

Plant Contributed Papers

P-1000

Expressing the Gibberellin Catabolizing Enzyme *AT-GA-ox1* in a Low-input Turfgrass (*Paspalum notatum* Flugge) Improves Turf Quality and Field Performance. P. LOMBA, K. Kenworthy, and F. Altpeter. Agronomy Department, Plant Molecular and Cellular Biology Program, Genetics Institute, University of Florida - IFAS, Gainesville FL32611. Email: altpeter@ufl.edu

Bahiagrass (*Paspalum notatum* Flugge) is a popular forage and turf species in the southeastern US due to its persistence under low-input conditions. However, the turf quality of bahiagrass is limited by its open growth habit and prolific production of long seedheads. We recently reported improved turf characteristics of bahiagrass following constitutive expression of the gibberellin catabolizing enzyme, GA 2-oxidase (*AT-GA-ox1*). Here we describe a field evaluation of turf quality and drought tolerance of these transgenic bahiagrass lines. Transgenic bahiagrass and wildtype plants were established in 1 m×1 m plots under USDA-APHIS permit 06-219-01r in a split-split-plot design. Following establishment plants were evaluated under two different mowing environments (weekly and biweekly) and three different irrigation regimes (full, moderate and no irrigation) in four replications. Turf was evaluated by comparing establishment, persistence, turf density, number of inflorescences, clipping weights, root and rhizome weight under different mowing and irrigation conditions. Statistical analysis was performed according to the randomization structure using the MIXED-procedure of SAS. Transgene expression under field conditions was evaluated with RT-PCR. Bahiagrass over-expressing *AT-GA-ox1* produced significantly more tillers than wildtype. Transgenic plants also showed decreased stem length while root and rhizome biomass as well as drought tolerance and low input characteristics were not compromised. Delayed flowering and improved recovery from drought was also observed in some lines. These results suggest that suppression of bioactive GAs enhances tiller bud outgrowth, reduces apical dominance and delays inflorescence development in bahiagrass. Transgenic bahiagrass lines over-expressing the gibberellin catabolizing enzyme GA 2-oxidase (*AT-GA-ox1*)

display improved turf quality without compromising its persistence and low-input qualities.

P-1001

Genetic Engineering of Turfgrass and Rice with Two Novel Antimicrobial Peptides for Enhanced Disease Resistance. MAN ZHOU, Allison Cason, and Hong Luo. Department of Genetics and Biochemistry, Clemson University, 110 Biosystems Research Complex, Clemson, SC 29634. Email: mzhou@clemson.edu, hluo@clemson.edu

Turfgrass and rice, the two agriculturally and economically important crop species, are highly susceptible to a wide range of destructive fungal and bacteria pathogens, which cause a great decrease in quality and safety. Chemical pesticides not only add a lot of operational costs but also raise serious environmental problems. Thus, resistance to biotic stress is one of the most important targets in the improvement of turfgrass and rice. The antimicrobial peptides - PEN4 from shrimp (*Litopenaeus setiferus*) and Ib-AMP4 from Impatiens (*Impatiens balsamina*) have been reported to possess in vitro antifungal and antibacterial activity against various economically important fungal and bacterial pathogens including both plant pathogens and some multi-drug resistant human pathogens. Using transgenic approaches, we have studied the potential of using these two novel peptides for enhanced disease resistance in *Arabidopsis*, turfgrass and rice. Six different DNA constructs were prepared containing coding sequence of either a single peptide, or a cleavable chimeric polyprotein of PEN4 and Ib-AMP4. In some cases, the DNA region encoding the signal peptide of the tobacco *AP24* gene was N-terminally fused to the coding sequence of either a single peptide or a polyprotein. A corn *ubiquitin* promoter was used in all the constructs to drive gene expression. Transgenic turfgrass, rice and *Arabidopsis* plants containing different chimeric DNA constructs were generated by *Agrobacterium*-mediated transformation, and transgene expression was demonstrated. Pathogen challenge experiment is currently under way and the preliminary data demonstrate the great potential of these peptides for use in plant disease resistance. High level of foreign gene

expression in turfgrass also suggests the possibility of using perennial grass species as bioreactors to produce pharmaceutically active peptides for cost-effective molecular farming. Data resulting from our research would lead to the development of novel technologies for enhancing plant biotic stresses, thus contributing to agricultural production.

P-1002

Assessing RNAi Gene Targets of Root-knot Nematodes in Composite Transgenic Soybean. B. L. RAMBO-MARTIN¹, P. LaFayette¹, R. Hussey², and W. A. Parrott¹. ¹Dept. of Crop and Soil Sciences, The University of Georgia, Athens, GA and ²Dept. of Plant Pathology, The University of Georgia, Athens, GA. Email: benlm@uga.edu

Root-knot nematodes (RKN), *Meloidogyne* spp., account for losses of over \$200 million in soybean yield per year in the United States. RKN establishes a complex feeding site within plant roots by altering gene expression in root cells, causing those cells to enlarge and adjacent cells to proliferate, netting a result of visible galls. The recent identification of RKN genes involved in plant parasitism has opened the possibility for developing novel RKN resistant soybean by disrupting the expression of these genes by using RNA interference (RNAi). Accordingly, the effectiveness of targeting two RKN parasitism genes, 17H02 and 31H06, to confer resistance in soybean is being evaluated. Composite soybean (cv. 'Peking') plants were created by cutting and inoculating newly emerging radicals with *A. rhizogenes* strain K599 harboring one of various binary vectors designed to produce double-stranded RNA (dsRNA). Aside from targeting the two genes separately, targeting both simultaneously is also being tested. Finally, two promoters, a ubiquitin promoter from *Glycine max* (GmUbi) and a phosphate transporter promoter from *Medicago truncatula* (MtPt1) are being compared for their relative effectiveness at obtaining RKN resistance. Stable transgenic soybean (cv. 'Jack') with the same constructs are also being obtained.

P-1003

Highly Efficient Suppressor-dependent Protein Expression in Plants with a Foxtail Mosaic Virus Vector. ZUN LIU and Christopher M. Kearney. Department of Biology, Baylor University, Waco, TX 76798-7388. Email: zun_liu@baylor.edu

A new viral vector based on foxtail mosaic potyvirus (FoMV) was constructed by eliminating the triple gene block and coat protein genes, reducing the viral genome more than 2-fold. The resulting FECT vector (*Fomv*

Elimination of Coat protein and Triple gene block) is driven by a CaMV 35S promoter in a binary vector and was delivered via syringe agroinoculation of *Agrobacterium tumefaciens* to whole plants of *Nicotiana benthamiana*. Interestingly, agroinoculation of the vector alone results in only slight transient expression, whereas co-inoculation with silencing suppressor genes (carried in a separate agrobacterial strain) allows for highly efficient GFP expression of up to 40% TSP. Thus, the FECT vector provides high capacity expression coupled with a tight on-off switch which could be utilized in permanently transgenic plants. It is also a good model system for molecular biology and molecular virology studies. FECT transient gene expression system are especially useful to rapidly confirm that the foreign molecule of interest is correctly assembled and retains its biological activity before generating stably transformed transgenic plants. Full-sized HC and LC components of an anti-langerin IgG, each carried by a separate FECT vector, were able to produce immunologically functional antibody upon co-inoculation. This vector also addresses many environmental safety concerns: i) its genome is reduced by more than half, ii) it does not replicate efficiently unless the plant immune system is suppressed, iii) it lacks a coat protein and cannot form a virion, and iv) it is derived from a virus that in most hosts causes only mild infections (no symptoms observed in *N. benthamiana*).

P-1004

Regeneration of *Arachis paraguariensis* Through Different Morphogenic Pathways. O. O. AINA, K. H. Quesenberry, and M. Gallo. Agronomy Department, University of Florida, 304 Newell Hall, PO Box 110500, Gainesville, FL 32611-0500. Email: ainab@ufl.edu

Several wild species in the Genus *Arachis* represent important sources of novel genes for improvement of cultivated peanut. *Arachis paraguariensis* Chodat & Hassl. is a wild peanut that is highly resistant to *Cercospora arachidicola*, the causal agent of early leaf spot disease. Attempts of conventional hybridization between cultivated peanut and *A. paraguariensis* have failed due to hybridization barriers. Tissue culture and biotechnology techniques offer potential routes for overcoming these barriers, but as with many legumes, the *Arachis* spp. are generally recalcitrant to tissue culture regeneration. This study investigated the roles and interactions of different genotypes, explant sources and growth regulators in tissue culture regeneration of *A. paraguariensis*. Deembryonated cotyledon and embryonic axis explants dissected from mature seeds were grown in vitro under continuous light in modified MS media that has been supplemented with

auxin and cytokinins at different concentrations. Two factorial experiments in a complete randomized design led to the induction of embryogenic callus when combinations of 2,4-D and BA or 2iP were used. Somatic embryogenesis and direct shooting was also achieved with various combinations of TDZ and BAP or 2iP. Analyses confirmed a tri-directional pathway via direct organogenesis, somatic embryogenesis and callus-mediated embryogenesis. Deembryonated cotyledons explants produced higher number of shoots than the embryonic axis explants across all media treatments. Shoots were transferred into semi-solid MS medium containing either of IAA, NAA and IBA for adventitious root initiation before they were planted in soilless mix in the greenhouse. This regeneration system has been tested for six genotypes of *A. paraguariensis* and found to be reproducible; the procedure is genotype independent and can be utilized for improved introgression of genes from this wild species into the cultivated peanut.

P-1005

Exogenous Tocopherol and Ascorbic Acid Improve In Vitro Recovery of Cryopreserved *Rubus* Shoot Tips. ESTHER E. UCHENDU¹ and Barbara M. Reed². ¹Department of Horticulture, Oregon State University, Corvallis, OR 97331-7304 and ²USDA ARS, National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333-2521. Email: uchendue@hort.oregonstate.edu

Oxidative processes involved in stresses such as cold temperatures can decrease the viability of plant tissues. Antioxidants that counteract these oxidative reactions could improve plant viability following the stresses involved in cryopreservation. We studied the effects of exogenous vitamin E (Vit E) and ascorbic acid (AA) added at four critical points of the cryopreservation process (pretreatment, loading, rinsing, recovery medium). Shoot tips of two blackberry cultivars were cold acclimated, then cryopreserved using a PVS2 vitrification protocol. Shoot tips (0.8 – 1 mm) of two *Rubus* hybrid cultivars were cold acclimated, then cryopreserved. Vit E added to the preculture medium for 48 h prior to liquid nitrogen exposure or to the rinse solution following rewarming significantly ($P < 0.0001$) improved recovery of shoots compared to shoot tips without Vit E. There were no significant improvements with Vit E in the loading solution or the regrowth medium. Recovery of shoot tips treated with AA at three critical points was significantly better than untreated tissues. Recovery of shoot tips on standard regrowth medium with AA was significantly reduced compared to the recovery of untreated shoot tips. Regrowth medium without iron and with AA significantly improved regrowth compared to untreated shoot tips on either recovery medium. Combina-

tions of Vit E and AA produced significant improvements in recovery at the four critical points compared with the untreated shoot tips, but the combination treatment was not significantly different from treatment with AA alone. Although treatments were effective at several critical points, we recommend adding AA to the pretreatment medium as the most convenient for the PVS2 vitrification process.

P-1006

Host-delivered RNAi: An Effective Strategy to Silence Nematode Genes in Transgenic Hairy Roots of Soybean. JIARUI LI¹, Timothy C. Todd¹, William T. Schapaugh², and Harold N. Trick¹. ¹Department of Plant Pathology, Kansas State University, Manhattan, KS 66506 and ²Department of Agronomy, Kansas State University, Manhattan, KS 66506. Email: hnt@ksu.edu, jli2@ksu.edu

The soybean cyst nematode (SCN), *Heterodera glycines*, is the primary biotic factor limiting soybean production, accounting for 40% of total disease losses. Current methods to control this pest are not totally successful in part due to new SCN biotypes emerging that can overcome current resistant varieties. To control soybean cyst nematode (SCN) in soybean, our laboratory has been evaluating the expression of siRNAs against specific nematode genes in chimeric transgenic plants. Ten separate nematode genes were selected for this study. Gene fragments were cloned into siRNA expressing vectors by Gateway cloning strategy. The siRNA constructs of these ten genes were independently transformed into soybean using this hairy root system mediated by *Agrobacterium rhizogenes*. Transgenic roots were confirmed via PCR and Southern-blot analysis. Transgene expression was surveyed by reverse transcription PCR. SCN bioassays resulted in up to 85% reduction in eggs g⁻¹ root tissue, indicating that chimeric transgenic plants expressing specific RNA silencing vectors significantly suppressed the reproductive potential of *H. glycines*. Stable soybean transformation is in progress for some of the effective genes.

P-1007

Higher Accumulation of F1-V Fusion Recombinant Protein in Plants After Induction of Protein Body Formation. M. LUCRECIA ALVAREZ^{1,2}, Emel Topal^{1,2}, Federico Martin^{1,2}, and Guy A. Cardineau^{1,2}. ¹Center for Infectious Diseases and Vaccinology (CIDV), The Biodesign Institute at Arizona State University, 1001 South McAllister Avenue, Tempe, AZ 85287-5401 and ²The School of Life Sciences, 1001 South McAllister Avenue, Tempe, AZ 85287-5401. Email: Lucrecia.alvarez@gmail.com

Our main goal was to increase recombinant protein accumulation in plants in order to enhance the efficiency of orally-delivered plant-made vaccines. It is well known that oral vaccination requires substantially higher doses than parental formulations. Cereal grain evolved to store large amounts of proteins in tightly organized aggregates. In maize, γ -Zein is the major storage protein that accumulates in specialized organelles called protein bodies (PB). “Zera[®]” (γ -Zein ER-accumulating domain) is the N-terminal proline-rich domain of γ -zein that is sufficient to induce the assembly of PB formation. Fusion of the Zera domain to proteins of interest results in assembly of dense PB-like, ER derived organelles, containing high concentration of recombinant protein. As a part of a project to develop an orally-delivered plant-made plague vaccine, we expressed the *Yersinia pestis* F1-V antigen with and without a fused Zera domain in transgenic NT1 cells and alfalfa plants. We observed a slower regeneration in alfalfa plants and transgenic NT1 calli expressing ZeraF1-V compared to F1-V alone. However, the number of transgenic lines regenerated in selective media after transformation with Zera-F1-V vs. F1-V alone, was similar. We determined that the accumulation of F1-V fusion protein was at least 3 X higher in plants expressing Zera, according to western-blot, without affecting plant development and growth. The subcellular localization of Zera-F1-V protein inside PB-like structures was determined by immuno-electron microscopy. These results confirm the potential exploitation of Zera to substantially increase the accumulation of value-added proteins in plants, which might be particularly useful in the development of oral plant-made vaccines.

P-1008

Transposon Mutagenesis of Soybean (*Glycine max*) Using the Rice MITE *mPing*. C. N. HANCOCK^{1,2}, F. Zhang², D. M. Tucker¹, S. R. Wessler², and W. A. Parrott¹. ¹Dept. Crop and Soil Science and ²Dept. of Plant Biology, University of Georgia, Athens, GA. Email: cnhancock@plantbio.uga.edu

The recently sequenced soybean genome is predicted to have ~ 65,000 genes. Transposon tagging mutagenesis will facilitate the annotation and characterization of these genes. *mPing* is a miniature inverted terminal repeat element (MITE) from rice (*Oryza sativa*) that acts as a natural mutagen when it transposes. As a tagging tool it has the advantages of both the TNT (high activity and unlinked insertion) and the Ac/Ds (potential for activation tagging) mutagenesis systems. Our objective is to determine whether *mPing* is suitable for transposon tagging of soybean. *mPing* was previously shown to be mobilized in *Arabidopsis* when the Ping proteins, ORF1 and TPase, were expressed. We

transformed a similar construct into soybean somatic embryos and observed both excision and insertion of *mPing* for multiple transformation events. We found that these transposition events produce unlinked insertions with the same T/A rich insertion preference observed for rice. In addition, we found that for three independent lines, transposition is upregulated during late embryogenesis. Together these results confirm the potential for *mPing* to produce useful mutations in soybean. We hope to produce heritable *mPing* insertions by inducing transposition in the meristematic tissues that produce seeds. Thus, we are testing additional promoters and a mutant TPase that shows increased transposition in *Arabidopsis*. We hope to use the resulting plants to produce an *mPing* mutagenized soybean population for both forward and reverse genetic screens.

P-1009

TILLING for Peanut Improvement. J. E. KNOLL¹, M. L. Ramos², and P. Ozias-Akins¹. ¹Dept. of Horticulture/NESPAL, University of Georgia-Tifton Campus, P.O. Box 748, Tifton, GA 31793 and ²Dept. of Plant, Soil, and Agricultural Systems, Southern Illinois University-Carbondale, 1205 Lincoln Dr., Carbondale, IL 62901. Email: jknoll@uga.edu

Allergic reactions to peanuts (*Arachis hypogaea*) can cause severe symptoms and can even be fatal. Avoidance is the best means to prevent allergic reactions, but accidental consumption is still of concern due to the prevalence of peanut-derived products in processed foods. One strategy of reducing the allergenicity of peanuts is to alter the amino acid sequences of allergenic proteins in the seed, thus making them less reactive to IgE. Mutagenized peanut populations have been generated using EMS. Targeted Induced Local Lesions in Genomes (TILLING) is a reverse-genetics approach used to screen mutagenized populations for individuals carrying single-nucleotide mutations or small indels in specific genes. Two similar copies of a major allergen, ara h 2, have been identified, one in each sub-genome. The same situation has also been shown for major allergen ara h 1. For TILLING these genes, a nested PCR approach is used in which both copies are amplified in the first round, and then IRDye-labeled primers specific to only one gene are used in the second round. Heteroduplexes are then formed and digested with CEL1 nuclease. Cleaved PCR products representing mutations are detected on a Li-Cor DNA Analyzer. Putative mutations are then confirmed by sequencing. To date, we have identified several mutations in ara h 2.01, ara h 2.02, ara h 1 A and ara h 1 B; including a premature stop codon in ara h 1 A.

This work represents the first steps toward the eventual goal of creating a peanut cultivar with reduced allergenicity. This approach is also being used to alter seed oil composition through changes in fatty acid desaturases. We have found potential mutations in both copies of AhFAD2, genes which control the ratio of oleic to linoleic acid in the seed.

P-1010

Integration and Expression of *E. coli* L-aspartate-alpha-decarboxylase in Tobacco Chloroplasts Enhances Photosynthesis and Biomass Accumulation Following High Temperature Stress. W. M. FOUAD and F. Altpeter. University of Florida – IFAS, Agronomy Department, Plant Molecular and Cellular Biology, Laboratory of Plant Molecular Physiology, 3062 McCarty Hall, Gainesville, FL 32611. Email: altpeter@ufl.edu

Beta-alanine, a non-protein amino acid, is found in all living organisms and essential for normal growth. In prokaryotes, beta-alanine is a product of the alpha-decarboxylation of L-aspartate catalyzed by the *panD*-encoded L-aspartate-alpha-decarboxylase (AspDC). In plants, beta-alanine increases in response to high temperature and/or drought. Beta-alanine prevents protein aggregation and reactivates the thermally denatured enzyme in vitro. The objective of this study was to evaluate the role of beta-alanine protection to the photosynthesis during high temperature stress through the localized expression of the *E. coli panD* gene in the chloroplast genome. The constitutive *panD* expression cassette was co-introduced with the constitutive, selectable *aadA* expression cassette into the chloroplast genome of tobacco via biolistic gene transfer and homologous recombination. Site specific integration of the *E. coli panD* expression cassette into the chloroplast genome and generation of homotransplastic plants were confirmed by PCR and Southern blot analysis respectively, following plant regeneration and germination of seedlings on selective media. *PanD* expression was verified by assays based on transcript detection and in vitro enzyme activity. The AspDC activities in transplastomic plants expressing *panD* were increased by high-temperature stress up to six-fold compared to plants growing at 25°C. Transplastomic plants accumulated up to 4 times more β -alanine than wildtype plants. Analysis of chlorophyll fluorescence of plants subjected to four hours of 45°C under light verified that the photosystem II (PSII) in transplastomic plants had a higher thermotolerance than wildtype plants. The CO₂ assimilation of transplastomic plants expressing *panD* displayed increased high temperature tolerance, resulting in the production of 30–40% more above ground biomass than wildtype plants. These

results indicate that chloroplast engineering of the β -alanine pathway enhances thermotolerance of photosynthesis and biomass production following high temperature stress.

P-1011

Using Endogenous *Vitis* Genes to Produce Disease Resistant Transgenic Grapevines. S. A. DHEKNEY¹, Z. T. Li¹, T. W. Zimmerman², and D. J. Gray¹. ¹University of Florida/IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703 and ²University of Virgin Islands Agricultural Experiment Research Station, RR1 Box 10,000, Kingshill, St. Croix, VI 00850. Email: Sadanand@ufl.edu

Two endogenous genes, *vvtl-1* (*Vitis vinifera* thaumatin like protein) and *eg-2* (a proprietary gene) were isolated from grapevine ‘Chardonnay’ and ‘Merlot’ by PCR. Each gene was reengineered for constitutive expression by placing it with an EGFP/NPT II fusion gene under the control of a CaMV35S-derived bidirectional duplex promoter complex. Embryogenic cultures of *V. vinifera* ‘Thompson Seedless’ ‘Merlot’ and ‘Shiraz’ were transformed with *Agrobacterium* to regenerate transgenic plants. Transgene integration and copy number in transgenic plants was estimated by quantitative real-time PCR, while transprotein expression was determined by ELISA. Transgenic plants were screened for powdery mildew (*Uncinula necator*) resistance by comparing symptom development on leaves and stems with corresponding non-transgenic susceptible varieties and a resistant control variety. Among the transgenic *vvtl-1* plant lines tested, 5 ‘Thompson Seedless’ and 3 ‘Merlot’ and ‘Shiraz’ lines exhibited a 7–10 d delay in symptom development compared to susceptible controls. Resistance of transgenic plants was confirmed by repeated screening for 2–3 seasons. Among transgenic *eg-2* plant lines tested, no difference was observed in powdery mildew development on leaves and stems of transgenic and control plants. Transgenic *eg-2* ‘Shiraz’ and control plants flowered and produced fruit in greenhouse tests. Development of powdery mildew was significantly lower on transgenic berries compared to controls. Transgenic berries exhibited normal color development and ripening, whereas controls exhibited poor color development and failed to ripen. Selected transgenic *vvtl-1* ‘Thompson Seedless’ vines were planted in USDA/APHIS approved field sites in Florida and the US Virgin Islands. Two plant lines exhibited significantly lower symptoms of black rot (*Guignardia bidwellii*) compared to control plants. Promising transgenic ‘Merlot’ and ‘Shiraz’ lines currently are being propagated to test their response to fungal pathogens under field conditions.

P-1012

Green Genetic Engineering Technology: Rearrangement of Endogenous Functional Genetic Elements to Create Improved Grapevines. D. J. GRAY¹, Z. T. Li¹, S. A. Dhekney¹, D. L. Hopkins¹, and T. W. Zimmerman². ¹University of Florida/IFAS, Mid-Florida Research and Education Center, 2725 Binion Road, Apopka, FL 32703 and ²University of Virgin Islands Agricultural Experiment Research Station, RR1 Box 10,000, Kingshill, St. Croix, VI 00850. Email: djg@ufl.edu

Use of genetic engineering technology to add needed traits to otherwise desirable varieties is an attractive approach to improvement of *Vitis*. Endogenous genes and expression regulatory elements were isolated directly from grapevine, tested for functionality using various marker systems, and subsequently transformed into *V. vinifera* ‘Merlot’, ‘Shiraz’ and ‘Thompson Seedless’, plus *Vitis* hybrid ‘Seyval Blanc’. Studies showed that two promoters (EP and Alb) isolated from grapevine had expression efficiencies of up to 12% of a double enhancer-35S promoter. Other sequences operated as functional *Agrobacterium* T-DNA borders. Endogenous genes inferred to be active in fungal resistance did, indeed, provide enhanced resistance when constitutively expressed in donor plants. It is now possible to insert genetic constructs into grapevine that contain only native DNA and confer enhanced traits. Our adaptation of endogenous genes to modulate disease resistance is a first step in creating “green transgenic plants” that contain only DNA sequences from grapevine, thus eliminating concerns about incorporation of foreign genes in GMO’s.

P-1013

Facilitation of GFP Visualization in Green Tissues Using Bleaching Herbicides. J. FINER. Department of Horticulture and Crop Science, Plant Molecular Biology and Biotechnology Program, OARDC/The Ohio State University, Wooster, OH 44691. Email: finer.1@osu.edu

Fusion of GFP to upstream regulatory elements or protein coding regions in plants has provided valuable information on gene expression and protein localization. In plant tissues expressing GFP, visualization of GFP can be challenging if that tissues contains high amounts of chlorophyll, which interferes with GFP fluorescence. To eliminate chlorophyll interference, the bleaching herbicide, isoxaflutole, was added to plant tissue culture media at 3-10 mg/l. Addition of isoxaflutole did not affect the proliferation of transgenic and nontransgenic embryogenic soybean cultures and GFP visualization was tremendously improved. Soybean tissues, expressing GFP at low-moderate levels, showed clear GFP

expression following culture on isoxaflutole-containing medium, while GFP could not be easily detected in tissues grown without the herbicide. Induction of GFP, placed under regulatory control of inducible promoters was also easily observed with herbicide treatment. Tracking of GFP expression using automated image collection and inducible promoters allowed generation of time lapse animations, showing GFP induction followed by decline. Inclusion of the herbicide in a sunflower shoot induction medium also led to bleaching of the explant tissues, which formed large numbers of achlorophyllous shoots. This approach is useful for transformation tracking and gene expression/protein localization work in green tissues, where interference from chlorophyll is problematic.

P-1014

Over Expression of AtNHX1 Gene in Transgenic Salt Tolerant Cultivated Tomato. RAMA SWAMY NANNA¹, Praveen Mamidala¹, and Hongxia Zhang². ¹Plant Biotechnology Research Group, Department of Biotechnology, Kakatiya University, Warangal-506 009, INDIA and ²Institute of Plant Physiology and Ecology, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, Shanghai-200032, CHINA. Email: swamynr.dr@gmail.com

Tomato (*Lycopersicon esculentum* Mill.) is a highly nutritive vegetable crop that has achieved tremendous popularity over the last century. Tomato is rich in carotenoids especially lycopene an antioxidant which contains moderate amounts of α , β -carotene and vitamin-c. These antioxidants counteract the adverse effects of oxidative stress and lead to improved immune function and reduced risk of infectious diseases. Though it has importance in daily food, as vegetable and medicine the crop can’t be grown in drought prone areas. Hence, in the present investigations we have attempted to induce over expression of antiporter gene (AtNHX1) in salt tolerant cultivated tomato cv. PKM-1 through transgene technology. Molecular cloning was done using AtNHX1 gene with stress inducible promoter (SIP) and constitutive promoter (CaMV 35 S) with *bar* gene as a selective marker gene by replacing hpt gene in the pCAMBIA 1300/1301 cassettes. Two different vectors pMEX and pMS were developed to use in genetic transformation for engineering salt/abiotic stress resistance. Genetic transformation experiments were carried out by using *Agrobacterium tumefaciens* LBA 4404 harboring cloned vectors pMS and pMEX. The putative transformants were identified by following the techniques of PCR, RT-PCR, Southern and Northern blots. Two transgenic lines (AtNHX1 with CaMV 35S and AtNHX1 with SIP) have been screened in tomato cv. PKM-1 for the first time. Expression of these two promoters was studied in relation to different physiological parameters.

We found the differential expression levels in transgenic plants consisting of SIP and CaMV 35 S promoters and wildtype in conferring resistance against salt stress/drought stress. Salinity stress was carried out in hydroponics cultures maintained in green house. Phenotypic traits and biochemical characterization of the NaCl treated plants were recorded. Thus, the importance of OEX1 SIP/CaMV 35S promoters during the abiotic stress/ salt stress in tomato will be presented.

P-1015

Qualitative Analysis of *Aloe vera*: Commercially Important Medicinal Plant Through HPLC and Clonal Propagation of ChemoProfiled Material Through In Vitro Techniques. S. K. TIWARI, P. K. Shukla, Amit Pandey, S. Mishra, M. P. Goswami, and P. Bhargava. Forest Genetics Plant Propagation and Biotechnology Division, State Forest Research Institute, Polipathar, Jabalpur (M.P.), INDIA. Email: drsktiwari@rediffmail.com

Aloe vera is a commercially important medicinal plant of lily family. It has been used worldwide in pharmaceutical, food and cosmetic industries and in traditional medicine, due to many biological activities of some of its primary and secondary metabolites. The medicinal value of this plant is due to the presence of aloin which is an anthraquinone derivative. The content of aloin in plant varies with the geographical distribution. Therefore the germplasm of the species has been evaluated by the institute through a simple quick and accurate HPLC method using C-18 ODS-2 column. The plant material with highest aloin percentage was used as the source of explant for establishing in vitro culture. The shoot meristem or tips were used as explant and were cultured on MS medium supplemented with different combinations and concentrations of plant growth regulators. Multiple shoot formation was achieved within 20-25 d on M.S medium supplemented with BAP 4 mg/l + IAA 1.0 mg/l. The shoots were harvested and rooted under in vitro conditions.