery system was demonstrated by transient GUS expression following particle bombardment. The introduced DNA contained GUS coding sequence flanked by the 35S CaMV promoter and NOS terminator sequence. The number of mature embryo expressing GUS activity was higher in tetraploid and hexaploid buffalograss compared to diploid buffalograss.

P-1017

Genetic Transformation in Diploid Turkish *Brachypodium distachyon* Based on a Well-established Tissue Culture System. B. S. OZDEMİR and H. Budak. Sabanci University, Biological Sciences and Bioengineering Program, Orhanli 34956, Tuzla, Istanbul, TURKEY. Email: budak@sabanciuniv.edu

Brachypodium distachyon, with its diploid nature, serves as a model species for the grass family. Due to its biological and genetic properties, it is widely used in plant genetics and genomics studies. In our study; three different genotypes of *B. distachyon*, collected from different geographical regions of Turkey, were used to assess gene delivery using optimized tissue culture conditions. For callus induction, three levels of auxin hormone (2,4-D; 1.0, 3.0, and 5.0 mg l⁻¹) and two levels of cytokinin hormone (BAP; 0.0 and 0.5 mg l⁻¹) were experimented resulting in each genotype exerting different culture conditions. The callus formation percentages varied from 50% to 100% whereas regeneration percentages of the calli derived from mature embryos were between 11.7% and 52.3%. Different bombardment pressures (650, 900, and 1,100 psi) and distances (6 and 9 cm) were tested using microprojectile bombardment method. One thousand one hundred pounds per square inch pressure versus 6 and 9 cm distances, using gold particles of 1.0 and 1.6 mm diameter, respectively, caused an increase in GUS expression which was quantified by histochemical GUS staining. Transient transformation efficiency was almost 28% across all accessions. Furthermore, *Agrobacterium*-mediated transformation of these genotypes using different strains and injury conditions will be discussed in detail.

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An Efficient Protocol for Stable Transformation of *Glycin max* L. and *Capsicum annum* L. by agroinjection. ZIA MUHAMMAD. Department of Biotechnology, Quaid-i-Azam University, Islamabad 45320, PAKISTAN. Email: ziachaudhary@gmail.com

In planta *Agrobacterium*, mediated transformation via pollen tube pathway has been successfully carried out in

several plant species. Agroinjection to various fruits has been used for mainly transient expression of foreign gene in plant tissue. But there is no report of successful stable transformation by this method. In this report, the agroinjection method was developed for stable transformation of *Glucine max* (soybean) and *Capsicum annuum*. Overnight, grown *Agrobacterium tumefaciens* strain EHA105 harboring *Arabidopsis* (LEAFY) gene with GUS reporter and nptII marker was injected to immature soybean pods and capsicum fruits while still intact with plant. *Agrobacterium* was injected after 2 d of soybean pod formation and after 4 d of capsicum fruit formation. After ripening of fruit, seeds were collected and GUS assay was performed. These seeds were also germinated on plain agar medium containing 100 mg/l kanamycin. After 1 mo selected plants showed GUS expression in the stem and leaves. PCR analysis confirmed the presence of LEAFY and nptII genes. These transgenic plants showed retarded growth with normal leaves, but the leaves were less in number compared to normal plants. Transgenic plants produced flowers after about 4 wk of seed germination (which was about 2 wk earlier than the untransformed plants); however, these flowers dried before blooming and did not produce seeds. This procedure is easy and also avoids tissue culture steps; however, further work is required to optimize conditions like concentration of *Agrobacterium* culture and time of injection. Furthermore, the initial results of this study reveals that the *Arabidopsis* LEAFY gene enhances flowering in soybean and capsicum, but the flowers produced do not develop completely.

P-1019

Enhancing *Agrobacterium*-mediated Transformation Efficiency of Sugarcane: Progress Towards an Efficient, Genotype-independent Method. H. K. KHANNA, M. Bokan, M. Harrison, L. Kancherela, M. B. Dickman, and J. L. Dale. Centre for Tropical Crops and Biocommodities, Queensland University of Technology, 2 George Street, Brisbane QLD 4001, AUSTRALIA. Email: h.khanna@qut.edu.au

Several key issues need to be resolved before an efficient and reproducible *Agrobacterium*-mediated sugarcane transformation method can be developed for a wider range of sugarcane cultivars. These include loss of morphogenetic potential in sugarcane cells after *Agrobacterium*-mediated transformation, effect of exposure to abiotic stresses during in vitro selection, and most importantly the hypersensitive cell death response of sugarcane (and other nonhost plants) to *Agrobacterium tumefaciens*. Eight sugarcane cultivars (Q117, Q151, Q177, Q200, Q208, KQ228, QS94-2329, and QS94-2174) were evaluated for loss of morphogenetic potential in response to the age of the culture, exposure to *Agrobacterium* strains, and exposure to abiotic stresses during selection. Corresponding changes in the polyamine profiles of these cultures were also assessed. Strategies were then designed to minimize the negative effects of these factors on the cell survival and callus proliferation following *Agrobacterium*-mediated transformation. Some of these strategies, including the use of cell death protector genes and regulation of intracellular polyamine levels, will be discussed.