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Poster Abstracts

P-001

Distinct Fluorescent Labeling of Each Cell Type within the Arabidopsis Egg Sac

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In *Arabidopsis*, the female gametophyte is comprised of four unique cell types: one egg cell, a central cell, two synergids, and three or more antipodal cells. Each cell type is structurally distinct and each except the antipodal cell type has a well-characterized, essential function in sexual reproduction. Previous studies have focused on the gene regulatory networks that confer this high degree of specificity, and genes that are expressed in each of the four specific cell types have been identified and characterized via promoter/GFP fusions (Steffan et al., *Plant Journal* 2007). We have fused these and other cell-type-specific promoters to four different fluorescent proteins and stably transformed *Arabidopsis* plants. This allows simplified visual tracking of each cell type from early gametophyte cellularization through fertilization and zygote development. Transgenic lines with various combinations of these synergid, egg cell, central cell, and antipodal markers have been produced. These lines and derivatives of these constructs will be utilized not only for promoter characterization but also for tracking of cell fates or cell sorting for additional promoter isolation.

P-002

Hyperthermostable Xylanase Xyl10B Produced in Transplastomic Plants Converts Hemicellulose to Fermentable Sugars for Fuel Ethanol Production

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Biofuel production from lignocellulosic biomass depends on technology that efficiently and economically releases fermentable sugars from multi-polymeric cell wall components. Xylan is after cellulose, the most abundant polysaccharide in grass and wood biomass, and must be hydrolyzed to its component sugars (xylose or xylobiose) before fermentation to ethanol. Endoxylanases are the main enzymes involved in xylan hydrolysis. Transplastomic production of cell-wall-degrading enzymes will reduce costs of enzyme production. The coding sequence of the hyperthermophilic xylanase xyl10B was isolated from genomic DNA of *Thermotoga maritima* and subcloned under control rice prrn promoter, 5′UTR of gene 10 of phage T7 and first 14 amino acids of gfp and the rps16 3′ UTR. The partial rbcL and accD gene sequences flanked the xyl10B expression cassette and the selectable aadA expression cassette for site-specific integration into the chloroplast genome. Transplastomic tobacco plants were regenerated following biolistic gene transfer of the integrative chloroplast transformation cassette and selection on spectinomycin-containing media. To obtain homotransplastomic plants, two additional cycles of de-differentiation and plant regeneration were carried out on spectinomycin-containing media. Site-specific integration of the transgenes into the chloroplast genome was confirmed by PCR using primer combinations annealing to the chloroplast genome and the transgene. Xylanase expression was confirmed by Western blot analysis. Heat stability and functional activity of the recombinant Xyl10B were confirmed by Congo red assay with 2% birch wood xylan and fluorometric xylanase activity assay using 6,8-difluoro-4-methyllumbelliferyl β-D-xylobioside as substrate. Thin-layer chromatography of sugarcane or sweetgum xylan incubated with in-planta-produced recombinant Xyl0B at 85°C revealed that the

majority of xylan was converted to fermentable xylobiose and xylose, suggesting that in planta expression of hyperthermostable Xyl10B will enhance conversion of hemicellulose to ethanol.

P-003

The Relationship among Knowledge of, Attitudes Toward, and Acceptance of Genetically Modified Plants among Slovenian Teachers and Prospective Teachers

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The objective of this study was to investigate opinions on, attitudes toward, and readiness to accept genetically modified plants (GM plants) among Slovenian teachers and prospective teachers and their relation to other genetically modified organisms (GMOs). On average, they both have higher levels of knowledge in classical genetics, poor levels of knowledge about modern issues in biotechnology, and on average negative attitudes toward GM plants. GM plants and microorganisms are more acceptable than animals. The acceptability of a particular GM plant follows two patterns: GM plants are more acceptable if they cannot be used directly for consumption and/or produce something recognized as useful. The relationship among knowledge of classical genetics and modern issues in biotechnology, attitudes towards modern issues in biotechnology, and readiness to accept GM plants showed that there is a weak correlation between knowledge and attitudes, no correlation between knowledge and acceptance, and a solid correlation between attitudes and readiness to accept GM plants. The practical implication of our findings is that acceptance of GM plants (and other GMOs) will not be changed by providing new technical or scientific information to teachers and prospective teachers but by changing attitudes. Finally, we show how realization of a teaching unit concerning plant biotechnology in general secondary school by considering 14 generic competences can improve the level of students' scientific literacy on this topic.

P-004

In Vitro Plant Regeneration of Four *Agropyron* Species

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Agropyron species are native grasses of the semiarid region in Iran which is quite resistant to cool and droughty climate and can withstand heavy grazing. This species has a close phylogenetic relationship with *Triticum* and *Hordeum*. The present study investigated the effects of different explant types and growth regulators, supplemented to solid and liquid MS medium, on embryogenic and organogenic callus production, somatic embryogenesis, and plant regeneration of four *Agropyron* species, i.e., *Agropyron cristatum*, *Agropyron desertorum*, *Agropyron intermedium*, and *Agropyron trichophorum*. Different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and thidiazuron (TDZ) (from 5 to 25 μM), alone or in combination (20 μM 2,4-D + 5 μM TDZ), were tested to induce germination and callus production. Experiments were conducted both in darkness and under 16-h photoperiod, provided by cool daylight fluorescent lamps (16 $\mu mol m^{-2} s^{-1}$) in growth chambers. Germination rate of *Agropyron* spp. was always better when the medium was devoid of growth regulators (MS0). The results showed that callus production benefited from the inclusion of 2,4-D in the induction medium, 5 or 10 μM producing the best conditions for *A. cristatum*, *A. trichophorum*, and *A. desertorum*. *A. intermedium*, however, responded better when the medium was supplemented with a combination of the growth regulators. Different light conditions did not produce marked diversity in the percentage of regenerating seeds; however, the callus produced under a 16-h photoperiod was of superior quality, in terms of both embryogenic and organogenic characteristics. Development of embryos were observed when somatic embryos or

embryogenic callus masses of *A. cristatum* and *A. trichophorum*, obtained by subsequent subculturing on solid medium, were transferred to liquid MS0 medium and cultured under continuous agitation on a shaker at 86 rpm. Matured embryos and plantlets only occurred when calluses regenerated at 4.52 μm 2,4-D in MS medium. Indeed, there were significant differences between 4.52 μm (1 mgL^{-1}) 2,4-D and other treatments for producing globular and torpedo embryos, plantlet, rooted callus, and a number of roots ($p<0.05$), and there was no callus production and embryogenesis in control treatment without growth regulator. Moreover, callus masses originating from the other two species were of organogenic characteristics, from which elongated shoots were easily developed on MS0 medium. All the plantlets produced more shoot in MS medium with 2.69 μM α-naphthaleneacetic acid (NAA) + 6.66 μM BAP + 2.32 μM KIN and strong root in MS medium supplemented with NAA (5.38 μM) and Kn (0.49 μM). Regenerated plants were phenotypically normal; therefore, the plant regeneration system of the present research is efficient and suitable for reproduction and regeneration of wheat grasses.

P-005

Taxane Bryotechnology: Production of Anticancer Compounds Using Transgenic Moss

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Taxol® (generic name paclitaxel) is an expensive antineoplastic drug used in cancer chemotherapy. Originally isolated from the bark of Pacific yew, paclitaxel, whose complex structure defies commercial-scale total synthesis, is now manufactured from taxanes present in yew needles. However, the variable taxane content of yews provides a still unreliable supply of the drug (which translates into increased cost). To solve this problem, we are using the moss *Physcomitrella patens* as a taxane production platform by expressing in it the genes involved in the biosynthesis of taxanes. Constitutive expression of taxadiene synthase in *P. patens* resulted in the production of taxadiene, while coexpression of taxadiene-5-hydroxylase with taxadiene synthase resulted in the production of a compound with molecular weight consistent with a taxadienol. In addition, other unexpected taxanes were also produced when either taxadiene synthase is overexpressed by itself or in conjunction with taxadiene-5-hydroxylase. Neither of these transgenic *P. patens* exhibited detrimental phenotypes, unlike in higher plants where introduction of taxadiene synthase resulted in stunted growth. Furthermore, the amounts of endogenous diterpenoids (ent-kaurene and 16-hydroxykaurane) in *P. patens* were not significantly affected, which suggested that taxane production in this moss can still be increased by inhibiting the formation of ent-kaurene and 16-hydroxykaurane. Moss biotechnology (or "bryotechnology") is therefore a very promising approach in the production of both paclitaxel and new taxanes that may have anticancer properties.

P-006

Constitutive Expression of a Peach AP2/ERF Transcription Factor in Apple Confers Short-Day Cessation of Growth

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Cold acclimation and dormancy in *Prunus persica* (peach) are regulated by both photoperiod and temperature, whereas in *Malus* × *domestica* (apple) they are regulated solely by temperature. To understand the process of cold acclimation and dormancy regulation in fruit crops, we have begun a functional analysis of several environmentally regulated AP2/ERF family transcription factors. We report here on the molecular and phenotypic analysis of several lines of transgenic apple ("M.26") that constitutively overexpress a specific AP2/ERF transcription factor gene (*PpSD1*) obtained from a cDNA library constructed from "Loring" peach bark tissues harvested in December. Phenotypic analysis indicated that, compared to untransformed plants, the transgenic lines varied in levels of cold hardiness and their response to short-day (SD) photoperiod (8-h day: 16-h night). Molecular analysis indicated that the level of phenotypic change was associated with the level of expression of PpSD1. The highest expressing line exhibited several degrees (C) of improved cold hardiness in both the nonacclimated and acclimated state. Unexpectedly, the transgenic apple lines also exhibited photoperiod-induced cessation of growth. Transgenic lines of apple placed under

SD conditions for 1 to 4 wk and then returned to optimum growth conditions in the greenhouse did not resume growth but rather set terminal buds, and the leaves began to senesce. The rapidity of dormancy induction increased with the length of SD exposure. In contrast, while growth slowed in untransformed "M.26" apple plants under the same SD conditions, normal growth resumed once plants were returned to optimum growth conditions. This gene could provide a means to mitigate bloom damage from temperature swings during spring.

P-007

Micropropagation of Heterotic F1 Hybrids of *Eucalyptus* FRI-5 (*E. camaldulensis* × *E. tereticornis*), FRI-10 (*E. grandis* × *E. tereticornis*), and FRI-14 (*E. torelliana* × *E. citriodora*) and Their Field Evaluations

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Promising F_1 hybrids of *Eucalyptus* artificially and naturally produced at the Forest Research Institute plantation have displayed a very high degree of vigor (positive heterosis) both in height, diameter, and wood quality. In the present study, FRI-5, FRI-10, and FRI-14 heterotic hybrid combinations were selected for rapid and mass-scale production using in vitro technique. Micropropagated plants were field-evaluated. Explants were collected from selected mother plant and washed with liquid detergent (Teepol 5–8 drops per 100 ml). Surface sterilization was done with (0.15%) $HgCl_2$ (for 10–15 min) after treatment with bavistin and antibiotics streptomycin and chloramphenicol to control 80% contamination with good survival rate (60–75%). Axillary shoot bud proliferation has been achieved in these hybrids by using explants from a 28–30-yr-old mature tree of FRI-5, FRI-10, and FRI-14. Cultures were established on MS medium supplemented with cytokinin BAP along with auxin NAA. In vitro shoot multiplication was carried out on MS medium supplemented with BAP. In vitro multiplied shoots were elongated on basal MS medium with 2% sucrose. In vitro rooting was obtained on one-half-strength MS medium supplemented with IBA and NAA. Clonally multiplied stock retained the potential of these heterotic hybrids. Field study of these micropropagated hybrids (FRI-5 and FRI-14) performed at seven different agroclimatic sites of the country showed superiority for growth parameters like height, collar diameter, and clear bole length.

P-008

Cryopreservation of *Phalaenopsis gigantea* for Conservation

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Phalaenopsis gigantea is an endangered orchid of Borneo and listed in Appendix II in the Convention on International Trade in Endangered Species (CITES). Conventionally, the species is conserved by growing them in gardens and nurseries; the risk of using these methods is that the species is exposed to climatic and disease threats. To complement these conventional methods, a cryopreservation protocol using vitrification was developed using seeds as explants. Three cryopreservation parameters were evaluated, namely preculture conditions, loading, and cryodehydration durations. Seeds were precultured on NDM media supplemented with sucrose from 0 to 0.5 M prior to vitrification with PVS2. Loading durations between 0 and 60 min and cryodehydration duration in PVS2 from 0 to 7 h were tested. Following vitrification procedure, seeds were stored in liquid nitrogen for 1 wk before being recovered and cultured on the germination medium. Seeds precultured on the medium containing 0.3 M sucrose resulted in the highest germination (8.3%) while 10-min loading time gave the maximum germination (13.3%). Cryodehydration duration in PVS2 solution for 7 h supported the highest germination (13.9%) after liquid nitrogen storage. Plants formed from cryopreserved seeds showed normal morphology and successfully acclimatized.

P-009

Ethylene Perception is Involved in Female Cucumber Flower Development

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It is well established that ethylene promotes female flower development in cucumber. However, little is known about how the gaseous hormone selectively affects female flowers, and what mechanism it uses. Previously, we found organ-specific DNA damage in the primordial anther of female cucumber flowers. This finding led to a hypothesis that ethylene might promote female flower development via the organ-specific induction of DNA damage in primordial anthers. In this study, we tested this hypothesis first by demonstrating ethylene induction of DNA damage via the ethylene signaling pathway using cucumber protoplasts. Then, using representative component genes of the ethylene signaling pathway as probes, we found that one of the ethylene receptors, CsETR1, was temporally and spatially downregulated in the stamens of stage 6 female cucumber flowers, especially along with the increase of the nodes. Furthermore, by constructing transgenic *Arabidopsis* plants with organ-specific expression of antisense CsETR1 under the control of an AP3 promoter to downregulate ETR1 expression in the stamens, we generated *Arabidopsis* "female flowers," in which the abnormal stamens mimic those of female cucumber flowers. Our data suggest that ethylene perception is involved in the arrest of stamen development in female cucumber flowers through the induction of DNA damage. This opens up a novel perspective and approach to solve the half-century-long puzzle of how gaseous ethylene selectively promotes female flowers in the monoecious cucumber plant.

P-010

Tnt1 Mutagenesis Study by In Vitro Transformation in Soybean

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Soybean (*Glycine max*) is one of the most important and widely cultivated crops in the USA and in many parts of the world. Seeds are rich sources of oil and protein, a valuable product for human and animal nutrition. Its diverse applications urges for more research to develop new varieties of this legume. For the past few decades, progress has been made in search of novel mutants in soybean. Recently, transposon mutagenesis has been widely used to generate insertional mutants to study the functions of genes. However, generation of large-scale insertional mutants is a challenge in soybean because it is still not trivial to generate such a large mutant population due to the lack of high-throughput soybean transformation process. In the present study, Tnt1, an active retrotransposon from *Nicotiana tabacum*, is used to study insertion mutagenesis in soybean. The aim of the study is to introduce multiple independent insertions (high copy number) per plant via genetic transformation to generate a population of Tnt1 insertional mutants. To achieve this, two *Agrobacterium* strains (EHA101 and AGL1) containing a vector carrying *Tnt1* gene and bar gene as a selectable marker were used to transform soybean cotyledonary explants, and transgenic events developed through organogenesis-based tissue culture. Experiments regarding generation of Tnt1 mutant soybean lines are in progress. In addition to the above experiments, explants were subjected to various levels of sucrose pretreatment, to test the impact on Tnt1 copy number in soybean. Standardized protocol with optimal sucrose pretreatment was currently used to reactivate Tnt1 copy number using T0 Tnt1 soybean seeds. More detailed studies will be presented.

P-011

Exposure to Gibberellins Eliminates the Cold Stratification Requirement of *Penstemon grandiflorus* Seeds

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Penstemon grandiflorus, large beardtongue, is a native perennial plant that prefers full sun, dry conditions, and rocky or sandy soil. *P. grandiflorus* is classified as endangered in Illinois, and it is only found in the wild in a few counties of northwestern Illinois. Although *P. grandiflorus* can be grown from seed, dormant seeds require 30 d of cold stratification for germination. Embryo dormancy is often due to the presence of inhibitors such as abscisic acid (ABA) and the absence of growth promoters such as gibberellic acid (GA). Germination is often associated with a drop in the ratio of ABA to GA. We studied methods for reducing or eliminating seed dormancy by seed treatment with GA_3. Seeds were soaked in GA_3 (0, 250, 500, 1,000, and 1,500 mg/L) and then placed at 4°C for 0, 5, 10, 15, 20, 25, or 30 d in petri dishes containing moistened filter paper. As dishes were removed from 4°C, they were placed in growth chambers at 15°C or 20°C in 24-h darkness, and germination was monitored. GA_3 treatment was shown to reduce and even completely

eliminate the requirement of a 30-d cold stratification. Additionally, seeds maintained at 20°C germinated at a faster rate than the seeds at 15°C.

P-012

Analysis of Stress-Induced Transcripts and Biogenic Volatile Organic Compound Emissions in Tropical *Hymenaea courbaril* and Coniferous *Pinus ponderosa*

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Changes in the composition of biogenic volatile organic compound (BVOC) emissions and stress-responsive gene transcripts were investigated in the tropical tree species *Hymenaea courbaril* and the coniferous species *Pinus ponderosa*. Heat stress and insect wounding have been associated with an increase in the release of stored and de novo synthesized BVOCs. These BVOCs are released into the atmosphere, where they can interact with nitrous oxides to create ozone and alter cloud properties via the formation of secondary organic aerosols (SOAs). To determine the source of the BVOCs being released (stored vs. de novo), we subjected both species (*Ponderosa* and *Hymenaea*) to heat stress and methyl jasmonate (MeJa) treatment. The temperature in the growth chamber containing the trees was increased from 24°C to 40°C (*Ponderosa*) or 27°C to 42°C (*Hymenaea*), and BVOCs were collected using leaf cuvette enclosures. Leaves were collected at 0 and 24 h for transcript analysis. Heat- and MeJa-responsive gene transcripts were detected using SeeGene's GeneFishing™ technology. These differentially expressed genes were then classified using BLAST analysis, and expression of 25 selected gene transcripts were examined by qPCR. These stress-inducible genes have potentials to be transferred to plants for trait enhancements.

P-013

Regeneration of Plants from Black Ash (*Fraxinus nigra* Marsh.) Hypocotyls

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Black ash is an important North American tree species with significant ecological and ethnobotanical importance to American Indians of the eastern USA. The seeds are eaten by wildlife, and the strongly ring-porous wood is preferred for making splints for basketry. Black ash has irregular seed production, immature embryos at seed set, and a complex stratification requirement, making it difficult to regenerate naturally from seed. Because of inadequate natural regeneration and the threat of the emerald ash borer (EAB), an in vitro adventitious shoot regeneration and rooting protocol would be beneficial for propagation and genetic improvement of this species. Such a system will provide the basis for an *Agrobacterium*-mediated transformation system for developing resistance to the EAB. Hypocotyls extracted from aseptic seeds were cultured for 4 wk on a Murashige and Skoog (MS) medium containing 13.3 μM 6-benzylaminopurine (BA) plus 4.5 μM thidiazuron (TDZ) for shoot induction. Shoots were then successfully regenerated on MS medium with Gamborg B5 vitamins (MSB5) plus 6.7 μM BA, 1 μM indole-3-butryic acid (IBA), and 0.29 μM gibberellic acid (GA_3), followed by transfer to MSB5 medium with 13.3 μM BA, 1 μM IBA, 0.29 μM GA_3, and 0.2 gL^{-1} casein hydrolysate for shoot elongation. Elongated shoots were successfully micropropagated using MSB5 medium with 13.3 μM BA, 1 μM IBA, 0.29 μM GA_3, and 0.2 gL^{-1} casein hydrolysate. Rooting of shoots (85%) was successful using woody plant medium containing 4.5 μM IBA plus 5.7 μM indole-3-acetic acid with a 10-d dark incubation. There was an average of 6.3 roots per shoot, and 85% of rooted shoots survived acclimatization to the greenhouse. This protocol will be used for experimental studies to produce transgenic black ash with resistance to the EAB or mass propagation for conservation.

P-014

Overproduction and Large-Scale Production of the Phytohormone Coronatine, a Methyl Jasmonate Mimic

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The phytohormone coronatine (COR) is a low molecular weight compound produced by several pathovars of the plant pathogenic bacterium, *Pseudomonas syringae*. COR has structural and functional similarity to the jasmonates, which are endogenous plant signaling molecules that function in response to biotic stress. Several commercial applications of COR have been explored, including the use of COR as a harvest aid in agriculture and as an elicitor of taxol synthesis. The structure of COR is not conducive to chemical synthesis; thus, the main source of the compound is via fermentation of *P. syringae*, which is a limiting factor in commercial production. In this study, we develop a strain of *P. syringae* that overproduces coronatine and explore methods for overproducing COR using various matrices, including alginate beads and silica-based carriers. When Cytoline was used as an immobilization matrix, the yield of COR by *P. syringae* approached 0.5 g/L, which is within a range acceptable for large-scale fermentation. The production of COR on immobilized beads of silica-based carriers shows promise for industrial production of this novel molecule.

P-015

In Vitro Propagation of the Medicinal Plant *Eremanthus erythropappus* (DC.) Macleish

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The aim of the present work was to establish appropriate conditions for the in vitro micropropagation of *Eremanthus erythropappus* (DC.) MacLeish through shoot multiplication on apical and nodal bud explants. Explants were excised from in-vitro-grown seedlings and incubated on Murashige and Skoog medium containing different combinations of 6-benzylaminopurine (BAP) and 1-naphthalene acetic acid (NAA) (for apical buds) and gibberellic acid and NAA (for nodal segments). The proliferation of apical shoots was successfully achieved in the presence of BAP and NAA, each at 1.0 mgl^{-1}, while the elongation of apical shoots could only be attained on medium containing 1.0 mgl^{-1} NAA. Elongation of nodal shoots was induced in the presence of 2.0 mgl^{-1} NAA. The most suitable medium for inducing root proliferation on explants of *E. erythropappus* was 1.0 mg/l NAA.

P-016

Reduced Oxalic Acid Levels Following Infection with *Sclerotinia sclerotiorum* in Transgenic Soybean

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Sclerotinia sclerotiorum is a common plant pathogen that infects soybean (*Glycine max*) and causes millions of dollars in losses due to reduced yield and poor seed quality in eastern Canada and northern USA. Partially resistant commercial soybean lines continue to exhibit considerable damage from white mold. Transgenic soybean lines 20B7 and 80(30)1, previously generated in our lab using *Agrobacterium* and biolistics techniques, express the wheat germin gene for oxalate oxidase (OxO) and show a high degree of resistance to invasion by *Sclerotinia*. The transgenic enzyme OxO allows plants to metabolize the pathogenicity factor oxalic acid (OA) that is produced by the invading fungus and enables these lines to avoid major damage to leaves and stems beyond the formation of lesions at the site of infection. Here, we present data illustrating how the presence of OxO leads to reduced OA accumulation in transgenic plants in relation to the parental lines that do not contain the wheat germin gene. We compared the levels of OA in flowers and leaves of both pairs of plants that were inoculated with *Sclerotinia*. OA determination showed that the transgenic lines had significantly less OA accumulation in their tissues 3 d postinoculation compared to their nontransgenic parents, by as much as a fourfold difference. The 20B7 transgenic line, which produces more OxO, accumulated less OA in the infected flowers compared to 80(30)1. The presence of OxO plays an important role in a plant's defense mechanism against invasion by *Sclerotinia sclerotiorum*.

P-017

Induction of Vegetative Pigmentation in Cymbidium Orchids by Transformation with Heterologous Anthocyanin Transcription Factors

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Flower and leaf colour are important traits of the aesthetic appeal and therefore commercial value of ornamental plants. Pigmentation in both vegetative and floral tissues can be modified by the introduction of heterologous transcription factors. Transcription factors (TFs) are useful because they can influence multiple steps in a pigment biosynthetic pathway, thus avoiding the need to introduce multiple biosynthetic genes. Vegetative pigmentation has been induced by the introduction of flavonoid TFs in various plant species including petunia, *Arabidopsis* and tomato. Our aim was to produce a red-leaved *Cymbidium* orchid by introducing the maize leaf colour (Lc) transgene, which encodes for a bHLH-type anthocyanin TF in combination with the maize colorless1 (C1) transgene, which encodes an MYB-like transcription factor. At the same time, we sought to avoid the negative impact on tissue growth, shoot regeneration, or plantlet survival associated with high accumulation of anthocyanins in vegetative tissue, as seen in some other monocot crops, e.g. sugarcane. Here, we report on the biolistic introduction of the Lc, C1 and gfp transgenes into leaf bases of *Cymbidium* × *hybrida* cultivars and their co-localised transient gene expression. These TF transgenes, the selectable marker nptII and gfp were then introduced biolistically into rapidly dividing protocorm-like bodies (PLBs) of cv. Red Nelly Devil, and immediate selection with geneticin or hygromycin was applied. The bombarded PLBs were regularly monitored for green fluorescent protein (GFP) and Lc/C1-induced anthocyanin pigments. Red-coloured tissues and PLBs that also stably express GFP have been obtained to date. PLB multiplication and shoot regeneration are being monitored.

P-018

Stable, Long-Term Expression of a Reporter Transgene in a *Cymbidium* Orchid Exhibiting Somaclonal Variation

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Molecular breeding is an option to introduce novelty to cymbidium orchids, a major cut flower and pot plant crop exported from New Zealand. Long-term, stable expression of introduced transgenes is an absolute requirement for this venture. The production of transgenic plants is an initial hurdle, but other factors such as transcriptional interference between promoters controlling different transgenes or somaclonal variation that alters nontargeted phenotypic traits can cause complications. Here, we report on the successful long-term expression of a reporter gene concomitant with the development of nondesirable traits. A particle inflow gun transformation and two-step selection protocol, which included geneticin as the selection agent, was developed for cultivar Fuss Fantasy. Preconditioned protocorm-like bodies were transformed with DNA of a nptII selectable marker transgene and a gusA reporter transgene, coated onto gold particles. Stable expression of the gusA transgene was observed in leaves and some flowers collected from nine transgenic plants growing in a containment greenhouse, approximately 9 yr after bombardment. Presence of nptII and gusA transgenes was shown in leaves by PCR. The occurrence of somaclonal variation among lines involved ploidy shifts, delayed flowering, abnormal flower orientation, leaf stripes, abnormal leaf width, leaf thickening, leaf color changes, and stunted plant growth. We have determined that cultivar "Fuss Fantasy" is susceptible to somaclonal variation in culture. We conclude that the agronomic background and stability in tissue culture are important attributes to consider for cymbidium orchids when choosing a target cultivar for molecular breeding.

P-019

Microscopic Study of Floral Pieces of Artichoke, *Cynara scolymus* L., in Order to Develop a Haploid Production Technique

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Haplomethods are nowadays commonly used for several agronomic plants and allow the fast production of perfectly homozygous individuals. For artichoke, a commonly vegetatively propagated species, the selection of interesting recessive agronomic traits would be of great interest. In this work, we tried to obtain haploids in artichokes, by either gynogenesis or androgenesis—techniques previously test-

ed. At the present time, no successful technique is available. Using protocols described for other members of the Asteraceae family, gerbera and sunflower, we obtained structures after in vitro plating of unpollinated ovaries, but microscopic observations showed that they probably resulted from embryo sac abortion. In androgenesis, we were able to repeat previous results obtained for culture of isolated microspores, with a development stopped after the first division of the proembryo. The microscopic techniques described here are now used for the characterization of different behaviors of cells, according to culture conditions. This work allowed a better understanding of tissue development in vitro needed for further research for producing dihaploids of artichokes.

P-020

Comparison of Fish and Mammalian Cell Line Responses to Multiple Chemicals Using Electric Cell-Substrate Impedance Sensing (ECIS)

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The US Army for Environmental Health Research is developing an Environmental Sentinel Biomonitor (ESB) system to test Army drinking water supplies for the presence of toxic industrial chemicals (TICs). One of the major components of this system is an electric cell-substrate impedance sensing (ECIS) toxicity sensor. ECIS provides a sensitive noninvasive response measure of the impedance of a monolayer of vertebrate cells on fluidic biochips (Agave Biosystems) after exposure to water samples that may contain TICs. In this study, bovine lung microvessel endothelial cells (BLMVECs) and rainbow trout gill epithelial cells (RTgill-W1) were seeded onto fluidic biochips for ECIS testing of 20 TICs and six potential water interferences. Combined ECIS testing with both cell lines resulted in detection of all 20 TICs; 10 out of 20 were detected within 60 min between Military Exposure Guideline levels and estimated human lethal concentrations. The RTgill-W1 cells had comparable chemical sensitivity to the mammalian BLMVECs but showed greater potential for low maintenance and long-term storage capabilities on the fluidic biochips. These features of the RTgill-W1 cells make them good candidates for use in portable toxicity sensors and as one component of the ESB system.

P-021

Creation of Recombination Variability Form Interspecific Crosses in *Secale* Genus and Analysis of Genetic Similarity of Parents From F_1 Progeny

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The aim of the research was the new genetic variability development of rye from interspecific hybridization in *Secale* genus. The plant material used in this work consisted of species, subspecies, and cultivars of rye: *Secale cereale* ssp. *afghanicum*, *S. cereale* ssp. *ancestrale*, *S. cereale* ssp. *dighoricum*, *S. cereale* ssp. *segetale*, *Secale strictum strictum*, *S. strictum* ssp. *africanum*, *S. strictum* ssp. *anatolicum*, *S. strictum* ssp. *ciliatoglume*, *S. strictum kuprianovii*, *Secale vavilovii*, *Secale sylvestre*, and *S. cereale* ssp. *cereale*: cv. Walet, Kier, Amilo, Dankowskie Diamant, Scat—as reference species. Efficiency of crossability ranged from 0.0% to 51.4% in single and reciprocal crosses. Variability of investigated genotypes was assessed using RAPD molecular markers. Three groups of *Secale* were distinguished according to the different degrees of genetic similarity. Perennial species were classified in one group of similarity and annual species in two distinguished groups. Analysis of genetic similarity of parental species and their F_1 progeny using RAPD and AFLP markers proved similarity in the range 0.3–0.8 and 0.6–0.9, respectively.

P-022

High Leaf Carotenoid Content Corresponding to AFLP Markers, Growth, and Seed Yields of Cashew and *Jatropha*

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Cashew (*Anacardium occidentale*), a nut-producing tree, and jatropha (*Jatropha curcas*), a biodiesel feedstock tree, are both known as excellent candidates for planting in arid areas. However, even within a species, drought tolerance traits differed widely between varieties. The objectives of our study were to investigate the relationships between genetic and morphological characteristics as well as crop performances in several varieties for both species under rain-fed conditions. In our research, we firstly conducted a field survey and DNA polymorphic analyses and then determined carotenoid contents of shoots from various cashew and jatropha varieties which were growing under diverse ecological conditions. The obtained results indicated that higher carotenoid content in shoots was closely correlated to the more drought-tolerant varieties, which in turn produced higher seed and oil yields even under nonirrigated condition. Using molecular marker approaches, we found certain AFLP markers, which corresponded strongly to higher carotenoid content trait and fruit yield of the two species. Our data and observations further strengthen evidence that nonchlorophyllic pigments probably play a very important role in conferring abiotic stress tolerance in plants. The genetic and biochemical markers, linked to performance and drought tolerance, discovered will be useful for the future improvement of both tree crops.

P-023

The Development of a Regeneration Protocol for Capsicum Chinense Jacquin cv. Scotch Bonnet Pepper.

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The Jamaican Scotch Bonnet pepper, *Capsicum chinense* Jacq., is a very important crop with significant potential for successful and competitive production within the region. This potential has been hindered by its susceptibility to pathogens and pests and the occasional discrepancies in the shape and size of the fruit. The development of efficient regeneration protocols is a requirement for subsequent biotechnological procedures which may be used to improve crop productivity, yield, disease resistance, and the conservation of germplasm. We have developed a reliable protocol for the regeneration of Scotch Bonnet from nodal and shoot tip explants. We have found that the addition of silver nitrate to the multiplication and elongation medium increases regeneration frequency and reduces tendencies to callus and the production of brittle ill-defined shoots which plague the development of tissue culture protocols for *Capsicum* sp. Explants from seedlings of 4–6 wk old were first placed on multiplication medium MS + 4.44–22.19 mM BAP with the addition of 0–20 mM $AgNO_3$, where a maximum of 4.75 shoots per explant were recovered. Shoots recovered from this step were then placed on elongation medium for maturation and elongation of small shoots and buds, before rooting on MS + 2.46–4.9 mM IBA. Other noteworthy developments arising from this work were the induction of multiple buds from the cut surface of shoot tip explants and the complete regeneration of explants on basal media. These developments have increased our understanding of the regenerative potential of the species and have great implications for further biotechnological research.

P-024

Improving Switchgrass Transformation Using a Novel Media, LP9

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Switchgrass (*Panicum virgatum*), a summer perennial grass native to North America, is currently being explored for its potential use in the production of biofuels. With these

interests, genetic manipulation of switchgrass to produce plants that are easier to digest, have an increased resistance to diseases and stresses, and maintain viability longer in the field is required. Therefore, it is necessary to develop a reliable and efficient tissue culture system for the transformation of switchgrass. Current switchgrass tissue culture requires months for regeneration of transformants with relatively poor transformation efficiencies and are limited to derivatives of a single variety, Alamo. We have developed a tissue culture system, utilizing a novel media, LP9, not MS-based, which has demonstrated decreased time in the production of whole transgenic plants and increased efficiency. LP9 is comprised of both N_6 macroelements and B_5 microelements with the auxin, 2,4-D, and does not include any cytokinin. After 1 mo on LP9 media, callus can be selected and used for *Agrobacterium tumefaciens*-mediated transformation or particle bombardment, and plants can be regenerated within 3 wk of callus initiation. Unlike prior switchgrass tissue culture systems that yield type I callus only, LP9 cultures types I and II callus. Type II friable callus, which has been shown to produce higher transformation efficiencies in other monocots, is visually selected and used and might provide up to 10 times greater transformation efficiency of prior systems.

P-025

Transcriptional Networks of Phosphate Acquisition

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Fundamental questions in systems biology address how gene regulatory networks control processes in development, adaptation to the environment, and maintenance of homeostasis in living organisms. In agriculture, the mechanisms by which plants control nutrient acquisition and utilization are particularly relevant. Due to the environmental and fiscal costs associated with the use of phosphate (P_i)-rich fertilizers, we have chosen to study the genetic basis of acquisition, use, and storage of this nutrient in the model system *Arabidopsis thaliana*. We paired two experimental approaches with the Affymetrix ATH1 microarray platform to test our hypothesis that roots respond to P_i deprivation by rapidly initiating transcriptional programs within distinct tissue types. First, wild-type seedlings were exposed to low P_i conditions for 0, 1, 3, 6, 12, 24, and 48 h, and root tissue was assayed for differential gene expression. Second, reporter plants exhibiting cell-type-specific GFP expression were exposed to low P_i conditions for 3 h, and root protoplasts were enriched using fluorescence-activated cell sorting. Differential gene expression data between whole root and sorted root cells (endodermis, cortex, stele, and columella) revealed unique but overlapping expression profiles. Direct network connections between candidate transcription factors and putative gene targets (based on *cis*-regulatory motif analysis) are currently being tested by RT-qPCR. Upon validation of direct regulatory relationships with a combination of ChIP-qPCR and yeast one-hybrid analyses, we aim to establish a network-level understanding of the early transcriptional events of P_i acquisition in roots.

P-026

Doubled Haploids, a Tool for *Brassica* L. Improvement and Developmental Studies

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Brassica is the most important genus in the Brassicaceae family. Among the *Brassica* crops, oilseed rape is today the world's third leading source of vegetable oil and fodder protein in oil extraction meal. Nowadays, the classical breeding methods of oilseed rape are complemented by biotechnological methods and molecular markers. The production of doubled haploid (DH) plants is one of the most important technique useful in plant breeding, aiming at the development of homozygous lines with stable expression of desired characters. Oilseed rape generally displays high response to microspore culture technique, and therefore the use of doubled haploids in breeding programs has become common practice. The most important application of androgenic haploids is the production of homozygous lines in one generation. It allows the rapid fixation of segregating genotypes, very important in case of winter oilseed rape which needs 5 to 7 yr for development of inbred lines. Current application of *Brassica* doubled haploids will be discussed in this work on the basis of investigations realized at the Department of Genetics and Breeding of Oilseed Crops in Plant Breeding and Acclimatization Institute in Poland. One of the examples is the new cultivar of winter oilseed rape Monolit, licensed lastly in Poland, which is selected as one DH line developed from isolated microspore culture. It is the canola quality cultivar, characterized by high

and stable yield of seeds, high fat content, and relatively good tolerance to drought.

P-027

Exogenous Morphogenetic Signals in the Genus *Hypericum*: Differences in Morphogenetic Potential and Ability to Synthesize Hypericins

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The use of in vitro culture of *Hypericum* spp. as an experimental system with prospective biotechnological application was triggered by the discovery of new activities of hypericin and its derivatives. From among the representatives of this extensive genus, a complex research including in vitro culture and biotechnology was performed only with *Hypericum perforatum*. Besides the knowledge we have from this model *Hypericum* species, there are some partial but promising results from other species of the genus which can be considered as candidates for further investigations and possible future application. Root, stem, and leaf cuttings of 6-wk seed-derived plantlets of several representatives of the genus *Hypericum* were cultured on MS medium supplemented with auxins (IAA, IBA, NAA, and 2,4-D) and cytokinins (BAP and 2iP), each in two concentrations. Morphogenetic response was evaluated in regular intervals. The species capable of shoot differentiation were subjected to analyses of hypericin content. This comparative study revealed the best morphogenetic response on plant growth regulators of *Hypericum annulatum* explants. Rhizogenesis was effectively induced especially by IBA in *H. annulatum*, *Hypericum tomentosum*, *Hypericum maculatum*, *Hypericum tetrapterum*, and *Hypericum pulchrum.* Biomass of shoots was promoted by BAP rather than 2iP in *H. annulatum*, *H. tomentosum*, *H. maculatum*, *H. tetrapterum*, *Hypericum humifusum*, and *Hypericum monogynum*. The best candidates for in vitro production of hypericins by shoot cultures are *H. humifusum* due to its ability to bring flowers under in vitro conditions but also *H. annulatum*, *H. tetrapterum*, and *Hypericum rumeliacum* in which the content of hypericin exceeds that in *H. perforatum.*

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P-028

Establishing the Genetic Integrity of Tissue-Culture-Derived Plantlets of *Fortunella polyandra* Using a RAPD-Based Assessment

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Random amplified polymorphic DNA (RAPD) analysis using arbitrary 10-mer oligonucleotide primers was employed to investigate the genetic fidelity of in-vitro-derived plants of *Fortunella polyandra* and their mother plants from two different locations. A total of nine primers that gave consistent, strongly amplified fragments were selected for further screening with the DNA of these plants. Calculation of the diversity index for each primer ranged from 0.1964 to 0.2907, indicating a low degree of polymorphism among the materials. A UPGMA dendrogram generated using the NTSYS program showed the genetic similarity among the in-vitro-derived plants and its two mother plants. Almost all the in-vitro-derived materials fell into two groups according to their source locations at a genetic similarity value of 75.5%. A mean Jaccard genetic similarity value of 0.83 was obtained between the regenerants and their mother plants. These findings support the functionality of RAPD, being a useful tool for establishing the genetic integrity of micropropagated plantlets and for devising future conservation strategies.

P-029

Production of Triterpenoids in In Vitro Cell Cultures of Medicinal Plants

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Production of bioactive compounds from cell cultures has been a subject of extended research lately. Compared to whole plants, it offers advantage of faster growth and metabolic rate with a condensed biosynthetic cycle. This leads to rapid formation of secondary metabolites. Also, production is tunable, controlled, and unaffected by the wrath of environmental fluxes. We examined the production of a powerful biopesticide, azadirachtin, from anther-culture-derived six haploid lines of *Azadirachta indica* and anticancerous triterpenoids, betulinic (BA), oleanolic (OA), and ursolic acids (UA), from leaf-derived aseptic lines of *Lantana camara*. Cultures were maintained on Murashige and Skoog medium. Cells were subjected to solvent extraction and reverse-phase high-performance liquid chromatography analysis. Azadirachtin eluted at 6.39 min and was confirmed in all six haploid lines. The highest amount was found in redifferentiated lines on MS + BAP (2.2 μM) + NAA (0.05 μM) (728.41 μg/g DW), and the least amount was present in dedifferentiated lines on MS + 2,4-D (1 μM) + Kn (10 μM) (49 μg/g DW). In vitro haploid leaves contained 700 μg/g DW of azadirachtin. Calibration curve of the standard showed linearity with a correlation coefficient (R^2) of 0.989. From the leaf-disk cultures of *Lantana* maintained on MS + BAP (5 μM) + NAA (1 μM) + 2,4-D (1 μM), BA, OA, and UA eluted at 10.8, 12.61, and 13.2 min, respectively. In this case, standards showed linearity with R^2 values of 0.987, 0.989, and 0.991 for BA, OA, and UA. Cells contained 3.1% BA, 1.88% OA, and 4.12% UA per gram DW. This is the first report on azadirachtin detection in androgenic haploid lines and coexistence of three triterpenic acids in leaf cultures. Cytotoxicity of leaf extract against HeLa cells further accentuated the importance of cultures.

P-030

Salt-Tolerant Potato Developed via Plant Tissue Culture

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Potato is one of the world's most important crops next to wheat, maize, and rice. It is highly sensitive to soil salinity and needs fresh water irrigation for tuber production. Salinity is one of the major factors that affect potato production in arid countries. Development of salt-stress-tolerant lines by traditional breeding technique is difficult because cultivated potatoes are tetraploids and highly heterozygous. Attempts were made to develop salt-tolerant potato lines through tissue culture techniques. Potato cultivars Spunta, Diamond, Ajiba, and Mondial were established in tissue culture via meristem culture and tested for their salinity tolerance in vitro. Among the four cultivars tested, none of them showed tolerance to above 1,000 ppm NaCl in vitro. The stem callus of potato cultivar Spunta also showed 1,000-ppm salinity tolerance in vitro. Through repeated subculture on high-salinity culture media and in vitro selection, callus tolerant to 5,000 ppm NaCl was developed and multiplied under the same NaCl concentration in MS culture media containing an auxin. Plantlets were regenerated from the salt-tolerant potato callus in the same saline media containing cytokinin and multiplied in hormone-free culture media through stem nodal multiplication system. All the plantlets survived and showed tolerance to 5,000 ppm NaCl in vitro. Well-developed plantlets were isolated and multiplied for the tuberization experiments both in vitro and in vivo conditions. The rooted plantlets produced microtubers on MS medium containing 5,000 ppm NaCl and 80 g/l sucrose in vitro and minitubers under brackish water irrigation in the greenhouse. This newly developed salt-tolerant potato line via tissue culture is under large-scale multiplication for the field trial.

P-032

Cloning of Genome-Specific Repetitive DNA Sequences in Wild Rice *O. rufipogon* and Developing Novel Molecular Markers for Distinguishing Indica and Japonica in Rice

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Common wild rice (*Oryza rufipogon* Griff.), known as the ancestor of Asian cultivated rice (*O. sativa* L.), carries abundant genetic variations. As retrotransposons exist in high copy and accumulate abundant evolution information in rice genome, it could be used to develop subspecies-specific (SS) markers to distinguish indica from japonica. We isolated four BAC clones and digested these clones into small fragments to sequence in Dongxiang wild rice

(*O. rufipogon*.) and obtained 19 subclones (sc1-19). Compared with NCBI and TIGR repeat database, sc1-16 showed high homology with the long terminal repeat (LTR) of Ty3-gypsy-interspersed retrotransposon, and sc17-19 is homologous with high-copy tandem repeat. These retrotransposons can be classed into RIRE subfamily, including sc1-11 belonging to RIRE3 subfamily, sc12-14 belonging to RIRE2, and sc15 belonging to RIRE8 by LTR sequences. Subclones sc1 and sc12 were selected as Southern probes to hybridize with AA, BBCC, CC, CCDD, and EE genome. The result showed that they all exist in high copy in AA genome are but very low in BBCC, CC, CCDD, and EE. The subclones sc1 and sc12 were utilized to develop SS markers by retrotransposon-based molecular marker systems. The anchored primers were r2-1, 2 designed based on sc-12 to display RIRE 2 inserted position polymorphism (IPP) and r3-1, 2 designed based on sc-1 to display RIRE 3 IPP. The total 22 SS markers (ssj1-13, ssi1-9) were identified, in which ssj1-13 belonged to japonica-specific type and ssi1-9 belonged to indica-specific type. The average accuracy in distinguishing two subspecies is over 85%. The ssj-10 could completely distinguish indica from japonica at 100% accuracy. Principal component analysis (PCA) showed that these markers were also suited to distinguishing indica from japonica varieties from different geographical regions. Small fragment insertion or deletions inside LTR was the major reason for causing sequence divergence between indica and japonica. This results indicated that molecular markers based on retrotransposon should be very useful in the study of indica–japonica differentiation and the origin of *O. sativa*.

P-033

Molecular Characterization of a Cold-Regulated Gene BnCor25 Involved in Cell Tolerance to Cold Stress

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Cold stress, which causes dehydration damage to the plant cell, is the most common abiotic stress that adversely affects plant growth and agricultural productivity. To improve cold tolerance, plant cells often enhance some cold-related gene expression. One gene encoding cold-regulated protein was isolated from *Brassica napus* cDNA libraries using a macroarray analysis and designated as BnCor25. RT-PCR analysis demonstrated that BnCor25 was mainly expressed in hypocotyls, cotyledons, stems, and flowers, but its mRNA was found at low levels in roots and leaves. Northern blot analysis revealed that BnCor25 transcripts were significantly induced by cold and drought treatment. Overexpression of BnCor25 in yeast (*Schizosaccharomyces pombe*) significantly enhanced the cell survival probability, suggesting that the BnCor25 protein is involved in cell freezing tolerance.

P-034

Effects of Thermotherapy and Chemotherapy on Viruses and Viroids Infecting Stone Fruits (*Prunus* sp.)

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Thermotherapy and chemotherapy, alone or in combination, were applied to in vitro cultures of *Prunus* to eliminate viruses and viroids. Treatments were applied to apical and axillary buds collected from infected stone fruit trees maintained in a screen house. Thiouracil and azauracil were more phytotoxic among nine antiviral agents tested at a 100-μm concentration in the medium. High temperature (28–38°C) for thermotherapy was detrimental to two species (*Prunus mahaleb* and *Prunus persica* "GF305") but stimulated growth of *Prunus salicina*. When subjected to elevated temperatures, *P. salicina* produced more shoots than the other two species although the leaves were smaller and narrower than those produced at 23°C. The effect of antiviral agents on pathogen survival varied depending on the target virus/viroid. Cherry virus A (CVA) was eliminated by the addition of ribavirin without heat treatment; however, there were fewer CVA-free explants derived from treatments of salicylic acid, amantadine hydrochloride, or guanidine hydrochloride. Ribavirin was also effective in eliminating peach latent mosaic viroid, but only when combined with heat treatment. Both oxytetracycline and quercetin eliminated cherry necrotic rusty mottle virus albeit with low efficiency (28% with oxytetracycline and 50% with quercetin). The effect of thermotherapy and chemotherapy on elimination of Hop stunt viroid (HSVd) was unclear in initial trials as virus-free shoots were produced in all treatments (including untreated control). This may result from seasonal titer variation and erratic distribution of HSVd in the host plant.

P-035

Tissue Culture Propagation for High Resveratrol-Expressing Plants of Taiwan Native *Polygonum cuspidatum* and Secondary Metabolite Production

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Traditional Chinese medicinal herb, *Polygonum cuspidatum* Sied. et Zucc., widely spread in China and Taiwan, belongs to the family Polygonaceae. Several studies have highlighted various properties of resveratrol such as antiatheromatosic, potential oncogene tumour inhibitor, and antioxidant against the peroxidation of low-density lipoprotein in the prevention of many cardiovascular diseases. Highly expressing *trans*-resveratrol content of Taiwan native *Polygonum cuspidatum* plants has been selected to the 051111 line (5.01 mg/g DW) by HPLC analysis. In vitro somatic clone from the line has been established. Node explants inoculated into the MS basal medium adding 0.1 mg/L NAA and 0.5 mg/L BA inducted more efficiency regeneration and multiplication of adventitious buds after 4 wk of culturing. In this study, the 051111 foundation stocks were replanted in the Highland Experimental Farm, National Taiwan University (above sea level 2,500 m), and MingDao University, ChangHua (below sea level 5 m). Although the roots grew much more slowly in Highland Farm than in MingDao, the *trans-resveratrol* was quantified to 3,371.3 and 522.9 μg/g DW using HPLC analysis after 6 mo of culturing.

P-036

Key Factors for the Successful In Vitro Establishment and Micropropagation of Commercially Valuable Clones of *Gmelina arborea* (Verbenaceae).

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The ten most productive and valuable operational clones of melina property of Colombian forestry company Pizano S.A. were introduced in vitro for mass micropropagation in order to produce suitable material for further studies on cryoconservation. Previous reported procedures and results on melina micropropagation published in the scientific literature were taken into consideration in our study without any success. Significant difficulties faced during the early stages of establishment and micropropagation were undesired callus growth, hyperhydration, no shoot elongation, and very slow growth, with hyperhydration being the most serious problem. In order to solve those difficulties, the following factors were assessed: type of donor material, type of culture vessel closure, basal medium salt composition, type and concentration of gelling agent, incubation temperature, type of explant, and type and concentration of growth regulators. Successful in vitro establishment and micropropagation were achieved for all the ten clones in our study, having overcome the initial difficulties, when the first nodal segments collected from shoots produced in a clonal minigarden were established in MS medium with BAP 0.5 mg/L and then subcultured into a BAP-free medium alternated with BAP 0.1 mg/L, gelled with Sigma agar 6 g/L, culture vessels closed with cotton plugs, and cultures incubated at a temperature and photoperiod regime of 16-h light at 28°C/8-h darkness at 14°C. Significant differences between clones on susceptibility to hyperhydration, shoot proliferation, and shoot growth are attributed solely to the genotype. The highest micropropagation rate achieved so far in a clone is of six nodes per shoot produced per nodal cutting after 3 wk of subculture.

P-37

Biological Roles of Antioxidative Compounds in *Agrobacterium*-Mediated Transformation of Tomato cv. MicroTom

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Tissue browning and necrosis, which are two of the major obstacles in many *Agrobacterium*-mediated plant trans-

formations, are associated with reactive oxygen species. We have investigated the effects of 12 antioxidative compounds on tissue browning, transient expression, and stable transformation frequencies in *Agrobacterium*-mediated MicroTom transformation. These compounds are phenolic compounds, protein or enzyme inhibitors, isoflavones, and coenzymes. Among the 12 compounds, eight of them significantly ($P<0.05$) reduced 1.2- to 2.0-fold the tissue browning and two of them significantly ($P<0.05$) increased 1.7- to 1.8-fold the transient GUS expression with the comparison to a control. Five compounds significantly ($P<0.05$) increased 1.2- to 1.6-fold stable transformation frequencies and reduced 1.4- to 8-fold escape shoots compared with a control. Detailed information of the compounds and their application in transformation and enhancement of transformation frequency will be discussed in this poster.

P-038

Antioxidative Compounds Enhancing Plant Transformation

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Necrosis of *Agrobacterium*-mediated transformed cells is one of the major challenges in plant transformation and severely limits the number of transgenic plants that can be regenerated. Nineteen antioxidative compounds were investigated on their effect on tissue browning, necrosis, transient expression, and stable transformation. Of the 19 antioxidative compounds, 11 have significantly reduced tissue browning (twofld to fivefold; $P<0.05$). Nine of the compounds have highly significantly increased stable transformation frequency in MicroTom and soybean (up to 2.6-fold; $P<0.01$). Furthermore, the nine compounds that increased stable transformation frequency also reduced the escape frequency up to eightfold. We have demonstrated that tissue browning and necrosis occur consistently in the presence of *Agrobacterium* infection and that this is not due to the effects of wounding or in vitro conditions. The amount of browning increased with increasing *Agrobacterium* titer and time of coculture. Our data suggest a curvilinear relationship between browning and transient GUS expression, in that expression was highest for moderate levels of browning and lower with increasing or decreasing levels of observed browning. The optimal stage in transformation to apply the selected compounds was investigated. Preliminary studies of a biochemical mechanism underlying necrosis of *Agrobacterium*-mediated transformed cells and the application of compounds to reduce this effect will be discussed in the presentation.

P-039

Comparative Analysis on the Somatic Embryogenic Systems of Two Pineapple (*Ananas comosus*) Varieties: Red Spain (Commercial Variety) and Tabë Canä (Indigenous Venezuelan Amazon Variety)

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A somatic embryogenesis protocol was developed to propagate both a commercial variety (Red Spain) and the Amazonian indigenous variety (Tabë Canä) of pineapple (*Ananas comosus*). The fruits from the latter represent a nutritional element and a source of economical benefit for the Piaroa community in Betania del Topocho (Venezuela). The tissue culture was initiated from leaf base sections. Four different media were tested, all of them prepared from Murashige and Skoog (1962) salts, supplemented with 0.4 mg/L thiamine, 100 mg/L myoinositol, and 30 g/L sucrose. The pH of the media was adjusted to 5.8 and solidified with 8 g/L agar. All four media had different concentrations of growth substances: MS_1 (control medium) without growth substances; MS_2 with 2.5 mg/L 2,4 D and 1.0 mg/L BA; MS_3 with 10 mg/L picloram and 2.0 mg/L TDZ; and MS_4 with 3 mg/L picloram. Embryogenic calli were observed in explants from both varieties grown in the three supplemented media after 4 to 5 wk. Different stages of the somatic embryogenesis process (globular, enlarged

embryos, and converted embryo to plantlet) were found in media MS_2, MS_3, and MS_4. Tabë Canä did not develop embryos in MS_2 and MS_4; in contrast, Red Spain developed an average of 4.4 embryos per explants in MS_2 and an average of 0.96 embryos per explants in MS_4. In MS_3, Tabë Canä produced an average of 5.6 embryos/explants, and Red Spain produced an average of 3.7 embryos per explants. Only direct somatic embryogenesis was observed in Tabë Canä, whereas Red Spain was produced solely by indirect embryogenesis. This demonstrates different embryogenic potency in both varieties. In fact, the presence of a highly potent cytokinin such as THZ was necessary to induce direct somatic embryogenesis in the indigenous variety.

P-040

Effects of Explant Types and Different Cytokinins on Kohlrabi Regeneration

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Brassica oleracea var. *gongyloides* is the least studied species of the *Brassica* genus. In order to establish efficient in vitro regeneration system of kohlrabi, different cytokinins (CKs) benzyladenine (BA), thidiazuron (TDZ), *trans*-zeatin (transZ), and *cis*-zeatin (cisZ) were tested. Root and hypocotyl fragments of 2-wk-old in-vitro-grown seedlings of Vienna White (VW) and Vienna Purple (VP) cultivars were placed on MS media containing tested CK. The highest number of buds on hypocotyl segments of VP was obtained on transZ- and cisZ-containing medium while VW was more affected by TDZ. Using root explants proved to be less successful than the use of hypocotyls and resulted in bud formation only on media with transZ in VW or cisZ in VP. Kohlrabi seeds were also tested, and their regeneration efficiency was over ten times higher than in the explants mentioned above. The strongest effects were induced by transZ in VW and BA in VP. Regeneration occurred in a lower extent on medium containing cisZ. Regenerated shoots were further placed on BA (0.5 mg/L) for multiplication, and results showed their stability in culture and statistically significant differences in average numbers of newly formed VP shoots in response to individual CK pretreatments. Our data demonstrate that regeneration efficiency was dependent on genotype, media, and explant type as well as that all tested CKs including cisZ rather specifically participated in kohlrabi regeneration.

P-041

Overexpression of AP1-Like Genes from Asteraceae Induces Early Flowering in Transgenic Chrysanthemum Plants

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MADS box genes play an important role in plant ontogeny, particularly, in the regulation of flower development. In the model plant *A. thaliana*, AP1, belonging to the MADS box gene family, is required for determining the identity of the floral meristem and for proper development of first and second whorl organs. The AP1-like genes are only found in the core eudicots clade, probably linked to the origin of the eudicot flower. Functional studies using gain- or loss-of-function mutants revealed that putative AP1 orthologs have slightly diverged over evolution with respect to redundancy and gene function. We had cloned three AP1-like genes from chrysanthemum (CDM111) and sunflower (HAM75, HAM92) and have become interested in the generation of chrysanthemum transgenic plants that overexpressed these genes. In total, 37 independently regenerated plants carrying integrated transgenes were produced and used for subsequent experiments on flowering induction. Chrysanthemum (*Chrysanthemum morifolium*) is a short-day plant. The majority of chrysanthemum varieties is facultative for flowering initiation, but all need a short-day photoperiod for flower development. We have demonstrated that overexpression of Compositae AP1-homologous genes in

transgenic chrysanthemum had no effect on flowering time and vegetative development under long-day conditions. Under inductive short-day conditions, most of the transgenic plants grow faster and start bud initiation 2 wk earlier than nontransgenic controls. There were no obvious changes in florets morphology of transgenic plants. Our results indicate that the dominant role of analyzed Compositae AP1-like genes is the determination of floral meristem identity.

P-042

Crop-to-Crop Pollen-Mediated Gene Flow from Genetically Modified to Cultivated Wheat Plants

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One of the most discussed environmental effects associated with the use of transgenic plants is the flow of genes to plants in the environment. Potential risks of gene escape from transgenic crops through pollen and seed dispersal have slowed down the full utilization of gene technology in crop improvement. Wheat is primarily self-pollinated; however, some cross-pollination can occur depending on biological, agronomic, and environmental factors. In 2004, 2005, and 2008, crop-to-crop gene flow in spring wheat was investigated. As the pollen source, transgenic homozygous line expressing recombinant DNA encoding *bar* and *gfp* genes was used. The *bar* gene, conferring resistance to herbicide ammonium glufosinate, is particularly suitable for investigating gene flow in controlled experimental field trials. Analyses of phenotypic and molecular data showed that the average frequencies of pollen-mediated gene flow in nontransgenic receptor plots growing at 1 m from transgenic were 0.416% in 2004, 0.134% in 2005, and 0.225% in 2008. A strong asymmetric distribution of the gene flow was detected in different parts of plots and the maximum outcrossing in individual samples were recorded following the direction of the dominant wind. The frequency of pollen-mediated gene flow declined with increase of distance from the transgenic circle plot to recipient wheat plants. In 2008, gene flow declined from 0.225% (1-m isolation distance) to 0.021% (3-m isolation distance) and 0.009% (5-m isolation distance). Our results suggest that the risk of crop-to-crop gene flow grown under open-filed condition of the western area of Russia would be low even if wheat plants were separated by a distance of a few meters.

P-043

Constitutive and Fruit-Specific RNAi-Mediated Silencing of the Tomato ACC Oxidase for Prolonged Fruit Shelf Life

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Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable worldwide because of its overall contribution to nutrition and its important role in human health. Tomato fruit has a rather short postharvest life. In climacteric fruits such as tomato, ripening is associated with a characteristic burst of respiration, this correlates with an increase in ethylene production. Besides the important economic value, tomato is a good model system for studying the role that ethylene plays in ripening. For reduction of ethylene biosynthesis, which delayed ripening of the transgenic fruits and extended their storage life, inverted repeats of the fourth exon of LeACO1 gene in both orientations driven by constitutive cauliflower mosaic virus 35S promoter were expressed in tomato plants cv. Yalf. The fruits of transgenic tomato plants carrying DNA fragments in an antisense–sense orientation had a prolonged shelf life of more than 3–4 mo. But transgenic lines with sense–antisense orientation of hairpin RNA expression cassette did not differ from the nontransgenic control plants and did not show an increase of fruit shelf life. We studied the expression of the *LeACO* gene family during various stages of fruit ripening of transgenic and control plants and such physiological properties as flowering period, onset of breaker stage, color development, pigment contents, titratable acidity, pH, and weight loss. For manipulation of gene expression to specific tissues, we used 1,191-bp fragments of the polygalacturonase promoter, which demonstrated the highest fruit and ripening-specific GUS activity in previous experiments.

P-044

Nonfunctional Delta-12 Fatty Acid Desaturase Allele (SBFAD2) Decreases Linoleic Acid Contents in Transgenic Sorghum

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Fatty acid desaturase (FAD) is a membrane-localized enzyme of the endoplasmic reticulum that is involved in the desaturation of oleic to linoleic acid. In previous studies, cotton transformed with the canola nonfunctional delta-12 fatty acid desaturase (FAD2) allele produced seed with 8% less lipids, diminished embryos, and increased sucrose levels and fiber production. Altering lipid biosynthesis levels in grain sorghum could reduce the amylase–lipid complex in grain mash, a negative factor in the bioconversion of grain to ethanol. In an effort to accomplish this objective, we synthesized a similar nonfunctional *fad2* (Sbfad2) allele and engineered it into sorghum using the seed-specific promoter Glob-1. Semiquantitative PCR, Northern analysis, and fatty acid analysis were performed in transgenic and wild-type sorghum (Tx430). We observed that wild-type FAD2 expression in seed of transgenic plants decreases as compared to nontransformed sorghum. Transgenic seeds of different transgenic lines showed altered levels of unsaturated fatty acids. Notably, linoleic acid levels were decreased by 10% in transformed plants. Our data suggest that the Sbfad2 allele silences endogenous FAD2 alleles possibly by feedback inhibition. The strategy provides a useful tool for genetic modification of seed quality and possibly other traits for improvement of agricultural crops.

P-045

A Preliminary Investigation into the Genetic Transformation of White Pine for Study of Resistance Against White Pine Blister Rust

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Western white pine is one of the most significant conifers of North America for its economic, social, and ecological value. *Cronartium ribicola* J.C. Fischer is a destructive forest pathogen that has decimated white pine plantation in the region, causing white pine blister rust. The results from our field screening program in British Columbia, Canada, indicated partial resistance against *C. ribicola* with low incidence in some populations. Several candidate genes with expression rate regulated differentially between the resistant and susceptible families at the time of infection have been identified. *RGA618* gene has been selected based on its homology to known *R* genes from other plants and its expression profiling. To examine the functionality of the gene in a closely related species, selected *R* candidate gene was introduced to SE cell cultures of *Pinus strobus*, originating from immature seed embryos. Transformation with the RGA gene was done through cocultivation of embryogenic tissue with *Agrobacterium tumefaciens*. From our first attempt, we recovered 16 kanamycin-resistant sublines. Of the first 16 sublines, we determined a considerable expression level of the candidate *R* gene in six sublines using absolute qPCR. Our preliminary results indicated a successful transformation and expression capability in the new host plant. In the next stage of this experiment, the protein content of transgenic embryogenic tissues and transgenic plants will be analyzed.

P-046

Proper Shoot Apical Meristems Activity Is Required for the Establishment of Embryogenic Competence in Culture

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Competence acquisition is an initial and a crucial event during in vitro morphogenesis: embryogenesis and shoot organogenesis. We hypothesized that the size and activity of the shoot apical meristem (SAM) is a key factor in determining embryogenic competence in culture. To test this hypothesis, we isolated two *Brassica napus* SAM-specific genes: *BnSTM* and *BnCLV1*, homologous to the respective *Arabidopsis* genes *SHOOTMERISTEMLESS* (*STM*) and *CLAVATA 1* (*CLV1*). These genes regulate SAM size and function antagonistically, with *STM* increasing stem cell number by maintaining the SAM cells in an undifferentiated state and *CLV1* inducing differentiation of the meristematic cells, thereby reducing the number of stem cells. RNA in situ hybridization and qRT-PCR analyses confirmed the SAM-specific localization and action of *BnSTM* and *BnCLV1*. To assess the role of these genes during in vitro morphogenesis, we transformed *Arabidopsis* and *Brassica napus* (*canola*) with *BnSTM* or *BnCLV1* and evaluated their performance in culture. Overexpression of *BnSTM* in *Arabidopsis* improved embryo yield during somatic embryogenesis and shoot organogenesis from root segments. Similar improvements were also observed during canola microspore-derived embryogenesis with enhancements in embryo number and postembryonic performance. A reduction in stem cell number obtained by either a downregulation of *BnSTM* or an overexpression of *BnCLV1* had negative effects on in vitro embryogenesis and organogenesis. Structural analyses, hormonal profiling, and molecular work using full-genome *Arabidopsis* cDNA microarray and *Brassica* oligo array were conducted on the transformed lines. These studies revealed the presence of precise structural and physiological changes associated to stem cell number and related to improve in vitro morphogenesis.

P-047

Effects of Pretreatments and Hormone Regime on Green Plant Production in Barley Isolated Microspore Culture

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Isolated microspore culture (IMC) allows the production of a great number of doubled haploid (DH) barley plants in just a few months. Doubled-haploid plants are considered very useful for plant breeding because they allow us to shorten the duration of the breeding cycle and reduce its costs. As a method of obtaining DH barley plants, we noticed that IMC is several times more efficient than anther culture. However, the high frequency of albino plants obtained with some genotypes remained a challenge, particularly for six-row spring genotypes, the most popular barley types grown in Québec. Spring six-row barley cultivars have been reported to be particularly recalcitrant to regeneration and to produce predominantly albino plants. In order to try to overcome this problem, we performed comparative studies considering different pretreatments and culture media which differed in the osmoticum and hormonal composition. The incidence of hormonal composition has very rarely been investigated in IMC optimization. We found important and significant effects provided by these parameters on both the number of embryo-like structures and the proportion of green plants. Subsequently, a new protocol was developed which combines cold and heat during the pretreatment of tillers, a culture medium characterized by a relatively high osmotic pressure, and the use of the growth regulators thidiazuron and dicamba.

P-048

Cell-Penetrating Peptides: Trojan Horse for DNA and Protein Delivery in Plant

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Cell-penetrating peptides (CPPs) are a class of short peptides with a property to translocate across cell membranes and recently reported in plant by three independent groups. Despite fundamental differences between animal cell and plant cell composition, the CPP uptake pattern between the mammalian system and the plant system is very similar. $Tat_{49–57}$ RKKRRQRRR basic domain, one of the shortest known cell-penetrating peptide, Tat-2, pVEC, transportan internalisation in protoplast, somatic and gametophyte triticale and wheat cells are concentration dependent and nonsaturable, enhanced at low temperature (4°C) and receptor independent. The permeation properties of CPP change upon complexing with the cargo, e.g. DNA and protein, and according to recipient cell; as a result,

cargo is internalised either through endocytosis or macropinocytosis. Synthetic or in-vivo-produced nucleic acid, proteins, and CPPs are blocks that conjugate to form nanocomplexes in a relatively predictable manner. Plant cell wall surrounding the cell membrane poses challenges in the uptake of CPP–cargo complex but can be overcome with permeabilisation pretreatment of targeted cells and tissues. The distinct ability of CPPs to deliver macromolecules cargo that are otherwise restricted to cross the membrane has lead to development of novel peptide-mediated gene and protein delivery methods in somatic cells and microspores. Stable DNA integration has been achieved following CPP-mediated delivery of reporter gene constructs in wheat and triticale microspores. Inheritance has been documented in subsequent generations. CPP-mediated transfection in plant microspore opened new possibilities for genetic engineering of this unique cell type and crops of commercial importance.

P-049

Hairpin RNA-Derived Immunity to Wheat Streak Mosaic Virus in Transgenic Wheat Plants

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Wheat streak mosaic virus is a relatively a new incursion into Australia (2003) and New Zealand (2009). It is one of the most destructive viral diseases of wheat and may lead to 100% yield losses. The temperature-sensitive sources of natural resistance against WSMV have prompted scientist to create a durable transgenic resistance to minimize losses. We describe the development of the first successful transgenic immunity in wheat to WSMV. Wheat Bob White 26 was stably cotransformed by biolistics with two plasmids: pStargate-NIa expressing hairpin RNA (hpRNA) including WSMV sequence and pCMneoSTLS2 with nptII selectable marker. Transgenic plants were characterized by using genomic PCR, Southern hybridization, virus bioassay, and Northern blot of small RNAs. Efficacy of resistance was characterized by detection of WSMV RNA in inoculated transgenic lines and test inoculation to detect infectious virus in leaf sap from transgenic plants. The resistance in T_1 progeny segregated with the transgene and was classified as immunity by four criteria: absence of disease symptoms, low ELISA readings as in healthy plants, no detection of viral sequences in leaf extracts by RT-PCR, and leaf extracts failing to give infections in susceptible plants when used in test inoculation experiments. Southern blot hybridization analysis indicated integration of hpRNA transgene into the wheat genome. Moreover, accumulation of small RNAs derived from the hpRNA transgene sequence positively correlated with immunity. We also showed that the selectable marker gene nptII segregated independently of the hpRNA transgene in some transgenics. We have demonstrated the development of WSMV immune transgenic plants. This is the first report of immunity in wheat to WSMV using a spliceable intron hpRNA strategy.

P-050

High-Frequency Micropropagation and Somatic Embryogenesis from Axillary Bud and Leaf Explants of *Clerodendrum inerme*

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Clerodendrum inerme (L.) Gaertn. (blue glory bower) belongs to Verbenaceae and is one of the mangrove associate evergreen shrubs with elliptic-ovate leaves and white flowers. It is found in saline localities near the sea and often grown as a hedge plant. The leaves and roots are used in the folklore medicine in the treatment of skin infections, psoriasis, flu, headache, cough, boils, and eye infections. Phytochemical studies reveal the presence of inerminosides, iridoid glycosides, and phenylpropanoids. The leaves have systemic antiviral resistance-inducing proteins which inactivate ribosomes. Since the plant is used in biopesticides, it is overexploited, and the natural habitats are dwindling which warrants the need for large-scale propagation. This species is propagated by seeds and cuttings. The present report deals with an efficient system for high-frequency micropropagation from axillary buds and regeneration through somatic embryogenesis from leaves. The explants were collected from field-grown plants, surface-sterilized and cultured on Lloyd and McCown Woody Plant Medium (WPM) supplemented with different concentrations and combinations of plant growth

regulators such as 2,4-D, indole-3-butyric acid (IBA), 6-benzylaminopurine (BAP), and 2-ip (iso-pentenyladenine), 3% sucrose, 0.8% agar, and pH 5.6. The cultures were incubated at 25±2°C with 16-h light. A high-frequency of axillary bud sprouting was induced with BAP-supplemented medium, while shoot multiplication was achieved with IBA + BAP. Rooting occurred when the shoots (2 cm) were subcultured on PGR-free WPM medium with 2% sucrose. The regenerated plantlets were acclimatized and transplanted in the pots with 70% success.

P-051

From Microspores to Plants in the Apiaceae

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The Apiaceae family includes vegetables, as well as herb and spice crops. Compared to major crops, there have been few breeding or genetic improvement programs for any of the Apiaceae, especially the herb and spice species. Haploidy technology is used to develop uniform, true breeding lines which are used to accelerate crop improvement programs. Microspore-derived embryos were obtained from 11 of the 20 Apiaceae species screened and doubled-haploid (DH) plants were obtained from 10 of the species that generated embryos. Various donor plant conditions, basal media, and culture conditions were evaluated for their efficacy in regeneration of microspore-derived embryos. Field trials of dill (*Anethum graveolens* L.), caraway (*Carum carvi* L.), and fennel (*Foeniculum vulgare* Mill.) DH lines were conducted from 2003 to 2008. Significant differences between the DH lines and the parental lines were observed for plant height, rate of crop development, and seed yield, but few differences were observed for seed oil content or composition. Several of the DH dill lines had desirable agronomic characteristics such as short uniform stature along with early maturity that would be useful for crop improvement. Seed yields and seed oil of the best-performing dill lines were either equal to or higher than the parental line. The essential oil composition of the dill DH lines was comparable to the parental lines. A DH annual caraway line that produced higher seed yields than the industry standard was identified . Fennel DH lines exhibited differences in height but were too late in maturity for seed production under prairie conditions.

P-052

Altering Lignin Content in Bahia Grass (*Paspalum notatum* Flugge) by Downregulation of 4-Coumarate–CoA Ligase

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Bahia grass is the primary warm-season forage grass in the southeastern USA. In Florida alone, it is grown on more than five million acres to support the beef cattle industry. However, the high lignin content of the Bahia grass biomass significantly reduces its forage quality. RNAi suppression of lignin biosynthetic enzymes in various plant species resulted in reduced lignin content that was accompanied with increased digestibility of forage biomass. The objective of this study is the improvement of the forage quality of Bahia grass by RNAi-mediated suppression of its total lignin content. The 4-coumarate–CoA ligase (4CL) is a key enzyme in the lignin biosynthetic pathway. Four partial genes coding for 4CL were cloned from tetraploid Bahia grass cv. “Argentine.” RNAi vector targeting highly conserved domains of two Bahia grass 4CL genes was constructed using 200 bp of the coding sequences. The 4CL-RNAi construct was subcloned under transcriptional control of three different promoters: the constitutive e35S promoter, the OsC4H promoter from rice for xylem-specific expression, or the ZmdJ1 promoter from corn for expression in the green tissue. Each of these cassettes was cotransferred with the neomycin phosphotransferase (*npt-II*) selectable

marker expression cassette to Bahia grass callus by biolistic gene transfer. Following selection, regenerated plants were confirmed as transgenic events by PCR and Southern blot analysis. Significant reduction of 4CL gene expression was confirmed in several transgenic lines by Northern blot analysis. RNAi suppression of 4CL was more effective under transcriptional control of the xylem-specific OsC4H promoter than under control of the e35S or ZmdJ1 promoters. Klason lignin analysis was conducted on transgenic lines growing in the green house in 2 m×1 m×0.6 m soil bins in a randomized block design of three replications. Data correlating the 4CL gene expression level with the lignin content and in vitro digestibility analysis will be presented.

P-053

Resistance and Susceptibility Expression Profiles of *Coffea arabica* During Root-Knot Nematode Early Infection

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The damage caused by *Meloidogyne* spp. root-knot nematode infection is a serious problem to coffee production and quality. This study aims to better understand the molecular response of coffee plants to *Meloidogyne incognita* infection. The expression pattern of selected genes of two resistant (R) and susceptible (S) *Coffea arabica* genotypes were analyzed in 4, 5, and 6 d after infection (DAI). Approximately half of the 91 genes amplified by quantitative real-time PCR had an altered expression profile that seemed to correspond to the infection time course: two genes in 4 DAI, 17 in 5 DAI, and 39 in 6 DAI. Notably, at 5 and 6 DAI, it was observed that most of the assayed transcription factors (77%) are activated in both R and S genotypes, suggesting an active response to the parasite's presence. Furthermore, 16 genes were altered exclusively in the resistant genotype and 19 genes in the susceptible one. This work is the first report on the molecular understanding of the physiological response of coffee to nematode infection. These results are the groundwork for the identification of candidate genes that may be involved in *C. arabica* resistance to *M. incognita*. Moreover, it also provides tools for the assessment of the mechanisms that participate in plant response to sedentary phytoparasites.

P-054

Effects of Blue-Light Photoreceptor Cryptochrome 2 on Chloroplast Genome Expression in CRY2-OX Tomato Genotype

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Light is one of the most important environmental signals perceived by plants. Light changes activate, by photoperceptive proteins, a cascade of biochemical and molecular processes which change the physiological state of plants. Some of these changes may regard the chloroplast genome expression in photosynthetically active tissues. The cryptochromes, UV-A/blue light photoreceptors, play an important role in the monitoring, capturing, and transmitting of the light stimuli. In tomato, four cryptochrome genes have been identified and characterized so far: CRY1a, CRY1b, CRY2, and CRY3 (or CRY-Dash). CRY2 gene has a central role in tomato plant development. Its overexpression detected in the transgenic CRY2-OX line is of high relevance for the overproduction of anthocyanins and chlorophyll in leaves and fruits, suggesting its role in promoting the photosynthetic performance. In this study, we are analyzing the effects of

CRY2 overexpression in the mutant CRY2-OX on chloroplast genome transcription in tomato, by using a genome tiling array. High-density arrays containing 90 k 35-mer oligonucleotide probes were constructed using Combimatrix technology. The tailing array consisted of 30-nt overlapping probes, covering the entire chloroplast genome on both strands. Total chloroplast RNA extracted from wild-type and CRY2-OX plants was used to hybridize the microarrays. Investigation of chloroplast transcript profiles in CRY2-OX genotype vs. wild type is under way.

P-055

Functional and Kinetic Characterization of the *Arabidopsis* Sulfotransferase AtSOT12

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Sulfonation is an important reaction involved in the biotransformation of various endogenous molecules and xenobiotics and thereby can either activate or inactivate a biological response. Sulfonation of small molecules in the cells is catalyzed by cytosolic sulfotransferases. Arabidopsis possesses a family of cytosolic sulfotransferases, but their biochemical and physiological functions have been obscure. Here, we report a functional analysis of the *Arabidopsis* cytosolic sulfotransferase AtSOT12. AtSOT12 can sulfonate salicylic acid (SA), a phytohormone crucial for pathogen resistance and systemic response. Loss-of-function mutant sot12 is hypersensitive to SA, which supports the in vivo function of AtSOT12 in SA sulfonation. sot12 accumulates less but AtSOT12 overexpressor accumulates more SA in response to pathogen infection. Consistent with more SA accumulation, AtSOT12 overexpressor exhibits enhanced PR gene expression after pathogen infection. These results suggest that sulfonated SA may serve as a mobile signal molecule to trigger local and systemic SA production and promote pathogen-related gene expression. In addition to modifying endogenous SA, AtSOT12 also exhibits activity to exogenous compounds and xenobiotics. AtSOT12 has strong activity towards the toxic protein translation inhibitor cycloheximide, and sot12 mutant is hypersensitive to cycloheximide. This suggests that AtSOT12 functions as a detoxification enzyme. Screening for exogenous substrates of AtSOT12 revealed that AtSOT12 can sulfonate nitrophenols, ethylphenols, and cresols. The substrate affinity is strongly influenced by the type and position of the side groups of the phenol derivates, which provides a platform to study enzyme substrate recognition and the reaction chemistry of AtSOT12.

P-056

Insecticidal Activity of Saponins

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Although saponins are mostly associated with antifungal activity, some reports indicate that saponins are also toxic for insects. In this study, we investigated the effects of artificial diets supplemented with saponins on caterpillars (the cotton leafworm *Spodoptera littoralis*) and aphids (the pea aphid *Acyrthosiphon pisum*). At relatively high doses (1% *w*/*v*) of saponins, strong growth reduction was observed in the case of caterpillars. Aphids were much more responsive, and 100% mortality was observed within the first days of application. Because of the extreme sensitivity of aphids to *Quillaja* bark saponins, we investigated its mode of action. First, we determined the repellent activity by allowing aphids to choose between a saponin-coated and uncoated glass surface. There was no preference for either of the surfaces, indicating that saponin did neither attract nor repel. To determine the antifeedant or deterrent activity, artificial food was composed with or without 1% saponin. Here, a clear preference was apparent in that over 90% of the insects were feeding on saponin-free preparations. Methyl-blue-stained food supplemented with saponin was defecated more slowly than regular food, suggesting a reduced digestion or alternatively slowed down intake. To analyze the impact of saponin on the digestive tract, thin sections of the gut were prepared at different times after saponin intake. The epithelial cells from the digestive tract were damaged or absent in saponin-

treated aphids, suggesting that the primary target of saponin is the gut membrane. We therefore suspect that saponins irreversibly damage the gut, which prevents the uptake of nutrients. In the case of caterpillars, this leads to growth retardation whereas in aphids this appears to be lethal.

P-057

A Cell Culture Derived from the Red Flour Beetle, *Tribolium castaneum*

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The red flour beetle, *Tribolium castaneum*, has become a model organism for agricultural and medical research (e.g., Li et al. 2010). We are in the process of establishing a continuously replicating *T. castaneum* cell line. Coupled with the recently completed genome sequence (Richards et al. 2008; Kim et al. 2010), this established line will become an important research tool. We used egg, pupa, and adult stages for tissue sources. We tested numerous cell culture media (including EX-CELL 420™, Shields and Sang, L-15, TNM-FH, IPL-41, and Schneider's medium with 10% fetal bovine serum and antibiotics) to determine their ability to sustain the viability and encourage the replication of *T. castaneum* cells. Our most promising culture was initiated by coculturing pupal and adult tissues in EX-CELL 420 medium containing 10% FBS. We surface-sterilized the beetles, minced them in medium, and then incubated the tissues in trypsin to dissociate cells. After centrifugation (800×*g* for 10 min), we resuspended the cells in fresh medium in T_{25} flasks. Thereafter, we replenished half the medium every 7–10 d. After approximately 6 mo, we began completely replacing the medium weekly. This culture has now been through seven passages. We performed DNA fingerprinting using PCR on the *T. castaneum* cell culture and its source insect to confirm identification. The cell culture contains a variety of possible cell types, based on morphology. This represents the first report of a nearly continuously replicating cell line from *T. castaneum*. We hope to make this cell line available to other researchers in the near future.

P-058

BDS: An Alternative Medium for Regeneration and Growth of Multiple Plant Species In Vitro

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The basal salt formulation of the medium is a vital but often overlooked component in many in vitro applications as it regulates the growth and morphology of plant tissues with essential nutrients. The Murashige and Skoog (MS) formulation is one of the most widely used basal media, yet it is also recognized to be suboptimal for many species. The B5 (Gamborg et al.) formulation was widely used for a period of time, but it seems to have fallen out of favor in recent years. The objective of this study was to evaluate the B5 as modified by Dunstan and Short (BDS) basal salt formulation as an exemplary medium choice for in vitro applications using rice, maize, soybean, cotton, onion, tobacco, muscadine, raspberry, and gerbera daisy. The applications included micropropagation, biomass production, plant regeneration, hairy root growth, or production of secondary metabolites. BDS was compared to the MS and B5 formulations along with BCA, a version of BDS with higher calcium (440 mg/l $CaCl_2$). Performance of these test systems on BDS and/or BCA was either comparable to or superior to results on the two controls. Because of the wide range of plant systems (cereals, a grain legume, a fiber crop, a nonfood crop, woody plants, and horticultural plants) and in vitro applications involved in this study, we conclude that BDS—or simple variations of BDS such as BCA—represents a basal medium that is better balanced for a variety of uses in plant biotechnology, research, and production systems compared to MS and B5.

P-059

Characterization of Hairy Root Cultures and Elicitation of Coumaric Acid in Raspberry, *Rubus idaeus*

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Inoculations of red raspberry (*Rubus idaeus*) and purple raspberry (*R. idaeus* × *Rubus occidentalis*) leaf tissue from in vitro micropropagated cultures with *Agrobacterium rhizogenes* 15,834 yielded stable independently growing hairy root cultures. We tested five raspberry varieties: Encore, Prelude, MRR—NY99-42VF, MRS—NY99-17VF, and PRR—NY03-01pVF, for susceptibility to *A. rhizogenes* and found that large numbers of root tips from inoculation sites yielded few independent lines. Stable hairy root lines confirmed with PCR for the presence of *rolC* and *aux1* genes grew predictably in liquid phytohormone-free modified B5 media (BDS-0, Gamborg's B5 as modified by Dunstan and Short). A selected line of hybrid raspberry, PRR-,E showed better growth response in BDS-0 and BCA-0 (modified BDS with $CaCl_2$ increased to 440 mg/l) than standard B5-0 or MS-0 (Murashige and Skoog) based on biomass accumulation of 21-d cultures. To study the production of inducible and secreted metabolites, the PRR-E hairy root cultures were elicited with methyl jasmonate and hydrogen peroxide for 24 h. HPLC analysis of ethyl acetate extracts from the medium showed accumulation of coumaric acid after elicitation with hydrogen peroxide. Interestingly, the roots grown in standard B5 medium yielded more coumaric acid while showing the least amount of biomass. Future goals include screening for other secondary metabolites with nutraceutical value from these hairy root cultures.

P-060

Transformation of Linseed (*Linum usitatissimum* L.) with Wax Moth (*Galleria mellonella* L.) Silk Proteinase Inhibitor Gene GMSPI2 Conferring Potential Resistance to the Flea Beetles (*Aphtona euphorbiae* and *Longitarsus parvulus*)

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Proteinase or amylase inhibitors of animal/plant origin introduced and expressed in plant genome may confer resistance to insect pests. Silk proteinase inhibitor 2 from the wax moth *Galleria mellonella* has been proven to inhibit bacterial and fungal proteases. Its potential to interfere with insects was studied. Linseed line AGT-917 was transformed with *Agrobacterium tumefaciens* strain LBA 4404 that carried plasmid pRD400 containing *gmspi2* gene fused to the sequence for green fluorescent protein under the control of CaMV 35S promoter and ocs terminator and nptII selectable marker gene. The presence of gmspi2::gfp fusion in kanamycin-selected putative T_0 transformants was confirmed by PCR. Possible insecticidal effect was examined in adult flea beetles (*Aphtona euphorbiae*, *Longitarsus parvulus*) that were collected in the field after linseed harvest and placed into clip-cages fixed to the shoot tips of the control and GM plants (ex vitro T_0 plants in soil with PCR-proved presence of gmspi2::gfp in the leaves) grown at 20°C and 16-h photoperiod. Mortality of flea beetles consuming linseed was followed in two independent experiments. The number of dead beetles was recorded twice a day for 1 mo. The significant difference in mortality was recorded after 16 d which resulted in 92.15% mortality in GM plants as compared to 63.34% in non-GM controls. Finally, T_1 germinating GM seedlings with developed cotyledonary leaves were exposed to beetles in clip cages for 5 d in the third experiment. The loss/damage of assimilation area of cotyledons was analyzed by image analysis. The results showed significantly lower damage of cotyledons in GM plants than in controls. Acknowledgement: Supported by grants 1M06030 and MSM 2678424601 of the Czech Ministry of Education.

P-061

Isolation and Functional Characterization of UCEA1.7: A New Plant Promoter that Drives High Levels of Expression in Root and Flower Tissues

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Cotton (*Gossypium* spp.) is one of the most important crops worldwide, and Brazil is one of the largest cotton producers in the world. However, insect pests and plant pathogens severely reduce productivity, and control management represents approximately 25% of total production costs. Generation of genetically modified (GM) crops is an effective tool for protection against insects and pathogens that does not hinder sustainability. The isolation and characterization of regulatory sequences, which spatially and temporally control transgene expression, are crucial to the development of new GM crops. TAIL-PCR was used to isolate the promoter of the cotton ubiquitin-conjugating enzyme (E2). The amplified sequence, named uceA1.7, was subjected to expression analysis in transformed *Arabidopsis thaliana* plants, and spatial and temporal β-glucuronidase (GUS) activity patterns were determined. The uceA1.7 promoter drives high levels of expression in all tissues tested (leaf, stem, floral bud, and root). In fact, this promoter induces expression levels comparable to or greater than the levels obtained with the widely used CaMV35S promoter. In addition, quantitative PCR analyses in nontransformed cotton plants confirmed that the cognate E2-related gene is expressed at high levels of mRNA abundance in leaf, stem, branch, flower, and especially root tissues. To our knowledge, the uceA1.7 promoter (US Patent Appl. US2009/0320153A1) is the first strong cotton promoter capable of directing expression in root and flower tissues. This finding represents an important biotechnological tool for generating high levels of transgene expression in GM cotton.
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P-062

Characterization of Stress-Responsive CBL-CIPK Signaling Network Genes for Stress Tolerance Improvement in Rice

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Calcium plays a crucial role as a second messenger in mediating various defense responses under environmental stresses. In plants, the calcineurin B-like protein (CBL) family represents a unique group of calcium sensors and plays a key role in decoding calcium transients by specifically interacting with and regulating a family of protein kinases (CIPKs). In this study, 10 *CBL* genes and 12 *CIPK* genes were obtained by silico cloning in rice genome. Our results showed that 22 genes were differentially induced by at least one of the stresses, including drought, salinity, cold, PEG, and ABA treatments. Homozygous T_3 transgenic lines with sense *OsCBL8* and antisense *OsCBL8* obtained by agro-transformation were used to investigate the function of *OsCBL8* in rice "Nipponbare." Semiquantitative RT-PCR showed that the expression of *OsCBL8* greatly increased in sense transgenic lines and decreased to some extent in antisense transgenic ones. By evaluation of tolerance to 150 mmol/L NaCl, 20% PEG6000 and low-temperature treatments, and relevant physiological index at the seedling stage of transgenic lines, one sense line 8F12 with high salt tolerance and one antisense line 8R14 with high drought tolerance were obtained, respectively. These data indicate that CBL-CIPK signaling network plays a key role in various stress responses in plants, and these signaling network genes have great significance in crop genetic improvement for stress tolerance.

P-063

In Vitro Plant Regeneration in *Lysimachia vulgaris* L. (Yellow Loosestrife)

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Lysimachia vulgaris L. (Primulaceae) has been used in the treatment of diarrhea, ulcers, and fever, as an analgesic, cytotoxic, and anti-inflammatory agent. In addition, within the genus *Lysimachia*, several species have also antineoplastic and cicatrizant properties. Studies were initiated to establish an in vitro culture protocol for *L. vulgaris* L. Explants (leaf, stem, and root) were cultured on Murashige and Skoog minimal organics medium with various concentrations of kinetin, benzyl adenine (BA), or TDZ (thidiazuron) in combination with indole-3-acetic acid (IAA) and also concentrations of zeatin or BA in combination with IBA (Indole-3-butyric acid). More shoot development was obtained with stem explants at 1.0 mg/l BA and 2.0 mg/l IAA and also 1.0 mg/l BA and 0.5 mg/l IBA. Shoots were transferred to rooting media containing different levels of IAA, naphthalene acetic acid, 2,4-dichlorophenoxyacetic acid, and IBA. Most of the shoots formed roots on media with 0.5 mg/l IBA. Plants were transferred to potting media and maintained in the growth chamber.

P-064

Positive Feedback Regulation of an *Arabidopsis* Sphingosine Kinase by Phosphatidic Acid in ABA-Mediated Stomatal Closure Signaling Pathway

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Phosphatidic acid (PA) and phytosphingosine-1-phosphate (phyto-S1P) are both identified as lipid messengers mediating plant response to abscisic acid (ABA). We investigated the direct interaction of PA with an *Arabidopsis* sphingosine kinase that phosphorylates phytosphingosine to generate phyto-S1P. A sphingosine kinase (SK) from *Arabidopsis thaliana* was expressed in *E. coli*. SK interacts directly with PA as demonstrated by liposomal binding, lipid immunoblotting, and surface plasma resonance. Surface dilution kinetics analysis indicates that PA–SK interaction stimulates SK activity by promoting the binding of lipid substrate to the catalytic site of the enzyme. We also found that phyto-S1P-promoted stomatal closure was abolished in pldα1, indicating that ABA signal is mediated by SK and PLDα1, and SK acts upstream of PLDα1. Our results suggest that an *Arabidopsis* sphingosine kinase is regulated by PA through a positive feedback loop in ABA-mediated stomatal closure signaling pathway.

P-065

Identification and Expression Analysis of Cold-Regulated Genes in Fingered Citron (*Citrus medica* var. *sarcodactylis* Swingle)

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Fingered citron (*Citrus medica* var. *sarcodactylis* Swingle) with highly ornamental and medicinal values is mainly distributed in South China. However, *C. medica* is very sensitive to low temperature which limits its application. Thus, more and more attention has been paid to improvement of its cold tolerance. In this study, semilethal temperatures (LT_{50}) and relative electric conductivity were determined in leaves of *C. medica* cv. "Qingpi" and cv. "Aihua" after 24-h treatments with a series of low temperatures. Results showed that 12–24 h is a critical period for cold injury of *C. medica* at the LT_{50}. Alterations of gene expression patterns were detected in *C. medica* cv. "Qingpi" treated by 24-h treatments with −4°C using differential display reverse transcription (DDRT) and semiquantitative RT-PCR (sqRT-PCR). One hundred twenty-one cold-regulated cDNA fragments were cloned through DDRT, 67 of which are genes of known functions and 54 have no known functions through BLAST analyses. Thirty-eight ESTs were selected to further analyze their expression by sqRT-PCR. Thirty-four positive fragments were obtained including 29 upregulated and five down-regulated genes. Further functional analysis of these 34 differential fragments showed that eight genes are related to plant defense/stress responses, nine are metabolism-related genes including eight photosynthesis-related genes, and 17 other genes are associated with cell wall modification,

signal transduction, transcription, oxidative resistance, protein, fat and carbohydrate synthesis, and unknown functions. These data provide a new insight into molecular mechanisms underlying cold regulation of plant defense/stress responses and photosynthesis in *C. medica*.

P-066

Modeling Recombinant DNA Technology in Pre-collegiate to Sophomoric Classrooms

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For many students, biotechnology and genomics concepts are hard to grasp. When trying to visualize the invisible and mind-boggling world of the molecule, students are often confronted with abstract theory. Like everything else around us, the fields of biotechnology and genomics are changing rapidly. To ensure that secondary students understand such phenomena, modeling is often used. One of the biotechnological modeling techniques utilized at Booker T. Washington High School is Recombinant DNA technology. Inserting genes that originated in one organism into another organism is proving indispensable in agriculture and other fields. In agriculture, adding genes to plants to make them draught or insect resistant is already common practice. Recombinant DNA is used widely today to create large amounts of protein for treating certain illnesses. Students at Booker T. Washington High School have been introduced to the laboratory technique of recombinant DNA using paper models. Restriction enzymes *Eco*RI, *Hin*dIII, or *Bam*HI is used for scanning and cutting the gene of interest from foreign DNA at the selected site. The chosen restriction enzyme is then used to scan and cleave circular plasmid DNA containing antibiotic resistance kanamycin, tetracycline, or ampicillin for gene of interest insertion. The paper models allow for ligation at sticky ends. In order to make sure the inserted length of DNA is actually in the plasmid, simulated gel electrophoresis of the recombinant plasmid and the original plasmid is run. Such hands-on activities have helped students think critically and analytically to grasp complicated biological concepts involving biotechnology and genomics. Work supported by USDA/CSREES GRANT and Tuskegee University GWCAES.

P-067

Plant Regeneration and Transformation of *Anthriscus sylvestris* L. (HOFFM.)

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Anthriscus sylvestris (L.) Hoffm. (Apiaceae) is common to northwest Europe and accumulates considerable amounts of deoxypodophyllotoxin. The closely related podophyllotoxin, obtained from Podophyllum species, is used for the semisynthesis of the anticancer drugs etoposide phosphate and teniposide. Podophyllotoxin and its new derivatives are now very much investigated for novel anticancer therapy especially in combination treatment, and approximately 60 clinical trials are in progress. An alternative and more sustainable source of podophyllotoxin may be obtained by (biotechnological) hydroxylation of deoxypodophyllotoxin at the C7 position. This conversion has been achieved in *E. coli* DH5α transfected with the human cytochrome P450. We used an *Agrobacterium* transformation to introduce P450 3A4 in the callus cells of *A. sylvestris*. In this approach, regeneration of the callus cells is needed after the transformation. Callus of *Anthriscus* was grown in Gamborg B5 medium supplemented with 4% sucrose, 2 mg/L 2,4-dichlorophenoxyacetic acid, and 1 mg/L 6-benzylaminopurin. We then obtained shoot cultures in Gamborg B5 medium containing 2 mg/L zeatin and 1.0 mg/L indole-3-butyric acid. Roots were obtained after 1–2 mo in Gamborg B5 medium with 1% sucrose. We are the first to report on the complete regeneration of *A. sylvestris*. The callus tissue has also been transformed with *Agrobacterium* containing the P450 3A4 gene and is now growing in a medium containing kanamycin 100 mg/L and carbenicillin 250 mg/L for 5 mo. PCR will be carried out to prove the presence of the P450 3A4 gene as soon as the callus is free from *Agrobacterium*. The activity of the P450 3A4 protein will be determined by a precursor feeding experiment.

P-068

Genetic Engineering of Barley—Methods and Applications

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Barley belongs to the most important crops worldwide. For this species, numerous genomics tools and resources such as specific cDNA libraries, EST databases, molecular markers as well as physical and genetic maps have been developed. As a result, many DNA sequences are available for which a detailed functional analysis is desirable. Therefore, a powerful cereal transformation platform based on the use of *Agrobacterium tumefaciens* has been established in our laboratory. Either immature embryos or isolated microspores stimulated to undergo embryogenic development have been routinely used as gene transfer targets. The employment of these methods have resulted in the transformation of various spring- and winter-type cultivars of barley. Functional gene analyses and biotechnological approaches further require cell-specific promoters. In this respect, we are facing the general problem that most promoters from dicotyledons are not useful in monocotyledonous plants. The recent identification and utilization of several monocot-compatible promoters have resulted in the establishment of a number of valuable expression systems for barley, including those with specificities for the leaf epidermis, the endosperm, or the egg cell. Examples are presented for the functional analysis of genes involved in interactions of barley with pathogenic fungi as well as for the production of recombinant proteins in barley grains.

P-069

Establishment of Amicrospore-Specific Expression System and GFP-Based Visualisation of Sub-cellular Structures in Viable Microspores to Unravel Initial Mechanisms of Pollen Embryogenesis

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The generation of doubled-haploid plants through pollen embryogenesis provides an important tool for crop improvement which permits the generation of particularly valuable, instantly true-breeding plant material such as mapping populations, (multiple) mutants, transgenic and near isogenic lines as well as parental lines for hybrid breeding. Because of the limited knowledge about the initiation of pollen embryogenesis, the development of highly efficient, less genotype-dependent methods of doubled-haploid production has been hampered tremendously. Therefore, we are aiming to test by microspore-specific overexpression a number of candidate genes that putatively influence initial mechanisms of pollen embryogenesis. However, a microspore-specific expression system is not yet available for monocotyledonous plants. Consequently, we have generated tobacco reporter lines carrying the gfp gene driven by the *Ntm19* promoter so as to provide a microspore-specific expression system in a dicot model species which is also well amenable to pollen embryogenesis. In addition, we try to elucidate ultrastructural changes during early pollen embryogenesis in barley by means of live cell imaging. To this end, transgenic lines expressing chimeric transgenes coding for GFP either targeted to the nucleus or attached to actin, a major component of the cytoskeleton, have been generated.

P-070

Rapid Functional Characterization of Soybean Promoters in Transiently and Stably Transformed Tissues

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Promoters are DNA sequences located upstream of gene coding regions and are largely responsible for controlling gene expression. Discovery and analysis of novel promoters will lead to a better understanding of gene regulation in native contexts, and new promoters are useful for the development of transgenic crops. Despite their importance and the high value of soybean, only a few soybean promoters have been characterized. For this research, we have characterized 10 Gmubi (*Glycine max* ubiquitin) and 10 GmERF (*G. max* ethylene response factor) promoters using two distinct validation tools, which were optimized

for this research. Promoters from these two distinct families were identified from the soybean genome, PCR-cloned, and fused to the gfp gene. For the first validation tool, transient GFP expression was quantified using image analysis following introduction of DNAs into lima bean cotyledons via particle bombardment. For the second validation tool, GFP expression in stably transformed soybean hairy roots was also evaluated. Soybean cotyledon explants, inoculated with *Agrobacterium rhizogenes* harboring promoter constructs, showed numerous GFP-positive independent hairy root events 14–20 d after inoculation. GFP quantification in hairy roots revealed considerable variation in GFP expression among different hairy root events, but expression was consistent for roots derived from individual events. GFP intensity in hairy roots seemed to be associated with transgene copy number. Strength of promoter activity was generally consistent using transient expression and stable transformation. The most highly expressing soybean promoters were Gmubi3 followed by Gmubi1, Gmubi5, Gmubi9, Gmubi7, and GmERF10.

P-071

Development of an Efficient and Exclusive Temporary Immersion Bioreactor for Plant Production in Commercial Scale

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Application of usual methods in plant micropropagation in comparison with new techniques is expensive and time consuming. Therefore, development of new methods for production of less expensive in vitro plants has steadily increased in recent years. In this study, development of an efficient and exclusive temporary immersion bioreactor (TIB) has been carried out. In order to decrease the primary investment, application of disposable polyethylene-terephthalate-based tank instead of glass-based tank is considered. As this kind of container is not autoclavable, a special chemical sterilization has been applied. It does not require much time for preparing medium and transplanting of multiplied plantlets under aseptic condition. Therefore, it does not need any special equipment such as air flow cabinet and autoclave. In addition, the mentioned TIB system is light and easily transferable. Already, a pilot scale of this TIB system has been setup for the commercial production of banana in multiplication and rooting phases in HPTC laboratory.

P-072

Characterization of Aintegumenta-like Gene in Coconut (*Cocos nucifera* L.) and Its Expression During Embryogenesis

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Coconut (*Cocos nucifera* L.), an outbreeding perennial which is grown throughout the tropics, can be propagated by somatic embryogenesis. Little is known about the genes expressed during coconut embryogenesis. APETALA2 (AP2)/ethylene responsive element binding protein (EREBP) multigene family members have been shown to promote embryo development in several plant species. In order to investigate the process of embryogenesis in coconut, we tried to identify the gene homologs of the members of the AP2/EREBP transcription family and analyze their expression during development. A homolog gene *CnANT* that encodes two AP2 domains and a conserved linker region similar to those of BABYBOOM transcription factor of AP2 subgroup was cloned using RACE and characterized, and its tissue-specific expression was identified. The full-length cDNA comprised 1,780 bp containing a 1,413 bp open reading frame (ORF) that encoded a putative peptide of 474 amino acids. The exon/intron organization of CnANT was similar to the homologous genes in other plant species. Phylogenetic analysis indicated that CnANT showed extensive homology with the AP2 subfamily *AINTEGUMENTA-like* genes of *Elaeis guineensis* Jacq. and *Oryza sativa*. Real-time RT-PCR results showed that CnANT was expressed in all tested tissue types except leaves. Notably, CnANT showed the highest level of expression in more mature zygotic embryos, indicating that CnANT mRNA levels peaked at this stage of development. Expression then decreased soon

after embryo germination. In a study of somatic embryogenesis in vitro, a higher level of CnANT expression was recorded in callus (both embryogenic and nonembryogenic) and somatic embryos compared to other vegetative tissues.

P-073

Analysis of Essential Regions of the NSS Protein of Watermelon Silver Mottle Virus for Pathogenicity by Attenuated Strain of Zucchini Yellow Mosaic Virus with Point Mutated in HC-Pro Gene

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Watermelon silver mottle virus (WSMoV), a member of the genus *Tospovirus*, is a major economically important plant virus affecting the production of watermelon, melon, wax gourd, and other cucurbits in tropical and subtropical countries. A conserved region in the nonstructural protein NSs (aa 89~125, denoted WNSscon) targeted by a mouse monoclonal antibody was previously identified. In this study, the WNSscon was analyzed for its role in pathogenicity using attenuated strains of *Zucchini yellow mosaic virus* (ZYMV) with point mutations in the helper-component-proteinase (HC-Pro) gene. The attenuated strain ZYMV-GAC, possessing two amino acid changes in HC-Pro (R180→I and E396→N), induces mild symptom on the systemic host zucchini squash. However, it does not induce local lesion on the local lesion host *Chenopodium quinoa*. The infection of the hosts by ZYMV-GAC was verified by GFP expression in leaf tissue. In the local lesion *C. quinoa*, ZYMV-GAC showed an unusual mode of spreading to the edge of the leaf 16 dpi. Different deletion and point-mutated variants of NSs ORF were constructed and expressed by ZYMV-GAC to analyze the essential region of the NSs protein for its function in complementation of the mutated HC-Pro protein. The recombinants derived from ZYMV-GAC carrying full-length NSs ORF complemented the function of mutated HC-Pro in inducing local lesions on *C. quinoa* and severe systemic symptoms on squash. However, the ZYMV-GAC recombinants carrying NSs ORF lacking WNSscon or those with K109A mutation did not induce local lesions on *C. quinoa*, though they induced mild symptoms on squash. Hence, our results indicate that WNSscon region of the NSs protein plays important roles in the induction of local lesions on *C. quinoa* and virulence enhancement on squash and that Lys109 of WNSscon the NSs protein is crucial for virulence enhancement.

P-074

A Plastid-Localized AtGDPD1 Is Involved in Membrane Lipid Remodeling Under Phosphate Deficiency in *Arabidopsis*

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Glycerophosphodiester phosphodiesterase (GDPD), which hydrolyzes glycerophosphodiesters into *sn*-glycerol-3-phosphate and the corresponding alcohols, has been demonstrated to play an important role in various physiological processes in both prokaryotes and eukaryotes. However, little is known about the physiological significance of GDPD in plants. We characterized the *Arabidopsis* GDPD family that can be classified into canonical GDPD (AtGDPD1-6) and GDPD-like (AtGDPDL1-7) subfamilies. In vitro analysis of enzymatic activities showed that AtGDPD1 and AtGDPDL1 hydrolyzed deacylated phospholipids such as glycerophosphocholine and glycerophosphoethanolamine, but the maximum activity of AtGDPD1 was much higher than that of AtGDPDL1 under our assay conditions. Analyses of gene expression patterns revealed that all AtGDPD genes except for AtGDPD4 were transcriptionally active in flowers and siliques. In addition, the gene family displayed tissue-specific or overlapping

expression patterns in roots, leaves, and stems, indicating functional redundancy as well as specificity of GDPD genes. AtGDPDs but not AtGDPDLs are upregulated by inorganic phosphate (P_i) starvation. Loss of function of a plastid-localized GDPD, AtGDPD1, leads to a lower GDPD activity and slower growth rate than the wild type (WT) under P_i starvation. Quantitative profiling of polar glycerolipids revealed that the level of major digalactosyldiacylglycerol (DGDG) species in roots of P_i-deprived seedlings was lower in the AtGDPD1-KO mutant than WT. Thus, our results provide several lines of evidence showing that the GDPD-mediated lipid metabolic pathway is a new alternative route for membrane lipid remodeling in plant response to P_i starvation.

P-075

Genomic Approaches to Plant Defense Research and Crop Pest Management

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This paper describes on the recent development of crop genomics for studies of plant–pest interactions and functional understanding of plant defense responses against insect pests in my lab and elsewhere. In our lab, sorghum, *Sorghum bicolor* (L.), and greenbug aphid, *Schizaphis graminum*, are being used as a model to investigate crop–pest interactions. Genome-scale methods are being employed in the research, which are revolutionizing the study of plant–pest interactions and are revealing a complex process (including direct resistance genes and important regulatory networks) involved in plant defense responses. Recently, gene expression profiling has been employed to comprehensively examine gene activities in host plants in comparison with nonhost plants. Evidently, a few resistance genes and a large number of regulatory factors were identified in the crop plants in response to greenbug feeding, which are involved in the salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signal transduction pathways. A variety of functional analysis tools are also being used to associate these differentially expressed genes with functions in host defense and regulatory networks and factors that regulate interactions between the host plants and the important pest aphids. Further, mapping projects were also executed recently using an $F_{2:3}$ population containing 277 individuals in order to dissect the genetic resistance to greenbug into sorghum chromosomal regions. Single-marker analysis suggests that six SSR markers spread over five chromosomes are significantly linked to host response to greenbug feeding. Composite interval and multiple interval mapping procedures indicated that one major QTL and a minor QTL resided on chromosome 9 are responsible for resistance to greenbug attack. In summary, the discovery of novel genes, determination of their expression patterns in response to pest attack, and a better understanding of their roles in host defense will provide the bases of designing either new pest-resistant crops or more environmentally friendly pesticides for crop protection.

P-076

Corn Modified with Rye CBF Genes to Improve Abiotic Stress Tolerance

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Maize, an important crop globally, is sensitive to drought stress which results in major yield reductions. Benefits of improved drought tolerance would include increased yield under water stress conditions and a reduction in demand for freshwater required for crop productivity. CBFs are members of the AP2/ERF transcription factor family that function in low temperature and dehydration stress response signal transduction pathways. Expression of cold responsive DREB1/CBF-type cDNA clones isolated from rye (Mendel Biotechnology Inc.) in transgenic maize provided protection against drought stress but not cold stress. During cold stress, root and shoot growth rates were significantly inhibited equally in both CBF homozygous and null plants. During drought stress, although shoot growth was inhibited in CBF and null lines, root growth rates were maintained at prestress levels in CBF plants. In corresponding null plants, the root growth rate was substantially reduced. Under field-grown conditions, the CBF plants showed earlier flowering, delayed chlorophyll depletion during grain filling, and delayed senescence. We are using a PCR-select cDNA subtraction method (SSH) to profile genes expressed in the

roots and leaves of the CBF transgene homozygous and null plants to characterize the mechanisms of improved root growth under water stress conditions.

P-077

Shoot Culture Micropropagation of *Sassafras albidum*

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Shoot cultures of *Sassafras albidum* were initiated using explants from three mature trees. The explants, a mix of shoot tips and nodal cuttings, were obtained from young, nonlignified shoots (1–5 cm long) isolated both at the time of natural bud break in late May and from forced branch samples (stored under refrigeration from December to March). A conventional bleach sterilization protocol was employed, and the explants were placed on hormone-free MS medium (2% sucrose, 0.2% Gelrite) for 7–10 d to screen for sterility prior to transfer to the experimental media; MS (2% sucrose) with either benzyladenine (BA) or zeatin (ZEA), at 10 μM, in combination with agar (0.8%) or Gelrite (0.2%), for a total of four treatment combinations. Hormone-free medium served as the experimental control. Stabilized cultures were obtained for all three trees, but only from the forced branch samples. Multiplication has generally been achieved via the induction of axillary branching from shoot tip cultures, a manipulation for which BA has been proven superior to ZEA for most of the shoot lines. However, ZEA appears suitable for the induction of axillary branching from stem cuttings as well as from shoot tips with injuries to their apical meristem regions. The frequencies of culture contamination were modest at the end of the screening period (<5%), but de novo bacterial contamination, presumably of endogenous origin at least in part, has been observed during subculturing, at frequencies up to 10–20% per transfer cycle. Rooting experiments employing auxin applications of variable duration (continuous vs. quick dip) have proven wholly unsuccessful to date, so we have started to experiment with more unconventional rooting strategies. We have also initiated experiments assessing the efficacy of disinfection protocols employing PPM™ for the elimination of overt bacterial contaminants.

P-078

Abscission Studies Using an Inducible System in Poinsettia

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Abscission is an important developmental process which is highly regulated and coordinated in plants. Understanding how to control abscission would enable us to reduce yield losses in the field and increase the ornamental value of flowers and potted plants. At the Norwegian University of Life Sciences, we have established a reliable method of abscission induction of flowers in poinsettia to study the process in a comprehensive manner. By correctly decapitating buds of the third order, we can induce abscission in 1 wk. We have elucidated the events of abscission by the use of differential display (AFLP) to find genes regulating abscission, now down to two candidate genes through the use of qRT-PCR and RNA in situ hybridization. We have also used antibodies (JIM5, JIM7, LM5, and LM6) to demonstrate the changes in esterification of pectins in the cell walls, together with FT-IR and FT-Rahman to show the breakdown of pectins and the buildup of lignins in the abscission zone. We discovered the importance of precise decapitation for successful abscission to occur and have applied IAA, GA3, ABA, and ethylene to elucidate the effect of altered hormone contents at the proximal end of the decapitated bud. This resulted in our current hypothesis on abscission regulation, resulting from changes in the distribution and transport of hormones in the poinsettia buds. Present work on IAA distribution in poinsettia at the University of Minnesota has extended this hypothesis.

P-079

Improvement of *Coptis japonica* Growth by Chimeric Repressor of *Arabidopsis* Transcription Factors that Regulate Growth and Development

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Coptis japonica (Cj) has been used in Kampo medicines for gastroenteritis because its rhizome highly accumulates antimicrobial berberine. In addition, it has recently been reported that berberine showed the effect on metabolic syndrome (e.g., a low-density lipoprotein-cholesterol-lowering effect). Despite the importance of berberine, Cj is a slow-growing plant, and more than 5 yr is required to obtain crude drug. To improve the productivity of berberine, we have established Cj transformant (CjHE4′) introduced with one of the key enzymes in berberine biosynthesis, 3′-hydroxy-*N*-methylcoclaurine-4′-*O*-methyltransferase (4′OMT) gene. CjHE4′ showed up to twofold increase of berberine by overexpression of 4′OMT. However, CjHE4′ plants exhibited slower-glowing phenotype than nontransformed Cj (CjWT). Therefore, we attempted to improve Cj growth by introducing transcription factors that regulate growth and development. Since genetic information on medicinal plants is insufficient, candidate genes were screened in *Arabidopsis thaliana* (At) by chimeric repressor silencing technology that could dominantly suppress downstream genes. We introduced candidate genes into CjHE4′ callus by *Agrobacterium tumefaciens*-mediated transformation and selected transgenic Cj plants expressing At chimeric repressor (HR0169-SRDX). We evaluated the efficacy of HR0169-SRDX in hydroponics. As expected from At screening, leaf size and height of transgenic plants showed approximately 1.5-fold increase compared with that of CjHE4′. Furthermore, expressing HR0169-SRDX had little effect on berberine amount or expression levels of biosynthetic enzyme genes. These results indicate that introduction of at chimeric repressor into medicinal plants is an effective tool for improvement of the productivity.

P-080

In Vitro Maintenance and Cryostorage of Plant Variety Protection Voucher Germplasm of *Solanum tuberosum* Cultivars

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The USDA-ARS, National Center for Genetic Resources Preservation maintains the voucher germplasm of Plant Variety Protection (PVP) potato (*S. tuberosum*) cultivars registered with the US Plant Variety Protection Office. The potato cultivars are deposited at the Center as tissue culture and are maintained in vitro as shoots and tubers as well as in long-term liquid nitrogen tanks as shoot tips. Voucher potato germplasm at the Center remains the property of the PVP Office and is securely maintained and kept viable for the duration of the donor's intellectual property rights. After expiration of the PVP, the potato genetic resources are publically released through the National Plant Germplasm System. The cryopreservation procedure has evolved over the years. Currently, the droplet vitrification method developed by Haeng-Hoon et al. (2006) is the widest protocol used. In the early years of the program (2000 to 2001), the post-cryo-shoot viability ranged from 24.7% to 39.5%; however, in later years, standards were changed so that a minimum of 40% was required for an accession to be considered successfully cryopreserved at the center. Procedural modifications are made for genotypes not responding to the established protocols. Since the year 2000, 94 PVP potato accessions have been successfully placed in long-term storage and 12 additional accessions are in different stages of the cryo procedure. The PVP cultivars originated from six different countries; however, most (>43%) of the cultivars came from US donors.

P-081

Genetic Manipulation of the Lignin Pathway in Triticale Using RNAi Technology

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Triticale lignin synthesis genes 4CL, CCoAOMT, CAD, and OMT were the target for silencing using RNAi technology. Cell-penetrating peptide (CPP)-mediated transfection was applied to isolated microspores. Linearized GUS construct was also delivered to the microspore to confirm transfection frequency using CPP-mediated transfection method. Embryoids transfected with GUS gene were tested with GUS histochemical assay for constitutive expression and was GUS positive (blue color) in developing embryos. A total of 247 independent putative green transgenic plants were produced. Forty-four plants derived from co-transfection with 4CLi and CCoAOMTi constructs, 30 plants from co-transfection of scramble-4CLi and scramble-CCoAOMTi constructs, 138 plants derived from co-transfection with CADi and OMTi constructs, and 35 plants from scramble-CADi and scramble-OMTi constructs. All the candidate transgenic plants were analyzed for the presence of the RNAi genes by normal PCR using primers in the region of the promoter, the ORF, and terminator. qRT-PCR was also used to detect reduced abundance of transcripts for the respective genes and confirm silencing in T0 lines. Ploidy level was estimated using flow cytometry 2 wk after transplanting plants in soil. The spontaneous doubling reached 37.5% for CADi and OMTi co-transformation treatment. Some transformants exhibited a phenotypic difference compared to control plants maintained under similar growth conditions. It indicated that RNAi strategy was effective and in some cases lethal. The method established will be a valuable tool in functional genomics as well as for the improvement of triticale.

P-082

Micropropagation of *Ariocarpus agavoides* with Thidiazuron and Characterization of DNA Variability by AFLP

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Ariocarpus agavoides (Cactaceae) is an endemic species from México, endangered under the Convention on International Trade in Endangered Species, which has been looted. The objective was the micropropagation of this species from seed collected from type localities in Tula, Tam, and Guadalcázar, SLP, México, and the characterization of the genetic diversity of in situ populations, compared to those obtained from in vitro cultures, using AFLP markers. Thidiazuron (TDZ) is a phenylurea, considered as a cytokinin because of its effect on the induction of in vitro shooting in several species; however, TDZ showed a dramatic effect on in vitro tissues of *A. agavoides*, but its presence induced callus formation and, when it is eliminated from the culture medium, induced shoot formation. Apices from germinated seeds on an organic substrate were aseptically established, and callus formation was induced with TDZ at concentrations from 0.11 to 11.0 mgL^{-1}. *A. agavoides* callus were maintained in Eriksson (1965) culture medium with TDZ 4 mgL^{-1}, obtaining an increase of fresh weight of 12.18 g per culture bottle at 6 wk of subculture. TDZ elimination promoted shooting, which was evident at 20 wk of subculture, resulting in 12 shoots per culture bottle; these shoots rooted spontaneously in this medium. AFLP analysis of DNA revealed a high genetic variability within each of the in situ populations, which were maintained in those plants obtained in vitro. This variability was from 2% to 14% within the two localities of Tula, Tam, from 2% to 24% in Guadalcázar, SLP, and from 2% to 27% within the plants obtained in vitro.

P-083

Oviposition Behavior of Pest Insects Can Keep Bt Cotton Durably Resistant: Consequences for Refuge Policies

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The major lepidopteran insect pests of cotton and maize harbor intra-specific variation for behavior determining the selection of host plants for oviposition. Yet the consequences of behavioral adaptation for fitness have neither been modeled nor monitored for Bt cotton and maize crops, the most widely grown transgenic herbivore-resistant plants. Here, we present a general two-locus heuristic model to examine potential outcomes of natural selection when pest populations initially have low frequencies of alleles for both physiological and behavioral adaptation to Bt crops. We demonstrate that certain ecological conditions allow for the evolution of behavioral choices favoring alternative oviposition hosts that limit the increase in resistance alleles, even when they are phenotypically dominant. These results have implications for current refuge policies, which should be adapted to promote the evolution of certain behavioral choices for alternative oviposition hosts in addition to dilution of physiological resistance alleles. Collection of data on oviposition host preference as a component of monitoring schemes will provide important insights into mechanisms underlying the durability of Bt-transgenic host-plant resistance.

P-084

Isolation, Characterization, and RNAi Suppression of *COMT* and *4CL* Genes in Sugarcane

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Sugarcane (*Saccharum* sp. hybrids) is a highly productive C_4 grass used as the main source of sugar and more recently to produce ethanol, a renewable transportation fuel. Down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. In the lignin pathway, 4-coumarate–CoA ligase (4CL) and caffeic acid 3-*O*-methyltransferase (COMT) are key enzymes that catalyze the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates or the methylation of 5-hydroxyconiferaldehyde to sinapaldehyde, respectively. However, sugarcane has a complex polypoid genome, and these genes belong to a large gene family with individual members showing a distinct substrate affinity and specificity. In this study, *COMT* and *4CL* family genes were isolated from the commercially important sugarcane cultivar CP 88-1762 by a combination of cDNA library screening and PCR-based approaches. Expression analysis allowed identification of candidate genes with differential expression in different tissues. For further characterization, RNAi suppression vectors were generated and stably introduced into immature leaf-derived callus of sugarcane cultivar CP 88-1762 by biolistic gene transfer. Transgenic events were confirmed by PCR and Southern blot analysis and NPTII-ELISA, following regeneration of plants and selection with geneticin for expression of the co-transformed selectable NPTII gene. RNAi suppression of the targeted lignin biosynthetic genes was confirmed by Northern blot analysis. Plants are currently grown to maturity and will be analyzed for lignin content by Klason lignin determination.

P-085

Identification of Isothiocyanates and Their Change by Heat Treatment in Leaves of *Brassica rapa* L. cv. Nakajimana

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Brassica rapa L. cv. Nakajimana (Nakajinana), family Cruciferae, is a traditional vegetable eaten in Ishikawa Prefecture in Japan. Edible parts are usually leaves and flower buds, which are normally eaten with boiled greens with soy sauce dressing. It is known that cruciferous vegetables produce glucosinolates (GLSs) and isothiocyanates (ITCs) which have been regarded as potentially cancer protectives. Salted and short-boiled Nakajimana leaves exhibit slightly hot taste similar to wasabi, not capsicum. Since it has not been identified as pungent compounds, extraction was performed by using solvent-assisted flavor evaporation (SAFE) of solvent extracts from fresh leaves. The extracts were analyzed by glass chromatography–mass spectrometry (GC–MS). From the data of GC–MS, Allyl ITC, 3-butenyl ITC, 4-pentenyl ITC, 5-hexenyl ITC, benzyl ITC, and β-phenethyl ITC were identified, and the major components were 3-butenyl, ITC, 4-pentenyl ITC, and β-phenethyl ITC. Fresh leaves were heat-treated directly in distilled water for 3 or 30 s, at the temperature of 50°C to 100°C. After treatment, samples

were cooled quickly in ice water. It was revealed that the treatment at 70°C for 30 s was optimal to obtain the highest concentration of the ITC in fresh leaves treated by different heat conditions.

P-086

Plant Tissue Culture of *Swertia japonica* Makino

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Swertia japonica is a Japanese folk medicine used to improve stomach disease and hair growth problem. Swertiamarin is one of the main constituents of *S. japonica* and a bitter seco-iridoid glucoside. To produce this compound in large-scale culture, we have found the optimal conditions for the induction of *S. japonica* callus. At first, we investigated the effect of various phytohormones on callus induction of sterilized stems of *S. japonica*. We used the combination of auxin (2,4-dichlorophenoxy acetic acid (2,4-D), naphthylacetic acid (NAA), or indolebutyric acid (IBA)) and cytokinin (kinetin (KIN), 6-benzyladenine (BA), or thidiazuron (TDZ)) on Murashige Skoog's medium (MS). The callus induction rate was the highest value (89%) on MS containing NAA and KIN. Next, to investigate the effect of various basal medium and decrease the contamination, the seedlings of *S. japonica* were placed on MS, Gamborg's B5 medium (B5), or Woody Plant medium (WP) containing the combination of auxin (NAA) and cytokinin (KIN or TDZ) and 3% sucrose. The callus induction was remarkably promoted on WP containing NAA and TDZ, and we found that the adventitious root obviously redifferentiated on B5 or WP containing NAA and KIN. We also investigate the optimal conditions for the cell growth and the production of swertiamarin in the callus.

P-087

Analysis of a T-DNA Insertional Mutant of Opium Poppy with an Altered Alkaloid Composition

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Opium poppy (*Papaver somniferum* L.) is one of the most important medicinal plants, which is used as a sole commercial source of the narcotic analgesic morphine. The mutant of opium poppy, established by the infection of *Agrobacterium rhizogenes* strain MAFF03-01724, showed aberrant morphology and altered opium alkaloid composition. The major alkaloid produced by this primary mutant was thebaine (16.3%, opium dry weight) instead of morphine. As thebaine is used as a starting material for the synthesis of potent analgesic oxycodone and it also has a great potential as a lead compound for semi-synthesis of novel pharmaceuticals, this mutant can be a future candidate as a source of thebaine. It is likely that this high thebaine phenotype was caused by T-DNA insertional mutagenesis. To gain an insight into the mechanism of thebaine accumulation and to establish genetically stable "thebaine poppy," we carried out a genetical analysis and a compositional analysis on alkaloids in selfed progenies. Analyses on T-DNA-integrated loci and copy number performed by inverse PCR, adaptor-ligation PCR, and real-time PCR revealed that T-DNAs were integrated into the genome DNA in highly complicated manner and their copy number, which was estimated to be 14 to 16 in primary T_0 plant, decreased to seven in one of the T_3 progeny lines. The average content of thebaine in dried opium from these T_3 plants analyzed by HPLC was 2.46 times higher than that of wild-type plant. On the other hand, morphine content was one fifth of wild-type plant. These results implicate that the high thebaine phenotype is being stabilized as the number of T-DNA-integrated loci has decreased by selfing. For the establishment of "thebaine poppy," inbreeding and analysis of T_4 progeny are underway.

P-088

The Role of Tissue Resident Mesenchymal Stem Cells in Injury Response and Repair

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In vitro methods provide a variety of tools to supplement traditional methods for collecting, propagating, and preserving endangered plant species. In this study, an efficient protocol was developed for in vitro propagation of *Colutea gifana*, a rare and endangered plant species with limited reproductive capacity that grows in a very narrow area of Iran. Single-node explants were used for a series of experiments to select appropriate disinfection method and growth regulators for establishment, proliferation, and rooting stages. Explants showed the highest establishment percent after 15-min treatment with 2% sodium hypochlorite (NaOCl) cultured in MS medium plus 2.2 μM 6-benzylaminopurine and 1 μM indole-3-butyric acid (IBA). BA was a more effective cytokinin in comparison to thidiazuron and kinetin in proliferation stage. In vitro rooting of proliferated shoots were induced in half-strength MS medium with both tested auxins, i.e., IBA and α-naphthaleneacetic acid. Eighty percent of the plantlets were successfully acclimatized to ex vitro conditions, exhibiting normal development. These plantlets can be used to replenish declining populations in the wild to conserve *C. gifana* from extinction and also for further studies about this species.

P-089

Hydrolysis of Hemicellulosic Sugarcane Residues to Fermentable Sugars by In Planta Expression of the Hyperthermostable Xylanase Xyl10B from *Thermotoga maritima*

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Sugarcane (*Saccharum* sp. hybrids) is the main source for production of table sugar and is the most efficient photosynthesizer in the plant kingdom. The sugarcane plant consists of about 75% stalks from which the juice for sugar crystallization is extracted. The other 25% of the plant consists of leafy material accounting for 10 to 25 tons per hectare which is typically reduced by open-air burning. Sugarcane bagasse, a fibrous residue of cane stalks left over after the crushing and extraction of the juice from sugarcane, is one of the largest cellulosic agro-industrial by-products. Both bagasse and sugarcane leaves represent an inexpensive and abundant cellulosic feedstock for fuel ethanol production. Acetyl-4-*O*-methyl-glucuronoxylan (xylan) is after cellulose, the major polysaccharide in sugarcane residues. This polymer must be hydrolyzed to its component sugars (xylose or xylobiose) before fermentation to ethanol. Endoxylanases (1,4-β-*d*-xylan xylohydrolase; EC 3.2.1.8) are the main enzymes involved in xylan hydrolysis. Constitutive, apoplast, or chloroplast targeted expression cassettes of the codon-optimized, hypothermostable GH10 xylanase from *Thermotoga maritima* (xyl10B) were generated for in planta expression. Transgene integration and expression following biolistic co-transfer of the xyl10B and the selectable nptII expression cassettes were evaluated by Southern blot analysis, PCR, RT-PCR, ELISA, Western blot analysis, flourometric xylanase activity and TLC. Co-integration of both *nptII* and *xyl10B* was confirmed in 83% of the transgenic lines. Seventeen transgenic sugarcane lines showed clearly detectable xylanase activity. The in planta produced enzyme was purified and sweetgum xylan was used as a substrate. TLC analysis confirmed that directly fermentable xylobiose and xylose were the main degradation products, consistent with the activity of the native *T. maritima* enzyme. Ongoing experiments will reveal pretreatment requirements and efficiency of ethanol production from transgenic sugarcane biomass expressing xyl10B.

P-090

Construction and Validation of the Novel Panic Vector Set by Stable and Transient Expression Using In Vitro Cultures of Switchgrass (*Panicum virgatum* L.) and Rice (*Oryza japonica* L.)

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Switchgrass is a native, perennial, warm-season pasture grass and grows nearly ubiquitously throughout the USA. The US Department of Energy selected switchgrass as a renewable cellulosic biomass crop for the production of ethanol and as a candidate for genetic improvement via stable transformation, primarily to reduce its recalcitrance

to saccharification. Currently, improved monocot vectors are needed. Therefore, 16 Gateway-compatible destination vectors designated "pANIC" were constructed to facilitate more efficient screening and expression of transgenes. The pANIC series allows for the insertion of any DNA sequence of interest, including open reading frames (ORF), and allows overexpression (OE) or knockdown (KD) of gene targets, the latter using RNA interference (RNAi). Vectors were designed to facilitate biolistic and *Agrobacterium tumefaciens*-mediated transformations of monocots. Our technology is driven by three main components: a Gateway cassette for OE or KD of a gene target driven by ZmUbi1, as well a hygromycin B (hph) or basta (bar) plant selection cassette driven by OsAct1 and a visual reporter cassette driven by a novel switchgrass ubiquitin promoter PvUbi1, which drives the expression of either GusPlus™ or a novel red fluorescent protein gene (pporRFP). The vector designated pANIC-7A was successfully used to stably transform rice and switchgrass callus cultures which validated the functionality of the pporRFP and hph cassettes in stable transformation of both species. The functionality of GusPlus™ has also been validated in both species. The pANIC vectors will be used in the future in combination with desired gene candidates to improve biomass feedstocks.

P-091

Cryopreservation of Sempervirens by Droplet-Freezing Method and Evaluation of Post-thaw Tissue Damage by Histoanatomical Observations

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Cryopreservation refers to storage of specimens at ultralow temperatures, where almost all biological reactions in cells are hampered, allowing maintenance of the material for theoretically unlimited periods of time. Among the different methods available, "droplet freezing" is the recently developed one-step freezing method, repeatedly proven to be very effective in inducing cryotolerance of plant tissues. Present study investigated the effect of droplet freezing on cryopreservation of apical and basal buds of *Sequoia sempervirens*, i.e., a threatened germplasm which has not been cryopreserved before, neither by traditional slow cooling nor other one-step freezing methods. The procedure is as follows: cold hardening of in vitro shoot cultures; preculturing of the apical and basal buds, excised from in vitro cultures, on sucrose-rich media; treatment of vitrification solutions (PVS2, PVS3, DMSO, from 15 to 180 min) at 0°C by droplet-freezing approach; storage of explants in liquid nitrogen; thawing; and washing and recovery. Histoanatomical observations of the buds, surviving or not after storage in liquid nitrogen, shed a light to the tissue damage caused by such cryoprotective applications, as well as to the conditions that help the cells maintain their integrity and viability. Indeed, results of the study highlighted the importance of sucrose preculturing step, as only the cells that absorbed sugars in their membranes were viable, while the others were sallow with their cytoplasmic content released to intercellular area and revealed the possibility to conserve *Sequoia* germplasm following a careful optimization of each cryoprotective step. To our knowledge, this is the first study to report successful cryopreservation of such valuable germplasm, which is currently being conserved only in biosphere reserves.

P-092

Optimizing Adventitious Rooting of Woody Plant Micro-cuttings Through the Use of Nitric Oxide

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Clonal propagation of plant species via adventitious de novo root generation from stem tissue is widely utilized in the nursery industry. The speed at which rooting occurs is most often the limiting factor. Many species rapidly deteriorate prior to rooting, or root development is impaired, subsequently reducing propagule vigor. To remedy this, attempts have been made to utilize in vitro

tissue culture as a rapid tool for plant propagation, especially for the more difficult-to-root species. Many synthetic and naturally occurring compounds have been used in these efforts. One of the most promising, but little known, of these compounds is nitric oxide (NO). The role of NO has been demonstrated in regulation of root development and, of great importance to plant propagators, is likely operating in the auxin signaling pathway. The objective of this study was to determine if exogenous application of NO in in vitro tissue culture of the difficult-to-root Manzanita (*Arctostaphylos* sp.) resulted in a viable method for mass propagation. This species is a widely sought after, drought-tolerant California native shrub. Explants were cultured axenically on full-strength WPM following surface sterilization with 20% NaOCl, 75% EtOH, and 0.2% PPM. Media were supplemented with 2.0 μM 6-benzylaminopurine, 1.0 μM a-naphthaleneacetic acid, and/or 2.5 μM indole butyric acid with 3% *w/v* sucrose and solidified with 0.65% TC agar. Sodium nitroprusside was used as the NO donor at 0, 25, 50, 75, or 100 μM. Cultures were maintained under 16/8 h (day/night), 50 μM $m^{-2}s^{-1}$ at 25°C. Data from this study will significantly increase our understanding of the factors involved in de novo rooting and further develop our understanding of NO-auxin-rooting signaling pathways in root development.

P-093

Nitrogen Fixation in Poplar: Increased Efficiency of Bioenergy Crop Production. An Introduction to the Diazotrophic Endophytes of *Populus trichocarpa*

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Ethanol is a main component to the bioenergy push in the USA. It is an effective additive to gasoline, cutting the overall consumption of fossil fuels while reducing the greenhouse gas emissions. Nearly half of the cost of bioethanol production comes from the expense of growing the biomass. Current production of ethanol relies on agricultural crops that require the input of fertilizer and intensive agricultural practices. Endophytic bacteria capable of fixing nitrogen have been identified and studied in a number of non-leguminous species including rice and sugarcane. The exploitation of these endophytes has resulted in higher-yielding crops with little or no input of fossil-fuel-derived fertilizer. Diazotrophic endophytes have recently been isolated from *Populus* sp. (Doty et al. 2005, 2009; Xin et al. 2009). Poplar provides a regionally responsible and sustainable resource of lignocellulosic material that has the potential to be a more efficient feedstock in the production of bioethanol. The aims of our study are focused on further demonstrating the benefits of these naturally occurring endophytes within the poplar system and their potential to benefit additional bioenergy crop species.

P-094

Establishment of Efficient Protocol for Producing Culture Plantlet of *Coptis japonica*

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A plant factory is the cultivation system which can produce high-quality crops efficiently. However, the plant factory is unprofitable because its initial and running costs are high and expensive crops cannot be produced for its weak light condition. Therefore, the benefit-expecting crop cultivation is required. *Coptis japonica*, a kind of medicinal plants, is one of the suitable crops for the plant factory cultivation. It contains berberine, which is effective for gastrointestinal coordination, antibacterial function, and so on. It is difficult to obtain the sufficient amount of *C. japonica* seedlings by germination, so that tissue culture is considered to be a suitable method. But the protocol to produce efficiently a lot of cultured seedlings is not yet established, and so we studied about establishing the effective culture seedling production method. In this study, we investigated about an

appropriate part for using explant, a suitable hormone condition for dedifferentiation, a suitable culture condition for callus propagation, and an effective propagation condition of the cultured seedling. By the results of experiments, it was proven that the flower stalk was the ideal explant for callus induction, under 10^{-5} M of 2,4-D and BA hormone conditions. The callus propagation was advanced by liquid culture by the standard or a half concentration MS medium. It was revealed that the effective propagation method of the cultured seedling was to divide the flower bracts individually and to plant each flower bract onto 1/2 MS agar medium. With these results, the protocol for producing the culture seedling of *C. japonica* was almost confirmed. Future work should investigate the effective redifferentiation method from a callus.

P-095

Genetic Diversity in a Collection of Serbian Wheat (*Triticum aestivum* L.) Cultivars Released in Twentieth Century as Revealed by Microsatellite Markers

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Simple sequence repeats (SSRs) were used to study the genetic diversity within wheat (*Triticum aestivum* L.) varieties released in Serbia during the twentieth century. The DNA fingerprinting profiles of 180 bread wheat cultivars, developed in Serbia, and six check cultivars (Chinese Spring, Obrij, Fundulea 4, Hays 2, Mexico 3, Madrigal) were obtained by using 18 wheat microsatellites and one secalin-specific marker for rye chromosome arm 1RS. The results were obtained by using the Applied Biosystems 3130 Genetic Analyzer. A total of 151 alleles were detected at 18 wheat microsatellite loci, resulting in an average allele number per marker of 7.95. The number of alleles for the individual markers ranged from four (Xgwm680) to 17 (Xgwm577). For seven markers, null alleles were detected. The occurrence of rare alleles (frequency <2%) was observed for 17 markers. The values of polymorphism information content (PIC) ranged from 0.32 for Xgwm408 to 0.79 for Xgwm437. An average PIC value of 0.619 for all markers indicated the high level of detected polymorphism. The dendrogram, which was constructed on the basis of a similarity matrix using the UPGMA algorithm, clearly discriminated all varieties from each other, based on the information obtained from the 18 markers. The results demonstrate the utility of microsatellite markers for detecting polymorphism leading to genotype identification and for estimating genetic diversity.

P-096

Identifying New Genes Influencing the Cell Wall of Grasses Using a Forward Genetic Approach

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In recent years, plant cell walls and the polymers that constitute them have received increased attention as a potential highly abundant renewable resource for biofuel production. The feasibility of using plant feedstocks will in part be dependent on the optimization of plant wall composition, as it can directly influence conversion yield. Therefore, the understanding of plant cell wall polymer biosynthesis and metabolism and the genes involved therein will be essential. Multiple members of the Poales order have been proposed as potential bioenergy feedstocks as they combine multiple desirable traits such as C4 photosynthesis, large biomass yield, and fast growth. Examples include switchgrass, *Miscanthus*, or sugar cane. Also, crop residues like corn stover or wheat straw could be utilized. We performed a forward genetic approach facilitating monosaccharide composition analysis via GC analysis of alditol acetate derivatives to identify mutants with alterations in their cell wall monosaccharide composition. Mutagenized lines of *Zea mays* (chemical mutagenesis) were analyzed, and multiple lines with altered monosaccharide composition have been identified. A summary of

the screen and data on promising candidates will be presented and discussed.

P-097

Isolation of an Embryogenic Line from Non-embryogenic *Brassica napus* cv. Westar Through Microspore Embryogenesis

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Brassica napus cultivar Westar is substantially non-embryogenic under all standard protocols for induction of microspore embryogenesis; however, a few embryos are produced in Westar microspore cultures when brassinosteroids are added to the induction medium. When these embryos are grown to plants, they were found to develop into heritably stable embryogenic lines after chromosome doubling. One of the Westar-derived doubled-haploid lines, DH-2, produced up to 30% the number of embryos as the highly embryogenic *B. napus* line, Topas DH4079. Expression analysis of marker genes for embryogenesis in Westar and the derived DH-2 line, using real-time reverse-transcription PCR, revealed that the timely expression of embryogenesis-related genes such as *LEAFY COTYLEDON1* (*LEC1*), *LEC2*, *ABSCISIC ACID INSENSITIVE3*, and *BABY BOOM1* and an accompanying downregulation of pollen-related transcripts were associated with the commitment to embryo development in *Brassica* microspores. Microarray comparisons of 7-d cultures of Westar and Westar DH-2, using a *B. napus* seed-focused cDNA array (10,642 unigenes; http://brassicagenomics.ca) identified highly expressed genes related to protein synthesis, translation, and response to stimulus (GO Ontology) in the embryogenic DH-2 microspore-derived cell cultures, whereas transcripts for pollen-expressed genes were predominant in the recalcitrant Westar microspores. Besides being embryogenic, DH-2 plants showed alterations in morphology and architecture as compared to Westar, for example, epinastic leaves, non-abscised petals, pale flower color, and longer lateral branches. Various mechanisms accounting for the increased capacity for embryogenesis in Westar-derived DH lines are considered.

P-098

Interruption of New Proteins Synthesis Delays Flower Senescence: A Molecular Approach

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The upregulation of several genes and their protein products is well known at the onset of flower senescence. Protein synthesis inhibitor, cycloheximide has been found effective in delaying flower senescence earlier. Here, we chose molecular biology approach to interrupt new protein synthesis. *Petunia hybrida* was transformed with a construct allowing for chemically inducible silencing of ribosomal protein gene. The silencing of a ribosomal protein was carried out by treating the flowers of three growth stages (bud, folded petal, and fully open flower) with the chemical inducer. The folded petal stage was found most perfect to initiate the ribosomal protein gene silencing which delayed the flower senescence by 2 d as compared to the control (transgenic flowers kept in water lasted for 5 d). These results indicate that after a certain growth stage some specific proteins are synthesized which plays important role in flower senescence.

P-099

RNA Virus-Amplified Heterologous Protein Expression in Plant Tissue Culture

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We are developing a rapid and scalable plant-tissue-culture-based platform for the production of therapeutic proteins. The rapid sequencing of novel pathogens, including pandemic strains of influenza, has created a new market for the on-demand production of recombinant vaccines. These products can be expressed in plant tissue culture in the controlled environment of a bioreactor with minimal lead time. Despite our successes in process scale-up, product titer limits economic feasibility. Thus, we engineered plant RNA viruses, including Potato Virus X (PVX), with the objective of achieving high level expression of the reporter gene beta-glucuronidase (GUS). In shake flasks, cell suspensions and hairy root cultures of *Nicotiana benthamiana* were co-cultured with an *Agrobacterium tumefaciens* auxotroph for the targeted delivery of the viral cDNA to the plant cell nucleus. In vivo replication of the transcribed viral RNA amplifies both native and heterologous gene products. A gene replacement construct, where the GUS gene replaces the coat protein gene of PVX, was found to be optimal in hairy roots. In contrast, a minimal amplicon which also includes a triple gene block deletion resulted in the highest GUS expression in plant cell suspensions. A viral suppressor protein, p19, suppressed post-transcriptional gene silencing to a greater extent in leaves than in cell suspensions. As a means of enhancing transient GUS expression in cell suspensions, we investigated the *in trans* complementation of movement-deficient PVX vectors via co-delivered constructs. This work explores the limitations of PVX-based expression vectors in hairy roots and cell suspensions as well as presents preliminary results of our efforts to overcome these challenges with vectors derived from alternative RNA viruses.

P-100

Large-Scale Insertion Mutagenesis Using Tobacco Retrotransposon (TNT1) in *Medicago truncatula*

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Autonomous long-terminal repeat (LTR) retrotransposons are retrovirus-like elements which encode functions required for their own replication and transposition and move in the genome via a "copy and paste" mechanism. Retrotransposons can be activated by tissue culture to transpose in multiple copies. The absence of excision during transposition makes retrotransposons ideal tools for saturation mutagenesis with stable tags. We are using tobacco retrotransposon Tnt1 to mutagenize and tag the whole genome of model legume *Medicago truncatula*. We have generated over 18,000 independent Tnt1-containing lines encompassing approximately 450,000 insertion events. Over 20,000 Tnt1 flanking sequence tags (FSTs) have been recovered. We have pooled genomic DNA from 14,000 lines for customized reverse-genetic screening, and the frequency of insert identification in this pool for an average-sized gene is approximately 90%. All FST sequences have been deposited in a publicly available database (http://bioinfo4.noble.org/mutant/database.php). Mutant screening workshops are open to the scientific community on an annual basis. The range and diversity of mutant phenotypes suggest that *M. truncatula* offers a great opportunity to dissect symbiotic and developmental pathways for a comprehensive understanding of legume biology.

P-101

Towards Development of TNT1-Insertion Mutants in the Model Grass *Brachypodium distachyon*

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Brachypodium distachyon is a small grass that shares many characteristics with *Arabidopsis* and is genetically closely related to important crops such as wheat, barley, and switchgrass. It has a rapid generation time, small genome, diploid accessions, small stature, and simple growth requirement. Brachypodium possesses physical and biological qualities necessary to serve as a model system. Currently, several genomic resources are available including cDNA libraries, BAC libraries, EST sequences, BAC end sequences, a physical map, genetic markers, a linkage map and complete genome sequence. However, a large collection of stable tagged mutants is not available. Retrotransposons are retrovirus-like elements which encode functions required for their own replication and transposi-

tion. Retrotransposons can be activated to transpose in tissue culture, can transpose long distances, and show preferences for gene-rich regions. The absence of excision during transposition makes retrotransposons ideal tools for saturation mutagenesis with stable tags. We are using tobacco retrotransposon Tnt1 to mutagenize and tag the whole genome of the model grass *B. distachyon*. Because Tnt1 transposes in multiple copies (on average 15 per generation), we envision that saturation mutagenesis in *Brachypodium* is feasible. Our progress towards developing tagged mutants in *Brachypodium* will be presented.

P-102

Patatin-like Phospholipase A Regulates the Accumulation of Salicylic Acid and Immune Response to *Pseudomonas syringae* DC3000

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Phospholipases are ubiquitous and diverse enzymes that induce changes in membrane composition and produce lipid-derived messengers in various cell signaling cascades. Phospholipases can be classified into A, C, and D types based on their site of phospholipid hydrolysis. Phospholipase A2 (PLA2) consists of secretary and intracellular types. In mammalian cells, calcium-independent intracellular phospholipase A2 plays a critical role in pathogen defense, and its catalytic site shares high sequence similarity with plant patatins. There are 10 genes encoding patatin-like phospholipase As in *Arabidopsis*, which are classified into three groups. However, their functions in pathogen defense are largely unknown. We have isolated eight individual T-DNA knockout mutants and eight double T-DNA knockout mutants of patatin-like phospholipase As in *Arabidopsis*. Three single knockout mutants and five double mutants are compromised in resistance to bacterial pathogen, *Pseudomonas syringae* DC3000. The five double mutants also showed attenuated accumulation of salicylic acid (SA) after bacterial inoculation. SA is a primary factor responsible for exerting diverse immune response in plants and is synthesized in response to attack by a wide range of pathogens. This study suggests that PLA2 regulates the accumulation of SA and positively mediates plant defense against pathogens.

P-103

RNA Interference-Mediated Silencing of 4-Coumarate/Coenzyme A Ligase Gene for Reduced Lignin in Switchgrass

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For improvement of switchgrass (*Panicum virgatum* L.) as a major biofuel crop, we developed a high-throughput transformation protocol for cv. Alamo and two newly released, elite cultivars Performer and Colony with the transformation efficiency ranging from 50% to 90%. To reduce lignin content to improve biofuel production using lignocelluloses of switchgrass, we employed RNA-interference-mediated silencing of 4-coumarate/coenzyme A ligase (4CL) gene, a key enzyme of the lignin biosynthesis pathway. Near 200 transformation events were obtained from cv. Alamo and Performer. By quantitative RT-PCR analysis, up to 90% of mRNA of the targeted 4CL genes was reduced. Phenotype changes were observed among many transgenic plants. Stem bending at nodes regions are often observed. In addition, about 80% of the transgenic plants showed abnormal inflorescence development. Lignin content and composition of transgenic plants are under analysis.

P-104

Interactome Analysis of Cotton 14-3-3S that are Preferentially Expressed in Fibers and Involved in Fiber Cell Elongation

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Proteins of 14-3-3 family regulate a diverge set of signaling pathway in all eukaryotic organisms. In this study, six

cDNAs (designated as Gh14-3-3L, Gh14-3-3a, Gh14-3-3e, Gh14-3-3f, Gh14-3-3 g, and Gh14-3-3 h) encoding 14-3-3 proteins were isolated from cotton fiber cDNA library. The Gh14-3-3 genes share high sequence homology at nucleotide level in the coding region and at amino acid level. Real-time RT-PCR analysis indicated that the expression levels of these Gh14-3-3 genes were developmental-regulated in fibers, and the expression reached the highest level in the elongation stage of fiber development of cotton. Overexpression of Gh14-3-3 L, Gh14-3-3a, and Gh14-3-3e in fission yeast (*Schizosaccharomyces pombe*) promoted atypical longitudinal growth of the host cells, whereas RNA interference significantly suppressed fiber elongation in transgenic cotton plants, suggesting that 14-3-3 proteins may participate in regulation of fiber elongation. Yeast two-hybrid analysis revealed that the interaction between cotton 14-3-3 proteins is isoform selective. Through yeast two-hybrid screen, over 50 novel interaction partners of the five 14-3-3 proteins were identified in cotton fibers, suggesting that Gh14-3-3 proteins may play important roles in signal transduction related to fiber development.

P-105

Preliminary Study on the Lower Mutation Rate of Unreduced Microspore in *Ginkgo biloba* Induced by Colchicines

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Triploid plants have fast growth, huge organs, and high content of secondary metabolites, etc. The use of 2n pollen is a kind of time-saving breeding of triploid in an efficient way, for which this experiment carried out relevant studies. The unreduced microspore (2n pollen) in *Ginkgo biloba* were induced by the colchicines on our experiments, but 2n pollen mutation rate was lower than in other plants which can induce unreduced microspore; the biggest mutation rate of 2n pollen was only about 7% in the *G. biloba*. To understand the reasons for this phenomenon, we conducted the following experiments funded by project of NSFC (30771747). The samples of the androsporangium were treated for 2 d with colchicines (concentration 6 and 8 $mgmL^{-1}$); the samples of the microsporocyte were treated in various times (0.5, 1, 3, 6, 12, and 24 h) with colchicines (6 and 8 $mgmL^{-1}$); the samples of the microsporocyte protoplasm were co-cultured in various times (0.5, 1, 3, 6, 12, 24 h) with colchicines (6 $mg{\cdot}mL^{-1}$ and 8 $mg{\cdot}mL^{-1}$), after they were treated or co-cultured by the colchicines, the residue amount of the colchicines in their interior of all the samples were analyzed by RP-HPLC. The results: There were colchicine residues in the treated samples of the androsporangium and the microsporocyte; and the colchicine residue amount of the treated samples by 8 $mgmL^{-1}$ colchicines was bigger than that by 6 $mgmL^{-1}$ colchicines. The colchicine residue amount of the protoplasm–colchicine mixtures were nearly same with the residue amount of water–colchicines control mixtures. Conclusion: All the androsporangium, the microsporocyte wall, and the protoplasm in *G. biloba* are not the major cause which lead to the lower mutation rate of 2n pollen in *G. biloba* induced by the colchicines. So we can put the research emphasis on the impacts of the related proteins on the lower mutation rate of 2n pollen in the next step. The result can provide the basis for exploring farther how to increase the mutation effects of 2n pollen in *G. biloba* and other gymnosperm.

P-106

Molecular Characterization of the PR-1 Gene Family and Discovery of a Novel Expanded Subfamily in Grapevine (*Vitis* spp.)

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Group 1 of plant pathogenesis-related 1 proteins (PR-1) have been shown to play a key role in host defense against pathogen attacks. Using public genomics databases, we identified and analyzed a total of 21 putative PR-1 proteins and genes from grapevine. These PR-1 proteins have molecular sizes ranging from 10.9 to 29 kDa and p*I* values from 4.6 to 8.25 and are composed of a signal peptide and a mature protein with a highly conserved eight-cysteine motif. A cluster of 14 PR-1 genes was found within a 280-kb region on chromosome 3. A particular PR-1 gene encoding a basic isoform, herein named VvPR1b1, was further analyzed via PCR amplification and DNA sequencing. Restriction and sequence alignment analyses of amplified DNA confirmed the high degree of sequence conservation in all 14 examined genotypes of *Vitis* spp. However, an additional subfamily of VvPR1b1-related genes was detected from several genotypes including a highly disease-resistant Florida hybrid bunch grape selec-

tion, BN5-4, and its parents with wild species background. Unlike VvPR1b1 gene, members of this subfamily contained a wide range of DNA mutations leading to amino acid substitutions and deletions throughout signal peptide and mature protein regions and consequently resulting in significant changes in mature protein acidity and p*I* values. Phylogenetic analysis of DNA/protein sequences with available PR-1 genes from *V. vinifera* and other plant species suggest that all isoforms of the VvPR1b-1 subfamily are previously unidentified PR-1 proteins in the genus *Vitis*, which are entirely absent from some of the major *V. vinifera* cultivars.

P-107

Utilization of the Anthocyanin Biosynthesis Regulator Gene VvMYBA1 of Grapevine as a Versatile and Dynamic Reporter Marker for Plant Transformation and Gene Expression Studies

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The VvMYBA1 gene controls anthocyanin biosynthesis in berries of red grapes. Overexpression of this regulatory gene can lead to ectopic production and accumulation of anthocyanins in non-fruit tissues. A copy of the VvMYBA1 gene was isolated from *Vitis vinifera* cv. "Merlot" and tested for use as a visible reporter marker, along with the EGFP gene to indicate transformation status and promoter activity. Using transgenic tobacco plants, localization and relative efficiency of anthocyanin accumulation were compared with four promoter constructs, including double-enhanced CaMV35S and double-enhanced CsVMV promoters in their single and bidirectional dual-promoter (BDDP) complex formats. Under in vitro culture conditions, anthocyanins accumulated throughout leaf tissues on par with expression of the EGFP gene and the relative amount of anthocyanin pigments correlated well with promoter strength, whereas accumulation of anthocyanins in root hair cells showed promoter synergy in BDDP constructs. However, unlike the relatively uniform EGFP expression, anthocyanins produced in the majority of greenhouse-grown tobacco plants were localized mainly in vascular tissues on the abaxial side of leaves and all tissues of the inflorescence, probably due to the unstable nature of anthocyanins and/or other overriding factors that regulate tissue-specific gene expression. Nevertheless, variations in promoter activity associated with test constructs were still evident based on comparative pigmentation patterns and intensity. Similar discernable patterns of anthocyanin accumulation in somatic embryos (SE) of grapevine transformed with various promoter constructs were also observed. Unlike tobacco, stably transformed grape SE with strong promoter construct displayed intense anthocyanin pigmentation in all types of cells. Accordingly, the VvMYBA1 gene can be used as an effective and dynamic reporter maker to identify transformants in transformation studies and facilitate continuous tracking of promoter activity at the whole plant level.

P-108

Overexpression of a Novel Arabidopsis Phosphatase Results in Accelerated Growth and Increased Seed Yield

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Overexpression of a novel phosphatase in *Arabidopsis* was found to speed up plant growth by altering its carbon metabolism. The growth phenotype can be seen at http://www.youtube.com/watch?v=8HdI708ojVY. The inflorescences of overexpression (OE) lines emerged earlier (5–6 d under LD, 14–16 d under SD) than that of the wild-type (WT) and T-DNA lines. The number of rosette leaves of the OE lines were fewer (five to six leaves fewer under LD and nine to 13 leaves fewer under SD) than the WT during the emergence of inflorescence. At maturity, the OE lines produced 38–57% more siliques and seeds. The amount of sucrose and glucose at the end of day in the shoots of 21-day-old soil-grown plants, determined by LC–MS–MS, were found to be significantly higher than that in WT (1.3–1.8× for sucrose; 1.9–2.6× for glucose, $p<0.01$). Carbohydrate starvation experiment by extended darkness treatment (no photosynthesis for 12 d) showed that the OE lines were more energy rich than the WT and T-DNA lines. Transcriptome analysis revealed that key enzyme genes in sucrose biosynthesis, nitrogen metabolism, and potassium uptake are positively modulated ($p<0.05$). Interestingly, while high extracellular sucrose treatment was reported to induce a number of genes in the anthocyanin synthesis pathways, most of these genes were downregulated in the OE lines, which had higher internal sucrose. This novel phosphatase may have positive effects on C, N, and K metabolisms of plants, which resulted in the accelerated growth and higher yield phenotypes.

P-110

Phosphatidic Acid Interaction with GLABRA2 (GL2) in Transcriptional Regulation

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The homeodomain-containing transcription factor GLABRA2 (GL2) regulates plant developmental and biochemical processes, including root hair patterning and seed oil accumulation. We have found that GL2 interact directly with phosphatidic acid (PA), an emerging lipid mediator in plant development and in plant response to biotic and abiotic stresses. To investigate the function of the interaction, we have determined the amino acid region of GL2 responsible for the interaction by sequential deletion and point mutation. GL2 belongs to a large family of transcription factors, which share many critical motifs in common, including this binding region. Our work may help unveil a key mediator in transcriptional regulation in plants.

P-111

Physical Mapping of the Rice Bacterial Blight Resistance Gene Allelic to XA7 to a 125-kb Segment of Rice Chromosome 6

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Rice bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a serious disease in rice production worldwide. Rice cultivar Zhenhui084, a newly developed strong indica restorer line, exhibits high resistance to most of the Philippine races of BB and has been widely used in rice hybrids in China; however, the resistance gene has not been cloned yet. Here, we showed that the resistance of Zhenhui084 to *Xoo* strains was similar to that of IRBB7 containing *Xa7*, a durable and broad resistance dominant gene for BB. To map the resistance gene in Zhenhui084, a F_2 population with 331 highly susceptible individuals derived from a cross between Chenghui448 and Zhenhui084 was built. We finely mapped the target R gene to a region between two proximal markers RM20576 and MY4 in rice chromosome 6. Marker-based physical map of chromosome 6 was used to construct the contig covering the genomic region between two markers RM20576 and MY4. The target gene was assumed to be in an interval of approximate 200 kb. To shorten this mapping region, another F_3 and BC_2 F_2 populations of Chenghui448/Zhenhui084 were constructed, and 1,300 and 900 highly susceptible individuals were selected for the fine mapping. The target gene was further delimited into a 125-kb physical distance, in which 10 candidate genes were predicted. Our results will greatly facilitate the isolation and characterization of the target R gene allelic to *Xa7*. Additionally, two PCR-based markers, tightly linked to the target R gene locus, will be a useful tool for the marker-assisted selection of the target R gene allelic to *Xa7* in breeding programmes.

P-112

Construction and Analysis of EST Libraries to Facilitate *Panicum virgatum* L. Switchgrass Genomics

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Switchgrass (*Panicum virgatum* L,) is a warm season C4 perennial grass widely grown in North America. It has been recognized as a promising feedstock for bioenergy industry by the U.S government. Genomic resources for analyzing basic biological processes important for switchgrass production are not well developed. Seed dormancy is one of

the major problems that potentially hinder the large-scale production of switchgrass. Genomic resources for analyzing the molecular basis of dormancy in switchgrass are lacking. To facilitate this process, we are conducting expressed sequence tag (EST) analysis of dormant and sprouting switchgrass seeds. Field studies of switchgrass have indicated that tiller number and plant height are important factors contributing to increased biomass in switchgrass. Emerging tillers from 1-mo-old seedlings and leaves from 4- to 8-wk-old plants are being used for constructing EST libraries. We are in the process of sequencing approximately 10,000 clones from these EST libraries. Preliminary analysis of EST sequence data and their eventual use in constructing switchgrass cDNA-based microarrays will be discussed.

P-113

Evaluation of Temporary Immersion Methods and Prototypes of Temporary Immersion System (SIT) Applied in *Coffea arabica* L. Somatic Embryogenesis

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The temporary immersion systems have demonstrated to increase significantly the index of multiplication in several plant species. However, the high cost of the commercial flasks for automated temporary immersion (RITA®) has limited its use in developing countries. In this research, we used a prototype of double flask temporary immersion system (SIT) constructed in the Universidad del Zulia (patent pending), with a new system of filtration that permits its application in processes such as somatic embryogenesis. The objective of the present research was to evaluate the application of SIT prototypes for the in vitro culture and germination of somatic embryos of coffee (*Coffea arabica* L.), and compare this system with RITA®, suspension cultures, and solid media. Embryogenic calli were induced from leaf sections cultured 12 wk in media MS/2 with 1 mg/l 2,4-dichlorophenoxiacetic acid and 8 mg/l 6-bencyladenine (BA). The media tested to induce somatic embryogenesis and embryo germination were composed of MS/2 salts with 0.8 mg/l naphthalene acetic acid, or 5 mg/l BA, or without hormones. The optimal system for production of primary and secondary somatic embryos was the suspension culture, producing up to 1,484 somatic embryos after culture of embryogenic calli during 4 mo in media with 5 mg/l BA. The next best yield was obtained in SIT, with an average production of 276 somatic embryos. In the stage of germination and conversion to plants, the systems RITA®, SIT, and solid media MS/2 gave better results than liquid cultures. These results showed that the liquid suspension culture was the best system for induction and multiplication of coffee somatic embryos, but germination was better in the temporary immersion systems RITA and SIT.

P-114

Microsatellite Polymorphism in Hybrids of *Coffea arabica* (L.) Produced Industrially by Somatic Embryogenesis

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Plant propagation by tissue culture should theoretically result in the production of individuals identical to the original mother plant. Nevertheless, genotypic changes have been observed during the process of tissue culture, in a phenomenon called somaclonal variation. Somaclonal variation is undesirable in cases where uniformity of agronomical characteristics is to be maintained. The present research was done to verify the genetic conformity of hybrid coffee plants produced industrially by somatic

embryogenesis for commercialization. In previous studies, abnormal phenotypes have been characterized and quantified, but molecular markers permit an early evaluation of the variation and can detect non-visible characteristics; for this reason, we evaluated the molecular polymorphism by the method of microsatellites. Ten microsatellites were tested by the technique of capillary sequencer with the DNAs of 273 plants, including the parents of the hybrids, genetic and technical witnesses, somaclones of hybrids exhibiting normal phenotype, and morphological variants. We report for the first time the application of microsatellites to evaluate somaclonal variation in coffee and detected low polymorphism. The next stage of the research will compliment the analysis by the use of other molecular markers such as AFLP and MSAP. As was already shown for other species, no correlation was observed between the detected microsatellite polymorphism and the somaclonal variants tested in this study.

P-115

Improving Water-Use Efficiency by Regulating Stomatal Density

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Improving plant drought tolerance and water-use efficiency (WUE) is global priority to maintain biomass production and/or yield stability in the face of decreasing fresh water availability. The *Arabidopsis* GT-2 like 1 (GTL1) trihelix transcription factor acts as a negative regulator of WUE by controlling stomatal density and, in turn, transpiration through transrepression of stomatal development and distribution1 (SDD1). *gtl1* T-DNA insertional mutations (*gtl1–4* and *gtl1–5*) resulted in reduced stomatal density on the abaxial leaf surface, leading to lower light period but not dark period transpiration rates, without a reduction in CO_2 assimilation or biomass accumulation, resulting in improvement of WUE. The reduction in transpiration and subsequent maintenance of leaf water content significantly enhanced the capacity of gtl1 plants to survive water-deficit stress. *gtl1* mutations up-regulate the expression of *SDD1*, a negative regulator of stomatal density. The pathway activated by SDD1 and that regulates stomatal density is activated by gtl1. In vitro DNA binding and in vivo chromatin-immunoprecipitation assays revealed that GTL1 binds to a GT element (GT3-box: GGTAAA) in the *SDD1* promoter. These results indicate that GTL1 functions as a transcriptional repressor of *SDD1* to regulate stomatal density, transpiration, and WUE. Natural variation for WUE that is associated with altered stomatal density may result from differential regulation of stomatal development determinants.

P-116

Use of H2AX and Micronuclei Formation to Evaluate Genotoxicity in Cultured Human Skin Cells Following Sulfur Mustard Exposure

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Sulfur mustard (2-2′-dichlorodiethyl sulfide; SM) is a cytotoxic chemical warfare agent. The skin serves as a principal target site for in vivo toxicity of SM exposure resulting in the formation of blisters and inflammation. To elucidate genotoxic effects of SM, normal human epidermal keratinocytes (Lonza Corp., MD) served as an in vitro model to observe the presence of γ-H2AX and micronuclei formation. γ-H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double-stranded DNA break sites. Micronuclei, the existence of small DNA fragments, occur due to broken chromosomal remnants that remain in the cytoplasm during mitosis, indicating a clastogenic event. Cells were exposed to 0, 50, 100, or 300 μM concentrations of SM for 2 or 24 h. γ-H2AX was measured by flow cytometry and western blotting. Micronuclei formation was measured by flow cytometry and microscopy. SM exposure resulted in the formation of γ-H2AX, and preliminary data show the formation of micronuclei. When combined, these biomarkers of DNA damage are useful techniques for the study of SM toxicity and potential therapeutic approaches.

P-117

Improving *Agrobacterium tumefaciens*-Mediated Gene Delivery in *Sorghum bicolor*

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Grain sorghum (*Sorghum bicolor*), the fifth most important cereal worldwide, possesses multiple agronomic traits desirable for future crop development. It survives abiotic stresses like drought, flood, and high temperatures, while using fewer inputs than crops like maize. It is an important food in semi-arid regions of Africa and Asia, and its grain and stalks, and sugars from sweet sorghum, can be important for biofuel production. Despite these positive traits, it lacks certain amino acids, vitamins, and minerals, and is the least digestible of all cereals. Genetic engineering of sorghum could rectify some of these shortcomings; however, its recalcitrant response to in vitro culture has led to low transformation efficiencies. Using immature embryos as target tissue, our laboratory improved the transformation frequency of sorghum variety, P898012. Prior to *Agrobacterium* infection, we tested multiple treatment methods to induce a stress response in order to improve post-infection survivability. The most effective was a brief heat treatment, which led to higher immature embryo survival rates, increased callus induction frequencies, and ultimately an ~8% frequency of fertile, regenerable independent events. To expand the number of sorghum varieties amenable to transformation, current efforts focus on two other genotypes. To reduce the time to perform proof-of-concept experiments with genes of interest, we are performing in vitro culture and transformation with a short-season variety, N247. To facilitate functional genomics studies, we are also doing tissue culture and transformation with B623, the variety for which the genome has been sequenced. Both varieties respond positively to heat treatment; selection and regeneration of putative transgenics is currently underway.

P-118

Some Weeds Used by Rakhain, the Ethnic Group of Chittagong Hill Tracts, Cox's Bazar District, Bangladesh, to Treat Various Ailments

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A multicultural and ritual country Bangladesh is an alluvial plain land breaks the scenario by not only topographical but also human habitations. Each ethnic group has their own customs and traditions, religion, language, and culture. Rakhain is a small ethnic group of Arakan origin belonging to the Bhotbarmi community of the Mongoloids. For cure of diseases, they rely on their own ethnic group healers who are experts in the knowledge and use of weeds. The selection of weed is a closely guarded secret and is usually kept within the ethnic group. As a result, the use of weeds varies widely between ethnic groups of different areas within the country, and is based on both weed availability and the ethnic group's unique knowledge derived from practice. There is little information on the weeds used by the Rakhain ethnic group. The present paper deals with ethnobotanical study on weed species used for several common diseases by Rakhain, the ethnic group of Chittagong Hill Tracts, Cox's Bazar district, Bangladesh. Interviews were conducted in the native dialect, and weed samples as pointed out by the Rakhain ethnic group healers in guided field walks were collected and identified at the Bangladesh National Herbarium. Some of the weed names obtained in this survey included *Hygrophila auriculata*, *Heliotropium indicum*, *Bacopa monnieri*, *Amaranthus spinosus*, *Aristolochia indica*, *Hemidesmus indicus*, *Cen-*

tella asiatica, *Mikania scandens*, *Phyla nodiflora*, *Cassia occidentalis*, *Coccinia cordifolia*, *Cuscuta reflexa*, *Datura metel*, *Achyranthes aspera*, *Cynodon dactylon*, *Gloriosa superba*, *Cissus quadranglaris*, *Spilanthes acmella*, and *Lygodium flexuosum*. The botanical, vernacular, and family names, mode of preparations, and uses have been provided for further pharmacological and clinical evaluations.

P-119

Development of *Agrobacterium*-Mediated Transformation Method for Wild Sugarcane, *Saccharum spontaneum*

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Sugarcane, an important sugar and energy crop, belongs to the genus *Saccharum.* This genus consists of six species, two wild and four cultivated species, and almost all modern cultivated sugarcanes are bred from inter-species hybrids between cultivated noble cane *Saccharum officinarum* and wild cane *Saccharum spontaneum*. The genome sequence of a crop provides indispensable information to improve the crop, but the complex and variable genome of sugarcane is an obstacle to genome sequencing. On the other hand, genome sequences have been reported in many crops and provide useful information to improve sugarcane. Genetic transformation of sugarcane with *Agrobacterium* with a binary vector, which is also employed for the transformation of genome-sequenced rice and sorghum, should be a powerful tool for sugarcane improvement. We developed an efficient *Agrobacterium*-mediated transformation method for sugarcane. This development was divided into two parts. Initially, we evaluated the frequency of plant regeneration from calli of 13 sugarcane varieties. These calli were induced from plantlets cultured in a growth chamber. The plantlets were generated from sterilized auxiliary buds and were used to induce calli. We selected one variety of wild cane *S. spontaneum* by the highest frequency of regeneration among 13 varieties. Next, we modified an efficient *Agrobacterium*-mediated transformation method for rice to use for calli of the wild cane (Toki et al., Plant J 47: 969–976, 2006), and obtained regenerated transformed plantlets, which are growing on a medium with Hygromycin B by transformation with the *Agrobacterium* containing a binary vector including the *hygromycin phosphotransferase* (*hpt*) gene.

P-120

Lactogenic Immunization of Piglets with Transgenic Carrot Vaccines Produced in Bioreactor

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In an attempt to develop the plant vaccine against diarrhea in piglet, the transgenic carrots with ETEC K88ac and PEDV antigens were produced in vitro. The Pillin and COE genes from ETEC and PEDV, respectively, were cloned with some modifications in sequence and structure. The antigen genes were transformed via Agrobacteria into carrot hypocotyls to generate transgenic cells. Levels of the antigen expressed were estimated by real-time PCR and western analyses and the selected cell lines were propagated and differentiated into various tissues such as callus, embryo and root. The transgenic tissues for vaccine were produced in bioreactor and freeze-dried. The transgenic carrot was fed to pregnant swine to induce oral immunization, and then a lactogenic immunization via colostrum to her offsprings was examined. Piglets were orally challenged twice (in 12-h interval) with ETEC K88ac at 7 d after birth. The number of suckling piglets showing diarrhea was three times lower in the offsprings from pregnant swine fed with

carrot callus than those from mothers immunized by commercial vaccine. The result indicates that an active immunization of the female parent through oral delivery of vaccine plant callus cell could induce a passive immunization of their offsprings.

P-121

Polyembryonyin Non-apomictic Citrus Genotypes

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Adventitious embryony from nucellar cells is the mechanism leading to apomixis in citrus. However, singular cases of polyembryony have been reported in non-apomictic genotypes as a consequence of $2x \times 4x$ hybridisations and in vitro culture of isolated nucellus. The origin of the plants obtained as a consequence of these two processes is still unclear. In this work, we systematically analyzed the genetic structure (ploidy and allelic constitution at SSR *locus*) of plants obtained from polyembryonic seeds arising from $2x \times 4x$ sexual hybridisations or regenerated from nucellus culture in vitro of different non-apomictic citrus genotypes. Histological studies were also conduced to try to identify the initiation process of polyembryony in non-apomictic genotypes. We demonstrate that all plants obtained from the same undeveloped seed in $2x \times 4x$ hybridisations resulted from fission of the original zygotic embryo. Also, the plants obtained from in vitro culture of nucellus were recovered by somatic embryogenesis from cells having the same genotype as the zygotic embryos of the same seed. It appears that in non-apomictic citrus, proembryos or embryogenic cells are formed by fission of the original zygotic embryo and that the development of these adventitious embryos, normally hampered, can take place in vivo or in vitro as result of two different mechanisms that prevent the dominance of the initial zygotic embryo.

P-122

Use of a Model Monocotyledon Species, *Setaria italica* (Foxtail Millet), and Virus-Induced Gene Silencing to Evaluate the Recalcitrance Potential of Target Genes for Biofuel Production

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Cellulosic biomass from perennial grasses (e.g. switchgrass and miscanthus) represents a potential high energy and renewable fuel source. To take full advantage of this energy source, the difficulty in accessing and converting the useful polymers in the cell wall to ethanol, or other biofuel, must be overcome. To help address biomass recalcitrance to biofuel production, we are utilizing virus-induced gene silencing (VIGS) to evaluate the function of putative recalcitrance genes in foxtail millet, a model species very closely related to switchgrass. VIGS is a transient RNA silencing method that provides very rapid results (target gene knockdown and analysis of silencing phenotype within 2 mo of inoculation). A *Brome mosaic virus* (BMV) clone was modified to serve as a vector to express plant gene fragments to be targeted for silencing. The BMV vector was capable of inducing target gene silencing (both marker and potential recalcitrance-associated genes) as determined by quantitative RT-PCR. The visible and biochemical phenotypes of the silenced tissue from inocu-

lated plants are being evaluated and compared with baseline biochemical phenotypes of tissue from un-inoculated plants. These results, along with efforts to modify the BMV vector to reduce screening costs and optimize silencing, will be presented.

P-123

Biolistic Transformation of Tobacco (*Nicotiana tabacum* L.) with Antigen Gene

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Many vaccines and biopharmaceutical proteins have been expressed at high levels via the chloroplast genome. Production of antigen proteins in chloroplast eliminates the expensive fermentation technology, purification steps, cold storage, cold transportation, and delivery via sterile needles, thereby further decreasing their cost. Biolistic transformation was carried out on the high-biomass tobacco variety VietNam 2 (V2) for production of human immunodeficiency virus type 1 (HIV-1) p24 antigen. Tobacco variety V2 offers the prospect of higher yields of intact functional protein per unit floor area. Leaves from 4-wk-old shoot cultures were bombarded with vector harboring aadA and HIV-1 p24 genes. Selection of transgenic shoots on MS salts and B_5 vitamins (Sigma) medium containing 1.0 mg/L BAP, 0.1 mg/L NAA, 500 mg/L spectinomycin, and 500 mg/L streptomycin. Molecular biology analysis was used to confirm the presence of aadA and HIV-1 p24 genes in transgenic tobacco plants. T0 plants were then transferred to soil and allowed to self-pollinate and set seeds.

P-124

Use of In Vitro Propagation in Maximizing Germination of *Lobelia monostachya* (Rock) Lammers

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The remote isolation of the Hawaiian Islands has produced a unique terrestrial biota with an extraordinarily high rate of endemism. Urbanization, habitat degradation, invasive species, and predation by pests have critically impacted the native habitat and populations of most of the endemic Hawaiian flora. *Lobelia monostachya* (Rock) Lammers (Campanulaceae), a federally endangered species, is found in only two extant populations (with a total of 10 plants) on the island of Oahu (Hawaii). The purpose of this study was to develop a protocol to maximize the germination rate of *L. monostachya* using in vitro propagation, with the goal of producing viable seedlings for restoration and germplasm banking. Propagules used for this study were intact immature fruit, intact mature fruit, and loose mature seed. Both the immature and mature fruit were surface-sterilized using an ethanol dip and flame protocol. Excised seeds were then placed onto an initiation medium of 1/2 MS (Murashige & Skoog, 1962) resulting in a germination rate of 32% for immature fruit and 23% for mature fruit. Mature seeds sterilized using a bleach protocol were placed on an initiation medium and had a germination rate of 29%; however, mature seeds sterilized using a gas sterilization protocol and placed on the same initiation medium had the highest germination rate of 80%. This study demonstrates that in vitro propagation protocols can be used successfully to disinfest both intact fruit and loose, mature seed of *L. monostachya*. Furthermore, the high rate of germination from gas-sterilized seeds can significantly increase the number of viable seedlings that can be produced in vitro for future restoration work and germplasm banking.

P-125

Screening Sweet Potato Cultivars as a Potential Source of Feedstock for Bio-ethanol Production

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Growing attention on bio-ethanol as a renewable alternative or additive to gasoline is mainly due to recent increases in crude oil prices because of increased demand and/or political instability in oil-producing countries. Fossil fuel sources are finite, and there is increased pressure to reduce atmospheric emissions of CO_2, thereby positively impacting global climate changes. One approach being explored is the use of bio-fuels from biomass, which adds little or no CO_2 to the atmosphere. Sweet potato storage root carbo-

hydrate and ethanol yield are approximately three times that of corn, and white fleshed high dry matter clones are less likely consumed as food in the USA. Experiments were conducted to screen 10 sweet potato cultivars for ethanol production based on extractable starch yields, dry matter content, amylose–amylopectin ratios, and ethanol yields. Samples of sweet potato slurry comprising a solid/liquid ratio of 0.12 were hydrolyzed using diastatic barley malt and fermented with yeast (*Saccharomyces cerevisiae*) for 40 h at 30°C. Results showed that amylose/amylopectin ratio ranged from 0.24 to 0.44, dry matter from 19.6% to 35.9%, extractable starch yield from 10.9% to 25.3%, and ethanol yield from 32.4 to 66.0 g/L. There was a strong positive correlation between dry matter and starch, dry matter and ethanol yield, and starch and ethanol yield, indicating for example that dry matter could be a predictor of both extractable starch and ethanol yield. This study provides new information on the relationship among dry matter, extractable starch, and ethanol yield. The overall results suggest that sweet potato can be a viable biomass candidate for starch ethanol in Alabama.

P-126

Expression of Bacterial Dihydrodipicolinate Synthase in Transgenic Barley

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Nutritional quality of human and animal foodstuffs is determined by the content of essential amino acids. Cereal grains such as barley contain insufficient levels of some essential amino acid, especially lysine. Dihydrodipicolinate synthase (DHPS) is the key enzyme in the regulatory step for lysine biosynthesis. Two constructs pBract214::sTPdapA and pBract214::mdapA containing the *dap*A gene from *Escherichia coli* coding for the bacterial DHPS were used for transformation of barley. The vector pBract214::sTPdapA in addition includes the transit peptide Rubisco *Hordeum vulgare* (rbcS). An *Agrobacterium*-mediated technique was used for transformation of immature embryos of spring barley cv. Golden Promise. Transgenic barley plants of the T_0 generation were evaluated by PCR, real-time PCR, and Western blot. Amino acid content was analyzed by HPLC after HCl hydrolysis. The lysine content in leaves was 15–25% higher than in wild-type plants. This work was supported by project 1M06030 MEYS Czech Republic.

P-127

Expression of *Aspergillus niger* Phytase Gene in Barley

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Cereals, oil crops and legumes contain phytic acid, *myo*-inositolhexakisphosphate (IP_6) that is an antinutritional compound. Ions of iron, manganese, magnesium, zinc and calcium, which are bound to phytic acid, are difficult to

digest. These complexes negatively affect the usability of phosphorus and bound cations in human and animal foodstuffs. This antinutritional effect can be addressed by overexpressing, in plants, the fungal phytase gene (phyA). Zygotic immature embryos of spring barley cv. Golden Promise were co-transformed by particle bombardment with combinations of plasmids pAL51/pAMFIT, containing the gene phyA from *Aspergillus niger* (from Prof. C. Fogher, Catholic University of Piacenza, Italy). PCR positive diploid and tetraploid lines were homozygotized by anther culture. The regenerated plants were evaluated by PCR and RT-PCR methods. Some independent transgenic lines with higher expression of the phytase gene showed increased phytase activity. This work was supported by project 1M06030 MEYS Czech Republic.

P-128

Phytotoxicity of New Complex of Lawsone with Cu(II) Ions

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Naphthoquinones demonstrate a wide range of biological actions (inc. allelopathy), which are based on generation of reactive oxygen species or DNA intercalation. Their complexes with heavy metals ions represent important modification of their biological properties. For this purpose, a new complex of naphthoquinone lawsone—Cu $(Law)_2(H_2O)_2 1/2H_2O$—was synthesized and tested on tobacco BY-2 cells. Cells were exposed to complex in concentrations 0–1,000 mM; samples were taken in time intervals from 0 to 120 h. Cell viability and nuclear morphology were determined. Application of Cu $(Law)_2(H_2O)_2.1/2H_2O$ complex led to significant changes in BY-2 cells structure and viability as well as changes of nuclear architecture, mitotic disorders, and manifestation of symptoms of programmed cell death, including cytoplasm shrinkage, chromatin condensation, and formation of apoptic-like bodies, which are manifestations of DNA fragmentation. We can conclude that synthesized and tested complex demonstrated significant phytotoxicity. This work was supported by projects GA CR 522/09/0239 and 1M06030 MEYS Czech Republic.

P-129

Micropropagation, Organogenesis and Somatic Embryogenesis from Different Yam Explants

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Three in vitro regeneration systems for yam (*Dioscorea alata*) were established from different explants. To establish a micropropagation system, 1-cm-long stem sections, with one to two lateral buds, were extracted from greenhouse plants. For organogenesis and somatic embryogenesis, micropropagated plants were used as sources of explants (microcuttings, leaves, and roots). For micropropagation, Murashige and Skoog (1962) (MS) medium supplemented with BA 0.5 mgl^{-1} was used. After 45 d, 4.9 buds per explant were obtained. Mass multiplication was achieved on MS medium supplemented with BA 2 mgl^{-1}. After 90 d, an average of 5.75 plants per explant was obtained. MS medium supplemented with BA 1 mgl^{-1}+ANA 0.5 mgl^{-1} was used for the establishment of organogenic processes. After 105 d, an average of 25.15 buds per microcutting explant was obtained (direct organogenesis). Callus tissue production was observed on 10% of microcutting explants cultured on the same medium with an average of 5.3 buds

per 1 cm^2 callus fragment (indirect organogenesis). Somatic embryogenesis from root explant was achieved on MS medium supplemented with 2,4 D 1 mgl^{-1}. After 42 d, an average of 7.6 embryos per explant was obtained. On the other hand, an average of 23 somatic embryos per explant was obtained from leaf explants after 70 d using MS medium supplemented with 2,4 D 4 mgl^{-1}. Micropropagated *D. alata* plants were obtained after 4.5 mo of culture; meanwhile, *D. alata* plants, which had originated through direct organogenesis, took 6 mo to develop. *D. alata* plants regenerated through micropropagation and organogenesis processes were potted on a mixture of soil and river sand 1:1, and 70.69% of plant acclimatization was obtained.

P-130

Factors Effecting In Vitro Bulblet Maturation in *Fritillaria imperialis* and *F. persica*

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The earliest cultivated ornamentals, *Fritillaria imperialis* and *Fritillaria persica*, are native to wide stretch from Anatolia to Himalayan foothills. They have attractive and large flowers in whorl shape facing downward at the top of the stem. Bulbs of *Fritillaria* species have also been used as herbal remedies in Turkish, Japanese, and south-east Asian folk medicines for centuries. However, endangered species *F. imperialis* and *F. persica* are threatened by extinction, and low natural propagation rate often inhibits the large-scale cultivation of these plants. Recently, we have developed an efficient in vitro bulblet regeneration system from immature embryos of both species and produced average 20 bulblets per explant on different media. Since these regenerated bulblets are small and immature, they did not grow into plants when they were transferred to soil. In order to increase the diameter and maturation of bulblets in vitro, $CaCl_2$, NaCl, KCl, and active charcoal were added to MS medium, and different jelling agents and culture room temperatures were used. Increase in salts in the medium and low temperatures in the culture room improved the diameter and maturation of bulblets. These bulblets were finally transferred to soil in greenhouse.

P-131

Peanut Mutants Deficient in Allergen Proteins

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Allergy to peanuts (*Arachis hypogaea* L.) affects 0.6–1% of the US population. Allergic reactions result from ingestion of foods containing whole or processed peanut seeds and are triggered mainly by seed storage proteins. Three major allergens fall into two protein classes, Ara h 1 (a vicillin) and Ara h 2 plus Ara h 6 (conglutins). Knockdown of Ara h 2 and Ara h 6 expression has demonstrated that a significant reduction in these proteins has no detrimental effect on seed viability or enhanced susceptibility to fungal (*Aspergillus flavus*) invasion. Elimination of Ara h 1 and Ara h 2 is being pursued by knockout of corresponding genes through ethyl-methane sulfonate mutagenesis and mutation detection through targeting induced local lesions in genomes. Such an approach becomes more complex in peanut because of its polyploid nature where Ara h 1 is encoded by two genes, Ara h 2 by two genes, and Ara h 6 by three genes. Screening of ~3,500 M2 lines has resulted in the recovery of a spectrum of Ara h 1 and Ara h 2 mutants, two of which are knockouts. A mutation in the B-genome gene encoding Ara h 2 eliminated the start codon and resulted in absence of protein expression. The Ara h 1-encoding mutant has a premature stop codon and the effect on protein production is under investigation. Knockout mutations in the second gene for each of Ara h 1 and Ara h 2 must be identified to make breeding for specific allergen-free peanuts feasible.

P-132

MSP Domain-Containing Protein Reveals A New Level of Regulation of Stomatal Signaling in *Arabidopsis*

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Stomata are specialized epidermal structures that mediate gas exchange between plant and environment. The formation and patterning of stomatal complexes in *Arabidopsis* result from molecular interactions within a complex web of functionally interconnected regulators. To date, several components of signal transduction pathway, including ligands, receptors, and transcriptional factors involved in stomatal patterning, have been identified. One of the most crucial regulators of stomatal patterning is the *TOO MANY MOUTHS (TMM)* gene. *TMM* encodes a receptor-like protein localized at the plasma membrane in stomatal lineage cells. TMM plays a central role in the deciding whether a cell will enter the stomatal pathway; mutations in this gene result in violation of major patterning rules governing stomatal development. The aim of this study was to identify specific modulators of the TMM receptor. We have designed and performed a forward genetic screen and identified specific suppressors of the *tmm-1* mutant. Here, we present molecular characteristics of the *MST1* (MSP domain-containing suppressor of *tmm* 1) gene, which specifically modulates the phenotype of *tmm-1* but not other stomatal signaling mutants.

P-133

Micropropagation of *Syngonanthus elegans*, a Brazilian Native Wild Flower

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Syngonanthus elegans is an important plant commercialized as dry cut flower with its production occurring mainly by extrativism. In this context, this research aimed at establishing methodologies for in vitro propagation of this species. Seed germination was evaluated on WPM medium with 0%, 25%, 50%, 75%, and 100% salt concentrations. For plant establishment, MS and WPM media with 50% and 100% salt concentrations were tested. The most adequate sucrose level for plantlets growth was evaluated in WPM medium supplemented with 0, 5, 10, 15, 20, 25, and 30 gL^{-1} sucrose. For in vitro multiplication, plantlets were inoculated on WPM medium supplemented with 0.0, 0.5, 1.0, 2.0, and 4.0 mgL^{-1} TDZ and 0.0, 0.5, and 1.0 mgL^{-1} NAA in all possible combinations. The influence of pre-acclimatization was also evaluated by using sand substrate, plantmax, and vermiculite on flasks with plantlets maintained on growth room. The results showed that germination is not affected by culture medium salt concentrations; however, the germination rate decreases with the increase of nutrient concentration. WPM medium with its original composition provided the best in vitro plant establishment, as well as the addition of 17 gL^{-1} sucrose, which promoted the best in vitro plantlets growth. The highest callus induction occurs in the absence of TDZ or using the same proportions of auxins/cytokinins. This species requires the addition of growth regulators for sprout formation, in concentrations of 0.5–1.0 mgL^{-1} TDZ and 0.5–1.0 mgL^{-1} NAA. There is no difference between the substrates on pre-acclimatization. This work was financially supported by FAPEMIG and CNPq.

P-134

In Vitro Culture of *Byrsonima intermedia*, an Endangered Brazilian Native Species

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The *Byrsonima* genus, especially *Byrsonima intermedia* species, is an interesting shrub from the Brazilian Cerrado, which has been extensively used for both feed purposes and therapeutic activities. The objective of the present study was to gather information about *B. intermedia* cell callus induction, cell suspension cultures, and their growth. For this, we established a protocol for callus induction at different environmental conditions (under light and dark conditions). Cell suspensions culture protocols were established, and the effects of explant type which originated calli and inoculums densities were studied. Moreover, growth curves of cell suspension cultures were established, and their different aspects were observed (heterogeneous and

homogeneous). We were able to successfully induce calli for *B. intermedia* species, and when cultured at different light conditions, different characteristics were observed. Growth curves of *B. intermedia* cell suspensions with exponential and stationary phase were established. Independent of the inoculum's density, growth rates during the exponential growth were similar, and growth was from day 0 on going into the exponential phase. No lag phase was thus observed. Growth suspensions were different. Red suspensions grew more slowly than yellow ones, and homogeneous cell suspensions grew slower than the heterogeneous ones. This work was financially supported by FAPEMIG and CNPq.

P-135

Regeneration of Plants from White Ash (*Fraxinus americana* L.) Hypocotyls

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White ash trees (*Fraxinus americana* L.) provide both economical and ecological benefits. White ash is a commercial hardwood used in the production of baseball bats, tool handles, furniture, flooring, doors, and cabinets. The seeds are consumed by wildlife, and the trees provide cover and nesting sites for various bird species. The emerald ash borer (*Agrilus planipennis*) is an invasive species that poses substantial risk to the ash resource in North America. There are no known means of complete eradication or of any innate resistance in white ash. The threat from this wood-boring beetle becomes more urgent with each growing season, making the development of an in vitro plant regeneration and genetic transformation system a valuable goal. Hypocotyls excised from mature embryos were cultured on Murashige and Skoog (MS) medium containing 13.3 μM 6-benzylaminopurine (BA) plus 4.5 μM thidiazuron (TDZ). Sixty-six percent of hypocotyl segments produced adventitious shoots, with a mean of 3.5±0.9 adventitious shoots induced per hypocotyl explant. Adventitious shoots were established as proliferating shoot cultures following transfer to MS medium with Gamborg B_5 vitamins (MSB5) containing 10 μM BA plus 10 μM TDZ. For in vitro rooting, woody plant medium with indole-3-acetic acid (IAA) at 2.9, 5.7, or 8.6 μM in combination with 4.9 μM indole-3-butyric acid (IBA) were tested for a 5- or 10-d dark treatment followed by culture in the light. Preliminary results show best rooting (75–87.5%) of in vitro shoots when exposed to a 5-d dark pulse on medium containing 2.9 to 8.6 μM IAA plus 4.9 μM IBA with an average number of 2.8±1.6 to 4.2±2.9 roots per shoot. Rooted plants were successfully acclimatized to the culture room. This regeneration system will be used for experimental studies to produce transgenic white ash with resistance to the emerald ash borer.

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Cloning and Sequence Analysis of HVCIPK2 in Wild Barley

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It has been proposed that binding of Ca^{2+} with calcineurin B-like proteins (CBLs) induces interaction of CBLs with CBL-interacting protein kinases (CIPKs) and in turn activates downstream response genes in plants. Multiple lines of evidence suggest that CIPKs play a regulatory role in various stress responses such as drought, salt, cold, low K^+, and abscisic acid in *Arabidopsis*. Recent studies showed that *OsCIPKs* are involved in various stress responses in rice. However, wild barley *HvCIPKs* still have not been identified and cloned so far. In this study, a wild barley homologue of *Arabidopsis CIPK2* was isolated using PCR-based strategy. Sequence alignment analysis indicated that wild barley *CIPK2* is 57.4% identical with *AtCIPK2* and 77.1% identical with *OsCIPK2* at the DNA level, respectively, whereas its deduced amino-acid sequence shares 59.9% identity with AtCIPK2 and 81.7% identity with OsCIPK2, respectively. Similarly, DNA and amino acid sequences of *OsCIPK2* share 58.4% and 62.2% identity with those of *AtCIPK2*, respectively. These data

suggest that *CIPK2* is highly conserved in higher plants, and meanwhile wild barley *HvCIPK2* is evolutionarily much closer to monocot *OSCIPK2* than to dicot *AtCIPK2*.

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One-Step Regeneration of Complete Plantlets and Early Assessment of Clonal Fidelity in Sugarcane with RAPD and SSR Markers

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Direct regeneration protocols are the need of the hour as they reduce the time required in vitro, leading to cost efficiency and reduced chances of somaclonal variation. The present work takes the work on direct regeneration in sugarcane a step further, by obtaining complete plantlets on the same medium, overcoming the need for separate medium for shoot and root induction. Immature leaf disc explants pre-treated on MS medium supplemented with different amounts of 2, 4-D in the dark for 0, 3, 5, 8, and 10 d when transferred on regeneration medium developed 15–22 shoots per explants in 3 wk. These induced shoots, if left on the same regeneration medium for 2 wk, developed healthy root system. Early assessment of clonal fidelity of micropropagated plants using molecular markers aids in fine tuning protocol parameters and gauge suitability of regeneration protocol for large-scale applications. Clonal fidelity of sugarcane plants regenerated through direct organogenesis was assessed using RAPD and SSR markers. Analysis of RAPD banding patterns generated by PCR amplification using 20 random primers gave no evidences for somaclonal variation, and the percent of polymorphic bands in a total of 110 amplicons was 0.02%. Meanwhile, analysis of SSR banding pattern generated using 15 primers (112 amplicons) gave no evidences for somaclonal variation. Lack of genetic variation confirms the genetic stability of tissue culture plants of sugarcane raised through direct organogenesis in leaf roll explants, and confirms the suitability of overall regeneration protocol. This is the first report describing direct regeneration and assessment of fidelity using RAPD and highly informative SSR markers in sugarcane.

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Modulating Carbon Flux During Soybean Seed Development as a Means to Impact Total Oil and Protein Levels

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Soybean is a valuable source of both protein and oil for food and feed applications. Total protein and oil levels of soybean typically are present at approximately 40% and 20%, respectively. We introduced into soybean a number of genetic constructs designed to attempt to perturb carbon flux during seed development, and thereby influence the protein to oil ratio of seed. The first constructs carry the *Arabidopsis* homologs of the yeast Mg-dependent phospatidate phosphatase, designated Atlipin1 and Atlipin2. The second set of constructs harbor the kinase domain of general control non-derepressible-2 (GCN2), both wild type and three constitutive active variants. The lipins act upstream in the synthesis of phospholipids and triacylglycerol, while GCN2 plays a role in global control of protein translation. The respective genes were codon optimized for soybean and placed under control of the seed specific promoter ß-conglycinin. Preliminary molecular and phenotypic analyses of the derived transgenic events that carry the respective constructs will be presented.

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Drought-Related Gene Expression in Upland Cotton

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Cotton is the world's primary fiber crop and is a major agricultural commodity in over 30 countries across the world. Like many other world commodities, sustaining cotton production while also adapting to changes in climate is expected to increase agricultural water demands. In response to the anticipated increased demand on natural water supplies, a major research objective is to develop crops that use less water or that use water more efficiently. In this study, our objective was to study the expression of genes in response to water deficit stress in cotton. Two gene expression profiling experiments were conducted to compare expression profiles between root and leaf tissue of non-irrigated (water stressed) and irrigated field-grown samples using a cultivar Siokra that is known to be a relatively drought-resistant line. First, a global expression analysis using cDNA-AFLP profiling was conducted to identify a global set of differentially expressed transcript derived fragments. Second, the aquaporin gene family of cotton, previously identified in our laboratory, was assayed using semi-quantitative RT-PCR to identify differentially expressed aquaporin genes. These expression data will identify genes that are transcriptionally up- or down-regulated in response to water deficit stress. These data will also aid efforts to understand the complex genetic signatures related to cotton water use both at global and specific gene family levels. The genes identified in this study will provide potential targets to manipulate the water use characteristics of cotton at the molecular level.

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An *Arabidopsis* Trichome-Specific Promoter Drives Reporter Gene Expression and Retains Its Specificity in *Brassica juncea*

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In several cases, tissue-specific expression of a transgene in plant species is desirable. Trichomes are specialized epidermal cells that produce secretions and that are thought to provide a first line of defence against pests and pathogens. Many trichome-secreted compounds are used commercially as flavourings, medicines, etc. The trichome-specific promoter was isolated from the upstream sequence of ethylene response factor gene (*At5g11190.1*). Hypocotyles of *Brassica juncea* were used to develop an in vitro culture system for plant regeneration and *Agrobacterium*-mediated transformation. Plants were regenerated from hypocotyle-derived callus precultured on Murashige and Skoog medium supplemented with 2 mg/L 2,4-dichlorophenoxyacetic acid. Transgenic plants were obtained by inoculation of precultured explant with a disarmed *Agrobacterium* strain (GV3101) containing the binary vector pBI101-TSP, which carried the genes encoding β-glucuronidase under a trichome-specific promoter. This promoter is shown to direct the specific expression of the reporter gene, ß-glucuronidase (GUS), in trichomes of in vitro regenerated *Brassica* plantlets. The expression of gus gene was predominantly more in trichomes of young leaves, petiole and stem. PCR and RT-PCR analysis revealed the presence and expression of gus gene in transgenic plants, respectively. This promoter may provide efficient bioengineering to enhance pest and pathogen resistance, and for molecular farming.

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Promoter Element of an ERF Gene of *Arabidopsis* Drives Trichome-Specific Expression and Retains Its Specificity in *Brassica juncea*

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In several cases, tissue-specific expression of a transgene in plant species is desirable. Trichomes are specialized epidermal cells that produce secretions and are thought to provide a first line of defence against pests and pathogens. Many trichome-secreted compounds are used commercially as flavourings, medicines, etc. The trichome -specific promoter was isolated from the upstream sequence of ethylene response factor gene (*At5g11190.1*). Hypocotyles of *Brassica juncea* were used to develop an in vitro culture system for plant regeneration and *Agrobacterium*-mediated transformation. Plants were regenerated from hypocotyle-derived callus precultured on Murashige and Skoog medium supplemented with 2 mg/L 2,4-dichlorophenoxyacetic acid. Transgenic plants were obtained by inoculation of precultured explant with a disarmed *Agrobacterium* strain (GV3101) containing the binary vector pBI101-TSP, which carried the genes encoding β-glucuronidase under trichome-specific promoter. This promoter is shown to direct the specific expression of the reporter gene, *ß-*

glucuronidase (GUS), in trichomes of in vitro-regenerated *Brassica* plantlets. The expression of gus gene was predominantly more in trichomes of young leaves, petioles and stems. PCR and RT-PCR analysis revealed the presence and expression of gus gene in transgenic plants, respectively. This promoter may provide efficient bioengineering to enhance pest and pathogen resistance, and for molecular farming.

P-142

Facilitating Biology Studies and Advancing Transgenic Technologies for Crop Plants at MU Plant Transformation Core Facility

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Since its establishment in 2000, the University of Missouri (MU) Plant Transformation Core Facility has been providing cutting-edge research support services for the genetic transformation of maize (*Zea mays*), soybean (*Glycine max*), as well as model plant species *Arabidopsis* and tobacco (*Nicotiana* ssp.). Located at the heart of the MU campus in the Sears Plant Growth Facility, the goals of the Plant Transformation Core Facility are to provide transformation services, improve transgenic technology, and offer transformation training opportunities for the public. The facility offers varied services, including standard as well as customized stable and transient transformations for maize or soybean, based on the user's request. The maize transformation utilizes *Agrobacterium*-mediated approaches, using immature zygotic embryos as the starting explants. For soybean experiments, we employ an *Agrobacterium*-mediated cot–node transformation system. This *Agrobacterium* approach has been the major method of transformation for the Plant Transformation Core Facility since 2003 and has yielded very desirable results. Current researches of the facility focus on developing high-throughput transformation systems, improving the quality of transgene integration as well as effective gene regulation through RNAi, and transposon tagging, geared towards meeting the needs of crop improvement and functional genomics. Our specific interest in soybean genetic engineering is to regulate several economically important genes conditioning soybean seed traits, abiotic stress tolerance, virus resistance, etc. Two of the facility's most recent transformation projects focus on improving switchgrass (*Panicum virgatum*) and sorghum (*Sorghum bicolor*) transformation to meet the need of biofuel crop engineering. As a result, the facility will soon utilize the established transformation systems to provide the transformation services for these two crops upon requests. Some of the facility's researches are conducted as collaborations with on- and off-campus researchers. More details of the facilities' activities will be presented at the conference.

P-143

In Vitro Regeneration of the Fibre-Rich Amazonian Species *Ananas erectifolius*

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The aim of the study was to develop a method for the in vitro propagation of *Ananas erectifolius*, a fibre-rich Amazonian species. In vitro cultures were established from axillary buds of field-grown plants cultured on medium without plant growth regulators (PGRs). Stumps were excised from in vitro plantlets and incubated under dark conditions on medium supplemented with different combinations of 1-naphthaleneacetic acid (NAA), kinetin and gibberellic acid (GA_3). The most efficient induction of etiolated shoots occurred on explants cultured in the presence of NAA at 10.74 mM (T1 medium) or NAA at 5.37 mM+GA_3 at 3 mM (T2 medium). Apical tips and nodal segments of the etiolated shoots were re-cultured under a 16-h photoperiod in medium without PGRs, and the effects of residual PGRs were evaluated by determining the numbers and lengths of plantlets that regenerated within 30 d. Residual PGRs exhibited no effect on the length of the regenerated plantlets but significantly affected the number of plantlets regenerated from nodal segments but not from apical tips. Nodal segments and apical tips derived from etiolated shoots produced, respectively, on T2 and T1 medium were most appropriate for plantlet regeneration. Nearly all (98%) regenerated plantlets formed roots when cultured in liquid medium without PGRs, and all plantlets survived acclimatization under greenhouse conditions. The stumps originating from etiolated shoots regenerated new etiolated shoots when re-cultured in the dark on medium without PGRs, thus providing a supply of new explants for plant regeneration.

P-144

Delivery of Multiple Proteins Using Multi-gene Expression Vehicles

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We have developed multi-gene expression vehicles (MGEVs) based on the modification of multi-domain proteinase inhibitors from *Nicotiana alata*. The *N. alata* proteinase inhibitors (NaPIs) are produced as precursor proteins which form a circular conformation via disulfide bonds between the N- and C-terminals. Proteolytic processing of the precursor protein occurs in a linker region between each domain resulting in the release of mature 6-kDa proteinase inhibitors, which have an inhibitory activity against chymotrypsin or trypsin. We have produced MGEVs, which are either circular or linear in conformation. Insertion of additional proteins such as plant defensins or different PIs, or the replacement of the native NaPI domains, has allowed us to produce precursors that contain from two to potentially eight individual proteins. We demonstrate the expression and processing of multiple proteins from single MGEV constructs using stable and transient gene expression systems and show that MGEVs are a promising strategy for protein stacking in transgenic plants.

P-145

Light-Colored, Low Acrylamide Potato Chips

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Potato tubers are stored at cold temperatures to prevent sprouting, minimize disease losses, and increase the marketing window. Cold storage also causes an accumulation of reducing sugars, a phenomenon referred to as cold-induced sweetening. Unacceptable, dark-colored chips and fries are formed during high-temperature frying when reducing sugars in cold-stored tubers react with amino acids in a non-enzymatic Maillard reaction. These fried products also accumulate acrylamide, a toxin and potential carcinogen. The mechanisms regulating sugar accumulation in the cold are, therefore, of commercial interest, but remain poorly understood. Vacuolar acid invertase converts sucrose into reducing sugars during cold storage. We hypothesized that by suppressing invertase gene expression, we could address simultaneously the cold-induced sweetening and acrylamide problems. An RNAi-based silencing approach was used to develop lines in which invertase gene expression was silenced either partially or completely. RNAi lines grown in the field or in greenhouses did not have obvious phenotypic abnormalities or negative effects associated with silencing of the invertase gene. Chipping experiments performed on RNAi lines stored for up to 6 mo at 4°C produced dramatically light-colored, industry-acceptable potato chips after deep-frying. Potato chips from some lines had 15–20-fold less acrylamide than potato chips processed from control tubers after 2 wk of cold storage.

P-146

Stimulation and Inhibition of Axillary Bud Outgrowth in *Alstroemeria* Cultured In Vitro: Evidence for a Role of Strigolactone

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In *Alstroemeria*, strong apical dominance causes a low propagation rate in vitro. Study of apical dominance mechanisms is very useful for improvement of micropropagation. Moreover, because *Alstroemeria* has a very different architecture compared with the model species used in research on apical dominance, this study will increase knowledge about apical dominance. We investigated the effect of 2,3,5-triiodobenzoic acid (TIBA), GR24 (an analog of the novel branching–inhibiting hormone strigolactone, which is carotenoid-derived), fluridone (a caroten-

oid biosynthesis inhibitor that supposedly inhibits strigolactone biosynthesis), and imazalil (IMA, an imidazole herbicide that interferes with cytochrome P450 enzymes). Medium containing 9 μM 6-benzylaminopurine was used as standard medium. Explants consisting of an intact rhizome with two consecutive aerial shoots were examined, and the outgrowth of axillary buds at the rhizome node was observed. TIBA and fluridone increased axillary bud outgrowth when they were added individually in the medium. However, when TIBA and fluridone were added together, TIBA decreased the promotion by fluridone. This corresponds with the proposed mode of action of strigolactone via reducing auxin transport (Bennett et al., Curr Biol 16:553–563, 2006). The strigolactone analog GR24 inhibited axillary bud outgrowth, but the inhibitive effect of GR24 was reduced in the presence of fluridone. IMA strongly promoted axillary bud outgrowth. Experiments on auxin transport are in progress.

P-147

Heat Increases Rhizome Growth and Propagation in *Alstroemeria* Cultured In Vitro

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This research was motivated by observations in the greenhouse that rhizome formation of greenhouse grown *Alstroemeria* is strongly enhanced after stress. In this study, heat stress is of particular interest. Rhizome explants were exposed to heat stress induced by two different techniques: hot water treatment in a water bath (HWT; explants were just submerged) and hot air treatment in an incubator (HAT). HWT between 36°C and 39°C for 1 or 2 h had positive effects on rhizome growth and multiplication; 38°C was the optimum resulting in 50–100% increase of growth (fresh-weight increase). For HAT, temperatures between 30°C and 40°C (1 h) did not influence the number of new rhizomes, but HAT at 35°C (optimum) resulted in the highest rhizome fresh weight. When explants were treated with hot water or hot air at 38°C for 1–3 h, both heat treatment methods increased rhizome growth and multiplication, but HWT gave somewhat better results. As the increase of growth was caused by stress, we were interested whether treatments that protect tissues from stress would reduce the positive effects. After a pre-treatment with moderate stress (hot water or hot air at 28°C for 1 h), the severe stresses resulted in the same enhancement. Furthermore, pre-treatment with stress-protectants (including putrescine and trehalose) did not interfere with the promotion by the severe stress. This study showed positive effects of heat stress, which may be used to develop new procedures for improvement of micropropagation of *Alstroemeria*.

P-148

In Vitro Conservation and Multiplication of *Stevia rebaudiana*—Bio-sweetener of the Future

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The plant cell and tissue culture has been successfully exploited for in vitro conservation of several important medicinal plants. *Stevia rebaudiana* (Bertoni) is a small herb of Asteraceae family and emerged has as bio-sweetener of the future. The leaves of this plant possess intense sweet taste, i.e. 200 to 300 times more than the sugar. The main constitutes are steviosides (St) and rebausioside A, which are non-caloric and heat stable at 200°C, and hence also used in bakery products. There is a high market demand for *Stevia* in India due to the increased incidence of diabetes and growing concern over the safety of chemical sweeteners. Since the plant is of commercial importance, many farmers are coming forward for commercial cultivation of this species. But, the major constraints being experienced by farmers are high cost of planting material and low seed set and poor germination. Thus, the in vitro production and multiplication of this species is of great importance. In the present study, nodal segments were collected from young branches of field grown plants, surface-sterilized and inoculated onto MS media with various concentrations of BAP. Direct shoot regeneration was observed within 1 wk of inoculation. Among all, high frequency of plant regeneration (83.4%) was obtained on MS with 3 mg/l BAP concentration. The number of shoots varied from six to eight per explants, and also, the length of the shoots is more at this concentration. Shoots were multiplied by sub-culturing

on lower concentration of BAP (1.0 mg/l BAP) for every week in first cycle and 3 wk after second cycle. Through this procedure, a total of 60–80 resulted from a single nodal segment. Regenerated shoots were transferred to half-strength MS medium supplemented with different concentrations of IAA and IBA. Among all, 5 mg/l IAA induced a high frequency of rooting (60.2%). The plantlets were transferred to portrays and later established in the field with 80% survival rate.

P-149

Cloning and Identification of Phosphate Starvation-Induced Genes in *Brassica napus*

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Phosphorus (P) is one of the essential elements for plant growth and development. It plays an important role in an array of processes, including energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen fixation. Although abundant P is present in many soils, very little of it is present in Pi forms that are available to plants. To investigate genes which are involved in response to Pi starvation in *Brassica napus*, a comprehensive survey of genes induced in Pi starvation was done by macroarray analysis. Among the 5,000 cDNA clones, 102 clones were identified to be up-regulated, while some genes were detected to be down-regulated by Pi starvation in *B. napus*. The functional classification of the genes indicated their involvement in stress responses, Ca^{2+} signaling, Pi transport, hormonal response, various metabolic pathways, and other processes related to growth and development of *B. napus*. Several candidate genes were selected for further functional analysis. Using transgenic *B. napus*, which overexpress the candidate genes under the control of CaMV 35S promoter, we found that Pi uptake of transgenic plants was increased and the downstream Pi response genes were up-regulated obviously during Pi starvation. The data presented in this study will facilitate the understanding of molecular mechanism of *B. napus* in response to Pi starvation.

P-150

Development of Hyperhydricity in *Arabidopsis thaliana* Seedlings and Measurement of the Water Content of the Apoplast

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In vitro-cultured plants may develop a physiological disorder referred to as hyperhydricity. Hyperhydric plants have curled, brittle leaves and a glassy appearance. The essential characteristic of hyperhydricity is a surplus of water, supposedly located in the apoplast (cell walls and intercellular spaces). We investigated the effect of the gelling agent (microagar, gelrite, or liquid medium) and 6-benzylaminopurine (BAP; 2.2 and 8.8 μM) on the development of hyperhydricity in *Arabidopsis thaliana* seedlings. As judged by the appearance of the seedlings, gelrite and liquid media, and the combinations with BAP resulted in hyperhydricity. We measured the water content of the leaves as an objective parameter of hyperhydricity. Seedlings grown in liquid medium or gelrite contained 3.3 or 1.9 times more apoplastic water than seedlings grown in agar, respectively. Seedlings grown at 8.8 μM BAP accumulated more water in the apoplast (1.9 times) than seedlings grown without BAP. Centrifugation for 18 min at 3,000×*g* has been used to study apoplastic water. Centrifugation though may cause disruption of cells; measurement of the EC of water collected by centrifugation did not indicate contamination by symplastic water below 5,000×*g*. Experiments with a biochemical marker for cytoplasmic contamination, malate dehydrogenase activity, are in progress. Levels of chlorophyll and anthocyanins were also determined.

P-152

RNAi-Mediated Viral Resistance in Transgenic Wheat

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Wheat streak mosaic virus (WSMV) and *Triticum mosaic virus* (TriMV) are two viruses of the wheat mosaic complex affecting wheat in the Great Plains of the USA. The current disease management strategy incorporates the deployment of resistant varieties, mite vector control, and various cultural practices; however, it is not fully effective. As an alternative strategy, we evaluated the use of interference RNA to generate resistance to these wheat viruses. RNAi expression vectors were independently created from the sequences of the coat proteins (CP) of both WSMV and TriMV. Immature embryos of the wheat cultivar 'Bobwhite' were independently co-transformed by biolistic particle delivery system with RNAi expression vectors and pAHC20, which contains the *bar* gene for glufosinate selection. After tissue culture, putative transformed plants were analyzed through PCR for the presence of the appropriate RNAi CP gene. Transgenic T_1 seeds were collected, and each line was tested for transgene expression via RT-PCR. To determine viral resistance, T_1 progeny was mechanically inoculated with the corresponding virus. Viral presence was established by ELISA. In the T_1 generation, resistance was seen in up to 60% of the plants evaluated for both CP constructs, although some events that showed a transgene presence did not exhibit resistant phenotype. Analyses of transgene presence and expression in T_2 generation evidenced events of transgene silencing and deletion. Regardless of these phenomena, consistent resistance response in two lines of WSMV CP construct and one TriMV CP transgenic line was found.

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Phylogenetic Analysis of Set Domain in *Solanum lycopersicum* Trithorax SLX1

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The SET (Su(var)3-9, Enhancer-of-zeste, trithorax) domain exists in major counteracting epigenetic controlling factors. The SET domain has methyltransferase activity dedicated for specific histone lysines on the histone tails. We have characterized and cloned all domains of SLX1, a trithorax-like protein from tomato flower buds. SLX1 SET domain and downstream post-SET cysteine-rich motif were analyzed. The 85 amino acid long SET-post-SET domain was probed blastp in the GenBank to retrieve homologs along the plant kingdom. The threshold limit was set to 50% identities. In addition, repeated sequences from Arabidopsis, grapes, and moss were removed. The resulting 14 sequences were subjected to ClustalX multiple alignment. The aligned sequences were analyzed using neighbor-joining method and bootstrapped 500 times. The generated phylogenetic tree revealed two major clades: the higher plants clade and the moss clade. Consequently, the first clade sharply separated monocots from dicots into two clusters. The monocot clade showed tighter grouping of maize with sorghum than with rice. On the other hand, the dicot clade was further subdivided into smaller subclades. The SET-post-SET domain of tomato SLX1 was loosely subclustered in one subclade with the grape ortholog. Our earlier phylogenetic data, using whole plant trithorax protein sequences, showed a clear interchangeable clustering between monocots and dicots. The current investigation shed a light on the distinctiveness evolution of the SET-post-SET domain among monocot and dicot plants. These attractive functional features would be valuable in future plant phylogeny.

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Molecular Breeding of a Tryptophan-Fortified Rice via Homologous Recombination-Mediated Gene Targeting Based on Protein Engineering

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Gene targeting (GT) is a clean transformation technology which could introduce precise modifications into the endogenous genomic sequences of interest via homologous recombination. Molecular-designed breeding by site-direct mutagenesis via GT is thought to be much more efficient than conventional breeding that depends on natural variation

or random mutagenesis. Here, we present a successful example of production of novel high-value-added rice by GT based on the information of protein engineering. Rice is a poor source of the essential amino acid tryptophan (Trp): Trp fortification in rice is thus important for human food and animal feed. The activity of anthranilate synthase (AS), a key enzyme of Trp biosynthesis, is controlled by feedback regulation by Trp. Protein engineering of OASA2—an α subunit of AS in rice—showed that the S126F/L530D and Y367A/L530D mutations conferred Trp insensitivity and enhanced catalytic activity. Moreover, transgenic rice calli overexpressing modified OASA2 accumulated higher levels of free Trp. Thus, we attempted to introduce S126F/L530D mutations into endogenous OASA2 by GT to produce Trp-fortified rice. In GT experiment, we succeeded in obtaining two candidate regenerated plants. In one line, true GT, in which wild-type OASA2 was modified as expected, had confirmed. In this plant homozygous for modified OASA2, free Trp accumulated to levels >100-fold higher than in wild type. Moreover, we succeeded in obtaining a GT plant with Y367A or Y367A/L530D mutations. Thus, we demonstrated the effectiveness of molecular-designed crop improvement by combining GT with protein engineering.

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Genetic Mechanism of Wood Production in Silverleaf Sunflower

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Silverleaf sunflower (*Helianthus argophyllus*) is an annual plant that produces woody stems within a single growing season (i.e., 6–8 mo). Silverleaf sunflower is an interesting bioenergy candidate because of the increased portability of woody biomass relative to forage and silage type cellulosic feedstocks. Our objective was to gain insight into structure and genetic regulation of xylem differentiation and lignocellulosic accumulation in silverleaf sunflower. Anatomical images of silverleaf sunflower stems indicate vascular cambium divisions as early as 4 wk after transplanting. Chemical spectra generated using a pyrolysis coupled mass spectrometer (MS) will provide both quantitative and qualitative cell wall chemistry information for stem samples of *Helianthus annuus* (non-woody stems) and *H. argophyllus* (wood forming) sunflower. Non-normalized stem cDNA libraries were constructed using mRNA isolated from *H. annuus* and *H. argophyllus* stems over multiple developmental stages (i.e. 4, 8, 12, and 14 wk after transplanting). High-throughput sequencing was carried out on a Roche Genome Sequencer 454-FLX Titanium platform. The sequences were processed through a bioinformatic pipeline and assembled using CAP3. A BLAST search of this data set with the existing sunflower database and other sequenced genomes including *Arabidopsis thaliana*, *Poplus trichocarpa*, *Vitis vinifera*, *Mimulus guttatus*, and *Oryza sativa* will provide insights into genes and genetic pathways related to biomass and wood formation in silverleaf sunflower. A second-generation microarray is being built with the updated transcript assembly including 134,552 *H. annuus* and 35,721 *H. argophyllus* ESTs, in addition to the newly developed 305,900 *H. annuus* and 597,654 *H. argophyllus* stem ESTs. Time course experiments have been designed to study development-specific differentially expressed genes in woody and non-woody ecotypes. Further insights into genomic regions harboring QTL for wood formation and other cellulosic biomass traits will be gained by QTL mapping in a backcross (BC1) population developed from a hybrid of *H. annuus* NMS377 (PI 560145)×*H. argophyllus* 1820 (PI 494580). This BC1 population was grown in Georgia, IA and Vancouver in Summer 2008. Agronomic and biomass traits were recorded on 600 BC1 plants. This population will be genotyped on an Illumina 384-SNP array using the BeadXpress reader. Polymorphic SNPs in *H. annuus* NMS377 and *H. argophyllus* 1820 will be identified from the parental screening panel consisting of 10,640 SNPs on an Infinium array.

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Agrobacterium rhizogenes and Salicylic Acid Trigger Defense Responses in *Hypericum perforatum* Shoots

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Secondary metabolism of *Hypericum perforatum* represents a barrier against *Agrobacterium* infection. Changes in the levels of phenolic compounds and hypericin have been related to the recalcitrance to plant transformation. In this study, we evaluated the response of *H. perforatum* plants to the infection with *Agrobacterium rhizogenes* (AR) and compared with the treatment with salicylic acid (AS; 1,000 μM). In vitro shoots of *H. perforatum* were infected with *A. rhizogenes* (strains K599 and R1000) and in the presence of acetosyringone. Shoots in the control treatment were inoculated with distilled water. The activities of phenylalanine ammonia lyase (PAL), polyphenol oxidases (PPO), and peroxidases (POX), and the levels of phenolic compounds, flavonoids and hypericin, were determined at 0, 6, 12, 24, 48, 72, and 120 h post infection (hpi). Shoots inoculated with both *A. rhizogenes* and SA showed a decrease in PAL activity at 6 hpi and a subsequent increase at 12 hpi. The levels of phenolic compounds followed the changes observed in PAL activity, increasing after 24 hpi. Similar response was observed for flavonoids, although a significant difference was observed at 6 and 48 hpi with both AR infection and SA. Alterations in the levels of phenolics were also related to changes in the PPO and POX activities. SA fastened the response through PPO activity (6 hpi), while infection with AR K599 caused a peak of activity at 72 hpi. POX activity also increased at 72 hpi. The highest levels of hypericin were obtained in shoots treated with AS (6 hpi) and in the AR-infected shoots (at 24 hpi). The changes in the secondary metabolism indicate a defense strategy of *H. perforatum* against *A. rhizogenes*, posing obstacles for plant transformation. SA seems to be involved in the defense mechanism.

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Rhizobacteria Autochthonous Affect Growth of *Araucaria angustifolia* (Coniferae) Seedlings

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Araucaria angustifolia (Bertol.) Kuntze, known as Brazilian pine, is an endangered species with great ecological and economical importance. Despite many efforts to establish large commercial areas with this species, its cultivation shows slow and heterogeneous development with high mortality. This study evaluated the effect of isolated PM1, PM4, and PM9 actinomycetes autochthonous in the early development and metabolism of *A. angustifolia* seedlings. The enzymatic activity of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POX), and the levels of phenolic compounds, flavonoids and chlorophyll, were determined in extracts of roots and leaves from 50-d-old seedlings. Assays were carried out at 1, 3, and 9 d after inoculation of roots with rhizobacteria. Seedlings were also evaluated for length and fresh weight of shoots and roots, volume, and density of roots at 100 d after root inoculation. All actinomycetes evaluated showed rhizospheric competence and capacity to produce indole-3-acetic acid. At the beginning of colonization (1 and 3 d), the activities of PPO and POX were reduced in roots; however, no changes in the levels of phenolics and flavonoids were observed. An increase in volume, density, and length of roots was observed in seedlings maintained in contact with rhizobacteria for 100 d. The isolate PM9 caused significant changes in plant metabolism, increasing the shoot growth. Shoots did not show any change in the chlorophylls. PAL activity was increased only 9 d after root inoculation with PM9. Isolate PM9 is a promising plant growth promoting rhizobacterium.

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Characterization of Nuclear/Nucleolar Localization of *Arabidopsis* Ribosomal Protein L23A

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Ribosomes are two-subunit macromolecular enzymatic complexes comprised of rRNAs and ribosomal proteins (r-proteins). They are responsible for protein synthesis in all organisms. Ribosomal subunits are assembled in the nucleolus, a sub-nuclear compartment. Ribosomal subunit assembly is dependent on efficient targeting of r-proteins into the nucleus/nucleolus. Failure can be lethal to the organism. Nuclear localization of a protein is mediated by one or more stretches of basic amino acids, the nuclear

localization signal (NLS). The NLS drives the nuclear localization of a protein by interacting with negatively charged cytosolic nuclear transport receptors like importins. The mechanism underlying the transport of r-proteins from nucleus into, and their retention in the, nucleolus is not well understood. We are investigating the nuclear/nucleolar localization of a large subunit r-protein L23a, one of the 81 r-proteins of *Arabidopsis*. It is encoded by a gene family of two members, L23aA and L23aB. Isoform L23aA is essential for plant development under normal conditions, whereas L23aB is not. Both isoforms have nine putative NLSs. Site-directed mutagenesis studies have shown that mutations of any one of the NLSs had no effect on nuclear/nucleolar localization of L23aA. In fact, simultaneous mutation of all nine NLSs had no effect on nuclear localization. However, nucleolar localization was completely disrupted. Combinatorial mutation studies have shown that only five (^{10}KKAD^{13}, ^{17}KALK^{20}, ^{86}KK^{87}, ^{121}KK^{122}, and ^{133}KK^{134}) of nine NLSs are required for nucleolar localization. In light of the above results, we are currently investigating (a) if nuclear localization of L23aA is mediated by the classic importin pathway and (b) the mechanism of nucleolar transport and retention of L23aA (transportation by protein–protein interaction and retention by rRNA binding).

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The Role of NIA1 and NIA2 in Nitric Oxide Production Associated with Survival Under Hypoxia

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Research in recent years has suggested that nitric oxide (NO) not only is a signalling molecule but also participates actively in plant metabolism. Its role during stress is quite noteworthy. NO plays an important role in plant growth and development and in survival under abiotic stress. Numerous enzymatic and non-enzymatic pathways involving NO production have been proposed. Plants growing under flooded conditions survive under very low supply of oxygen. Oxygen concentration in such condition is extremely low, at times very close to the cytochrome *c* oxidase saturation level, which is defined as hypoxia. The condition where there is even less or no oxygen supply is termed as anoxia. Plants growing under hypoxia and anoxia have been shown to make use of the NO turnover to maintain the electron flow. It makes use of nitrate, which is reduced to nitrite, and the latter is reduced to NO. NO is toxic to plants above certain levels and, hence, is immediately scavenged. Mitochondria are the major source of NO in plants. Apart from mitochondria, nitrate reductase is a potential source of nitric oxide in plants. NO production decreases considerably, but is not abolished when different complexes of the electron transport chain are inhibited. Studies on tobacco nitrate reductase mutants have shown that there is total loss of NO production when the complex III and the alternative oxidase are inhibited in the mutants. *Arabidopsis* has two nitrate reductase genes, *NIA1* and *NIA2*. We discuss here the growth of *Arabidopsis* plants with mutated *NIA1* and *NIA2* genes and evaluate the contribution of these two nitrate reductase genes to NO turnover leading to survival under hypoxia and anoxia.

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Biotechnological Investigation on *Nothapodytes nimmoniana*: An Endangered Camptothecin-Producing Medicinal Plant

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Nothapodytes nimmoniana is an endangered medicinal plant from Western Ghats of India producing camptothecin (CPT), the topoisomerase I inhibitor, and considered to be one of the most promising anticancer drug of the twenty-first century. Among different sources, the highest CPT content was reported from *N. nimmoniana*. However, due to lack of synthetic process, the global demand of CPT is being fulfilled by natural *Nothapodytes*. Extensive deforestation and lack of conservation have made this plant endangered. Plant biotechnology has been accepted as an attractive alternative to enhance/improve the secondary metabolites accumulation in plants. Present investigation was carried out to enhance CPT content using this technique. Callus was initiated from immature seeds aseptically transferred on 'Murashige and Skoog's' (MS) medium supplemented with various growth hormones in

different proportions. Three-week-old callus initiated in MS medium supplemented with picloram+thidiazuron+gibberellic acid (1:1:4) and 3% sucrose was used for analysis. High-performance thin-layer chromatography method has been used for the quantitation of CPT using chloroform–ethylacetate–methanol (4:5:0.5 *v*/*v*) as the mobile phase. CPT content of callus was compared with CPT contents from different parts of *Nothapodytes*. Methanolic extract of callus highest percentage of CPT (5.74% *w*/*v*) than methanolic extract of fruits (3.56% *w*/*w*) followed by leaves (1.56% *w*/*w*), stem (1.19% *w*/*w*), and root (1.11% *w*/*w*). Present investigation revealed that the CPT content is higher in methanolic extract of in vitro grown callus as compared with different parts of naturally grown plant.

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Regeneration of Multiple Shoots from Nodal Segments of *Ephedra gerardiana* Cultured In Vitro

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Ephedra gerardiana is considered as an important traditional medicinal plant for more than 5,000 yr and is popularly known as Soma in Indian ancient Ayurvedic literature and Ma Huang in Traditional Chinese Medicine. It is widely used in treatment of various ailments such as cold, fever, flu, asthma, and nasal congestion. It is also recognized as a weight-losing component and efficiency enhancement drug. Due to overexploitation of the plant from wild populations, it is now listed as an endangered species. In India, *E. gerardiana* grows at high altitude. Micropropagation technique can be an aid to conserve this important medicinal plant. The present report describes investigations onto micropropagation aspects of this plant. Nodal segment of *E. gerardiana* were cultured onto MS medium containing various plant growth regulators. Addition of any of auxin alone or in combination of cytokinin in the medium resulted into callusing from nodes. Lower concentrations (0.5 to 5 μM) of TDZ induced auxillary bud break from the nodes. However, higher concentration of TDZ (8 to 20 μM) exhibited callusing. Induction of multiple shoots occurred onto medium consisting of various concentrations of BAP and/or Kn. Maximum percentage of cultures showing multiple shoot buds was onto 8 μM BAP-supplemented medium, and onto this, the average number of shoot buds per explant was also highest. These multiple shoot buds were further subcultured and transferred onto rooting medium. Rhizogenesis was achieved onto a half strength medium supplemented with different concentrations of IBA. The regenerated plantlets are further multiplied before transplantation.

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Bioreactor Design for Plant Propagation: Enabling Tissue Culture Productivity Enhancements

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Plant tissue culture provides a means to proliferate plants without the requirement of starting with seeds, thus enabling the rapid propagation of superior plants such as sterile hybrids or disease resistant varieties. We are developing this technology to increase the productivity of cultivated plants, including fungal resistant *Theobroma cacao* via embryogenesis and seedless watermelon via organogenesis. This work established the feasibility of utilizing temporary immersion bioreactors (TIBs) for watermelon shoot proliferation while setting the stage for process scale-up in next-generation reactors. Watermelon cotyledon explants were cultured on MS media supplemented with benzylaminopurine and indoleacetic acid to induce shoot formation. The primary cotyledon explants developed much slower in TIBs as compared with solid media possibly due to the presence of a thick, waxy cuticle. In contrast, secondary shoots with less pronounced cuticles were successfully propagated in TIB culture. To enhance productivity, we explored the use of media additives to suppress tissue oxidation and increase the 'wettability' of the explants during liquid immersion. Consistent with our goal of implementing low-cost bioreactors, a next-generation temporary immersion bioreactor was designed and prototyped. The prototype consists of a disposable plastic bag suspended from a reusable headplate. Gravity, rather than gas pressure, is used to transfer the media between the reactor and reservoir vessels. The overall design from materials to operation is evaluated in terms of

scalability, reliability, and economic feasibility relative to typical rigid-vessel designs.

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Cell Wall Properties and Genetic Engineering of Cinnamoyl-CoA Reductase for Improvement of Saccharification Efficiency in Switchgrass (*Panicum virgatum*)

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The switchgrass cultivar Alamo has been selected as a biofuel grass for bioethanol production in BESC (the BioEnergy Science Center, US Department of Energy). The lignified cell walls, and crosslinks between lignin and other phenolic compounds with the cellulosic polysaccharides have been considered as major obstacles for cellulosic ethanol production from switchgrass feedstock. Plant cell-wall recalcitrance and saccharification efficiency were studied in the lowland switchgrass variety Alamo. A comprehensive study was undertaken of cell-wall structure, soluble and wall-bound phenolic content and composition, lignin content and composition, and lignin biosynthetic gene expression in developing stems of greenhouse-grown plants harvested at different developmental stages. The major biochemical parameters that may affect the cell wall recalcitrance properties were determined to include lignin content, monolignol unit composition, and wall-bound phenolic acid content and composition. The relationships of these biochemical parameters to saccharification efficiency were determined for field-grown material. The data indicate that the lignin and ester-linked *p*-hydroxybenzoic acid contents showed strong negative correlations with saccharification efficiency, but ester-linked ferulic acid positively correlated with saccharification efficiency. Genes encoding the monolignol biosynthetic enzyme cinnamoyl CoA reductases (CCR) was cloned from switchgrass and functionally analyzed. Stable transgenic plants harboring CCR-RNAi constructs were generated by agro-mediated transformation. Biochemical analysis of the T0 transgenic plants indicated that the total lignin content had been reduced by about 40~50%. The content of wall-bound phenolic acids was also reduced. The impacts of these biochemical changes on saccharification and ethanol production efficiency are under evaluation by BESC.

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Small-Grain Cereal Model Plant for Homologous Recombination Studies

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Genetic engineering of cereal crops relies on non-homologous end joining and random integration. Site-targeted integration though homologous recombination (HR) would be a preferred strategy. For developing a HR model cereal biological system, a disrupted luciferase reporter gene (lu-uc) was designed through introduction of a short herbicide resistance genetic construct in the luc gene and a gene portion repeat. The LUC activity emerging from a lu-uc transgenic event would be an indication of HR. Three transfection treatments and two controls were applied to triticale isolated microspore culture, namely, control (no DNA), lu-uc only, luc+Tat_2 carrier, lu-uc+Tat_2, lu-uc+RecA+Tat_2. Regenerated haploid/diploid plantlets were screened using herbicide glufosinate ammonium. Thirteen percent of the plants obtained from luc+Tat_2 treatment were PCR positive. Treatments lu-uc+Tat_2 and lu-uc+RecA+Tat_2 led to 20.4% and 6.25% PCR positive plants, respectively. Real-time PCR analyses of 20 T0s and T1s plants produced from Tat_2-mediated transfection estimated one to four lu-uc copies. Distinct luciferase expression was observed on the leaves of T0, T1, and T2 plants, supporting the occurrence of homologous recombination in this model system. This study also demonstrates the benefits of cell penetrating peptide (Tat_2) transfection in microspores to produce stable transformants from microspore.

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Analysis of Gus Gene Expression Driven by Truncated Sporamine Promoter, SD221, in Transgenic Sweet Potato

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Sweet potato is one of the ideal crops for producing biomass raw materials and valuable substances such as edible vaccines because it has storage roots, which can accumulate a certain amount of starch and protein, and it can be cultivated from monsoon to tropical area even on arable lands. We induced the embryogenic callus from the apices or small young leaves of sweet potato (*Ipomoea batatas* L. Lam. cv. Kokei No. 14) to introduce chimeric GUS (beta-glucronidase) gene with CaMV 35S promoter and truncated sporamine promoter, SD221 in it, through *Agrobacterium* infection. Transgenic sweet potato lines were obtained, and we analyzed the GUS gene expression quantitatively and histochemically. We could use kanamycin or hygromycin B to select transgenic lines, but hygromycin B was more effective to exclude escape lines. GUS activity was expressed in each organ: leaf, stem, root, and root tuber, in transgenic sweet potato with CaMV 35S promoter, especially highly expressed in stems and root tubers. Interestingly, GUS activity using SD221 truncated sporamine promoter indicated a high expression in root tuber, but it was expressed poorly in the other organs. Moreover, we also examined and found that the GUS expression was accelerated by dipping the leaf into sucrose solution from transgenic sweet potato lines with SD221 truncated sporamine promoter. But, there was no difference or decrease of GUS activity in transgenic lines with CaMV 35S promoter. These results indicate that truncated sporamine promoter, SD221, could be considered to be applicable to the inducible production of useful substances in transgenic sweet potato.

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Transformation System Improvements in Cotton

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Agrobacterium-mediated transformation system via embryogenesis usually requires a period of 12 mo or longer for production of transgenic cotton plants and in comparison with other crops is rather inefficient. A liquid-based culture system that drastically increases production efficiency was developed and successfully implemented for hypocotyl transformation. In a new protocol, the callus initiation stage was reduced from about 2 mo to a few weeks. For induction of embryogenesis, several culture steps were eliminated, and timeframe for this stage was cut in half. Selection of a distinct type of embryogenic callus (EC) and the regular sub-culturing at a low density on medium covered with a nylon mesh, as a tissue support, also sped up the process of converting embryogenic callus to embryos. Short cultivation of embryos on a medium with a high concentration of gelling agent resulted in faster maturation and germination of embryos. Thus, in transformation experiments with NPTII/GFP and NPTII/GUS constructs (kanamycin selection), the timeframe for converting embryogenic callus lines to plants could be reduced to 2 mo with conversion frequencies of 94–100%. Overall, with an optimized liquid transformation system, the time frame for plant production could be cut in half. Further improvements are connected with the development of *Agrobacterium*-mediated transformation system based on using EC as initial explants. It was found that EC could be transformed with *Agrobacterium tumefaciens* only if EC was desiccated during co-culture with the bacteria. Using GFP as reporter marker, it was shown that transgenic plants could be produced in about 3 mo after transformation.

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Monitoring DS Transposition in Soybean Genome

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The maize two-component transposon system Ac/Ds has been used in many plant species as a means to generate

insertional and activation tagged mutants. The long-term goal of this program is to develop a repository of transgenic soybean events harboring mapped Ds elements positioned approximately every cM, thereby creating a collection of Ds soybean events that will have utility for local mutagenesis. The usefulness of the system will be influenced by the ability of Ds to transpose when stacked with Ac. To investigate the transposition of Ds in the soybean genome, we selected a set of eight soybean events harboring gene or enhancer trap elements delineated by Ds termini. The selected events carry either one or two transgenic loci. To induce transposition, we stacked the respective Ds events with an Ac cassette under control of either the constitutive 35S CaMV promoter or the reported meiosis specific promoter, DMC1, from *Arabidopsis*. We have generated over 200 crosses with these two Ac cassettes. In addition, a third Ac cassette has been assembled that carries the soybean strictosidine synthase promoter, which has been demonstrated to be floral specific. The data gathered from this study allow us to test the influence of both level, and tissue specificity of Ac expression on transposition of Ds in the soybean genome.

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Identification and Transferability of Sugarcane Microsatellites to Other Cereal Genomes

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The genus *Saccharum* comprises six species which are potentially useful as a source of genetic variability that can be introgressed into the worldwide cultivated sugarcane (*Saccharum* spp., hybrids) for increasing sugar and sugarcane productivity. With the objective of identifying a large set of polymorphic microsatellite markers designated as Unigene-derived sugarcane microsatellite (UGSM) and sugarcane-enriched genomic microsatellite (SEGMS), 351 UGSM and 36 SEGMS were tested to find out informative SSRs marker for sugar and sugar-related traits and their cross transferability in related genera. One hundred and fifty-eight (40.83%) of the microsatellite markers (144 UGSM/14 SEGMS) were found to be highly robust and polymorphic with PIC values ranging from 11% to 98%. A total of 460 polymorphic DNA bands were identified, with their fragment size ranging from 40 to 1,338 bp. In this study, cross amplification was estimated among 19 accessions of six sugarcane cultivars, one inter-specific hybrids, five related species, four related genera, and three divergent genera by using 27 UGSM primers. Analysis of 388 alleles, amplified by 27 randomly selected UGSM primer pairs, indicated the high number of observed allele that ranged from 2 to 26, with an average of 14.37 alleles detected per locus. The level of polymorphism detected by 27 UGSM markers among sugarcane species, genera, and cultivars was 96.3%, while cross-transferability rate was 98.0% within the *Saccharum* complex and 88.27% to cereals. A wide range of genetic diversity 0.13–0.79 with an average of (0.45) was assayed with these markers. As these loci represent transcribed region and recorded a high level of cross transferability and reliable amplification across the species, genera, and cultivars, the utility of these markers for functional and genetic analysis of *Saccharum* complex was demonstrated. These informative markers in sugarcane can be useful for exploiting the genetic resources of this genus, for detecting allelic variants in loci associated with important agronomic traits, and for monitoring alleles that introgressed from distantly wild relatives to cultivated sugarcane. Further work is underway for validation of these polymorphic SSR markers in different mapping population for the construction of genetic map for sugar traits.

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Expression of Antioxidative Genes in *Hypericum perforatum* L. Subjected to Cold Stress

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It is well known that exposure of plants to temperature extremes is likely to induce stresses that can eventually lead to severe injuries not compatible with life. The aim of this study was to examine the responses of *Hypericum*

perforatum L. plants subjected to cold treatment. Oxidative stress, considered as a major temperature stress in plants, has been selected as an indirect marker for a plant to low temperature response. Two of the key enzymatic antioxidants, catalase and superoxide dismutase, were chosen to monitor the oxidative status of the cold treated plants. In this study, a gene in *H. perforatum* L., encoding for chloroplast Cu/Zn superoxide dismutase, has been identified and sequenced for the first time. Expression of the genes was determined at the transcript and final product levels. Although we found that both catalase (CAT) and superoxide dismutase (Cu/Zn-SOD) genes were induced during low temperature treatment, according to our results, the major contributor to the overall expression is stress from wounding rather than that from cold. The enzyme activity tests showed an increase in activity of CAT and Cu/Zn-SOD proteins throughout the whole 60-min cold exposure. Hydrogen peroxide content is correlated and discussed with the latter, providing an insight into relationship between the studied levels and their contribution to the plant stress-response.

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Genetically Modified Sugarcane in South Africa: Research Approach and Legislative Requirements

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South Africa is the world's eighth largest grower of biotech crops. Activities involving research and both trial and commercial release of GM crops in the country are regulated by the GMO Act (Act 15, 1997), which is administered by the Directorate Biosafety of the Department of Agriculture, Forestry and Fisheries. Although no GM sugarcane is grown commercially in South Africa, research has been undertaken by the South African Sugarcane Research Institute (SASRI) for the last 18 yr. Genetic engineering research in laboratories and containment glasshouses requires registration of the facilities with the Directorate. Once a transgenic line has undergone preliminary molecular screening, field trials can be conducted on receipt of a Trial Release (i.e. Intentional Introduction to the Environment) permit. Vegetative propagation for field trials is accomplished using either stem sections or in vitro multiplication. Activities such as regular field inspections, submission of annual reports, appropriate monitoring and record-keeping, and destruction of test material on termination of the trial are stipulated in this permit. Examples of field trials at SASRI include evaluation of herbicide tolerance, insect resistance and sucrose metabolism perturbations. Field-grown transgenic material is subjected to agronomic characterization and molecular analysis to determine stability and heritability of the inserted characteristic over successive growing seasons/harvest cycles. To release commercial GM sugarcane, a General Release permit would be required, but such applications are in abeyance while intellectual property and market-related issues are under scrutiny.

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Applications of In Vitro Technologies to Sugarcane Breeding in South Africa

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Sugarcane germplasm bred at the South African Sugarcane Research Institute (SASRI) constitutes 95% of cultivars grown commercially in sub-Saharan Africa. However, the time taken to release a new cultivar is approximately 11–16 yr. The major constraint on this process is the considerable length of time required to vegetatively propagate sufficient material of the selected progeny to allow thorough agronomic performance and pest and disease resistance. Although in vitro technologies are well established for sugarcane, their application to the SASRI sugarcane breeding programme is currently limited to micropropagation of a few pre-release cultivars in the final stages of the selection process, as well as in the elimination of viruses (sugarcane mosaic virus and sugarcane yellow leaf virus) from imported parental germplasm. Both these applications make use of apical meristem

culture technique. There are several other in vitro culture interventions that have the potential to reduce the time frame to release a new cultivar and decrease demands on resources such as land and labour. These include (a) germplasm conservation; (b) in vitro screening for resistance to diseases (e.g. smut—a fungal disease caused by *Ustilago scitaminea*) and insect pests (e.g. eldana—a lepidopteran stem borer, *Eldana saccharina*); (c) mutational breeding for the induction of herbicide (imazapur) tolerance; and (d) introduction of novel traits via genetic engineering. In the latter instance, proof of concept has been obtained for herbicide tolerance to glyphosate and glufosinate ammonium, although currently there is no commercial cultivation of genetically modified (GM) sugarcane in Africa. Other GM applications such as the production of alternative products and enzymatic perturbations to effect increased sucrose accumulation are being evaluated and will be discussed.

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Endoplasmic Reticulum-Related Stress Response in Plants

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The management of stress in plants is important for crop productivity. Abiotic stress responses protect plants from adverse environmental conditions, but the responses may delay growth and development. Through its protein quality control system, the endoplasmic reticulum (ER) in plants plays an important role in perceiving environmental stress and mounting responses to mitigate stress conditions. The major ER stress response system in plants is related to the unfolded protein response (UPR). In plants, there are two "arms" to the UPR pathway—one arm involving membrane-associated transcription factors (TFs) and another involving IRE1. We have identified in the first arm of the pathway two membrane-associated TFs, bZIP17 and bZIP28, each of which perceives different stresses and activates different sets of stress response genes. In general, bZIP17 responds to salt stress, and bZIP28 is activated by heat and by ER stress-inducing agents that interfere with protein folding. In response to these stresses, the TFs localized to the ER are proteolytically processed by Golgi residing proteases s1p and s2p, released from the membrane and relocate to the nucleus. In the nucleus, bZIP28 binds to ER stress response elements (ERSEs) in the promoters of UPR-upregulated genes. In doing so, bZIP28 interacts with a general TF, nuclear factor-Y (NF-Y), to form active transcriptional complexes. NF-Ys are a class of heterotrimeric TFs, and bZIP28 interacts with a specific member of that class in which one of the subunits (NF-YC) is upregulated by UPR while another subunit (NF-YB) relocates to the nucleus in response to stress. Thus, both the membrane-associated bZIP TFs and the general NF-Y TFs are stress regulated and assemble on ERSEs in the nucleus to upregulate stress response genes and collectively alleviate the response. Supported by NSF grant IBN0420015 to SH.

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Sustainable and Optimized Production of Triptolide by *Tripterygium wilfordii* Cell Suspension Culture in Bioreactor

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In order to find an alternative method to traditional extraction of drugs from plants, we investigated in plant cell culture how to optimize the production of the desired compounds in pilot scale. Cell suspension cultures of *Tripterygium wilfordii* have been shown to accumulate triptolide, a diterpenic triepoxide found almost exclusively in the roots of the intact plants. *T. wilfordii* cells showed a very weak production of triptolide during the growth, and using growth hormones during subcultures strongly inhibited triptolide biosynthesis. By designing several experiments and surface response methodology, we could elaborate an optimized elicitation cocktail composed of known elicitors and terpene biosynthesis precursors. Other optimized culture parameters were aeration and medium composition. Secondary metabolite yields in cultured cells of *T. wilfordii* were significantly increased after a period of hormonal deprivation followed by a combined bioconversion and elicitation step: Triptolide concentration reached 65 mg/L in flasks, in stirred and wave bioreactors. Triptolide was mainly accumulated in the extracellular medium, allowing an easy and rapid recovery of the compound in comparison with traditional root extractions.

The results of the present study indicate that plant cell culture could be a valuable and potential source of pharmacologically active compound.

P-174

Hairy Roots Production of Kacip Fatimah (Labisia pumila) with Green Fluorescent Protein (GFP) Gene

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Kacip Fatimah (*Labisia pumila*) is a medicinal herb found mainly throughout South East Asia. *Agrobacterium rhizogenes*-mediated transformation combined with a visual selection for green fluorescent protein (GFP) has been applied effectively in Kacip Fatimah (*L. pumila*) transformation. Kacip Fatimah in vitro shoots were inoculated with LBA 9402 and ARquaI strains, all bearing *gfp* gene pGEM. Ubi-*sgfp*S65T. The results indicate that transformed adventitious roots can be visually selected solely based on GFP fluorescence with a very high accuracy. The method requires no selection agents like antibiotics or herbicides and enables a reduction labour and time necessary for tissue culture. Moreover, individual transformants can be easily excised from the host tissue and cultured separately. All Kacip Fatimah plantlets of produced transformed adventitious roots, and the frequency of shoots producing GFP expressing adventitious roots varied from 25% to 65%. The highest transformation rate was found for using ARquaI strain. The results encourage that visual selection of transformed, fluorescing adventitious roots can be highly effective and applied routinely for the production of Kacip Fatimah transgenic plants and hairy roots.

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The Molecular Basis of Adventitious Root Formation in Eucalyptus Stem Cuttings

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One of the obstacles of massive propagation of selected woody plants clones is the lack of rooting capability, or adventitious root (AR) formation, in stem cuttings as the plant ages. *Eucalyptus grandis* was selected as a model system to study the molecular basis of AR formation. This is a widespread species with a significant economical value, whose genome is currently being sequenced. Gene expression was compared between Juvenile or *easy-to-root* stem-cuttings taken from below node 5, and Mature or *difficult-to-root* stem-cuttings are the ones obtained above node 15 of *E. grandis* mother plants. We started by profiling expression of more than 20,000 genes using microarray chip technology, in the lower section (2 cm) of Juvenile and Mature stem cuttings before AR induction. Out of the genes printed on the chip, 0.28% and 0.32% of the genes, belonging to different functional groups, were up-regulated in Juvenile and Mature stem cuttings, respectively. Hormonal response, transport, cell wall organization and cytoskeleton were the main biological processes up-regulated in Juvenile cuttings, while transcription, lipid, flavonoid and sugar metabolism were up-regulated in Mature cuttings. Several genes identified are now being characterized, using qRT-PCR assays, during the different phases of the AR formation. In addition, their functional relevance to AR formation is studied in *Arabidopsis thaliana* and tobacco model plants. A possible model integrating our data with known signaling pathways leading to AR is discussed.

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In Vitro Propagation and Genetic Transformation of *Jatropha curcas*

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Jatropha curcas is an excellent biofuel crop because it is a drought-resistant non-food crop that could be grown on marginal land. This study aimed to develop an efficient protocol for direct and indirect in vitro regeneration of *J. curcas* to enable rapid cloning of elite germplasm and genetic transformation to impart resistance to cold stress. Callus was initiated from different explants on MS medium containing BAP (0–3 mgl^{-1}) and IBA (0–2 mgl^{-1}). Shoots regenerated from the leaf callus when transferred to MS medium supplemented with different combinations and concentrations of BAP, NAA, IBA, and GA_3. Axillary bud multiplication could be achieved on MS medium supplemented with 0.3–0.6 mgl^{-1} TDZ. Shoot elongation required 0.2–0.5 mgl^{-1} GA_3 in the medium. In vitro rooting was achieved on 1/2 strength MS medium supplemented with 0.1–0.5 mgl^{-1} IBA alone or in combination with NAA. Ex vitro rooting on vermiculite was less efficient compared with in vitro rooting. Transformation of leaf discs and leaf calli with GUS marker gene using *Agrobacterium* and biolistic methods are being carried out.

P-177

Direct Embryogenesis from Immature Leaf Explants Accelerates Genetic Transformation of a Commercially Important Sugarcane Cultivar

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Transgenic sugarcane plants with improved agronomic and value added traits have been reported in the past. Future developments are expected to lead to commercial release of transgenic sugarcane and may include its development into a biofactory for high value products. Our strategy to improve the genetic engineering protocol for a commercially important sugarcane cultivar (CP 88-1762) is aiming at reducing the time in tissue culture by eliminating a callus phase. This may positively affect the transformation efficiency and the performance of the transgenic plants. A factorial comparison of alternative auxin sources (1-naphthalenacetic acid; 2,4-dichlorophenoxyacetic acid, 4-amino-3,5,6-trichlorophyridine-2-carboxylic acid, 4-chlorophenoxy acetic acid) and cytokinin concentrations (6-benzylaminopurine at 0.4 or 4.0 μM) during culture initiation from immature leaf roll transverse sections, allowed the identification of a superior culture medium for induction of direct embryogenesis. Biolistic transformation of leaf-roll-disk explants of the commercially important cultivar CP-88-1762 was carried out 5–10 d following culture initiation with two alternative antibiotic resistance genes supporting lethal (nptII) or non-lethal (aadA) selection schemes with geneticin or streptomycin, respectively. The optimization of pre-culture time before bombardment, particle size, acceleration pressure, subculture scheme, and selection protocol supported a drastic reduction of the tissue culture period compared with a protocol that involves gene transfer to callus. Transgenic plants were confirmed by PCR, ELISA, and Southern blot analysis. We will provide details on the optimized protocol that supports producing stably transformed sugarcane plants from cultivar CP 88-1762 in soil within less than 3 mo.

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Expression of Specific siRNAs and Plum Pox Virus Resistance Stability in Plum

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Plum pox virus (PPV) is a serious viral disease of Prunus fruit crops, including peaches, plums, nectarines, cherries and apricots. The disease was first found in Europe and has spread across many countries in the world. The disease causes major economic losses to fruit industries. Few natural resistant sources to PPV have been found, which may be used to develop PPV-resistant varieties. Biotechnology via genetic engineering of fruit trees is a potential approach to develop and control the viral disease. We

introduced DNA constructs containing PPV HC-Pro sequence in an intron-spliced hairpin design into European plum via genetic transformation. The transgenic plum plants were inoculated with PPV virus using a chip bud grafting method. The plants were analyzed for PPV resistance via real-time polymerase chain reaction assay. Transgenic plants showed strong resistance to the viral infection. The plants were subjected to cycles of dormant/growth periods in contained greenhouse conditions. Plants were analyzed for PPV resistance after each cold treatment. After three cycles of cold treatment, many transgenic plants still exhibited high levels of resistance to PPV while all the control plants were PPV positive. Presence of siRNA molecules corresponding to the HC-Pro transgene were detected in transgenic lines that were highly resistant to PPV. The study shows that induction of RNA silencing via expression of intron-spliced hairpin PPV transgene is an effective approach for high level and stable resistance to plum pox virus in plum.

P-179

A Binary Vector for Sweet Potato Intragenesis

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As a strategy to reduce regulatory burden while increasing consumer confidence in genetically engineered crops, we developed a new binary vector that will allow the modification of sweet potato by transferring only the crop's own genome sequences. To that end, we used publicly available sweet potato sequences to assemble T-DNA borderlike sequences, modified loxP sites, and a multiple cloning site. A binary vector was thus constructed, using as a selectable marker the nptII gene flanked by the modified loxP sites and the cre gene, guided by the heat-inducible promoter hsp40. The intragenic vector was named pCIP97. To test the functionality of the modified loxP sites, we amplified the entire nptII-cre-loxP region using flanking primers and found two bands, corresponding to the expected sizes of the fragments with cre-loxP excision and without excision. The functionality of the T-DNA border-like sequences is being assayed by cloning the gfp gene into pCIP97 and agroinfiltrating into *Nicotiana bentamiana*. GFP expression, if observed, will demonstrate the transference of the gene to the plant. Stable expression in sweet potato of the gfp gene is being tested, and sequencing will be done to determine the percentage of events with non-sweet potato sequences due to transference of DNA regions beyond the borders.

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Agrobacterium-Mediated Transformation of *Impatiens walleriana* Ornamental with a Tomato Spotted Wilt Virus Resistance Gene

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The efficient genetic transformation using *Agrobacterium tumefaciens* strain was established for *Impatiens walleriana* plants. Axillary buds isolated from in vitro grown shoots were co-cultivated with *A. tumefaciens* C581C1 carrying pac1 gene. The transformation efficiency was 1.48%. The transgenic nature of regenerated shoots was confirmed by PCR and RT-PCR analyses. Comparison of phonotypical traits (shoot length, number of nodes per plant, number of leaves, leaf length, and number of axillary buds) of *I. walleriana* confirmed the lack of significant differences between transformed and control plants. The establishment of an efficient transformation method may facilitate the improvement of ornamental plant in terms of the virus resistance.

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Genetic Transformation of *Centaurium erythraea* with AtCKX1 and AtCKX2 Genes Using *Agrobacterium tumefaciens*

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Cytokinins are plant hormones that regulate cell division and development. The degradation metabolism of cytokinins is an important process that controls the levels of cytokinin active forms and their distribution in plant tissues. Cytokinin oxidase/dehydrogenase (CKX) is an enzyme involved in cytokinins catabolism and widely distributed in plants. Two genes encoding CKX from *Arabidopsis thaliana* (AtCKX1 and AtCKX2) were utilized for transformation of *Centaurium erythraea* using *Agrobacterium tumefaciens* as transformation vector. Presence of AtCKX1 and AtCKX2 genes was shown by PCR amplification in transgenic clones. The expression of these two genes was analyzed by RT-PCR. Regeneration and increment of fresh weight of transgenic AtCKX1 and AtCKX2 *C. erythraea* shoots was investigated. The chlorophyll content of leaves in transgenic plants was also analyzed with respect to non-transformed plants. This is the first report on *Agrobacterium*-mediated transformation of *C. erythraea* with AtCKX1 and AtCKX2 genes. This study represents an efficient protocol for producing transgenic *C. erythraea* plants and opens up new opportunities for the use of these plants as a model system for further investigations about developmental and physiological processes that are under cytokinin control.

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Zinc Finger Nuclease-Mediated Gene Targeting in Plant Cells

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Zinc finger nucleases (ZFNs) are a new type of artificial restriction enzymes that are custom designed to recognize and cleave specific DNA sequences, producing double-strand breaks (DSBs) in living cells. DSBs can be repaired by homologous recombination or nonhomologous end-joining, which can lead to gene replacement, site-specific mutagenesis, and targeted DNA integration at the repair site. The deployment of ZFN technology for gene-targeting experiments in plant cells is hindered by the lack of versatile tools for the assembly, validation, and expression of novel ZFNs. To overcome these technological barriers, we have recently designed a set of constructs and procedures for the cloning, biochemical analysis, and in planta analysis of newly designed ZFNs. Cloning begins with de novo assembly of the DNA-binding regions of new ZFNs from overlapping oligonucleotides containing modified helices responsible for DNA-triplet recognition, and fusion of the DNA-binding domain with a *Fok*I endonuclease domain in a dedicated plant expression cassette. Following the transfer of fully assembled ZFNs into *Escherichia coli* expression vectors, proteins can be easily expressed, purified on nickel columns, and used for in vitro digestion analysis of palindromic target sequences. We have also developed a collection of three in planta activity assays suitable for validating the nucleic activity of ZFNs in plant cells. The assays are based on the reconstruction of a mutated *uidA* gene, its transient or stable delivery into plant cells, and monitoring for GUS activity in target tissues. We have further demonstrated the use of our tools for the analysis of various ZFNs and the recovery of mutated *Arabidopsis* seedlings.

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In Vitro Shoot Proliferation in Genus Endemic and Endangered Ornamental Plant *Neotchihatchewia isatidea* (BOISS.) Rauschert Using Different Organs

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Neotchihatchewia isatidea (Boiss.) Rauschert (=Syn: *Tchihatchewia isatidea* Boiss.) is a genus of Brassicaceae family and also an endemic and endangered genus in Turkey. The genus has interesting leaves forms and attractive flower colours with a nice smell and is in danger of extinction in the future. Irregular collection of *N. isatidea* plants from their habitat hampers existing of the genus. It needs exploitation for commercial propagation. The goal of this research was to obtain high-frequency shoot regeneration in the plant and to compare shoot multiplication capacity of different organs on the basal medium supplemented with different growth regulators. Hypocotyl, cotyledonary, and leaf explants were cultured on Murashige and Skoog (MS) medium supplemented with combinations of N^6-benzylamino-purine and indole-3-acetic acid IAA, kinetin, and thidiazuron. The best shoot multiplication capacity was obtained from hypocotyl explants on MS medium containing 1 mg/L BA and 0.50 mg/L IAA. Moreover, it was determined that hypocotyl explants had a regeneration capacity better than other explant types.

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Future Developments for Non-destructive 3D Plant and Root Imaging

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High-throughput screening and high-throughput phenotyping have become key technologies for research in and development of active ingredients for pharmacology, new plant protection compounds, and breeding for new traits in agricultural products. These technologies are fundamentally important for many fields of applied and basic research, enabling the examination and understanding of different plant gene functions and the overall effects of chemicals on various organisms. Most of these screening methods are measuring visible parameters of the plants such as color, shape, size, area, architecture, growth rate, performance, or movement. Therefore, digital imaging of plants has become a very important tool in plant research since modern image processing software algorithms are much better and more reproducible in quantifying these visual parameters than the human eye. Moreover, the spectrum of modern CCD cameras can be extended to lower or higher wavelengths far beyond the visual range of the human eye such as near infrared for measuring the water distribution and dynamics in plants during drought stress experiments. However, all these reflective measurements are just able to target the visible part of the plant, the shoot, while the root remains to be hidden in the soil or substrate. The goal of this joint study is to explore whether nuclear magnetic resonance imaging or (sub) terahertz imaging (THz) might be used for obtaining non-invasive and valuable information about plant roots in soil or substrate.

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Genetic and Epigenetic Changes Induced by Tissue Culture in Rye and Rice

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In vitro-regenerated plants of rye (R_S) and regenerated (R_O) and transgenic (T_O) plants of rice were studied to verify if genetic and/or epigenetic changes were promoted by the in vitro conditions and the transformation protocol. Inter-simple sequence repeat (ISSR) fingerprinting on *Hpa*II/*Msp*I-digested and uncut DNA were generated to assess the possible changes. DNA digested with the methylation-sensitive isoschizomers could reveal epigenetic modifications, while the modification of the ISSR patterns obtained with the undigested DNA indicates genetic changes. The frequency of genetic changes was not very high. In rye, 9% and 5% approx. of the studied markers were variable depending on the cultivar studied. These values were even lower in rice, with a frequency of 1% approx. The changes in methylation were more frequent, but again smaller in rice than in rye (rye, 25–28%; rice, 13% R_O–16% T_O). The frequency of plants with at least one variation was remarkable. In the case of rye, 73% and 30% of the R_S plants from the studied cultivars showed at least one genetic change, and 50% and 73% carried at least one methylation change. In rice, 16% and 56% of the R_O plants showed

genetic or epigenetic modifications, respectively; moreover, 57% and 45% of the T_O plants were variable. In rye, some plants presented no variation while others accumulated several modifications. In both species, we detected the appearance of hypervariable bands, the same marker change, genetically and/or epigenetically, in plants that were regenerated from different cell lines (rye) and/or the T_O plants (rice). We compared the obtained results in order to know if there was a relationship between the genetic and the epigenetic changes observed. Some of the variable amplicons in each species have been sequenced.

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High-Frequency Plant Regeneration via Direct and Indirect Somatic Embryogenesis and Cardiotonic Glycoside Production in *Digitalis ferruginea*

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An efficient protocol for the direct and indirect somatic embryo induction and plant regeneration system was developed using hypocotyl segments of *Digitalis ferruginea* (foxglove) belonging to the family Scrophulariaceae. The genus *Digitalis* are biennial or perennial herbs and contain important cardioactive compounds (glycosides) that are used to treat heart problems. Direct induction of somatic embryos was achieved on hypocotyl explants cultured on Murashige and Skoog (MS) medium supplemented with 0.05 mg/l NAA+2 mg/l BAP, 0.25 mg/l NAA+0.5 mg/l TDZ, 0.1 mg/l IAA+2 mg/l TDZ, 1/2 MS+0.1 mg/l zeatin, and 3% *w/v* sucrose and 0.8% agar. After 6 wk of culture, 30.6±0.29 somatic embryos per explant were induced, the highest response to somatic embryo induction achieved. For indirect somatic embryogenesis, callus was obtained from hypocotyl explants when cultured on MS medium supplemented with 0.1 mg/l NAA+2 mg/l BAP, 0.25 mg/l NAA+1 mg/l TDZ, 0.5 mg/l NAA+0.5 mg/l TDZ, 0.5 mg/l NAA+2 mg/l TDZ, and 0.25 mg/l IAA+1 mg/l TDZ. Five hundred milligrams of 30-d-old embryogenic callus was used for somatic embryo induction through suspension culture. A high frequency of embryogenic callus induction was observed on MS medium supplemented with 0.1 mg/l NAA and 2 mg/l BAP. After 6 wk of incubation, somatic embryos developed at the bottom of the suspension culture. MS medium containing a combination of 1 mg/l 2,4-D and 0.5 mg/l BAP was found to be the most effective for somatic embryo induction. Different samples were used for the determination of cardiotonic glycoside, viz. lanatoside C and digoxin content. For the lanatoside C, range was from 8.0 to 131.5 mg/kg DW; and for the digoxin, range was from 2.3 to 29.3 mg/kg DW, respectively. The somatic embryos obtained through both direct and indirect somatic embryogenesis were regenerated into plants on MS medium containing 2 mg/l BAP or 2 mg/l TDZ, achieving an average of 76.5% plant regeneration. The regenerated plantlets had a 100% survival rate in the greenhouse conditions.

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Highly Efficient In Vitro Multiplication of Staple Food Crops of the Pacific Region for Commercial Cultivation

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In vitro techniques are being increasingly applied to supplement conventional methods of vegetative propagation. The benefits include mass multiplication, production of disease free stock and stress-tolerant variants, and long-term storage of viable germplasm. In vitro techniques not only help in successful multiplication of the elite plantlets for raising food crops for sustenance and export but also provide a means for the conservation of germplasm in an inexpensive way. Banana, taro, and sweet potato are the most important staple food crops in the Pacific Region for local consumption as well as for export. These crops contribute significantly to the socio-economics and provide livelihood to almost all island people, and thus are crucial for ensuring nutritional and economic security. Staple food crops are placed on high agricultural priority, but limitations in availability of disease-free and elite seedlings are a major bottleneck in commercial production. Therefore, a study was undertaken to develop efficient in vitro protocols for 52 varieties of banana, taro, and sweet potato for mass multiplication. A series of experiments were performed to establish aseptic cultures and to develop efficient and reproducible in vitro multiplication protocols by manipu-

lation of various plant growth regulators, media composition, and culture conditions. Multiplication rate, shoot length, percentage of rooting, number of roots per shoot, and root length were recorded every 4 wk, and each experiment was replicated three times. The plantlets were produced at mass scale, acclimatized in the greenhouse, and then distributed and established in the field. This paper is based on a research work done on in vitro multiplication and utilization of multiplied plantlets for commercial cultivation.

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Callus Induction and In Vitro Propagation of Red Sanders (*Pterocarpus santalinus* L.) from Mature Nodal Explants

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A protocol was developed for callus induction and in vitro propagation of *Pterocarpus santalinus*, an endangered timber-yielding and medicinally important tree. The nodal segments from a 10-yr-old tree were used to study the in vitro response on MS medium with 15 g/l of sucrose. Among the various hormonal combinations used, the nutrient medium with 2 mg/l 2, 4-D and 1 mg/l KN was found to be more suitable for callus formation from the explants. The nodal segments responded very slowly in the nutrient medium, and white to pale green-colored callus was formed after 6 wk of culture. In vitro propagation was achieved through induction of organogenesis in callus tissue derived from nodal explants. Two to three shoots per culture were differentiated on an average when the nodular callus was sub-cultured on MS medium supplemented with 3 mg/l BAP.

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The Effect of Different Propagation Methods on Antioxidant Enzyme Activities of Lingonberry (*Vaccinium vitis-idaea*) Plants

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The activities of catalase and the enzymes of the ascorbate glutathione cycle, ascorbate peroxidase (APX), and glutathione reductase (GR) and dehydroascorbate reductase (DHAR) were monitored in the leaves of lingonberry cultivars: Regal, Splendor, and Erntedank. Each cultivar was propagated by three different methods: by conventional softwood cuttings, by in vitro shoot proliferation from nodal explants, and by shoot regeneration from excised leaves of micropropagated shoots. Significant differences were observed among the cultivars across all propagation methods. APX and GR activities were highest in Regal followed by Erntedank and Splendor, whereas catalase activity was highest in Erntedank. DHAR activity was the highest in Regal. Plants obtained from softwood cutting showed the lowest activity of all these enzymes, and those obtained from leaf cultures showed almost twice higher enzyme activity in all the cultivars except Erntedank, which was unaffected by propagation method for catalase and APX activity. Nodal cultures of all the cultivars showed significantly higher antioxidant activity of all the studied antioxidant enzymes except DHAR. Possible correlations between the growth and morphological characteristics of the studied cultivars and their antioxidant properties are discussed.

P-190

Comparison of Methods of Obtaining Carrot Doubled Haploids

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Carrot market is dominated by hybrid varieties. Traditional methods of breeding these varieties take about from 6 to 8 yr. It is assumed that application of anther cultures of isolated microspores shortens step of parental component homozygotization by about 1 yr. The experiments presented here deal with androgenesis induction, regeneration, and adaptation of carrot plantlets that resulted. The effect of

chosen factors on the time course of these processes was compared with the employment of the two methods of obtaining androgenetic plants. Through electrophoretic analysis of two isoenzymatic systems PGI (EC 5.3.1.9) and ATT (EC 2.6.1.1), homozygotisity of these plants was examined. Because of methodological differences between anther culture and isolated microspores technics, it was not possible to certainly state which of the two methods was more effective. The significant effect of considered factors on the process of androgenesis in anther cultures as well as in isolated microspores cultures was proved.

P-191

Soybean Transformation through Dicamba Selection

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A new selection system using dicamba as a selective agent has been developed for use in *Agrobacterium*-mediated transformation of soybean. Dicamba is a synthetic auxin herbicide widely used to control broadleaf weeds. Soybean plants are extremely sensitive to dicamba, resulting in characteristic auxin herbicide injuries. Dicamba monooxygenase (DMO), which was isolated from *Stenotrophomonas maltophilia* (previously *Pseudomonas maltophilia*), deactivates dicamba to 3,6-dichlorosalicylic acid, and soybean plants that transformed with the DMO gene were shown to withstand field use rates of dicamba. In the dicamba selection system, embryo axes isolated from imbibed soybean mature seeds were used as explants. After being infected by *Agrobacterium* harboring a binary vector containing the DMO gene, the explants were selected on dicamba-containing medium for direct shoot regeneration. Dicamba-resistant shoots were rooted on medium containing the same level of dicamba, and then grown in greenhouse to maturity. Plants containing the DMO gene and the gene transmission to the progeny were confirmed by molecular analysis. The results indicate that the DMO gene allows the use of dicamba as a selectable marker during transformation and production of dicamba-tolerant soybean plants that have high agronomic value.

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Phosphatidic Acid Interacts with WEREWOLF MYB in Regulating Root Epidermis Cell Fates in *Arabidopsis*

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WEREWOLF (WER), an R2R3-type MYB transcription factor, is regarded as a master regulator of *Arabidopsis* root-hair pattern formation. We show here that phosphatidic acid (PA) binds to WER, and the PA binding motif is essential to WER function. The PA-interacting region is defined to the end of the R2 subdomain of WER, and mutations of the residues Lys51 or Arg52, 58, and 60 lead to the loss of the WER binding to PA. In the introduction of the non-PA-binding, mutated WER into wer, the WER-knockout plants fails to complement wer in restoring the normal hair pattern formation. The non-PA-binding WER was also inactive in influencing the expression of GLABRA2, CAPRICE, and phospholipase Dζ1, which are involved in root-hair pattern formation. WER is detected in the cytoplasmic and nuclear fractions, but the mutated WER is present only in the cytoplasm. These results suggest that the PA–WER interaction is required for the intracellular translocation of WER into the nucleus and for the function of WER in regulating *Arabidopsis* root cell fate.

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Molecular and Cytological Characterization of Introgression Lines with Yellow Seed Derived from Somatic Hybrids between *Brassica napus* and *Sinapis alba*

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Polymerase chain reaction and genomic in situ hybridization techniques were applied to demonstrate the presence of *Sinapis alba* genomic introgressions in somatic hybrid progenies between *Brassica napus* and *S. alba*. Using minisatellite core sequence 33.6 as primer, an *S. alba*-specific band was amplified in progeny lines with yellow seed character. However, no hybridization signals were found when the genomic DNA of *S. alba* was used as a probe. In addition, according to the genes related to flavonoid biosynthesis of model plant *Arabidopsis thaliana*, degenerate primers were applied for the detection of genes, leading to the yellow seed phenotype, indicating a specific band of *S. alba* in the yellow seed lines. This study demonstrates that the new yellow seed germplasm derived from the backcrossed and self-crossed progenies of *B. napus–S. alba* hybrids are stable, and homozygous introgression lines with yellow seed color are different from existing yellow seed materials.

P-194

Assessment of Conditions Affecting *Agrobacterium*-Mediated Transformation of Soybean

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Transformation efficiency for soybean [*Glycine max* (L.) Merr.] using the dip-wounding infection method of the *Agrobacterium*-mediated cotyledonary node protocol was optimised by an orthogonal array. The soybean genotype "Bert" and the standard binary vector pZY102 carrying GUS as a reporter gene and the *bar* gene as a selectable marker were used. The transient transformation efficiency was determined by the GUS expression observed per explant after co-culture. The orthogonal array was designed, with three factors which included 4°C pre-treatment of explants, *Agrobacterium* reactivation by co-cultivation with acetosyringone, and the length of exposure to *Agrobacterium* inoculum. *Agrobacterium* reactivation was confirmed to be the most important factor, followed by the 4°C pre-treatment and the length of exposure to inoculum. When explants were held at 4°C pre-treatment for 1, 4, or 8 d before infection, it was observed those 4 d resulted in 22.5% more GUS expression than 1 d and 6.4% more than 8 d. *Agrobacterium* reactivation over a period of 2 h achieved 33.1% more GUS expression than 5 h, and 8.7% more than 16 h. Explants exposed to inoculum by the *Agrobacterium* droplet throughout co-culture stage yielded transformation efficiency, which was higher by 7.2% and 11.8% as compared with 0.5- and 1-h submersion in *Agrobacterium* suspension. This analysis suggests that a 4°C pre-treatment of cotyledonary node explants for 4 d, and the reactivation of *Agrobacterium* inoculum for 2 h with exposure to inoculum by the droplet throughout co-culture stage, can further improve soybean transformation efficiency. The approach is being tested on a range of recalcitrant genotypes.

P-195

Identification and Characterization of LeCAX1, a Tomato (*Solanum lycopersicum*) H^+/Ca^{2+} Antiporter

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Calcium (Ca^{2+}) concentration gradient established across the tonoplast is essential for Ca^{2+} fluctuations that are presumably involved in many aspects of plant growth, development, and signaling. Plant H^+/Ca^{2+} antiporters are thought to play a key role in establishing the Ca^{2+} concentration gradient. Ectopic expression of an activated *Arabidopsis* H^+/Ca^{2+} transporter (AtCAX1) in tomato plants displays an increased incidence of up to 90% blossom-end rot (BER) by disrupting the Ca^{2+} concentration gradient across the tonoplast. Here, we describe identification and characterization of a main tomato H^+/Ca^{2+} antiporter, *Lycopersicon esculentum* calcium exchanger 1 (*LeCAX1*). Full-length *LeCAX1* was cloned by 5′ and 3′ rapid amplification of cDNA ends according to a high similar EST sequence of *AtCAX1*. Bioinfomatics analysis showed that LeCAX1 is 64% identical to AtCAX1 at the amino acid level, and is predicted to have 10 transmemembrane domains. Similar with AtCAX1, the LeCAX1 has an autoinhibition domain in N-terminal, which can regulate the Ca^{2+} transport ability. The activated sLeCAX1 (short length LeCAX1, the truncate N-terminal regulation domain of LeCAX1) can suppress the Ca^{2+} sensitivity of the *pmv1 vcx1* double mutant yeast strains. *LeCAX1* transcripts appeared to be constitutively expressed in all tissues, but showed high expression level in leaf, flower, and stem, and low expression level in fruit and root. To clarify the regulation of LeCAX1, we examined *LeCAX1* RNA expression in response to various stimuli. The results

indicated that the *LeCAX1* was highly expressed in response to exogenous Ca^{2+}. The *LeCAX1* RNAi tomato plants are being generated, and we will evaluate how the *LeCAX1* RNAi lines affect the induction of BER.

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RNAi Suppression of the Lignin Biosynthetic Gene 4-Coumarate-CoA Ligase (4CL) in Sugarcane

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Sugarcane is the highest yielding biomass producer. It is the most efficient photosynthesizer in the plant kingdom, able to convert as much as 2% of incident solar energy into biomass. Typically, farmers reduce the sugarcane post-harvest leaf residue by open air burning, which negatively impacts air quality. Fuel grade ethanol can be made from sugarcane leaf litter residue. However, a major constraint for economic ethanol production from hemicellulosic sugarcane residues is lignin, which acts as a physical barrier to enzyme hydrolysis. Thus, down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. Therefore, the objective of this study is to reduce lignin content in sugarcane by suppressing 4-coumarate-CoA ligase (4CL), a key enzyme in lignin biosynthesis. Two 4CL partial sequences were isolated from the genome of sugarcane cultivar CP 881762. Two RNAi constructs targeting a conserved region in the two genes were constructed using 200 bp from each of the two genes. One or both Sc4CL-RNAi constructs, under the control of the xylem specific OsC4H promoter, were introduced by biolistic gene transfer into immature leaf derived sugarcane callus from cultivar CP 88-1762 along with a selectable nptII expression cassette. The transgenic nature of the regenerated sugarcane plants was confirmed by PCR, Southern blot analysis, and NPTII ELISA, following selection on medium containing geneticin. So far, a subset of 11 independent lines with integration of the 4CL RNAi construct was evaluated for target gene suppression by Northern blot analysis and determination of the lignin content. Data describing the level of 4CL suppression and its effect on lignin content will be presented.

P-197

Whole Genome Resequencing for Capturing Biodiversity, Rediscovering Domestication, and Beyond

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Obtaining the genome sequence of a wide range of individuals of a species will generate vast amounts of informative datasets and enable the rapid discovery of much greater genome-wide sequence variation than has been identified previously. With the decreasing cost of sequencing, the genetic maps of many species are getting increasingly dense, a great improvement for plant breeding and selection. Also, a wealth of knowledge will be gained from comparative genomic analyses within and across species, as how plants grow, function, and survive different ecological conditions and various environmental stresses. Whole genome resequencing approach has been successfully used in rice and maize studies to identify evolution patterns during domestication and to develop efficient ways to discover domestication genes. We re-sequenced 25 representative cultivated rice and 25 wild rice, and developed the genome variation maps containing about 8.4 million SNPs. With a combination of conventional population genetic methods and a new tree-thinking method, about 500 genes were detected with strong selection signals in cultivated rice and thus could be candidate domestication genes. Many of them have functions related to growth, architecture, maturity, productivity, or resistance and can be further applied in breeding programs. A similar study has been carried out in maize for whole genome resequencing of several maize inbred lines. A large number of SNPs and InDels were identified. Hundreds of genes that are present in one haplotype but absent in another were detected. More than 100 large

chromosomal intervals with low sequence diversity represent putative selective sweeps, which may be related with domestication. Limited amounts of intra-chromosomal recombination during pedigree breeding were identified. Whole genome resequencing will have far-reaching implications for improving breeding strategies and plant varieties to meet the world's growing demand on plant production.

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Whole Genome Resequencing for Crop Improvement

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Obtaining the genome sequence of a wide range of individuals of a species will generate vast amounts of informative datasets and enable the rapid discovery of much greater genome-wide sequence variation than has been identified previously. With the decreasing cost of sequencing, the genetic maps of many species are getting increasingly dense, a great improvement for plant breeding and selection. Also, a wealth of knowledge will be gained from comparative genomic analyses within and across species, as how plants grow, function, and survive different ecological conditions and various environmental stresses. Whole genome resequencing approach has been successfully used in rice and maize studies to identify evolution patterns during domestication and to develop efficient ways to discover domestication genes. We re-sequenced 25 representative cultivated rice and 25 wild rice, and developed the genome variation maps containing about 8.4 million SNPs. With a combination of conventional population genetic methods and a new tree-thinking method, about 500 genes were detected with strong selection signals in cultivated rice and thus could be candidate domestication genes. Many of them have functions related to growth, architecture, maturity, productivity, or resistance and can be further applied in breeding programs. A similar study has been carried out in maize for whole genome resequencing of several maize inbred lines. A large number of SNPs and InDels were identified. Hundreds of genes that are present in one haplotype but absent in another were detected. More than 100 large chromosomal intervals with low sequence diversity represent putative selective sweeps, which may be related with domestication. Limited amounts of intra-chromosomal recombination during pedigree breeding were identified. Whole genome resequencing will have far reaching implications for improving breeding strategies and plant varieties to meet the world's growing demand on plant production.

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Artificial MicroRNAs Targeting the Conserved Motifs of Replicase Gene Conferring Transgenic Resistance to Negative-Sense ssRNA Plant Virus

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MicroRNAs (miRNAs) have been shown to regulate the abundance of target mRNAs by guiding cleavage at the sequence complementary region. We use DNA sequences encoding *Arabidopsis* pre-miRNAs expressed from a CaMV 35S promoter to produce artificial miRNAs (amiRNAs) with sequence complementarily to a predetermined viral sequence to generate transgenic resistance against tospovirus, a positive-sense ssRNA plant viruses. Tospovirus is an enveloped virus with tripartite negative sense and ambisense ssRNAs, and it is propagated by thrips. Artificial miRNAs targeting conserved motifs A, B, C, D, and E of the L (replicase) gene of Watermelon silver mottle virus (WSMoV) were constructed, including six single amiRNAs (targeting motif A, B, C, D, or E) and two triple amiRNAs (targeting motif AB1E or B2DC). Processing of the pre-amiRNAs to produce mature amiRNAs was confirmed by agro-infiltration, and transgenic *Nicotiana benthamiana* plants expressing each amiRNA were generated. After upon WSMoV challenge, transgenic lines expressing amiR-LB2 or amiR-LD conferred resistance to WSMoV, but resistance was not observed in transgenic lines expressing amiR-LA or amiR-LB1. Expression levels of amiR-LC and amiR-LE were low, and no resistance was seen in transgenic lines carrying each of the two constructs.

Several transgenic lines expressing three amiRNAs, amiRLB2, amiRLD, and amiRLC, conferred high levels of resistance to WSMoV, without any indication of infection 28 d after inoculation. The transgenic resistance levels were positively correlated to amiRNA expression levels in single and triple amiRNA lines. Transgenic lines expressing amiR-LB2 showed delayed symptoms after challenge with Peanut bud necrosis virus, in which only one mismatch exists in the target site. However, all of WSMoV-resistant lines were susceptible to other four tospovirus species, in which two to four mismatches exist in the target sites. Overall, our results indicate that the amiRNA approach can also be used to generate transgenic resistance against negative-sense ssRNA plant virus.

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Modification of the Hybrid Poplar Defense Pathway for Enhanced Disease Resistance

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Cationic antimicrobial peptides (AMPs) have been used to generate transgenic resistance to pathogens in tobacco, potato, rice, and several other plant species. In most studies, AMP expression in plants was either driven by constitutive promoters such as the viral *CaMV 35S* promoter and its derivatives or by inducible, tissue-specific promoters with a known pattern of spatiotemporal activity. Because these promoters are not an integral part of natural defense system of the host plant, they may cause posttranscriptional silencing of AMP genes, altered metabolism, etc. In the present study, a different strategy that targets defense signal transduction pathways was used to enhance disease resistance in poplar. The nucleotide sequence encoding antimicrobial peptide MsrA2 (*N*-methionine-dermaseptin B1) was transcriptionally fused to the native poplar promoter, *win3.12T*, and introduced into commercial hybrid poplar *Populus nigra* L. × *Populus maximowiczii* A. Henry via *Agrobacterium*-mediated transformation. This promoter contains several pathogen-responsive *cis*-acting elements, exhibits strong systemic activity in response to a variety of pathogens, and is thought to be a part of the poplar defense system. Stable transgene integration into plants regenerated on selective medium was confirmed by PCR and Southern analyses. Northern analysis showed accumulation of *MsrA2* transcripts in response to pathogen infection. Most importantly, the expression level of the MsrA2 peptide in transgenic plants, regulated by the *win3.12T* promoter, was sufficient to confer resistance against the poplar-specific pathogen *Septoria musiva*. The *win3.12T*-driven accumulation of MsrA2 peptide in transgenic poplars had no deleterious effect on plant growth and development.

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A Novel MYBS3-Dependent Pathway Confers Cold Tolerance in Rice

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Rice seedlings are particularly sensitive to chilling in early spring in temperate and subtropical zones and in high elevation areas. Improvement of chilling tolerance in rice may significantly increase rice production. MYBS3 is a single DNA-binding repeat (1R) MYB transcription factor previously shown to mediate sugar signaling in rice. In the present study, we observed that MYBS3 also plays a critical role in cold adaptation in rice. Gain- and loss-of-function analyses indicated that MYBS3 was sufficient and necessary for enhancing cold tolerance in rice. Transgenic rice constitutively over-expressing MYBS3 tolerated 4°C for at least 1 wk and exhibited no yield penalty in normal field conditions. Transcription profiling of transgenic rice over- or under-expressing MYBS3 led to identification of many genes in the MYBS3-mediated cold signaling pathway. Several genes activated by MYBS3 as well as inducible by cold have previously been implicated in various abiotic stress response and/or tolerance in rice and other plant species. Surprisingly, MYBS3 repressed the well-known DREB1/CBF-dependent cold signaling pathway in rice, and the repression appears to act at the transcriptional level. DREB1 responded quickly and transiently while MYBS3 responded slowly to cold stress, which suggests distinct pathways act sequentially and complementarily for adapting short- and long-term cold stress in rice. Our studies thus

reveal a hitherto undiscovered novel pathway which controls cold adaptation in rice.

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Overexpression of Transcription Factor ZmPTF1 Improves Low Phosphate Tolerance of Maize by the Regulation of Carbon Metabolism and Root Growth

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A novel transcription factor that is involved in tolerance to Pi starvation and has a basic helix-loop-helix domain was cloned from *Zea mays* with an RT-PCR coupled RACE approach and named ZmPTF1. The cDNA sequence of ZmPTF1 contained a complete ORF encoding a putative protein of 481 amino acids. Sequence analysis showed that ZmPTF1 had identity with OsPTF1 in the basic region. Real-time PCR revealed that the gene was quickly and significantly upregulated in the roots under phosphate starvation conditions. Overexpression of ZmPTF1 in maize improved root development and enhanced biomass and changed root/shoot ratios under phosphate starvation conditions both in hydroponic cultures and sand pots. Overexpression of ZmPTF1 increased the expression of sucrose synthesis genes in the leaves and decreased in the root, and the decomposing of sucrose in the root seems reduced by enhancing the expression levels of ZmPTF1. This changes lead to the changing of carbon metabolism and carbohydrate distribution, especially the glucose and sucrose, thus promoting the growth and development of root. Under low phosphate condition, the improved root system provides more contact area of root and soil and increased phosphates absorb ability. Maize plants overexpressing ZmPTF1 also exhibited lower levels of total soluble sugars under phosphate sufficient and deficient conditions, and had more tassel branches and larger kernels in low phosphate conditions. Modification of physiology and morphology enhanced low phosphate tolerance and increased the yield in low phosphate soils. The research provides a useful gene for transgenic breeding of maize that is tolerant to phosphate deficiency and is helpful for exploring the relationship between sugar signaling and phosphate concentrations in cells.

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Agrobacterium-Mediated Genetic Transformation of *Cattleya* Orchid Using Protocorm-Like Bodies

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Transgenic plants of *Cattleya* orchid CM2450 were obtained by infecting transformation protocorm-like bodies (PLB) with *Agrobacterium tumefaciens* strains EHA101 (pIG121-Hm) and EHA101 (pIG121-Hm, pBBR*acdS*), both of which harbored genes for neomycin phosphotransferase II (*nptII*), hygromycin phosphotransferase (*hpt*), and β-glucuronidase (*gus*). EHA101 (pIG121-Hm, pBBR*acdS*) also contains 1-aminocyclopropane-1-carboxylate deaminase gene. PLB maintained in liquid New Dogashima (ND) medium were added with *Agrobacterium* suspension culture ($OD_{600}\approx0.6$) to give the dilution ratio of 1:10 or 1:40 and incubated for 30 min, 3 h, or 6 h. *Agrobacterium*-treated PLB were selected on 2.5 gL^{-1} gellan gum-solidified ND medium containing 10 gL^{-1} sucrose, 1.0 mgL^{-1} naphthaleneacetic acid, 0.1 mgL^{-1} benzyladenine, 10 mgL^{-1} hygromycin, and 20 mgL^{-1} meropenem. Hygromycin-resistant secondary PLB were produced after 4 wk and subsequently developed into plantlets within 3 mo after transfer onto PGR-free ND medium. The highest transformation efficiency was obtained when PLB were inoculated with 1:10 *Agrobacterium* liquid culture for 3 h. Transformation of the hygromycin-resistant plantlets regenerated from different sites of inoculated PLB was confirmed by histochemical GUS assay, PCR amplification of 1.2-kb *gus* fragment and 0.6-kb *hpt* fragment, and Southern hybridization using the PCR-amplified *gus* fragment as probe.

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Gene Expression Profiling of Developing Cassava Storage Roots Reveals an Active Process of Glycolysis/Gluconeogenesis

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Cassava storage root is a major sink for photosynthetic carbon assimilation. Its formation involves changes of transcriptional regulation and metabolic activities. For example, to accumulate starch in its parenchyma cells, it is supposed that carbon metabolisms should play important and dominate roles. Using Agilent Cassava Oligonucleotide Microarray 4x44K chip covering more than 20,000 cassava genes and transcripts, the transcription profiles from fibrous roots to mature storage roots were compared and analyzed. More than 900 differentially expressed genes were identified among fibrous roots, developing roots, and mature roots using MultiClassDif analysis. The identifications of these genes were annotated with Gene Ontology and could be catalogized into 20 important pathways. STC analysis also showed that the differentially expressed genes could be mainly grouped into six profiles. Among these pathways, the carbon metabolisms displayed as significant pathways by enrichment analysis, including *N*-glycan degradation, taurine and hypotaurine metabolism, pentose phosphate pathway, cyanoamino acid metabolism, fructose and mannose metabolism, glycan structures–degradation, carbon fixation, γ-hexachlorocyclohexane degradation, riboflavin metabolism, and glycolysis/gluconeogenesis. KEGG Atlas mapping of these pathways also revealed that glycolysis/gluconeogenesis is much more important one affecting other pathways. Pathways such as phenylpropanoid biosynthesis, starch/sucrose metabolism, and zeatin biosynthesis are also strongly regulated, indicating their important roles during cassava storage root formation. Inositol phosphate metabolism and phosphatidylinositol signaling system might participate in the process possibly through inositol phosphate–calcium signaling system. It is the first reported global transcriptional profiling of cassava storage root development and shed a light of important metabolic pathways in the process. Keyword: *Manihot esculenta* Crantz, storage root development, transcriptional profiling, oligonucleotide microarray

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Enhanced rstB Expression in Tobacco by Code Bias Modification

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We had demonstrated that the rstB gene (*Rhizobium* salt tolerance B, AF039956.1, GI: 2865454, open reading frame 2) could be used as a selectable marker gene in plant genetic transformation (Zhang et al. 2009). For better performance, a modified *rstB* (mrstB) was generated by modifying the rare code that may affect gene transcription, biasing the codes for expression in dicotyledonous plants. The hairpin structures, mirror repeats, and direct repeats in the sequence were also eliminated to avoid the potential effect of secondary structure on transcription. The mrstB was synthesized in vitro and inserted to expression vector for tobacco transformation. Transgenic tobacco plants were generated under the selection pressure of 170 mM NaCl. Higher expression of mrstB-GFP was tested by GFP fluorescence observation and Western blotting. The mrstB transgenic plants showed improved salt tolerance ability as expected than rstB transformation, indicating that the enhanced expression of rstB was achieved via code bias modification. Whereas retardation in growth, branching and early flowering, etc. phenotypes changing were also found. Thus, the mrstB gene may be used as an efficient tool for slat tolerance improvement under the control of salt-inducible promoter. Key words: code bias, selectable marker, salt tolerance, transgene

P-206

Improved Protein Quality in Transgenic Soybean Expressing a De Novo Synthetic Protein, MB16

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To improve soybean seed nutritional quality, a synthetic gene, MB16, was introduced into the soybean genome to boost seed methionine content. MB1 is an 11-kDa de novo

protein enriched in the essential amino acids methionine, threonine, lysine, and leucine. For efficient seed expression, constructs were designed using the soybean codon bias, with and without the KDEL ER target sequence, and β-conglycinin or cruciferin seed-specific promoters to drive MB16 (modified MB1 gene with improved protein digestibility). Homozygous lines, with single locus integration, were identified for several transgenic events. Southern and Western analyses showed the same transgene integration patterns and stable MB16 protein expression through to the T5 generation. Transcription varied during seed development. Transcription peaked in growing seed (size 5 to 6 mm), remained at this peak level to the full-sized green seed, and then was significantly reduced in maturing yellow seed. Transformed events carrying the construct with the rumen bacteria codon preference showed the same transcription pattern as those with the soybean codon preference, but transcription levels were lower at each developmental stage. The strongest Western blot signals were detected in full-sized green seed, but the MB16 protein was greatly reduced in mature yellow seed. However, amino acid analysis showed that methionine was increased by 8–16.5%, and cysteine was elevated by 30–58%, thus indicating that MB16 improved the essential amino acid profile. This study confirms that the de novo synthetic protein MB16 can improve protein quality in soybean.

P-207

Multiple Inserts of Gene of Interest and Selection Marker Gene Are Co-integrated and Stably Transmitted as a Single Genetic Locus in Transgenic Soybean Plants

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Particle bombardment has been used for soybean transformation for more than 20 yr, but the integration and segregation of transgene inserts in the soybean genome have not been clearly documented. Over the past 5 yr, we processed several hundred transgenic events. In each experiment, the expression cassettes of the gene of interest and hygromycin resistance marker gene were co-bombarded into soybean at a 1:1 molecular ratio. More than 75% of hygromycin-resistant events also carried the gene of interest. Molecular analysis of transgenic plants revealed that each event carried multiple inserts of the gene of interest and the marker gene. The gene of interest and the marker gene were co-linked in selfed T1 and T2 progeny. Segregation analysis of progeny indicated that multiple inserts of the gene of interest were integrated into the same genetic locus resulting in a 3:1 segregation ratio. Furthermore, multiple inserts of the gene of interest are co-transmitted into succeeding generations and no recombinants were found. These data indicate that multiple inserts of the gene of interest and the marker gene co-integrate and stably co-segregate as a single genetic locus in our transgenic soybean plants.

P-208

An Argonaute Family Gene, Lobbed Leaflet1, Regulates Leaf, Flower, and Root Development through the TAS3 Trans-acting siRNA Pathway in *Medicago truncatula*

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Small RNAs (sRNAs) have been shown to play important roles in regulating plant development. Trans-acting siRNAs (ta-siRNAs) are endogenous siRNAs that act *in trans* and direct the cleavage of complementary mRNA targets. Several key components, such as SGS3, RDR6, DCL4, and AGO7, are required for the biogenesis of ta-siRNAs. However, only TAS3-derived ta-siRNAs depend on AGO7. Recent studies show that AGO7 is involved into different processes of plant development. To further understand the roles of AGO7 homolog in development in legumes, we identify and characterize a leaf development mutant, *lobbed*

leaflet1 (lol1), from a Tnt1 retrotransposon-tagged mutant population of *Medicago truncatula*. lol1 mutant exhibits strong defects in leaf, flower, and root development. Further analyses show that LOL1 is the ortholog of *Arabidopsis AGO7* which is required for the biogenesis of *TAS3* ta-siRNAs. Microarray data indicate that three *MtARF* genes (*auxin response factor*) are negatively regulated by the *TAS3* ta-siRNA pathway. Moreover, we find that MtNAC1 which is the homolog of *Arabidopsis* NAC1, together with three target *MtARF* genes, are involved into the development of root in *M. truncatula*. In conclusion, LOL1 is required for the TAS3 ta-siRNAs in *M. truncatula*. The phenotype is caused by overexpression of its targets MtARF genes which are conserved between eudicots and monocots. Compared with the observation that *Arabidopsis* ago7 mutant has no phenotype in root, the defects of root system in lol1 mutant suggest that *TAS3* ta-siRNAs processing by LOL1 has a broader role in *M. truncatula* development.

P-209

An Efficient Protocol for Micropropagation of North American Ginseng

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North American (N.A.) ginseng is genetically and phenotypically heterogeneous due to mixing of different landraces, and as a result, the active chemical constituents of ginseng roots can vary considerably and evidence supporting its traditional health claims has been inconsistent. An initiative through the Ontario Ginseng Innovation and Research Consortium based at the University of Western Ontario is underway to exploit biotechnology to establish cultivars of unique Ontario ginseng with predictable quality, safety, and medicinal properties. N.A. ginseng has a long production cycle as seeds germinate only after an 18-mo stratification and are produced in adequate quantity only after a 3-yr cultivation. Clonal propagation of superior ginseng lines would contribute to its genetic improvement. A new efficient protocol for micropropagation of North American ginseng has been developed based on an established six-step in vitro tissue culture system. One- to 2-wk-old seedlings were used as starting material. Cotyledons and hypocotyls were excised from the seedlings and placed onto MS medium containing 1 mgl^{-1} 2, 4-D and 1 mgl^{-1} NAA. Compared to previous protocols, the frequency, number, and quality of embryos induced from seedling explants are higher. Embryogenesis frequency of seedling explants was 95%, and embryos from seedling explants are separate from each other and easily separable from explants. The quality of somatic embryos was improved by placing cotyledon-stage embryos on a reduced nutrient maturation medium containing activated charcoal and incubating for a prolongated period at lower (15°C) temperatures. With the protocol, lines can be established in 18 wk; 200 lines have been established from heritage germplasm, and phytochemical and molecular analysis of the lines is underway to select superior genotypes.

P-210

Significant Enhancement of Tolerance to Heat Stress in Soybean via Gene Engineering Approach

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High temperature in summer is an important factor influencing reproductive development of soybean plants in China. Plants have a large family of HSFs with different roles in the heat shock response by mediating the expression of HSP genes. The objective of the present study is to enhance notably soybean tolerance to heat stress via gene engineering approach. A novel GmHsfA1 gene was cloned from the genome of a soybean germplasm conferring tolerance to abiotic stresses by methods of bioinformatics, RT-PCR, and RACE. This gene with full-length cDNA sequence of 1,781 bp (ORF of 1,533 bp) encodes 510 amino acids. Multiple alignment showed that the amino acid sequence of GmHSFA1, matching best with LpHSFA1 (52.46% similarity), was obviously different from that of each of several HSFA1s from other plant species. *GmHsfA1* has a constitutive expression profile in different tissues of soybean plant examined. The expressive vector containing this gene and 35S promoter was constructed and transferred to genome of a heat-sensitive soybean variety by the *Agrobacterium*-mediated transfor-

mation method. We have obtained eight transgenic lines containing the sequence of 35S-*GmHsfA1*-NOS. The results of experiments on heat stress and Northern blot/RT-PCR indicated that (1) the heat-tolerant temperatures (52~58°C) of three of eight transgenic lines (their seed yield being higher than control) are remarkably higher than those (40~42°C) of non-transgenic plants and (2) the over-expression of GmHsfA1 activated the transcriptions of GmHSP22 and GmHSP70 genes in transgenic lines under normal conditions and enhanced obviously the expression levels of GmHSP23 and GmHSP70 genes in transgenic lines under heat stress conditions. The experimental results proved that the overexpression of GmHsfA1 led to significant enhancement of heat-tolerant level of transgenic lines by mediating the activation of transcriptions or improvement of expressions of several GmHSP genes.

P-211

Influence of Sucrose Concentration on Long-Term Sweet Potato Cultures

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Virus-free sweet potato plants are being maintained in culture to supply clean plantlets to local growers. However, between requests for plant material, a system was needed to control growth and increase the intervals between transfers. Long-term in vitro maintenance, on MS medium containing 0–12% sucrose, was used to evaluate shoot growth over time on four sweet potato cultivars. Sucrose levels above 6% were expected to impose an osmotic stress to suppress growth. However, sucrose levels from 2% to 12% had no influence on controlling in vitro growth and development over time. Having no sucrose in the medium resulted in minimal growth but was lethal to 50% or more of the cultures. The rate of root growth and leaf development was significantly reduces on sucrose levels from 0.1% to 0.3%. These low sucrose levels controlled the rate of growth and extended the interval between transfers from monthly, on 3% sucrose, to 8–12 mo on 0.1–0.3% sucrose. Shoots actively grew when transferred back to a 3% sucrose medium. Low sucrose concentrations can be used to control growth of sweet potato and extend the intervals between transfers in vitro.

P-212

Selection and Molecular Profiling of Salt Tolerant Tobacco Plants Obtained by Gamma Radiation

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Salinity is one of the important environmental stresses which influence plant growth and development. Plants have some biochemical and molecular strategies to mitigate the detrimental effects. Salinity tolerance is a complex mechanism that is regulated by multigene families. Mutation-based breeding studies are effective to improve the plant varieties well-adapted to desired traits. In this study, we used gamma radiation to obtain genetic variability in two oriental tobacco (*Nicotiana tabacum* L.) varieties (Akhisar 97 and İzmir Özbaş). The salinity tolerances of varieties are different. The seeds were irradiated with 100, 200, 300, and 400 Gy gamma radiation doses. M_1 and M_2 generations were grown under field conditions. The salinity tolerance selection studies were done in in vivo conditions at 250 mM NaCl concentrations at M_3 generations. Five plants from Akhisar 97 and five plants from İzmir Özbaş were selected as salt-tolerant plants. Genetic variability obtained from mutation breeding was examined through molecular marker techniques (RAPD).

P-213

Effects of Salinity Stress on Densities of Trichomes, Glandular Trichomes, and Stomata in Two Soybean Varieties

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In this study, the aim was to compare the salinity tolerance capacities of two soybean cultivars (Ataem-7 and Üstün-1) and to evaluate the ultrastructural effects of three different

concentrations of NaCl; 10-d-old soybean seedlings were exposed to salinity stress at 0, 50, 100, and 150 mM concentrations in in vivo experimental conditions. Chlorophyll quantities, guaiacol peroxidase activities were determined spectrophotometrically. Leaf and stomata area were done according to Scion Image 4.0.2. Measurements and counting of trichomes, glandular trichomes, and stomata were done on the adaxial and abaxial surfaces of the leaves on scanning electron microscopy. The results were given as per square millimeter. According to peroxidase activity results, Ataem-7 was found more salt sensitive than Üstün-1 variety. Chlorophyll quantities were decreased in accordance with the increasing NaCl concentrations. In both soybean varieties, total leaf and stomata area were decreased due to increasing NaCl concentrations. Trichome numbers showed increment in accordance with increasing NaCl concentrations on both surfaces of Üstün-1 variety. For Ataem-7, at 50 and 100 mM salinity treatments, trichome numbers increased, but at 150 mM, a decrement was observed on both surfaces of the leaves. Stomatal densities (stoma/mm^2) on the abaxial and the adaxial surfaces of Ataem-7 and Üstün-1 increased. In both soybean varieties, although the number of glandular trichomes per square millimeter on the abaxial surfaces of the leaves increased due to increasing NaCl concentrations subjected, on the adaxial surfaces, the density of glandular trichomes decreased.

P-222

In Vitro Establishment, Proliferation, and Rooting of an Iranian Native Olive (*Olea europaea* L. cv. Zard) In Vitro

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Olive (*Olea europaea* L.) is among the most ancient crops of the Mediterranean region. It is important for oil, table olives as well as pharmaceutical effects. To evaluate in vitro culture techniques for clonal propagation of an important native Iranian olive cultivar (zard), lateral buds were taken from olive trees, established on olive medium (OM) supplemented with 0.5 mg/l zeatin for 1 mo. Various concentrations of zeatin riboside (0, 0.5, 1, 2, 4 mg/l) were compared with this medium in establishment phase. The results showed that OM containing 0.5 mg/l zeatin trans was more effective for inducing shoot proliferation at this phase. Effects of various cytokinins including thidiazuron (0.1, 0.2 mg/l), 2iP (1 mg/l), zeatin (4 mg/l), and their combinations all supplemented in OM were investigated on shoot proliferation. The optimal shoot growth and proliferation was achieved in a combination of 4 mg/l zeatin + 1 mg/l 2iP. For rooting, the microshoots were then cultured in ten different treatments of various concentrations and combinations of IBA (0, 1, 2.5, 5, 10 mg/l) and NAA (0, 0.5, 2.5, 5, 10 mg/l) supplemented in 1/2 OM. The rooting percentages, callus formation, and plantlet growth quality index were evaluated 4 wk after culture. Among the ten rooting treatments, effect of three selected treatments with the highest rooting percentages including 2.5 mg/l IBA + 0.5 mg/l NAA, 5 mg/l IBA + 2.5 mg/l NAA, and 5 mg/l NAA were evaluated again. The results showed that the highest rooting (86.6%) and the longest roots (48.9 mm) were achieved on 2.5 mg/l IBA + 0.5 NAA 4 wk after culture.

P-224

Biotechnological Approaches to the Production of Elite Plant Material of *Jatropha curcas*

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Jatropha curcas (Euphorbiaceae) gains increasing attention as alternative bioenergy plant. Due to the non-domesticated state of *Jatropha*, the development of elite cultivars by conventional breeding will profit from biotechnological support, e.g., the production of molecularly characterized, pathogen-free accessions with specific traits. An in vitro germplasm collection of over 100 accessions from ten countries was established (a) to conserve the valuable genetic resources and (b) to serve as starting material for genetic improvement with emphasis on different breeding goals, e.g., pathogen resistance, increased oil, and decreased toxin content. For the purpose of molecular characterization, Eco-Tilling using 12 genes of interest related to stress tolerance, toxin, and oil metabolism was employed. We screened ~18 Mb of genomic sequences for natural nucleotide polymorphisms and identified several SNPs. Fifty ISSR markers were used for further differentiation of accessions. Since new plantations of *Jatropha* should be planted using healthy planting material, there is

an urgent need for the identification and subsequent elimination of pathogens with novel biotechnological methods. Due to the fact that the staple food crop *Manihot esculenta* also belongs to the Euphorbiaceae and might therefore host the same pathogens, a set of published and newly developed primers were employed in direct or RT-PCR for the detection of DNA and RNA pathogens. Several isolates of begomoviruses infecting cassava and *Jatropha* plants from Kenya were sequenced, allowing the design of vectors for pathogen-mediated protection. *J. curcas* can be improved through application of mutation, gene transfer through inter-specific hybridization, and biotechnological interventions.

P-225

The Effect of Planting Arrangements on Yield, Yield Components, and Some Agronomic Characteristics in Chickpea

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The objective of this research was to investigate the effects of plant density and row distance on chickpea (*Cicer arietinum* var. Philip) field performance. Treatments (row spacing × plant-within-row spacing, centimeters) were control (50×7) and 10×10, 12×12, 14×14, 16×16, 18×18, 20×20, 22×22, 24×24, 26×26, and 28×28. The design of experiment was a randomized complete block with four replications. The experiment was conducted in 2006–2007 on the research farm of Gonbad Agricultural Faculty, Iran. Row × plant spacing had no significant effect on seed yield or seed number per pod but it had significant effects on pod number per plant, seed weight, number of dead pods and number of lateral branches. The highest seed yield, 4,665 kg/ha, was obtained from the 18×18 cm spacing, which yield was 36.1% more than the control.

P-226

Potential Impact of Bt Tomato on the Infectivity of Some Lepidopteran Pest

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Tomato is considered as one of the most important vegetable crops grown in Egypt. Worldwide losses of tomato yield due to diseases have been estimated as 40%. Two caterpillars threat the tomato cultivation in Egypt: leaf miner (*Tuta absoluta*) and the black cutworm (*Agrotis ipsilon*). They both reduce yield and fruit quality of tomato grown in greenhouse and open field. Severely attacked tomato fruits lose their commercial value; 50–100% losses have been reported on tomato. It is difficult to achieve an effective control through application of chemical insecticides for these lepidopteran larvae due to the internal feeding of *T. absoluta*. Since *A. ipsilon* larvae have soiled dwelling habitat, they can rapidly evolve strains with reduced susceptibility to insecticides. Transgenic Bt tomato plants were developed with synthetic *cry*IAc using *Agrobacterium*-mediated transformation. Transgenic lines were evaluated for gene expression and revealed the expression of the transgenes. Bioassay tests were carried out on both leaf miner and cutworm larvae revealing a high mortality for small larvae (first and second instars).

P-227

An Evaluation of Plant Preservative Mixture on the Decontamination of Immature and Mature Explants of Citrus

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The genetic improvement of citrus relies on the establishment of clean explants for shoot regeneration and gene

transfer studies. One of the major obstacles toward generating in vitro material whether immature or mature wood explants is the presence of bacterial or fungal contaminants on the surface or within plants tissues. We have evaluated the broad-spectrum biocide/fungicide, Plant Preservative Mixture (PPM™), on reducing the contamination levels of citrus seeds for the production of epicotyl explants and decontaminating mature stem explants. Seeds of sour orange stored for 6–8 wk at 4°C after surface sterilization showed significantly less contamination when germinated on half-strength MS with 0.2% PPM (8% of 80 seeds contaminated) compared to treatments without PPM (81% of 108 seeds). Seeds pretreated for 2 d at 4°C after sterilization also exhibited less contamination in the presence of the same concentration of PPM (1% of 81 seeds) compared to non-PPM treatments (33% of 99 seeds). Furthermore, the presence of PPM in the medium improved the development and elongation of the epicotyl shoot (explant source) compared to treatments without PPM (0.2% PPM, epicotyl length 3.5±0.15 cm; no PPM, 2.8± 0.39 cm; mean ± standard error). In a separate study, mature stem explants of sweet orange cv. Hamlin previously showing that signs of bacterial contamination in culture were washed in 5% PPM and then cultured on DBA3 medium containing 0.2% PPM to aid removal of endogenous contamination were investigated. After 14 d, 8% of stems showed signs of re-contamination, 25% exhibited necrosis but the remaining 67% recovered. We will present the potential value of PPM on the future genetic improvement of citrus.

P-228

Cisgenic Barley with Improved Phytase Activity

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Genetic transformation is currently met with substantial skepticism among the general public in Europe and consequently also by the growers, the agro-industry, and the retailers. One major concern is the mingling of genetic material between species. In the light of this, we have initiated a project based on the cisgenesis concept. In contrast to transgenesis, cisgenesis implies that the plant is transformed only with its own or very closely related genetic material. Furthermore, all "helper" genes and gene sequences of foreign nature are removed from the transformed plant lines. Cisgenic crops are accordingly very similar to those generated by conventional breeding. The cisgenesis concept allows for the introduction of extra gene copies of a particular gene to accentuate the trait. We are using a barley purple acid phosphatase expressed during grain filling as candidate gene for cisgenesis. A genomic barley lambda library has been used to isolate the genomic clone of this phytase including 2.3 kb of the promoter region and 600 bp of the terminator region. The clone has been inserted into a cisgenic *Agrobacterium* vector where both the gene of interest and the selection gene are flanked by their own T-DNA borders in order to promote integration of the two genes at unlinked places in the plant genome. Transformed T0 plants show increases in the phytase activity of mature seeds from 1,400 in wild type to 8,950 FTU/kg in T0 plants. T1 plants of each transformant are currently screened with PCR for extra copies of the genomic phytase gene and the selection gene to identify segregation between the two genes. Presently, we have identified two cisgenic T1 plants without vector backbone and selection gene but with an extra copy of the genomic phytase gene.

P-229

Understanding the Role of the *Arabidopsis* Microtubule Plus-End Protein EB1 (End-Binding 1) in Plant Growth and Development

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The spatial organization of the interphase cortical microtubule array determines cell morphogenesis and adult plant stature. Microtubule array organization depends on regulated microtubule assembly, which principally happens at the microtubule plus-ends. The microtubule plus-end tracking proteins (+TIPs), which specifically bind to and track

growing microtubule plus-ends, are key regulators of microtubule assembly. End-binding 1 (EB1) is thought to be the master regulator of +TIP activity; however, its role in regulating cortical microtubule assembly dynamics and organization is still unclear. The *Arabidopsis* genome encodes three EB1 proteins designated EB1a, b, and c. Using a dominant negative approach, we found that the overexpression of GFP-EB1bC (the C-terminal protein interaction domain of EB1b fused to GFP) leads to developmental defects when compared to untransformed eb1 triple mutant plants. Specifically, we found a strong correlation between the level of GFP-EB1bC and the extent of growth defects in 17 independent T_3 lines. This result suggests a dose-dependent disruption of endogenous EB1 function, probably by GFP-EB1bC nonproductively binding to EB1-interacting proteins. To determine the effect of EB1 functional disruption on the dynamics and organization of the cortical microtubule array, independent dominant-negative lines will be crossed with the mRFP-TUB6 tubulin marker plants and examined using live-cell imaging. To identify new components of the +TIP complex, magnetic beads coupled to an anti-GFP antibody will be used to capture GFP-EB1a/b/c from plant extracts. The isolated protein complexes will be analyzed by mass spectrometry to identify EB1-binding partners. This work will provide new insights into fundamental mechanisms of plant growth and development.

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First Time Detection and Elimination of Begomovirus (*Ageratum enation virus*) Affecting Economically Important *T. dioica* (Parwal) Plant in India.

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Trichosanthes dioica Roxb. (family: Cucurbitaceae, commonly known as pointed gourd; local name: Parwal) is one of the most nutritive cucurbit vegetable that holds a coveted position in the Indian market during summer and rainy season. It is widely cultivated in north and eastern part of India. Parwal is a tridoshic vegetable and is excellent for balancing of all five fundamental elements. It is vine and perennial plant and highly acceptable due to its availability for 8 mo in a year. Being very rich in protein and vitamin A, it has certain medicinal properties and having an important role in circulatory system. It's also know to have antiulcer us effects. The fruits are easily digestible and diuretic in nature. Viruses are playing a major role in reducing the quality and quantity of the cucurbitaceous crops in almost every part of the world. Many viruses are reported on the cucurbitaceous crops. During survey of cucurbit-growing area of eastern U P, India, a severe mosaic disease affecting nearly 20% *Trichosanthes* cultivation was observed. Naturally infected plants exhibited mosaic mottling with severe mosaic symptoms, reduction of leaf lamina, and dwarfing of fruits and entire plants. White flies population were noticed in the field. On the basis of the symptomatology and presence of White flies population, begomovirus infection was suspected. So, to characterize the virus, total DNA were isolated from infected and healthy tissues of three symptomatic and non-symptomatic plants. The DNA samples were subjected to PCR with a set of primers designed from the coat protein region of begomovirus. An expected amplicon of ~800 bp was obtained from all the tested symptomatic but not from the symptomatic plant samples. Amplicon obtained from symptomatic plant samples was sequenced and was deposited in GenBank data base (account no. EU 867514). Basic local alignment search tool search analysis of the sequence data showed the highest sequence identity of 97% with *Ageratum enation virus* isolates. On the basis of phylogenetic analysis and sequence identity, the virus isolate is therefore proposed to be a begomovirus closely related to *A. enation virus* and tobacco curly shoot virus (TCSV) on the coat protein level analysis. This is the first report of natural occurrence of a begomovirus (closely related to *A. enation virus* and TCSV) in pointed gourd from the world. Cleaning programmes were assayed to produce virus-free material. Apical meristems measuring about 2 mm in length were dissected out, surface-sterilized and cultured on agar-gelled Murashige and Skoog's medium containing growth regulators for shoot induction. The established shoot cultures were multiplied through repeated subcultures on fresh media at 10–12 d interval. Further confirmation of elimination was checked through PCR analysis. Total DNA were isolated from healthy, infected and regenerated plants, and PCR was performed. No amplicon was found in gel electrophoresis in regenerated and healthy plants. Results agreed the previous report on the elimination of viruses on other host species and also suggested that tissue culture technique is the best method for the elimination of viruses and production of clean materials. Literature revealed that only limited attempts have been made to characterize viruses on *T. dioica*. However, in literature survey, no reports on viruses on *T. dioica* were found from India. About in vitro technique in other plant species, many works have been done to eliminate pathogen (viruses and phytoplasms) and found successful regeneration through apical meristem or meristem. Therefore, to the best of my knowledge, this is the first report of detection and elimination of begomovirus from *T. dioica* plant in India. These results will help to check the further spread of this virus through propagation and allow to

produce disease-free material and also to detect the infection at early stage.

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Shortening Flower Cycle of Cotton by Transforming the FT Gene into *Gossypium hirsutum* L.

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Many research groups around the world work on cotton in different areas of molecular biology (functional genomics, proteomics), evolutionary biology, and applied research in an attempt to improve cotton as a valuable crop. However, some of the problems that cotton researchers continue to confront include limited space and comparatively long-time periods for plant growth and fiber production. Smaller cotton plants with shorter life cycles would facilitate research efforts and speed up the process of overall cotton improvement. In order to accomplish this goal, researchers are focusing on manipulating the time to flowering, which is controlled by day length in many plants. It is known, for instance in *Arabidopsis*, that the day length response depends on the induction of the FLOWERING LOCUS T (FT) gene. The FT messenger RNA is transported to the shoot apex, where downstream genes are activated. Overexpression of this gene in cotton could result in earlier flowering and a shorter life cycle. The FT gene driven by 35S CaMV promoter is being used in transformation of cotton (cv. Coker 312) along with another construct with rsGFP driven by 35S CaMV promoter as a control. Cotton embryogenic callus, previously initiated from hypocotyls, has been transformed with both genes using an *Agrobacterium*-mediated method. Monitoring GFP expression in transformed embryogenic callus and somatic embryos helped to assure successful transformation of both genes during 3 mo of selection on kanamycin. Selection of putative transgenic somatic embryos resulted in production of small rooted plants on hormone-free medium. Putative transgenic plants will be transferred to soil, analyzed for the gene integration, gene expression level, and eventually for their flowering time, size, and number of cotton bolls and seed set. This research was supported by the Plant Transformation Laboratory at North Carolina State University with contribution from Cotton Incorporated, Cary, NC.

P-232

The Technology of Obtaining of Carrot Homozygous Plants Using the Anther Cultures

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At the Research Institute of Vegetable Crops in Skierniewice, the method was elaborated of obtaining carrot homozygous plants by application of anther cultures. Embryogenic varieties were found. Effective technique has been developed of plant regeneration from androgenetic embryos by inducing secondary embryogenesis and embryo conversion. This enabled to eliminate the step of rooting, which was rather uneffective. Over 90% of obtained plants had doubled the set of chromosomes. Similarity of the results of cytological and cytometric analyses allows to recommend the latter as a good tool for defining ploidy of androgenetic carrot plants. Anatomical observations of microspore development during course of anther cultures proved that embryos were formed directly from the microspores. Homozygosity of androgenetic plants was confirmed with isoenzymatic systems PGI and AAT. At present, the above stages of obtaining androgenetic plants are optimized.

P-233

Increased Fructan Biosynthesis by Over-expressing Rye 1-SST or Wheat 6-SFT cDNA in Developing Triticale Seeds

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Oligofructans are prebiotics with potential beneficial effects to human health. While oligofructans can be abundant in leaves and stems of cereals, particularly under abiotic stress conditions, cereal grains contain only small quantities of this carbohydrate. This work aimed to study the regulation of oligofructan synthesis and accumulation in triticale seeds by over-expressing a rye *sucrose–sucrose 1-fructosyltransferase* (1-SST) and a wheat *sucrose–fructan 6-fructosyltransferase* (6-SFT). 1-SST and 6-SFT cDNA, under the control wheat BX7 endosperm-specific promoter, were separately introduced into triticale by CPP-mediated microspore transfection. Gene profiling using quantitative real-time PCR showed that both 1-SST and 6-SFT were expressed at 10 d post-anthesis (PA) in developing seeds while at 15, 20, and 25 d PA, the genes were not expressed. Total fructan concentrations at 10 d PA in developing seeds of 1-SST transgenic lines ranged from 2.31% to 6.56% of grain fresh weight, representing 1.9- to 5.7-fold increases in fructans over that of the untransformed control (1.16%). A range of 3.27~4.74% was observed in 6-SFT transgenic lines representing 2.8- to 4.1-fold more fructan than in nontransformed plants. HPLC analyses of oligofructan in transgenic lines with the degree of polymerization (DP) ranging from 3 to 11 with obvious increases in quantity compared with the untransformed controls. However, no differences in fructan content from untransformed controls were observed at 15, 20, and 25 d PA. Results demonstrated that transient increased fructan content was achieved in the developing triticale endosperm following expression of 1-SST or 6-SFT under the control of the BX-7 promoter. Correlation of fructan concentration with 1-SST expression (r=0.83) suggests that this enzyme is rate limiting in oligofructan biosynthesis in cereals.

P-234

Differentiation of Multiple Roots and Shoots in Callus Cultures of *Antirrhinum majus* L.

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Antirrhinum majus L. belongs to family Scrophulariaceae and is commonly known as snapdragon. The plant has been identified as suitable material for studying functions of transposons. Generally, it is an ornamental plant and grows during spring season in Delhi. We have developed an efficient protocol for its multiplication by repeated subculturing of anther-derived callus masses. Among the various culture media and plant growth regulators used, the most suitable medium for multiple shoots and roots formation was NB containing either BA or 2,4-D. Numerous globular embryos developed directly on anther surface after 12 wk of culture on NB containing 1 mg/l 2,4-D. Prominent multiple roots developed from callus masses on NB + 1 mg/l 2,4-D medium during repeated subcultures. Multiple shoots developed during subculture of callus masses on NB basal medium alone or containing BA at 1 mg/l concentration. The plants were hardened using various methods first in culture room and then in glasshouse. Ultimately, plants were transferred to the field where they grew profusely and produced new branches, leaves, and flowers. The flowers were of various colors due to involvement of transposons. The details of experiments would be discussed in the present paper.

P-235

FLP-Mediated Site-Specific Gene Integration in Rice

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Site-specific recombination systems are powerful tools for plant transformation. They facilitate excision or integration of specific DNA fragments in site-specific manner. Of the tested recombination systems, the yeast FLP-FRT system has been widely used for excisional recombination but not for co-integration. Development of the FLP-FRT-mediated gene integration application requires an efficient FLP-FRT recombination system. This work focused on developing and experimentally validating an effective strategy for FLP-FRT-mediated transgene integration in rice. The strategy consisted of generation of target lines having a single FRT variant, FRT_L, embedded between the promoter and coding region of a FLP gene and transforming them with a gene construct containing a second FRT variant, FRT_R. FLP-mediated recombination between FRT_L $XFRT_R$ would generate a precise full-length integration of the introduced

DNA construct. Three different target lines were transformed, each of which produced site-specific integration lines. The successful integration, relied oncotransformation of an enhanced version of FLP gene, FLPe, as promoter-FRT-FLP construct failed to generate sufficient levels of FLP activity. Molecular analysis of the transgenic callus and plants revealed precise integrations without any additional random integration. The inheritance of the integration locus by T1 progeny will be studied in summer of this year. This work demonstrates that a modified FLP-FRT recombination system works efficiently for precise site-specific gene integration in rice.

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Genetic Engineering of *Lotus corniculatus* L. Forage Legume with Proteinase Inhibitor Genes

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Bird's foot trefoil (*Lotus corniculatus* L.) is a perennial forage legume which in many areas is preferred to alfalfa because of its tolerance to adverse environmental conditions and high nutritive value. The productivity of bird's foot trefoil could be increased by introducing stably inherited traits such as pest, disease, and herbicide resistance. Since the indicated traits are not available in natural *L. corniculatus* L. populations, a strategy for introducing insect and nematode control is to overexpress heterologous proteinase inhibitor (PI) genes in transgenic plants. PIs are proteins that occur naturally in a number of plant species and are characterized by varied specificity toward proteinases, among them insect digestive proteinases that fall into the serine, cysteine, aspartyl, and metalloproteinase classes. In this study, we engineered bird's foot trefoil plants with three different PI genes: *Beta vulgaris* serine PI, *Nicotiana alata* serine PI, and squash aspartyl PI. Detailed analysis of PI activities in transgenic plants will be discussed.

P-237

Sustainable Carbon Negative Rice Production

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Rice is the most important staple for more than half of the world population. Thus, reducing greenhouse gas emissions from rice cultivation while enhancing its productivity is an important strategy to mitigate climate change and advance food security. Between 1961 and 2000, Asian population increased from 1.7 billion to 3.7 billion, average rice (*Oryza sativa* L.) yield from 1.86 to 3.95 $t ha^{-1}$, total paddy area from 107 million ha (M ha; 93% of the world total) to 138 Mha (90% of the world total), and per capita rice consumption from 103 to 126 $kg person^{-1} yr^{-1}$. However, any future increase in rice yield is jeopardized by abrupt climate change. Rice production in Asia doubled since 1950s through the adoption of high-input technologies. However, any future increase in rice yield is jeopardized by abrupt climate change. Elevated temperature can decrease rice yield even in flooded paddy fields, which also accentuate methanogenesis through anaerobic decomposition of soil organic matter. The mean rate of gaseous emission from rice paddy is 179 kg of methane (CH_4) and 4.9 kg of nitrous oxide (N_2O) $ha^{-1} yr^{-1}$. Thus, C footprint of Asian rice is 1,980 kg C equivalent (CE) for producing 4,259 kg of rice and 9,463 kg of agricultural by-product per hectare per year. The C footprint can be reduced to 517 kg CE (reduction by 74%) by technological options such as aerobic rice, water drainage, and reduced tillage while also increasing rice yield by as much as 70%. Adopting recommended management practices of rice cultivation can reduce its C footprint and meet food demand of the growing population. Our results demonstrate that the C footprint of rice cultivation can be reduced by technological options such as growing aerobic rice, water drainage, and reduced tillage while also increasing rice grain yield. We anticipate our study to be a starting point for more region-specific management options of carbon negative which footprint reduction and increasing rice yield and production.

P-238

Induced Systemic Acquired Resistant with Glyphosate in Two Varieties of *Carica papaya* L.

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The papaya (*Carica papaya* L.) is one of the important delicious fruit crops grown in the tropical and subtropical regions of the world. This fruit is highly productive in vitamins, and its latex is used as a papain, quimiopapain, quitinase, and lycopene in food and medicinal industry. Several diseases and pests affected papaya crops which limits its production and caused great losses in the field. *Papaya ringspot virus* is the most limitations in papaya production worldwide and can destroy a crop if the isolation is not implemented. The purpose of this work was studying the incidence of ringspot virus through systemic acquired resistance in two varieties of *C. papaya* L.: Maradol and Tainung. In this case, glyphosate (*N*-(phosphonomethyl) glycine) as a systemic herbicide has been used in order to search plants that showed some resistant or tolerant to the virus. For this purpose, apical and axillary buds were used as explants for the clonal propagation. They were placed in the basal medium M&S (1962) supplemented with 2.32 μM kinetin, 2.016 μM gibberellic acid, 1 g of putrescine, 3 g of charcoal activate, and 8 g of agar-agar (Merck). For mutation induction of each variety, the plantlets were transferred to a media used for plant propagation with 0, 2–3.5 μM of glyphosate in where the abscission was detected in the two varieties. We used silver thiosulfate (0–5 μM) to solve this inconvenient, which one was efficient after 4 wk. The material obtained after glyphosate treatment was propagated in order to get whole plants (M_1V_4) for hardening. During the environment acclimation of plants, we observed some morphological variations that they are going for field evaluation.

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Somatic Embryogenesis and Plant Regeneration in Mango (*Mangifera indica* L.)

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The mango (*Mangifera indica*) is one of the most widely grown tropical fruit crops. In Colombia, this fruit is very important for the national consumers and export. The mango variety "Hilacha" is the most common in Colombia, and some genotypes exceed the required standards for pulp industry with vitamins and antioxidant properties. The advantage of efficient regeneration of tropical, perennial fruit trees from cell and tissue culture is one of the ways for improvement programs that have been studied. Litz et al. (1982) indicated the potential for ovule culture to produce large numbers of somatic embryos from the nucellus of naturally polyembryonic mango cultivars. In order to study somatic embryogenesis of Colombian cultivars, *M. indica* cv. "Hilacha" was selected; immature fruits of this variety were collected from southwest of Colombia. The effect of 2,4-diclorophenoxiacetic (0, 4.52, and 9.04 μM) and thidiazuron (TDZ; 2.27, 4.54, 6.81, and 9.80 μM) for induction of somatic embryogenesis was studied. Zygotic embryos and nucellus tissue from immature fruits were used as explants, and these materials were transferred in aseptic condition to a Pateña and Barba (2002) modified media with ascorbic acid, citric acid, and cysteine in each one 60 mg/L. The addition of gibberellic acid (AG_3 0, 1.44, 2.88, and 1.32 μM) for plant regeneration was evaluated. The induction medium in where reached a high proliferation somatic embryos was the media supplemented with 2.27 μM of TDZ and 4.52 μM of 2,4-D. The recommendation for plant regeneration should.

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Evaluation and Comparison of Sugarcane Genotypes for Transient and Stable Transformation Efficiency

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Sugarcane is an important commercial crop that is grown for sugar and alcohol production and in the future may also be used as feedstock for the production of cellulosic biofuel. Genetic improvement of sugarcane using transgenic technology is expected to play a crucial role in modifying the quantity and quality of sugar and biomass suitable for biofuel production. For this purpose, the establishment of a well-defined, rapid, and highly efficient genetic transfor-

mation system is an important prerequisite. However, like other monocot crops, sugarcane is considered recalcitrant for genetic transformation, and the efficiency of transformation is highly influenced by the source genotype. In this study, 11 sugarcane cultivars were tested for their tissue culture response and amenability to genetic transformation. Embryogenic callus was generated from all 11 genotypes using immature leaf roll disks as an explant source. Plasmid vector pCHR5798, containing selectable and screenable marker genes nptII and AcGFP, respectively, was used to transform the callus using particle bombardment. GFP expression was transiently observed 3 d after bombardment, with significant efficiency differences detected among the callus genotypes. Calli underwent four rounds of selection on MS-based medium supplemented with 30–50 mg/l G418 to identify stable transformed events. Following 3 to 4 mo of selection, resistant callus events were regenerated and rooted in the presence of G418 (25–40 mg/l). Transgenic plantlets have been produced from nine of the genotypes. Molecular and phenotypic analysis of the transgenic plants and estimation of transformation efficiency are in progress.

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Switchgrass (*Panicum virgatum*) Biotechnology and Transformation for Altered Cell Wall Biosynthesis

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Switchgrass (*Panicum virgatum*) is a leading candidate feedstock for biofuels in the USA and is a crucial model feedstock component of the BioEnergy Science Center (BESC). Biotechnology of switchgrass is important in screening potential cell wall biosynthesis genes and is being performed by six laboratories in three institutions within BESC. Within our lab, goals and milestones include improving tissue culture and transformation systems, isolating novel switchgrass promoters, developing a new versatile vector set for monocot transformation, and altering lignin and cellulose biosynthesis within switchgrass. Using a novel non-MS-based tissue culture medium for increased production and maintenance of type II callus, transformation efficiency has increased from less than 3% to 10–15%, making it feasible for high throughput applications. Additionally, switchgrass cell suspension cultures have been produced and characterized for mutant selection, mass propagation, gene transfer experiments via protoplast isolation, and cell biology in view of cell wall trait assessment. In order to coordinate gene expression within BESC and to facilitate more rapid screening of genes, we have developed a Gateway-compatible monocot transformation vector set (pANIC) for overexpression and RNAi-mediated knockdown with visual and selectable markers. BESC has facilitated the coordination of scientific expertise and research in switchgrass biotechnology that would have been otherwise impossible by one investigator with funding under a traditional grant.

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Generating Insect-Proof Cotton Plants Using RNA Interference Technology

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RNA interference (RNAi) is a gene regulation mechanism occurring widely in eukaryotes. Previously, we reported the development of a plant-based insect RNAi technology for protecting plants against herbivorous insects. *Arabidopsis* and tobacco plants were engineered to express the double-stranded RNA (dsRNA) targeting cotton bollworm (*Helicoverpa armigera)* CYP6AE14 gene, which encodes a P450 monooxygenase involved in the insect response to gossypol and related phytoalexins. Cotton bollworm larvae fed on the transgenic plants showed a lower level of CYP6AE14 expression and reduced growth on gossypol-containing diet (Mao et al. 2007). We then transferred the dsRNA construct into cotton (*Gossypium hirsutum*). Five lines of T1 transgenic plants exhibited an inhibitive effect on cotton bollworm larval growth. T2 plants with high dsCYP6AE14 levels were then selected, on which the bollworm larvae exhibited drastically retarded growth, and the transgenic plants were less damaged by bollworms than the control. Quantitative RT-PCR and Western blot assays showed that the *CYP6AE14* expression was obviously dropped. These results demonstrate that cotton plants expressing dsCYP6AE14 acquired enhanced resistance to

cotton bollworms and that RNAi technology can be used for generating insect-proof cotton plants. Reference: Mao YB, Cai WJ, Wang JW, Hong GJ, Tao XY, Wang LJ, Huang YP, Chen XY. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnology 25, 1307–1313.

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Expression Regulation of ABA 8′-Hydroxylase Gene by RNA Interference

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Pre-harvest sprouting (PHS) is a widespread problem, which results in significant losses in grain yield and decreased end-product quality. Dormancy at harvest is beneficial to enhance PHS resistance of grains under the cool moist conditions. Abscisic acid (ABA) is the primary mediator of seed dormancy. Recent research progress in both model plants and crop species has revealed the relationship between seed dormancy and ABA metabolism. ABA 8′-hydroxylase gene family (CYP707A) genes encoding ABA 8′-hydroxylases, which catalyze the first committed step in the major ABA catabolic pathway, play a key role in controlling grain dormancy. To further test the important role of the ABA 8′-hydroxylase gene and to develop an strategy for crop PHS resistance breeding by manipulating gene expression, a barley homolog (HvABA8′OH-1) was used to construct expression vector containing the *AtCYP707A2* promoter and a RNAi vector with rice endosperm-specific promoter, respectively. Compared to control *Arabidopsis* AtCYP707A2 mutant with delayed germination, transgenic lines displayed germination rate recovery up to 92%, much higher than that of mutant. Four of 18 transgenic rice lines germinated 5–10 d later in the primary germination test. Characterization of ABA 8′-hydroxylase gene expression profiling and endogenous ABA levels in transgenic rice seeds is underway.

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Development of Transformation Vectors Based Upon a Modified Plant α-Tubulin Gene as the Selectable Marker

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A plant transformation and selection system has been developed utilizing a modified tubulin gene as a selectable marker. The vector constructs carrying a mutant α-tubulin gene from goosegrass conferring resistance to dinitroaniline herbicides were created for transformation of monocotyledonous and dicotyledonous plants. These constructs contained β- and/or mutant α-tubulin genes driven either by ubiquitin or CaMV 35S promoter. The constructs were used for biolistic transformation of finger millet and soybean or for *Agrobacterium*-mediated transformation of flax and tobacco. Trifluralin, the main representative of dinitroaniline herbicides, was used as a selective agent in experiments to select transgenic cells, tissues, and plantlets. Selective concentrations of trifluralin estimated for each species were as follows: 10 mM for *Eleusine coracana*, *Glycine max*, *Nicotiana plumbaginifolia*, and *Nicotiana sylvestris* and 3 mM for *Linum usitatissimum*. PCR and Southern blotting analyses of transformed lines with a specific probe to nptII, α-tubulin, or β-tubulin genes were performed to confirm the transgenic nature of regenerated plants. Band specific for the mutant α-tubulin gene was identified in transformed plant lines. Results confirmed the stable integration of the mutant tubulin gene into the plant genomes. Using immunofluorescent microscopy, it has been revealed that the mutant α-tubulin subunit incorporates into native structure of cortical and mitotic microtubules conferring their resistance to antimitotic dinitroaniline herbicides in transgenic plant cells. It was found also that the dinitroaniline resistance inherits as dominant nuclear trait in F1 and F2 generations of transgenic plants. The present study clearly demonstrates the successful use of a plant mutant tubulin as a selective gene for plant transformation.

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Artificial MicroRNA Mediated Geminivirus Resistance in Transgenic Tomato

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MicroRNAs (miRNAs) are 20–24 nt small RNA molecules which modulate cellular gene expression by usually downregulating their target(s) in a sequence-dependent manner. Besides their diverse role in developmental and physiological processes, miRNAs are now being increasingly implicated in disease development and pathogenesis, including viral pathogenesis. It is known that some mammalian miRNAs can resist animal viruses, but such anti-viral miRNAs are not known in plants, till date. So, we explored the possibility of engineering artificial miRNAs (amiRs), targeting *tomato leaf curl New Delhi virus*—a member of geminivirus, which devastates tomato crop in tropics and sub-tropics. Two amiRs were designed to target conserved regions of viral replicase and two potent RNA silencing suppressors—AC2 and AC4. Computational programs were employed to ensure correct processing, right strand separation, and specific target recognition by amiRs. Precursor of an endogenous *Arabidopsis* miRNA was used as a vehicle for expressing amiRs of interest by replacing resident miR319a/miR*319a by amiR/amiR*, through a series of overlapping PCR reactions. AmiR biogenesis was validated by in vitro dicing reactions using *Arabidopsis* inflorescence and wheat germ extracts. Pri-amiRs were found to be consistently processed into small RNA species, reminiscent of hairpin-specific RNase III-type endonucleolytic processing. Subsequently, amiR precursors were used as transgene to generate CaMV35S:amiR transgenic tobacco and tomato lines. The mature 20-mer amiRs were detected by small RNA Northern blots in many T_0 lines. In viral challenge experiments, these lines exhibited remarkable resistance and reduced mini-viral DNA replication by approximately 60%. AmiRs offer an attractive strategy to engineer effective, broad-spectrum, and biosafe virus resistance in important crops.

P-247

Biotech Traits Improve Agronomic Nitrogen Utilization in Corn

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The development of enhanced B.t. insect protection traits can lead to indirect benefits such as ability to capture more soil moisture and increased nitrogen use efficiency. The objective of this study is to evaluate whether hybrids containing YGVT3 respond differently to N rates compared to non-insect protected corn and determine the optimum N rates. Field experiments were conducted at four locations across the Corn Belt during 2008 and 2009 growing seasons using a strip-plot design with four replications. Hybrids with and without traits were randomized in strips and N rates within hybrids to create a range of N supply. Corn N uptake and yield were determined at harvest. The optimum N rates were determined using a quadratic-plateau function. Preliminary results indicate that the two-way interaction of hybrid × N rate and the simple effect of hybrid were not significant at all the sites. However, the simple effect of N was significant at all sites. Hybrids with YGVT3 trait showed potential for lower optimum N rates relative to those without the trait. Plant N uptake data in addition with growing season weather data will be used to understand the differential levels optimum N rates. Differential levels on N requirements to optimize yield in hybrids with and without the YGVT3 traits have enormous implication toward a more precise and economical N management and potentially decrease groundwater N contamination.

P-248

Phosphatidic Acid Interaction with a Ribosomal Kinase in Plant Growth Regulation

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Phospholipase D (PLD) hydrolyzes membrane lipids to produce phosphatidic acid (PA), and PA participates in many physiological processes as a class of secondary messengers. Our recent data indicate that PLDε and its derived PA are involved in nitrogen signaling and growth promotion. Knockout and overexpression of PLDε result in decreased or increased, respectively, root growth and biomass production. The opposite effects are correlated with the level of phosphorylation of the 40S ribosome kinase (S6K) that regulates translation. To determine whether S6K is a target of the PLDε-mediated signaling pathway, we are investigating the effect of PA on S6K and found that PA interacts with S6K. The results may provide a direct link between the membrane sensing nutrient status and the translation regulation to promote growth.

P-249

Characterization of Non-specific Phospholipase C (NPC) in Soybean

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Phospholipase C (PLC) hydrolyzes phospholipids to generate diacylglycerol and a phosphorylated head group. Non-specific phospholipase C (NPC) differs evolutionarily and functionally from phosphoinositide-hydrolyzing PLC. NPCs have been implicated in different processes in *Arabidopsis* response to stresses. We have identified nine NPC genes in soybean. The function of the NPC family is investigated by genetic manipulation of specific NPCs and identification of molecular targets of the NPC-derived metabolites.

P-251

Genetic Analysis of *phyA′*—Anepiallele Associated with Exonic Methylation

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Genome-wide analysis of *Arabidopsis thaliana* DNA methylation revealed two major patterns of methylation: the presence of CG, CHG, and CHH methylation found largely on transposable elements and repetitive DNA and the presence of CG methylation clusters observed in the transcribed region of genome. We isolated an epi-allele of *A. thaliana* phytochrome A gene termed *phyA′*, which contains methylation in CG sites resident to transcribed region and is transcriptionally suppressed. These exonic modifications confer a strong *phyA* mutant phenotype, characterized by elongated hypocotyls in seedlings grown under continuous far-red light (FRc). Demethylation of *phyA′* in the DNA methyltransferase I mutant (*met1*) background resulted in restoration of the WT expression level and phenotype, confirming the pivotal role of the mCG in *phyA′* silencing. Genetic analysis revealed that a number of chromatin modification and RNAi genes have no significant role in *phyA′* silencing. This analysis covered DNA methylation genes (CHROMOMETHYL TRANSFERASE and DOMAINS REARRANGED METHYLASE), histone methylation gene (KRYTONITE), and RNAi genes (RDR2, RDR6, AGO 1, 4, and SGS3). To identify the novel genes involved in keeping *phyA′* suppressed, we took the approach of suppressor screening. Seeds of *phyA′* epimutant were mutagenized by EMS, and M2 populations were screened for the WT phenotype. Phenotypic screening of M2 populations resulted in an identification of the suppressor mutation, sps-1. Molecular and genetic analysis of sps-1 revealed that this second-site mutation reactivates *phyA* locus in spite of *phyA′* hypermethylation. Ongoing work on further characterization of sps-1 will be presented.

P-252

Corosolic Acid Production from *Lagerstroemia speciosa* L. Adventitious Root Culture: Next Generation Approach for Therapeutic Management of Diabetes

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Diabetes and obesity are two of the most common metabolic diseases in USA, affecting not only adults but also children and adolescents. In twenty-first century, these diseases are two of the main threats to human health. Insulin for diabetes and a variety of pharmacological and physiological as well as surgical interventions for obesity have been used for treatments. Plant-based therapeutic molecules are safer than synthetic and are relatively low cost. Corosolic acid, an ursan-type triterpene, which is naturally present in *Lagerstroemia speciosa* L. (banaba), has been clinically shown to have the potential effects of

anti-diabetic and anti-obesity. The aim of this study is to establish an adventitious root culture technology to produce natural corosolic acid. Adventitious root growth, elicitation kinetics, and mass spectrometry data will be discussed. These bioprocess data may be useful to establish bioreactor production of bioactive isomer corosolic acid to treat diabetes and obesity.

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P-253

Dual Trait Expression in Cassava Storage Roots for Accumulation of b-Carotene and Storage Protein

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Cassava (*Manihot esculenta*) is a major food source for millions of people in sub-Saharan Africa. Although its storage roots are rich in calories, they contain less than 2% dry weight (dw) protein and only 3–5 mg/g β-carotenes, leaving those who rely on this crop as a major component of their diet are at risk of malnutrition. We describe the production of cassava transgenically modified to accumulate β-carotene and nutritionally valuable storage protein within the same storage root tissues. The cultivar 60444 was genetically transformed with a single binary vector carrying two genes of the carotenoid biosynthetic pathway and one coding for sporamin targeted to form protein bodies within the ER. Expression of all three transgenes was under control of individual patatin promoters. Efficiency of transformation for this triple gene construct was not reduced compared to that of a single transgene, and RNA expression studies showed all transgenes to be expressed at comparable levels within the same transgenic event. Seven RNA expressing plant lines were established in the greenhouse and storage roots analyzed at 6 mo of age. Levels of total protein in these tissues were found to have increased from 2.5% dw in non-transgenic controls to reach 11% dw and to contain up to 45 μg/g β-carotenes, a 25 times increase compared to controls. Similar studies are presently ongoing under confined field trial conditions at the University of Puerto Rico.

P-254

Identification of Transcriptional Regulators of Phytosterol Biosynthesis in *Arabidopsis thaliana* Using a Data Mining Approach

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In plants, phytosterols function as essential membrane components and precursors to the plant steroid hormones brassinosteroids. Plant sterols are used industrially as value enhancing food and cosmetic additives whereas modulation of brassinosteroid metabolism could potentially impact numerous agronomically important plant traits. We analyzed various *Arabidopsis* microarray datasets for co-expression of genes putatively encoding transcriptional regulators with those known to be involved in the phytosterol pathway. As a second criterion, we identified statistical enrichment of characterized regulatory elements in the promoters of both phytosterol pathway genes and genes co-regulated with them. Using this approach, we identified several candidate basic helix-loop-helix transcription factors and an over-representation of MYC binding sites in the promoters of both phytosterol biosynthetic and co-regulated genes. The roles of two of the corresponding genes, those encoding regulator of membrane integrity (RMI)1 and RMI2, were further characterized. A phenotypic analysis of exonic T-DNA insertion mutants within our candidate genes and an rmi1 × rmi2 double mutant indicates pleiotropic developmental defects in both leaf and stem tissue. The rosettes of mutant plants exhibit increased membrane permeability in a chlorophyll leaching assay which cannot be attributed to cuticle deficiency. Rosette leaf tissue was analyzed using both targeted and untargeted metabolite profiling strategies. Rosette leaves of the mutants exhibit significant decreases in major membrane phytosterols and substantial alterations of the fatty acid profile. Constitutive over-expression of RMI1 under control of the CaMV 35S promoter revealed an unexpected “twin rosette” phenotype.

P-255

Agrobacterium-Mediated Transformation of Agricultural Varieties of Cassava (*Manihot esculenta* Crantz)

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Cassava is sub-Saharan African's second most important food crop. Our goal is to genetically enhance cassava for increased resistance to various diseases and for enhanced nutritional value of starchy tuberous roots. Transgenic technologies hold promise for stacking such beneficial traits within germplasm already preferred by farmers in this outcrossing, vegetatively propagated crop. While robust *Agrobacterium*-mediated genetic transformation systems are in place for the variety 60444, this cultivar is not longer grown by farmers in West Africa. Improved systems for the genetic transformation of a range of East and West African cultivars have been developed based on transgene integration into friable embryogenic callus and subsequent regeneration of plants. Factors that produced significant differences in frequencies of cassava transformation included duration of *Agrobacterium* co-cultivation, medium composition, type, and concentration of antibiotics in the medium used to control *Agrobacterium*. Information will be presented for the transgenic cultivars recovered.

P-256

Using Antioxidants to Improve Recombinant Protein Production in Transient and Stable Plant-Based Bioproduction Platforms

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With the projected introduction of the first plant-made human therapeutic into the marketplace by the end of 2010, the "pharming" of pharmaceutical proteins and industrial enzymes is gaining recognition as a major production platform alternative for manufacturing recombinant proteins. However, a limited understanding of fundamental mechanisms in play during heterologous protein expression in plants continues to hinder widespread commercialization of plant-made proteins. For example, the significant increase in metabolic activity during transient and stable plant-based recombinant protein expression creates significant cellular stress and results in feedback inhibition that suppresses host plant biosynthesis. The often lower-than-expected active protein yields have been attributed to altered protein translation, protein misfolding in the endoplasmic reticulum (ER) and cytoplasm, deficient protein transfer between organelles, or reduced export of secreted proteins from cells. We have leveraged the transient *Agrobacterium*-mediated system in *Nicotiana benthamiana* to enable rapid evaluation of different strategies for enhancing the recovery of our model recombinant, glycosylated, heterodimeric interleukin-12 proteins. Co-infiltration of a diverse class of antioxidants into the host plant has been shown to be very effective at reducing cellular oxidative stress, significantly enhancing IL-12 protein accumulation *in planta*, and increasing purified IL-12 protein recovery. Our studies are intended to improve ER and cellular health of the host plant, therein "equipping" the plant to better manage stress brought on during recombinant protein production.

P-257

Engineering the RTB Lectin to Impact Bioproduction, Trafficking, and Delivery of Associated Fusion Proteins into Mammalian Cells

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Whether it is vaccine antigens, cytokines, therapeutic proteins, nucleic acids, or bioactive compounds, mucosal surfaces and the cell membrane often represent formidable barriers for presentation of these molecules to their appropriate sites of action. We have been studying the potential of RTB, the non-toxic carbohydrate binding subunit B of ricin, to serve as a carrier to mediate the targeted delivery of payload antigens, effector proteins, and enzyme therapeutics. The biology of RTB uptake and

trafficking in mammalian cells is uniquely suited to mucosal delivery of antigens or therapeutic proteins. Heterologous plant-based bioproduction strategies ensure that high levels of lectin-active RTB can be produced in the absence of contaminating RTA toxin. We have demonstrated that tobacco-synthesized RTB retains its carbohydrate binding specificity and mammalian cell uptake and trafficking characteristics with payloads ranging from small molecules (e.g., fluorescein) to proteins exceeding 70 kDa. Computational protein modeling, transient expression in *Nicotiana benthamiana*, and mammalian cell uptake studies are being used to identify factors impacting plant-based bioproduction levels, lectin binding activity, and trafficking of RTB and RTB fusions to intended target cells and compartments within cells. Modifications of RTB, including C-terminal ER retrieval sequences, are being tested for their ability to enhance the accumulation of RTB fusions in specific organelles in both the plant production host and the mammalian cell target. These studies have significant implications for plant-based vaccine production that include greater vaccine efficacy and the potential to specifically bias immunity toward cell- or antibody-mediated responses.

P-258

Plant-Based Production Platform Compatible with Veterinary Vaccine Applications for Producing Bioactive Avian Interleukin-12

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Improved animal health is an important aspect in controlling zoonotic diseases such as influenza that transfer from animals to humans and continue to threaten pandemic outbreaks. Plants offer a low-cost production and delivery platform for veterinary vaccine reagents to target avian and animal hosts of these diseases. Using a rapid *Agrobacterium*-mediated tobacco plant-based transient expression system, we have produced purified chicken interleukin-12 (ChIL-12), a potent immune stimulatory protein with potential applications for veterinary vaccines and an immune health therapeutic in controlling bird flu. We have attained high yields of this complex, heterodimeric glycoprotein and confirmed the dominant product as a functional, 70-kDa single-chain ChIL-12. Signature bioactivity of this product was validated both by in vitro splenocyte assays and in vivo studies in 1-d-old chickens as well as turkeys. In addition to production of a ChIL-12 polyclonal, paired anti-ChIL-12 monoclonal antibodies against the ChIL-12 p70 and p40 subunit have been produced enabling the development of a ChIL-12 ELISA for the poultry research community and industry. Finally, stable transgenic *Nicotiana tobaccum* expressing ChIL-12 will be used to initially test seed-based delivery for poultry vaccines and immunotherapeutics. Introduction of ChIL-12 gene constructs into flax will support of our long-term goal of IL-12 for adjuvanted vaccination and therapeutic strategies to facilitate storage and delivery to both domesticated and wild avian species. Our results highlight the capacity of plants to provide a rapidly scalable source of very complex mammalian/avian cytokines with potential to powerfully impact livestock health management.

P-259

Efficient Production of Composite Soybean Plants via Disarmed *Agrobacterium* SSP

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All of the published protocols for production of composite plants rely on wild-type *Agrobacterium rhizogenes*, which induces hairy roots from hypocotyl/seedling explants either in vitro or ex vitro. Although hairy roots and/or composite plants with hairy roots have been used as an effective tool for transgene evaluation, some negative effects such as inconsistent transgene expression and abnormal phenotypes due to elevated auxin levels were often reported. To circumvent these problems, we have developed a simple, efficient, and rapid method for production of composite soybean plants with "non-hairy" transgenic roots via disarmed *Agrobacterium tumefaciens* AGL1 or disarmed *A. rhizogenes* SHA17 harboring a binary vector. Soybean hypocotyl/seedling explants were inoculated with *Agrobacterium* ssp. and co-cultured on media containing either 1% agar or MS medium supplemented with cytokinins for 5 to 7 d. Explants were then selected on medium with a selective agent for generation of transgenic roots. Composite plants with healthy root systems can be transferred to soil within 4 wk after inoculation. Transgenic roots and/or composite plants can be used for various root biology studies.

P-260

Two Interacting Half-ABC Transporters Are Essential for Arbuscular Mycorrhizal Symbiosis

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Most terrestrial vascular plants form symbiotic association with arbuscular mycorrhizal (AM) fungi, which help plants to gain access to mineral nutrients from soil. The major site of nutrient exchange between the two symbionts is in root cortical cells, where AM fungi form branched hyphae called arbuscules. Genes essential for arbuscule development are unknown. A *Medicago truncatula* mutant, "stunted arbuscule" (str), was identified from an ethyl methanesulfonate mutant population. str is impaired in arbuscule development and loses the AM symbiosis associated phosphorus benefit. STR encodes a half molecule ATP-binding cassette (ABC) transporter that represents a distinct clade in the ABCG sub-family. Putative orthologs of STR are highly conserved throughout the vascular plants but absent from *Arabidopsis*, which does not form AM symbiosis. A second "half-molecule" ABC transporter STR2 shares a similar phylogenetic profile as that of STR. STR2 shows an identical expression pattern to STR and is likewise essential for arbuscule development. STR and STR2 interact, as shown by bi-molecular fluorescence complementation, and both are located in the peri-arbuscular membrane where their activities are required for arbuscule development.

P-261

Cloning and Characterization of Genes Involved in ABA- and GA-Biosynthesis in *Camelina*

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The seeds of many plant species undergo dormancy before they germinate. The hormones abscisic acid (ABA) and gibberellin (GA) are known to regulate seed dormancy and germination, at least in part, by the balance between them in seed tissues. The summer annual oilseed crop, *Camelina sativa* is closely related to the model species, *Arabidopsis thaliana*, and is being used for studies of seed biology, plant nutrition, biofuels, and among other studies. *Camelina* seeds do not exhibit dormancy. To understand the mechanisms of germination in *Camelina* seeds, orthologs of *Arabidopsis* genes related to ABA and GA biosynthesis have been cloned form *Camelina*. Phylogenetic analysis of the sequences of these genes with ortholog sequences in other plant species revealed the highly conserved regions and ~86% to 96% amino acid sequence similarity with *Arabidopsis* genes. RT-PCR was used to monitor the accumulation of transcripts of these genes in leaves, roots, stems, inflorescences, flowers, in early development, mid-maturation, and mature seeds and in imbibed seeds of *Camelina*. Results revealed that ABA-related biosynthesis gene was expressed mainly in seeds, including developing seeds at early, mid, and maturation stages and imbibed seeds. In contrast, expression of GA-related biosynthesis gene was detected in all organs tested. These results and other data from expression analysis will be presented. Studies of transgenic *Camelina* plants that contain the cognate promoter confirmed that the transgene was detectable in seeds, suggesting that *Arabidopsis* homologous gene was also activated in *Camelina* seeds. These studies provide insight into the mechanisms regulating seed development, dormancy, and germination in *Camelina*.

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Role of Jasmonate Signaling During *Pseudomonas syringae* Pathogenesis in Tomato

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The *Pseudomonas syringae* virulence factor coronatine (COR) is a functional analog of the isoleucine conjugate of jasmonate (JA-Ile), an endogenous plant signaling molecule, and promotes pathogen virulence by modulating JA signaling within the host. For example, both *Arabidopsis thaliana* and tomato mutants impaired in JA signaling (e.g., *coi1* or *jai1* mutants) exhibit reduced susceptibility to *P. syringae*. Both COR sensitivity and disease susceptibility to *P. syringae* in *A. thaliana* require the activity of the JIN1/MYC2 transcription factor. It is unclear whether JA responses in other plants, such as tomato, are mediated

through the same signaling components identified in *A. thaliana*. We are examining the role of a tomato *JIN1*/*MYC2* homolog in jasmonate signaling and susceptibility to *P. syringae* and other pathogens by generating and characterizing transgenic tomato lines that are silenced for this gene (RNAi: MYC2). We have obtained 14 independent transgenic tomato lines and have begun to characterize them at the molecular and genetic level to verify the presence of the RNAi T-DNA. Preliminary analysis of T1, T2, and T3 plants of first three transgenic lines revealed that one line exhibits reduced expression of the tomato *MYC2* homolog in response to wounding and *P. syringae* infection. Further, this line appears to have reduced susceptibility to *P. syringae* infection, indicating an important role for the tomato MYC2 in mediating susceptibility to *P. syringae* in tomato. Future analysis of this and the remaining transgenic lines will enable us to further investigate the role of JA signaling and disease susceptibility in tomato and may lead to the development of tomato lines with reduced susceptibility to both *P. syringae* and necrotrophic pathogens.

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Brachypodium distachyon as a Model System for Studying Genes Involved in Cell Wall Synthesis

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Brachypodium distachyon was proposed as new model plant for grasses because of its small genome and its phylogenetic position between rice and Triticeae crops. Since, a range of genetic and genomic resources has been developed and the whole genome sequence is now available. We use *Brachypodium* as a model system for studying how to modify grass crops for increased biofuel production made from lignocellulosic cell walls. We are involved in developing many resources for *Brachypodium*: TILLING collection, natural accessions, different methods of phenotyping, and tools and method for gene transfer via *Agrobacterium tumefaciens*. Some lignin mutants from the monolignol biosynthesis pathway were already identified by phenotyping and TILLING, and their functional analysis is underway notably through complementation by overexpression of concerned gene in mutant background.

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Trans-epithelial Electrical Resistance (TEER) Measurement on In Vitro Cutaneous and Ocular 3-D Models: a Sensitive Parameter Needing Standardized Conditions

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Two major routes are involved in tissues barrier function (BF), thus driving small molecules flux. While transcellular route of chemicals depends on their lipophilicity, paracellular route takes place into the intercellular spaces and is under epidermis tights junctions (TJs) control. Together with the proteo-lipidic complex of the cornified layers, TJs are important effectors of tissues BF. When TJs are not mature or damaged, hydrophilic compounds (electrically charged) are able to diffuse from the upper layers to the basal one (for both skin epidermis and eye corneal epithelium). Consequently, modifications of BF-linked structures can influence the tissue trans-epithelial electrical resistance (TEER). Steady state and quality of the BF of in vitro models could be detected by using TEER measurements. This parameter could give some information about the tissue integrity modifications after chemical treatments. We studied three commercially available in vitro human epithelial engineered models: two epidermis models (EpiSkin large model, RHE small) and a corneal epithelial model (RHCE) supplied by SkinEthic Laboratories. A comparison between models on TEER measurements was made before and after surfactant treatment. TEER was measured by using a specific epithelial tissue Volt-Ohmmeter. TEER was monitored during a time period in order to define acceptable and standardized steps where TEER could be regarded as stable prior to measurements. TEER assays were carried out after topical surfactants treatments: SLS-treated EpiSkin/L or RHE small and Triton

X-100-treated RHCE. Results showed a sharp TEER decrease since the lowest concentrations tested reaching low resistance values at the highest surfactant concentrations. Complementary cellular viability assays showed clear effects only at the highest surfactant doses. TEER standardized method could be a useful endpoint for quality control assessments and comparisons between models. In addition, it could be used as a suitable easy-to-use tool, to describe BF, complementary to TEWL and other permeation studies.

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Testing Efficacy of Artificial MicroRNA to Control Cassava Brown Streak Disease

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Cassava brown streak disease (CBSD) is caused by the cassava brown streak virus (CBSV) of genus *Ipomovirus* and family *Potyviridae* with a monopartite positive ssRNA. CBSD is a major constraint to cassava production in East and Central Africa. To date, very few genetic sources of resistance to the disease are known. More recently, in addition to RNA interference (RNAi), artificial microRNAs (amiRNAs) have been employed to control plant viruses. MicroRNAs (miRNA) are 20–24 nt long non-coding RNAs that are highly conserved across plant and animal species involved in gene regulation and development. MiRNAs are expressed as precursors of ~263 nt and are further spliced to produce ~21 nt miRNAs. In this study, 21 nt of the *Arabidopsis* pre-mi159a were replaced with 21 nt sequences selected across the CBSV genome, generating 11 amiRNA constructs targeting different CBSV genes (P1 {CBSV-Ug and CBSV-Tz}, P3, CI, NIb, CP, and 3′-UTR). The modified precursors were then sub-cloned in a shuttle vector CGT11003-I and subsequently cloned into a binary vector AKK1420 with an RNAi cassette targeting green fluorescent protein, as an internal silencing control. Transient studies of these amiRNA constructs in transgenic *Nicotiana benthamiana* (16C) using *Agrobacterium* (GV3001) showed that Tz P1, Ug_P1 Tz, NIb, CP, and UTR constructs expressed miRNAs specific to the target nucleotides of CBSV. Transient protection assay showed varied levels of resistance to the homologous virus roughly corresponding to the level of siRNA accumulation using Northern analysis. In the future, the best amiRNA constructs will be used to transform *N. benthamiana* and cassava crop plant to establish if amiRNA technology can offer an additional efficient method to control the raging CBSD.

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Microalgal Oralvaccines for Fish

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We describe here the use of *Chlamydomonas reinhardtii* to deliver an antigen against infectious hematopoietic necrosis virus (IHNV) to salmon and trout. IHNV is the causative agent of an acute systemic disease inflicting high mortality, especially in young cultured and wild salmonid fish. The IHNV glycoprotein (G) protein has been identified as the antigen responsible for inducing protective immunity. We have used 12-mer phage display library to identify putative antigenic epitopes on the G protein. Based on our analysis of the phage sequence, we have identified eight putative G protein antigenic epitopes. These epitopes have been fused to the p57 protein from *Renibacterium salmoninarum*, which functions as a strong immunogen. The epitopes fused to the p57 protein were transformed into *C. reinhardtii* chloroplast, and p57 expression was confirmed by Western blot. These transgenic *Chlamydomonas* will be used to immunize the target fish species against IHNV. The vaccine will be delivered to the target species by either incorporating the transgenic *Chlamydomonas* in the diet or by immersing the fish in a culture of transgenic *Chlamydomonas* (immersion vaccination). Mucus and sera collected from treated fish will be used to screen Western blots of wild-type and transgenic algae expressing the IHNV-G fusion protein antigens to detect immune response. Individuals showing the desired immune response will then be used for conducting IHNV challenge trials to determine the efficacy of these vaccines.

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Non-specific Phospholipase C NPC4 Promotes *Arabidopsis* Response to Abscisic Acid and Tolerance to Hyperosmotic Stress

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Phospholipases are enzymes that hydrolyze phospholipids. In terms of the position of bond hydrolysis, phospholipases are classified into four major types: phospholipase C (PLC), phospholipase D, phospholipase A_1 (PLA_1), and phospholipase A_2 (PLA_2). PLC hydrolyzes phospholipids at the first phosphodiester bond, producing diacylglycerol (DAG) and a phosphorylated head group. Based on substrate specificity, PLC is divided into two distinctively different groups: phosphoinositide-specific phospholipase C and non-specific phospholipase C (NPC). There are six members of the NPC family in *Arabidopsis*, designated NPC1 through 6 that bear sequence homology to bacterial phosphatidylcholine hydrolyzing PLC. Here we show that knockout of the NPC, NPC4, results in a decrease in DAG levels and compromises plant response to abscisic acid (ABA) and hyperosmotic stress response. NPC4 hydrolyzes common membrane in a calcium-independent manner with distinguishable substrate preferences, producing DAG and a phosphorylated head group. NPC4-KO plants display reduced sensitivity to ABA-mediated stomata movement, seed germination, and root elongation, whereas NPC4-OE plants have increased sensitivities. In contrast, NPC4-KO plants display increased sensitivities to salinity and water deficiency, whereas NPC4-OE plants display increased tolerance and biomass production. The expression of ABA responsive genes is lower in NPC4-KO than wild-type plants. The addition of DAG or PA restores the ABA response of NPC4-KO to that of wild type, and the addition of a DAG kinase inhibitor does not rescue NPC4 phenotype. These data suggest that NPC4 positively modulates ABA signaling through its lipid product DAG that is converted to PA and NPC4 promotes plant growth under osmotic stress.

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Molecular Genetics of Soybeans with the High Oleic Acid Trait

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The alteration of fatty acid profiles in soybean [*Glycine max* (L.) Merr.] to improve soybean oil quality is an important and evolving theme in soybean research to meet nutritional needs and industrial criteria in the modern market. Soybean oil with elevated oleic acid is desirable because this monounsaturated fatty acid improves the nutrition, flavor, and oxidative stability of the oil. The objective of this work was to create the high oleic acid trait in soybeans by identifying and combining mutations in two omega-6 fatty acid desaturase genes, FAD2-1A and FAD2-1B. Soybean plant introduction (PI) germplasm lines that contained elevated oleic acid content in the seed oil were characterized for the sequence and genetic association of their FAD2-1A and FAD2-1B alleles. Three polymorphisms found in the FAD2-1B alleles of two soybean lines resulted in missense mutations. For each of the two soybean PI lines, there was one unique amino acid change within a highly conserved region of the protein. The sequence polymorphisms were developed into highly efficient molecular markers for the mutant alleles. The mutant FAD2-1B alleles were associated with an increase in oleic acid levels, although the FAD2-1B mutant alleles alone were not capable of producing a high oleic acid phenotype. When existing FAD2-1A mutations were combined with the novel mutant FAD2-1B alleles, a high oleic acid phenotype was recovered only for those lines which were homozygous for both of the mutant alleles. The high oleic acid soybean germplasm developed contained a

desirable fatty acid profile, and it was stable in multiple environments. The resources described here for the creation of high oleic acid soybeans provide a framework to efficiently develop soybean varieties to meet changing market demands.

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Trehalose Accumulation in Rice, Maize, and Wheat Plants Confers High Tolerance Levels to Different Abiotic Stresses

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Trehalose is a nonreducing disaccharide of glucose that functions as a compatible solute in the stabilization of biological structures under abiotic stress in bacteria, fungi, and invertebrates. With the notable exception of the desiccation-tolerant "resurrection plants", trehalose does not accumulate to significant levels in the vast majority of plants, in spite of the proliferation of plant trehalose pathway genes. However, recent studies show that trehalose metabolism is of immense importance in plant biotechnology, and its manipulation has great potential in crop improvement. Here we report our results on stress-inducible overexpression of *E. coli* trehalose biosynthetic genes as a fusion gene in rice, maize, and wheat plants for the purposes of improving drought stress tolerance and other agronomic traits. The fusion gene has the advantages of necessitating only a single transformation event and a higher net catalytic efficiency for trehalose formation. Compared with non-transgenic plants, several independent transgenic lines exhibited sustained plant growth and less photo-oxidative damage under drought-stress conditions. The observation that peak trehalose levels remain well below 5 mg/g fresh weight indicates that the primary effect of trehalose is not as a compatible solute. Rather, increased trehalose accumulation correlates with higher soluble carbohydrate levels and an elevated capacity for photosynthesis under both stress and non-stress conditions, consistent with a suggested role in modulating sugar sensing and carbohydrate metabolism. These findings demonstrate the feasibility of engineering cereal plants for increased tolerance of abiotic stress through stress-dependent overproduction of trehalose.

P-270

Effect of Different Pawpaw Cultivars on In Vitro Proliferation of Lung Cancer

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Pawpaw (*Asimina triloba* [L.] Dunal) is a temperate fruit tree belonging to the Annonaceae family and is native throughout the eastern half of the USA. Previous studies have indicated that pawpaw twigs and seeds inhibit the proliferation of several different types of cancer, including prostate and breast cancer. This study was conducted to determine the effect of fruit extract from five different pawpaw cultivars (Wells, 7–90, Susquehanna, Wilson, and Sunflower) on human lung cancer proliferation in male and female cell lines. Pawpaw juice was extracted from each of the five varieties, diluted to 400 mg/mL, and filter-sterilized. These were aqueous extracts, not alcohol extracts, as used in other studies of pawpaw's bioactive constituents. Human lung cancer cell lines H1793 and H2342, female and male respectively, were cultured in vitro and treated in triplicate with six concentrations of pawpaw extract ranging from 0 to 100 mg/mL for each cultivar. The entire experiment was repeated five times. In vitro proliferative assays were conducted at 48, 72, and 120 h post-treatment. The female lung cancer line appeared to be more sensitive to pawpaw extracts than the male cell line, but there was a cultivar effect as well. The cultivars Wilson and Wells had the least impact on cancer proliferation in male and female lines, while Sunflower and Susquehanna were the most effective at reducing cancer proliferation. Up to an 80% decrease in cell proliferation was observed. Decreases in cell proliferation appear to be both dose and time dependent. These results warrant further investigations for the use of pawpaw as a treatment for lung cancer.

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Targeted Gene Integration by Co-expression of Fluorescent Visual Markergenes GFP and DsRed in Transgenic Cassava

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Transgenic technology provides an effective means of modifying cassava (*Manihot esculenta* Crantz) for improved agronomic traits. However, random unpredicted integration of transgenes makes genetic modification relatively inefficient and time-consuming. This study is aimed at demonstrating proof of concept for zinc finger nuclease technology based on induced double-stranded breaks within highly specific DNA sequences. To develop this technology in cassava, our goal is to target an existing transgenic plant expressing a single copy of green fluorescent protein (GFP) and use zinc finger technology to integrate a novel DsRed sequence within the former transgene. Successful targeted events will thus be recognized as a switch from GFP to DsRed expression. As a first step toward development of this capacity, we assessed our ability to co-express and visualize both the GFP and DsRed visual marker proteins within the same tissues. To prove that recovery of DsRed expressing tissues is possible in cassava, friable embryogenic callus (FEC) was generated from cultivar 60444 and transformed with an ER-targeted version of DsRed driven by 35S promoter using *Agrobacterium* strain LBA4404. A total of 67 callus and 42 plant lines were recovered on selection medium while 55 callus and 28 plant lines expressed DsRed. Southern blot analysis was used to identify two previously produced highly expressing GFP plants with single copy integration. FEC was generated from these plants, the DsRed transgene was introduced by biolistics, and co-visualization of both fluorescent signals was detected. The application of DsRed in monitoring cassava transformation provides an alternative to GFP, an important first step in developing proof of capacity for targeted gene integration in cassava.

P-272

Identification of Transcription Factors Involved in Secondary Wall Thickening in *Medicago truncatula*

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Genes involved in secondary wall thickening have been shown to be regulated at the transcriptional level in *Arabidopsis*. To discover regulatory genes during *Medicago truncatula* stem development, we performed microarray and high throughput quantitative real-time PCR analysis. Our results showed that expression of some transcription factors co-clusters with expression of cell wall synthesis genes. Among these transcription factor genes, zinc finger, MYB, homebox, b-ZIP, and NAC domain family genes were over-represented. We also found some signaling pathway genes, such as receptor-like kinase and MAP kinase, which might also play roles in secondary wall development. To functionally characterize these candidate genes, we are employing in vitro analyses and reverse genetics approaches. Our long-term goal is to use transcription factor engineering to cause ectopic lignification in bioenergy crops, thereby increasing their energy content for gasification.

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Fusions of TAL Effectors to the FokI Endonuclease Confer Site Specificity in DNA Cleavage

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Transcription activator-like effectors (TALEs) are a class of DNA binding proteins used by plant pathogenic bacteria to colonize hosts. Upregulation of host susceptibility genes occurs when specific DNA sequences in the plant promoters are bound by a TALE protein. Recent advances in understanding the DNA binding specificity of TALEs reveal a novel DNA recognition mechanism and provide a basis for predicting specific TALE DNA associations. All TALEs contain a domain composed of a varying number of 33–35

amino acid (aa) near perfect repeats, in which the 12th and 13th residues are variable. The polymorphic aa residues in each repeat confer binding to specific DNA nucleotides. To test whether TALEs could be used to create site-specific nucleases, we fused the cleavage domain of the FokI endonuclease to the TALE DNA-binding domain. Three different types of TALE-FokI fusions were tested for cleavage activity in a yeast-based assay previously designed to test function of zinc finger nucleases. In this assay, two TALE recognition sites are cloned between a duplicated lacZ gene. TALE-nuclease (TALEN) binding of the recognition sites mediate cleavage, allowing recombination between the repeated sequences and restoring lacZ function, which can be measured in standard assays. The TALENs were found to cleave their DNA targets at predicted sites, but not at nonspecific sites, demonstrating activity as well as specificity. Fusion proteins with different TALE DNA-binding domains showed different levels of cleavage activity and different optimal spacer lengths separating the two TALEN recognition sites. To our knowledge, this work represents the first demonstration of a functional TALE-nuclease fusion protein and has potential for future genome engineering applications.

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Transgenic Strategies to Delay Onset of Post-harvest Physiological Deterioration (PPD) in Storage Roots of Cassava (*Manihot esculenta* Crantz)

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Cassava (*Manihot esculenta* Crantz) is an important staple crop for countries in sub-Saharan Africa, Asia, and Latin America. It is a primary food source for more than 250 million Africans and a significant source of calories in the diet of nearly 600 million people worldwide. The industrial utilization of the crop includes in the starch, animal feed, and fuel ethanol industries. However, the industrial utilization and marketing of cassava is limited by a serious constraint of short shelf life of its roots, a result of rapid post-harvest physiological deterioration (PPD). PPD begins 24 h after harvest and renders the roots unpalatable and unmarketable within 72 h. The short shelf life affects cassava value-added chains because it increases losses during processing and limits access to markets farther away from production sites. Various factors have been linked to the etiology of PPD; however, no solution has yet been provided. We explored a novel transgenic strategy to delay PPD in storage roots of seven cassava events. Our studies suggest that genetic improvement of cassava may provide an avenue to combat the constraints imposed by PPD.

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Zinc Biofortification of Cassava Tubers

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Biofortification is the process of enriching the nutrient content of staple food crops as a means of ameliorating micronutrient malnutrition worldwide. Cassava (*Manihot esculenta*) is a major staple food crop for more than 300 million people in Africa, but it lacks sufficient amounts of important micronutrients such as vitamin A, iron, and zinc. Intake deficiency of zinc is especially a widespread nutrition and health problem in the developing countries. The biofortification of cassava tubers using transgenic approaches is found to be more effective than other strategies such as food fortification or nutrient supplements. Although the mechanisms of Zn translocation in cassava plants are not fully understood, it has recently received increased interest. For efficient nutrient accumulation in cassava storage roots, both uptake and storage are essential. To achieve enhance zinc uptake and accumulation in cassava, *AtZIP1* (a Zn transporter that is localized to plasma membrane) under the control of the A14-root epidermal promoter and *AtZAT1* (a storage Zn protein in the vacuole that is localized in the vacuolar membrane) under the control of root-specific patatin promoter was introduced into cassava via *Agrobacterium*-mediated transformation. These constructs were also introduced into tobacco for a rapid assessment of zinc accumulation. Eleven independent transgenic lines were obtained in tobacco, and 71 independent transgenic lines were obtained in cassava. Molecular analyses to identify the presence of genes and ICP analysis to measure mineral concentrations in transgenic shoots and roots are in progress. We will present hypothesis of mechanisms for accumulation of zinc in storage roots of the cassava plant.

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Genetic Transformation of Cassava (Nwibibi)—a Farmer Preferred Cultivar in Nigeria

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Cassava is an important food security crop in the tropics and subtropics. Nutritionally, the low protein, vitamin, and essential mineral content of cassava are a source of concern for people whose diet is based mainly on this plant. In addition, the crop is susceptible to viral and bacterial pathogens that limit yield through sub-Saharan Africa. Biotechnology offers a powerful tool to overcome these limitations and complements efforts to improve this out-crossing vegetatively propagated plant via traditional breeding. In an effort to develop genetic transformation protocols for Nigerian farmer-preferred cultivars, the bacterial strain LBA4404 containing a pCAMBIA2300-based binary vector carrying the *npt*II gene as selectable marker and the green fluorescent protein (GFP) as a visual marker were used to transform friable embryogenic callus (FEC) of the cultivar Nwibibi. Subsequently, selection of transformed tissues on medium supplemented with cefotaxime and paramomycin resulted in the recovery of 169 GFP-expressing lines of FEC from which embryos and their subsequent 15 plants were regenerated. Comparatively, cv. 60444 embryos/plants were more robust in appearance and growth than those of Nwibibi cv. that exhibited slow growth with moderate establishment. Such a breakthrough signals the possibilities for the modification of the cultivar for enhanced resistance to disease and to biofortification through biotechnological methods.

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Evaluating Root Specific Promoters for Expression in Cassava Tuberous Roots

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Cassava (*Manihot esculenta* Crantz) is an important crop for over 500 million people worldwide. Its main constraints to production are viruses, root quality, post-harvest deterioration, and the presence of cyanogenic glycosides. To enhance its productivity and utilization, genetic engineering technology is being considered by scientists. A major drawback in incorporating new traits into cassava using biotechnology is the limited number of promoters that drive expression of transgenes in its tuberous roots. The aim of this work is to evaluate a number of root-specific promoters for gene expression in the tuberous roots of cassava. The promoters under this study include isoflavonone synthase (IFS), patatin, granule-bound starch synthase (GBSS), Rol B, and sporamin. Constructs have been made by positioning the β-glucuronidase (GUS) reporter gene under the control of each of these promoters at the 5′-flanking sequence. These constructs have been used to transform the West African variety 60444. Transgenic plants have been regenerated for some of the constructs. Preliminary histochemical GUS analysis has shown strong expression in the tuberous roots for patatin, GBSS, and IFS. Expression was more intense in the vascular areas of xylem, but patatin and GBSS also showed intense expression in xylem parenchyma. Expression patterns in the stems, petioles, and midrib sections were similar for all the promoters tested, with the strongest expression occurring in xylem and phloem tissues. GUS expression in transformed plants will be quantified using 4-methylumbelliferyl β-D-glucuronide assay and quantitative RT-PCR. It is expected that this work will yield preliminary results that will guide future work on cassava genetic modification.

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Identifying the Gene Expression Profile of Tuberization in Cassava (*Manihot esculenta* Crantz)

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Cassava has the unique capacity to store large amounts of starch in specialized root storages. To improve long-term cassava productivity, it is important to better understand the molecular mechanisms underlying initiation and development of these organs. To this end, histology, gene expression, and cassava transformation technologies are being used. Histological studies have been used to define the early stages of starch accumulation in specific cells of cassava roots at the earlier stages of the tuberization process and starch accumulation and sucrose transportation systems in tuberizing roots. Cassavas have been evaluated by studying transition of the vascular bundle anatomy. At the beginning of tuberization, xylem parenchyma is expanded to prepare for storing starch synthesis from sugars translocated from the stem via the phloem. The study of differential gene expression between tuberizing and non-tuberizing cassava roots during root development is considered a promising method to identify the unique sets of genes and/or pathways involved in cassava tuberization. Using microarray technology to study gene expression of leaves, stem, fibrous roots, and storage roots tissues, root-specific genes and putative promoters associated with the tuberization process have been identified for future study. Ultimately, this research is expected to facilitate the genetic improvement of cassava through manipulation of genes involved in key pathways regulating tuberization. In addition, it is also expected to identify new root-specific promoters for future genetic improvement of cassava.

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Functional Validation of Two Resistance Gene Candidates *RXam1* and *RXam2* to Cassava Bacterial Blight Employing RNAi

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Cassava bacterial blight (CBB) is one of the most important diseases affecting Cassava (*Manihot esculenta* Crantz) and is caused by the gram-negative bacteria *Xanthomonas axonopodis* pv. manihotis (Xam). Previous studies have identified two candidate genes that might confer resistance to CBB and have been named *RXam1* and *RXam2*. Mapping studies have demonstrated that *RXam1* and *RXam2* co-localized with QTLs that explain 13% of the resistance to Xam strain CIO136 and 62% of the resistance to Xam strain CIO151, respectively. *RXam1* encodes a protein with serine/threonine kinase and leucine-rich repeats (LRR) domains. *RXam2* codes for a protein containing a nucleotide binding domain and a LRR domain, which are domains typically present in proteins conferring resistance. In order to validate the function of these genes, we used interference hairpin RNA (ihpRNA) to silence the expression of these genes in cassava resistant plants and we are evaluating the loss of resistance in the transgenic silenced plants. We have cloned fragments of approximately 500 pb from *RXam1* and *RXam2* in pENTR-TOPO, a vector compatible with the gateway® system and transferred these inserts to pHellsgate12, an ihpRNA silencing vector developed by CSIRO that is compatible with gateway® system and contains the sequences sense and antisense spliced by the PDK intron that will produce gene silencing mediated by dsRNA. These constructs were transformed in *Agrobacterium tumefaciens* and subsequently in cassava plants cultivars TMS60444 (transformation model) and MBRA685 (highly resistant cultivar) to silence these candidate resistance genes. Currently we are conducting the molecular evaluation of putative transgenic plants, and further, we will evaluate the phenotype transgenic plant expecting to see an increased susceptibility to *Xam.*

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In Vitro and In Vivo Regulation of Antioxidant Potential of Fenugreek

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Plants have been the major source of nutraceuticals and phytoceuticals. Oxidative damage is induced by reactive

oxygen species, which can affect a specific molecule or the entire organism. Different plant antioxidants are difficult to measure separately due to the complexity of oxidation processes; therefore, a multi-method approach was necessary to assess the antioxidant activity. *Trigonella foenum-graecum* (fenugreek) of family Fabaceae was subjected to evaluation of anti-radical efficiency (AE), and seeds were used to raise the callus culture on MS medium supplemented with BAP/IBA (3:3 mgl^{-1}). The GI was found to be maximum in 6-wk-old tissue. Various plant parts (seeds and leaves) and calli were powdered, and different fractions (methanol fraction, hexane fraction (HF), ethyl acetate fraction (EAF), dichloromethane fraction) and total phenolic content as determined were the highest in EAF (130.43 GAE/mg of extract). Non-enzymatic AE were measured by different methods viz., free radical scavenging activity by DPPH, haemoglobin glycosylation assay, nitric oxide scavenging activity (NOSA) and lipid peroxidation (LPO) assay. HF of seeds (48.16) and EAF of leaves (58.03) gave maximum AE using DPPH and haemoglobin glycosylation assay, respectively. Fresh tissues (presoaked seeds, leaves, callus) of fenugreek were evaluated for NOSA, LPO, catalase, superoxide dismutase (SOD) and peroxidase assay. NOSA showed maximum activity in soaked seeds (14.66%); LPO content were the highest in leaves (5.30 $nmol ml^{-1}$). Maximum activity was of catalase in leaves (0.037 $\mu mol\ g^{-1} fw min^{-1}$), SOD in callus tissue (1.0067 $U ml^{-1}$) and peroxidase in soaked seeds (6.14 $\mu mol g^{-1} fw min^{-1}$). The isozyme profiling of antioxidant enzymes showed negative bands of SOD (EC.1.15.1.1), catalase (EC.1.11.1.6) and brown bands of peroxidase enzyme (EC.1.11.1.7). The effect of various elicitors on AE of the fresh tissues and callus using different treatment doses (0.025, 0.05, 0.075 and 0.1 mM) of salicylic acid (SA) and sodium nitroprusside after 6, 12, 18 and 24 h intervals were analysed separately. LPO increased in SA (0.05 mM) treatment after 12 h as compared to control. The highest SODA was observed in callus after 24 h at 0.025 mM and NOSA after 6 h in 0.05 mM. The activity decreased with increasing time of various treatment doses. There was no variation on SOD and catalase activity.

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Expression Profile of Soybean Promoters for Three Genes Relating to Embryogenesis

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For the production of transgenic plants, each gene of interest should be regulated by a promoter which provides the desired gene expression profile required. Although transgenic soybean (*Glycine max*) is grown on more acreage than any other transgenic crop, few soybean-specific promoters have been identified and characterized. In this study, promoters from specific genes of three distinct families, *actin*, *40S ribosomal protein S11*, and *heat shock protein 90-like protein*, were cloned to regulate gfp expression. After promoter constructs were reintroduced into soybean, transgenic plants were characterized. These three promoters were selected based on expression during early embryogenesis. Tissues from green fluorescent protein (GFP)-positive progeny of regenerated plants were sectioned and analyzed for GFP expression. The actin promoter displayed expression in root tips, cotyledons, and root meristems of seedlings and in the cambium and stem and leaf vascular tissues in plantlets. In contrast, expression of GFP with the S11 promoter and HSP90-like protein promoter was primarily observed in root tissues but was also seen in shoot apices. GFP expression from all three promoters was also observed in embryogenic tissue and in induced embryos. Southern hybridization analysis confirmed the presence of the introduced *gfp* gene in all of the transgenic plants. This research provides three additional soybean promoters that can be used for the regulation of gene expression in transgenic soybean.

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Diverse Metabolic Pathways Employed by the Green Sulfur Bacterium *Chlorobaculum tepidum*

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The anoxygenic green sulfur bacteria (GSBs) are known to assimilate CO_2 autotrophically through the reductive

tricarboxylic acid (RTCA) cycle. Some organic carbon sources (acetate, propionate, or pyruvate) can be assimilated during the photoautotrophic growth of GSB, although CO_2 is also required for growth. It is not clear why CO_2 is needed for incorporating organic carbon sources and how the organic carbons are assimilated. Also, fluoroacetate (FAc), an analog of acetate, has been reported to repress the autotrophic growth of the GSB *Chlorobium limicola*, but the mechanism of inhibition of FAc for the growth of GSBs has not been understood. In this report, we use multiple approaches to probe the carbon flux during autotrophic and mixotrophic growth of the GSB *Chlorobaculum tepidum*, including investigate the roles of FAc. Our studies suggest that the inhibition of FAc in *C. tepidum* does not arise from the RTCA cycle and that diverse metabolic pathways are employed by *C. tepidum*.

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Development of Efficient Micropropagation and Cryopreservation Protocols for *Thymus* Germplasm

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Thymus is one of the most important genus of the *Lamiaceae* family that serves as a natural source of phenolic oils, oleoresins and fresh and dried herbs, used all around the world. However, natural *Thymus* populations are far from being adequate to support such a great and even growing demand for its products. Interests focusing mainly on few selected chemotypes lead to the loss of the others in nature, which indeed should be preserved to make available the access to a wide range of genetic diversity to be used as a possible source of natural products. Recently developed biotechnological approaches provide unique alternatives for fast multiplication and long-term maintenance of such germplasm. Among these, 'in vitro micropropagation' enables propagation of plants under controlled environmental conditions, while 'cryopreservation' make available the cost-effective long-term storage of their germplasm at ultra-low temperatures. At such temperature, almost all the biological reactions in cells are hampered, paving the way to store the material for theoretically unlimited periods of time. This study was conducted to develop such protocols for *Thymus* germplasm. Optimizations for micropropagation involved use of cytokinins, auxins, silver nitrate and gibberellic acid for the induction of multiple shoots. Best shoot proliferation (97%) was obtained when MS medium was supplemented with 1 mg l^{-1} kinetin and 0.3 mg l^{-1} GA_3. Rooting was easily obtained on MS medium, either hormone-free or supplemented with auxins. Cryopreservation methods applied were (a) PVS2 vitrification, (b) encapsulation–vitrification and (c) droplet-freezing. All three methods showed to be effective to induce cryotolerance of thyme shoot tips, obtained from in vitro propagated plantlets.

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Insecticidal Properties of *Sclerotinia sclerotiorum* Agglutinin and Interaction with Insect Tissues and Cells

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During the last two decades, it was shown that plant lectins have high anti-insect potential. Although the effects of most lectins are only moderately influencing development or population growth of the insect, some lectins have strong insecticidal properties. Recently, some fungal lectins have also been reported to possess entomotoxic properties. This study illustrates the insecticidal activity of a fungal lectin from the sclerotes of *Sclerotinia sclerotiorum*, further referred to as the *S. sclerotiorum* agglutinin (SSA). Feeding assays with pea aphid (*Acyrthosiphon pisum*) nymphs on an artificial diet, containing the purified SSA, for 3 d resulted in mortality of the pea aphids. The LC50 value was calculated to be 66 μg/ml, demonstrating high toxicity of SSA toward this

pest insect. In an attempt to unravel the mode of action of SSA, the binding and interaction of the lectin with insect tissues and cells was investigated. Histofluorescent studies on sections from nymphs fed with a diet containing SSA labeled with fluorescein isothiocyanate (FITC) revealed that the midgut was the primary target for SSA. In addition, exposure of insect midgut FPMI-CF-203/2.5 cells to purified SSA-FITC for 4 d resulted in clear inhibition of cell proliferation. Fluorescence confocal microscopy demonstrated that FITC-labeled SSA was not internalized in the FPMI-CF-203/2.5 insect midgut cells but was bound to the cell surface. Internalization of SSA in the cells was achieved after incubation with saponin prior to SSA treatment. However, internalization of SSA into the insect cells did not increase SSA toxicity, suggesting that SSA interacts with specific carbohydrate moieties present on the cell surface. These results will be discussed in view of possible applications of SSA in insect pest control.

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Elderberry Agglutinins I and II Cause Cell Death via Caspase-Dependent Apoptosis in Insect Midgut Cells

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Sambucus nigra agglutinins I and II, further referred to as SNA-I and SNA-II, are two ricin-related lectins from elderberry. SNA-I is a chimeric lectin composed of an A-chain with enzymatic activity and a B-chain with carbohydrate binding activity and therefore belongs to the group of type 2 ribosome-inactivating proteins. In contrast, SNA-II consists only of carbohydrate-binding B-chains. The physiological effect of SNA-I was tested on different insect cell lines (midgut, ovary, fat body, embryo). In sensitive midgut CF-203 cells, SNA-I induced cell death with typical characteristics such as cell shrinkage, plasma membrane blebbing, nuclear condensation, and DNA fragmentation. The effect was dose dependent with 50% death of 4-d-exposed cells at 3 nM. SNA-I exposure induced caspase-3-like activities, suggesting that SNA-I can induce the apoptotic pathway. Interestingly, the hololectin SNA-II also induced apoptosis in CF-203 cells at similar doses with the same physiological events. SNA-I and SNA-II both induced caspase-dependent apoptosis at low concentrations (nanomolar order), leading to typical symptoms of cell death in sensitive cells. This effect seems independent from the catalytic activity of the A-chain but depends on the carbohydrate binding B-chain.