ABSTRACTS

Contributed Papers (Poster and Oral)

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The Abstracts are listed in order by Symposium number and presentation time. All abstracts are associated with a Symposium number and are coded as follows: Symposium number (S01, S02, etc.) type of presentation [oral (O), poster (P)], and the presentation number. The Author presenting the abstract is indicated by an asterisk. Presentation times are based on a 24-hour clock.

Symposium 1 (S01): Biotechnology of Horticultural Crop Improvement: Achievements, Opportunities and Limitations

Monday · August 12

Location: Crowne Plaza Hotel, Ballroom A

1100–1105
S01–O–1
BIOTECHNOLOGY IN HORTICULTURAL CROP IMPROVEMENT: ACHIEVEMENTS, OPPORTUNITIES AND LIMITATIONS

F.A. Hammerschlag*[1], P.K. Saxena[2]

[1]USDA/ARS, Fruit Laboratory, BARC-West, Building 010A Beltsville, MD 20705; [2]Dept. of Plant Agriculture, Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1

The applications of biotechnology to the improvement of horticultural crops have been considerable. In vitro propagation technologies, commonly referred to as micropropagation, are the methods of choice for propagating a wide range of horticultural crops. Of great importance has been the link between micropropagation and pathogen elimination protocols which has led to pathogen-indexed plants for worldwide distribution. The application of biochemical and molecular markers to horticultural crops has also developed rapidly, with advances in technology from isozymes to RFLPs, to RAPDs, AFLPs and SSRs. Genetic markers are now routinely used for classifying and identifying germplasm, analysis of genetic relationships among breeding materials, constructing genetic linkage maps, and selective breeding. Novelty horticultural crops have been developed through various tissue culture techniques including wide hybridization and embryo rescue, induced mutagenesis, somaclonal variation, somatic hybridization, and organelle transplantation. Closely aligned with tissue culture technology has been genetic engineering of plants. Numerous horticultural crops have been improved by selective insertion of specific genes for increased shelf life, disease resistance, novel morphological and floral attributes, and enhanced nutritional composition. This symposia will provide an overview of the impact of these advances on the horticultural industry with specific examples of successes.

1105–1200
S01–O–2
NAVIGATING THE SMORGASBORD OF DNA MARKER METHODS FOR IMPROVING HORTICULTURAL CROPS

Hilde Nybom*

Dept. of Crop Science, Swedish Univ. of Agricultural Fjalkestadsvagen 123-1, Kristianstad S-291 94 Sweden

About 20 years ago, methods for discrimination among horticultural crop cultivars were, if not always successful, at least relatively straightforward; you either relied solely on morphological features or, alternatively, on the more fashionable combination of morphological and isozyme analysis. At present, we instead find ourselves invited to a veritable smorgasbord of tempting and delectable DNA-based marker methods. This almost unlimited display of more or less sophisticated techniques brings its own problem, namely that of having to make choices. The requirements for equipment, expertise, time and funding vary considerably between methods, and the bewildered plant breeder often finds it difficult to identify the best alternative for the ever-increasing list of achievements that we now expect from our DNA-based marker methods. Identification and discrimination is only one of these tasks. We also require methods for gene tagging and genome mapping, for genetic analysis of character inheritance, for analyzing genetic relatedness, and for estimating variation in genetic resource collections. Recently, Southern blotting and hybridization-based methods (RFLP and minisatellite DNA analysis) have given way to PCR-based methods, employing either arbitrary primers (e.g. RAPD, AFLP, ISSR), or specific primers (e.g. SCAR, STMS). Whereas nuclear markers still are the mainstay of our smorgasbord, plastid-derived markers provide valuable information on maternal lineages. Several recent investigations have highlighted differences as well as similarities between the results obtained with these DNA-based markers. For maximum cost-effectiveness, it is essential to have realistic expectancies, based on up-to-date information about the strengths and weaknesses of the different methods. Acquiring sufficient knowledge about appropriate statistical methods for data evaluation is another daunting yet immensely important task.

1200–1240
S01–O–3
PROGRESS IN APPLICATION OF MOLECULAR MARKERS TO GENETIC IMPROVEMENT OF HORTICULTURAL CROPS

Sriyani Rajapakse*

Clemson Univ., Dept. of Biological Sciences, 132 Long Hall, Clemson, SC, USA, 29634

Over the last ten years molecular markers have entered the scene of genetic improvement of a wide range of horticultural species. Today, markers are being used for germplasm characterization, parental verification in crosses, gene tagging for marker-assisted breeding, and gene cloning for use in transformation. Among the major traits targeted are disease resistance, fruit quality, floral characteristics, and dormancy. Tomato and lettuce are in the forefront of the development and use of molecular markers, while fruit trees with long generation times stand to benefit the most from markers tightly linked to traits. Though DNA markers are still new to ornamental species and only a handful of species are used in marker development and testing, we see a surge of interest in genes that control characters preferred by consumers such as floral scent, petal color and plant shape.

1340–1440
S01–P–4
CONSTRUCTION OF A GENETIC LINKAGE MAP OF LYCHEE BASED ON RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS

Chengming Liu*[1], Mantong Mei[2]

[1]Dept. of Horticulture South China Agricultural Univ., Guangzhou, China 510642

[2]Genetic Engineering Laboratory, South China Agricultural Univ., Guangzhou, China 510642

An F_1 population of lychee was obtained from the cross of two highly divergent parents, *Maguili Litchi chinensis* cv. Maguili and Jiaohesanyuehong (a stenospermocarpy mutant of *Litchi chinensis* cv. Sanyuehong with 78 individuals. Based on this population, the analysis of randomly amplified polymorphic DNAs (RAPDs) was used to construct linkage map of lychee. A set of 500 random oligonucleotide primers were screened and 104 primers were selected to generate RAPD markers with DNAs from the 76 F_1 progenies. A total of 294 loci were identified. Among them, 130 loci had been found to be distortion from the normal 1:1 segregation, and only 135 loci of *Maguili* loci were used to construct lychee linkage map. The resulting linkage map consisted of 107 marker loci in 13 groups (four or more loci per group), 4 triples and 8 pairs, which covered the map distance about 1982.5 cm, estimated about 80% of lychee genome. This is the first molecular linkage map on lychee (*Litchi Sonn*) or on the family of Sapindaceae reported. Further study is underway to locate some important loci, such as fruit maturation period, seed abortion etc., based on this map.

1340–1440

S01–P–5

CYCLAMEN PERSICUM MILL.: SOMATIC EMBRYOGENESIS AND RAPD ANALYSIS OF EMBRYOGENETIC CALLUS

M. Laura, L. De Benedetti, A. Giovannini, S. Bruna, G. Burchi*, T. Berio, B. Ruffoni

[1]Ist. Sperimentale Floricoltura Propagation Dept. Corso Inglesi 508 18038 Sanremo; [2]Ist. Sperimentale Floricoltura Genetic Improvement Dept. Corso Inglesi 508 18038 Sanremo; [3]Ist.Sperimentale Floricoltura Propagation Dept. corso Inglesi 508 18038 Sanremo

In Cyclamen, a protocol of somatic embryogenesis has been established in order to produce artificial seeds. This seems to be applicable to a large number of genotypes; some phenotypic variations can be observed in the produced plants (Schwenkel, 1999). The use of 2,4-D to induce embryogenic competence is widely known and in this particular case, obliged. With the aim to study precociously the variability ascribed to genetic modifications, RAPD analysis have been made on the embryogenetic cyclamen callus. The primary callus was induced from immature ovules grown on a semisolid medium containing as growth regulators 2,4D (9.05 μM) and 2iP (2.39 μM). Cultures were grown in dark at 23 °C. After 30 days the embryogenic callus was transferred in the presence of increasing 2,4D concentrations. After 3 subcultures half of the calli were transferred onto GRF-free medium in order to detect the embryogenic potential related to the 2,4D levels. The other part was cryopreserved in liquid nitrogen, the genomic DNA was extracted and the molecular analysis was carried out by PCR amplification with random decamers. The best friable callus and the highest number of embryogenetic fragments were produced in the presence of 9.05 μM of 2,4D. After the molecular analysis, a total of 118 RAPD fragments were scored. Twenty out of 24 primers showed the same pattern, with the remaining 4 primers a polymorphic response (presence of absence of bands). In order to establish the applicability of the somatic embryogenesis propagation system, it is necessary to determine the rate of somaclonal variation that can be ascribed to the production protocol. This research investigates the occurrence of genome variants at different steps during the somatic embryogenesis protocol of Cyclamen persicum introducing the variable of different 2,4-D concentrations.

1340–1440

S01–P–6

HIGH FREQUENCY RECOVERY OF INTERGENERIC FUSION PRODUCTS OF *BRASSICA OLERACEA* (+) *LEPIDIUM MEYENII* AND THEIR MOLECULAR CHARACTERIZATION BY RAPDS AND AFLPS

U. Ryschka[1], E. Klocke*[1], S. Warwick[2], G. Schumann[1]

[1]Federal Centre for Breeding Research on Cultivated Plants, Neuer Weg 22/23, D-06484 Quedlinburg, Germany; [2]AAFC-ECORC, Ottawa ON, Canada K1A 0C6

Introgression of genes from distantly related donors within the family Brassicaceae is useful for augmenting the genetic base for achieving specific crop improvement objectives. Hypocotyl derived protoplasts of *Brassica oleracea* var. capitata cv. Latima were fused with non-irradiated mesophyll protoplasts of Lepidium meyenii and protoplasts of *B. oleracea* var. botrytis cv. Korso with X-ray irradiated mesophyll protoplasts of *L. meyenii* to produce symmetric versus asymmetric intergeneric hybrids. By using polyethylene glycol 76 plants were regenerated, 37 with symmetric fusion and 39 with asymmetric fusion. Regenerated plants were characterized by RAPDs and AFLPs. Due to the small amount of template DNA required (20 ng) the RAPD markers are suitable for detecting true fusion plants in very early stages of in vitro development, whereas the large number of markers provided with AFLPs proved useful for detecting a high degree of genetic polymorphisms in the fusion plants and also for quantifying the degree of asymmetric fusion. More plants with normal Brassica-morphology were obtained with asymmetric fusion.

1340–1440

S01–P–7

MARKER-ASSISTED SELECTION FOR APPLE SCAB RESISTANCE IN A SUBSET OF APPLE PROGENIES

Ernesto Huaracha[1], Mingliang Xu[1], Els Pauwels[2], J. Wannes Keulemans[2], Schuyler S. Korban*[1]

[1]Dept. of Natural Resources & Environmental Sciences, 310 ERML, Univ. of Illinois, Urbana, IL 61801 USA; [2]Fruitteeltcentrum, Katholieke Universiteit-Leuven, Willem De Croylaan 42, 3001 Heverlee Belgium

Six sequence-characterized amplified region (SCAR) markers linked to the apple scab resistance gene Vf have been evaluated for marker-assisted selection (MAS) in apple breeding. Of the six SCARs used in this study, ACS-6 is located left of the Vf gene, ACS-7 and ACS-9 co-segregate with Vf, and ACS-8, ACS-4, ACS-5 are located right of the Vf gene. Three families derived from crosses between scab-resistant and scab-susceptible cultivars, including 'Liberty' x 'Deljub', 'Liberty' x 'Delcorf', and 'Florina' x 'Delcorf', were evaluated for their phenotypic reaction to scab infection in the field. For each family, a subset progeny of 30 seedlings (propagated onto Malling 9 rootstock and 5 years of age) consisting of 10 seedlings with no visible scab sporulation and given a phenotypic score of 0 (deemed resistant), 10 seedlings with moderate scab sporulation and given a phenotypic score of 1.0 (deemed moderately resistant), and 10 seedlings with heavy sporulation and given a phenotypic score of 2.0 (deemed susceptible), were evaluated for presence/absence of all six SCAR markers. DNA was isolated from leaf tissue collected from all three subset progenies (a total of 90 seedlings), four parents (the scab-resistant 'Liberty' and 'Florina' along with the scab-susceptible 'Deljub' and 'Delcorf'), and *Malus floribunda* 821, the original source of the Vf gene, and screened with all six SCARs. The two scab-resistant parents and *M. floribunda* 821 showed all six SCARs; while the two scab-susceptible parents lacked all SCARs. For all three progenies, seedlings with a phenotypic rating of 0 (resistant) or 1.0 (moderately resistant) showed presence/absence of varying numbers of SCARs; while seedlings with a phenotypic rating of 2.0 (susceptible) lacked all SCARs.

1340–1440

S01–P–8

PATTERNS OF CYTOSINE METHYLATION DETECTED IN FIELD- AND IN VITRO-GROWN LEAVES OF APPLE

Xianqian Li, Mingliang Xu, Schuyler S. Korban*

Dept. of Natural Resources & Environmental Sciences, 310 ERML, Univ. of Illinois, Urbana, IL 61801 USA

Previously, we developed a method to detect cytosine methylation at the 5'-CCGG-3' sequence using isoschizomers and the amplified fragment length polymorphism (AFLP) technique. Following this protocol, DNA methylation profiles were investigated in leaf tissues of apple (*Malus* x *domestica* cv. Gala) subjected to two different growth conditions, field-grown adult trees and in vitro-grown shoot cultures. A total of 1,622 bands were detected in both sources of leaf tissue by using 32 primers pairs. For leaf tissues of field- and in vitro-grown apples, the ratios of types I, II, and III banding patterns to the total number of amplified fragments were 70%, 24%, 6 %, and 71%, 23%, and 6 %, respectively. Although the ratios of the three types of banding patterns were similar in both leaf tissues, a few bands specific to either field-grown trees or in vitro-grown shoots were observed. This study provided evidence that changes in DNA methylation occur when apples are subjected to different growth and environmental conditions.

1340–1440

S01–P–9

COMPARATIVE PHYSICAL MAPPING OF SEGMENTS OF THE GENOMES OF *BRASSICA OLERACEA* AND *BRASSICA CAMPESTRIS* THAT ARE HOMOEOLOGOUS TO SEQUENCED REGIONS OF CHROMOSOMES 4 AND 5 OF *ARABIDIDOPSIS THALIANA*

Jee Young Park[1], Chang Pyo Hong[1], Hyun Su Na[1], Ian Bancroft[2], Yong Pyo Lim*[1]

[1]Dept. of Horticulture, Agriculture College, Chungnam Nat'l Univ., Korea; [2]Dept. of Brassica and Oilseeds Research, John Innes Centre, UK

Due to their relatedness to *Arabidopsis thalinan*, the cultivated *Brassica* species represent the first group of crops with which to evaluate comparative genomics approaches to understanding biological processes and manipulating traits. We have constructed BAC library of *Brassica compestris* in oder to genome research. Using the *Arabidopsis* genome sequence and clones from the BAC library, we have analyzed aspects of gene conservation and microsynteny among a 222kb region of the genome of *Arabidopsis* and homoeologous segments of the genome of *B. oleracea* and *B. campestris*. All 19 predicted genes tested were found to hybridize to clones in the library, indicating a high level of gene conservation. Further analyses and physical mapping with the BAC clones identified allowed us to construct clone contig maps and analysis in detail the gene content and organization in the set of paralogous segments identified in the genome of *B. campestris*.

1340–1440

S01–P–10

DEVELOPMENT OF POLYMORPHIC PCR MARKERS FOR GENETIC MAPPING BASED ON BAC-END SEQUENCE AND EST IN CHINESE CABBAGE

Yong Pyo Lim*, Harui Cui, Su Ryn Choi

Dept. of Horticulture, College of Agriculture and Life Science, Chungnam National Univ., Taejon 305-764, Korea

PCR-based marker systems are relatively simple and easy to use, and various types of PCR-markers have been developed. Among the various classes of PCR-based markers currently available, the markers revealing co-dominant polymorphisms in specifically targeted sequences are the most informative, because they can be scored unambiguously and concern about the homology of markers among individuals or populations is greatly reduced. In this study, we developed the co-dominant polymorphic markers based on the BAC-end sequences and expressed sequence tags. SSR markers were developed using the known BAC-end sequence information. Out of 2,376 BAC-end sequenced clones, we found 58 clones containing repeat motif using Repeatmasker program, and designed 41 primer pairs with Primer 3 software. Thirty pairs out of 41 designed primer allow the polymorphism for SSR marker in *Brassica campestris*. For development of EST markers, we cloned and sequenced 1,736 ESTs. Some ESTs were selected to design primers for PCR. One hundred and fifty pairs of primers to specific ESTs have been designed. The amplified products were separated by 1.2% to 3.0% agrose gel to check polymorphism, such as obvious differences in band number and band size between the parents used to construct DH population. If no variation were observed, the differences of PCR products in pattern of restriction digestion would be further checked. Based on the band pattern, genotype of each DH line was determined. To date, about 30% of primers showed the polymorphism among the parents and 86 DH lines. We also found that the amplified segment containing the intron (s) was more polymorphic.

1340–1440

S01–P–11

PHYSICAL MAPPING USING BAC CLONE IN CHINESE CABBAGE: CONTIG ASSEMBLY

Yong Pyo Lim*, Chang Pyo Hong, Hyun Su Na, Zhong Yun Park, Ji Young Park

Dept. of Horticulture, College of Agriculture and Life Science, Chungnam National Univ., Taejon 305-764, Korea

The major benefit of physical map will be immediate access to any segment of the genome that can be defined genetically. Additionally, it will be a starting point for studying the large-scale organization of the genome. To meet the demands of large-scale sequencing, thousands of clones must be fingerprinted and assembled into contig. To determine the order of clones, a typical experiment is to digest the clones with one or more restriction enzymes and measure the resulting fragment. The probability of two clones overlapping is based on the similarity of their fragments. A contig contains two or more overlapping clones and a minimal tilling path of clones is selected to be sequenced. Interactive software with algorithmic support is necessary to assemble the clones into contigs quickly. Previously, a large-insert (115 kb) bacterial artificial chromosome (BAC) clone 56,592 was constructed. After fingerprintings were conducted using BAC clones, contigs were constructed using the software package FPC (Fingerprinted Contigs).

1340–1440

S01–P–12

MOLECULAR CHARACTERIZATION OF RIPENING FRUIT PROCESSES IN STRAWBERRY STARTING FROM A TRANSCRIPT GENOMIC DNA FRACTION

Giuseppe Martelli*, M. Rosaria Sabina, Anna Sciancalepore, Francesco Sunseri, Ivana Greco

Dept. Biologia, Difesa, Biotecnologie AgroForestali–Univ. of Basilicata–C.da Macchia Romana–I-85100–Potenza–Italy

The final results of the ripining fruit are determined by several factors such as genotype, crop management, nutrition etc. The genes involved in these processes could be considered as the milestones for starting to produce fruit of high quality. The organoleptic parameters that mainly influenced the fruit quality in cultivated and selvatic strawberry are flavor, acidity, sweetness as well as the fruit shape, color and firmness. The flavor is considered the trait most important in fruit quality and in several studied performed in the last 40 years, more than 300 different volatile compounds were identified. On the other hand, comparative studied among cultivars for its flavor were conducted more recently (Dirinck et al., 1981–J. Sci. Food Agric. 29, 316-321; Zabetakis, 1996–Food Sci. & Technol. 10, 157-159). The aim of the present paper is a characterization of ripening fruit processes in Fragaria spp. and the detection of DNA polymorphisms related to the traits presented above. Four different genotypes collected from the same field, 2 low quality and 2 high quality were considered in the experiments. During the ripening process, fruit was collected fruit at four different level of ripening, starting from 0.5 mm diameter to the complete development of the fruit. At the same stages leaves from all the genotypes were also collected. mRNA was extracted either from fruit or leaves collected and consequently cDNA was obtained starting from the expressed fraction of DNA. The molecular analysis by using molecular markers such as AFLP (Bachem et al. 1996–Plant J. 9, 745-753) and STS were directed to investigate ripining processes to detect switching (on/off) gene mechanisms during the processes in the same genotype. The results obtained showed differences in expressed DNA fraction among low quality and high quality genotypes. These preliminary results will be better investigated analyzing the sequences of the polymorphic fragments detected.

1340–1440

S01–P–13

IDENTIFICATION OF RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS LINKED TO THE GENE FOR *FUSARIUM OXYSPORUM LYCOPERSICI* (RACE 1) RESISTANCE IN TOMATO

Nam Jun Kang*, Hae Boong Jeong, Yong Seop Cho, Hoe Tae Kim

Busan Hort. Experiment Station, NYAES, RDA. Busan 618-300, Korea

Fusarium wilt caused by *Fusarium oxysforum lycopersici* is one of the most serious diseases in tomato. Bulked segregant analysis method was used to identify RAPD markers linked to the resistance gene. F_2 population was developed from the cross of Ohio MR (resistant to *Fusarium* wilt) and Yangjwa (susceptible to *Fusarium* wilt). A 440 primers was used to detect the polymorphism between bulked resistant parent and bulked susceptible parent. Two markers were found to be polymorphic for the bulked resistant and susceptible DNA. These two markers was screened with the genomic DNA from 102 F_2 individual progenies to detect genetic linkage between these RAPD markers and resistant gene loci using Mapmarker/EXP (version 3.0b). Markers of OPA18-834 and OPJ07-1137 were linked to the resistance gene to *Fusarium* wilt at 1.2 cm and 3.0 cm, respectively.

1340–1440

S01–P–14

RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS LINKED TO THE GENE CONTROLLING FERMENTATION IN CUCURBITA MAXIMA FOR ORIENTAL MELON (*CUCUMIS MELO* L. MAKUWA MAK) ROOTSTOCK

Nam Jun Kang*, Hae Boong Jeong, Yong Seop Cho, Hoe Tae Kim

Busan Hort. Experiment Station, NYAES, RDA, Busan 618-300 Korea

Grafting of cucurbitaceae crops for protected cultivation in cold season is a common practical method for stabilization of productivity. In grafting of oriental melon using *Cucurbita maxima* rootstocks, one of the most serious physiologi-

cal disorders is fruit fermentation influenced critically by rootstock. Bulked segregant analysis method was used to identify RAPD markers linked to the gene control fermentation trait in F_2 population from the cross of Chamdaemok (tolerant to fruit fermentation) and Greybanana (susceptible to fruit fermentation). A 440 primer was used to detect the polymorphism between tolerant bulk parent and susceptible bulk parent. Seven RAPD markers were found to be polymorphic for the tolerant and susceptible bulked DNAs. Seven RAPD markers were screened with the genomic DNA from 145 F_2 individual progenies to detect linkage position of fermentation trait between these RAPD markers and control gene of fermentation trait. Genetic distance between fermentation trait and identified marker was obtained using Mapmaker/EXP (version 3.0b). A total of seven markers were linked to the fermentation trait, and the fermentation trait was flanked by OPF09-538 and OPC16-554 markers with the distance of 12.8 cm and 13.9 cm, respectively.

1340–1440

S01–P–15

SEARCHING FOR A MOLECULAR MARKER TO SELF-COMPATIBILITY IN SWEET CHERRY

Lili Zhou*, Paul A. Wiersma, Guus Bakkeren, Cheryl Hampson, Frank Kappel

Agriculture and Agri-Food Canda, Pacific Agri-Food Research Centre, Summerland, BC V0H 1Z0 Canada

A stable mutation in sweet cherry (the S4'-allele) allows self-pollination to occur and is a very desirable characteristic for new commercial cultivars. Analysis of this trait, however, requires that the seedling be brought to full maturity (where a significant number of flowers can be tested) since there are no molecular markers available for this pollen part mutation. AFLP and RFLP methodologies were utilized in the search for a molecular marker linked to self-compatibility. No different AFLP band pattern was found between self-incompatible and self-compatible bulked DNA samples when nine pairs of primers (EcoR I + NN / MseI + NNN) were used for this study. Since the S-RNase for S4 is known to be closely linked to the self-compatibility mutation (no known crossover events) this gene was used as a DNA probe to look for genomic DNA fragment differences in the close vicinity of this gene. Bulked genomic DNA preparations from either self-incompatible or self-compatible cultivars were digested with 16 restriction enzymes. Fragment polymorphisms are being investigated for their consistency as a molecular marker in all self-compatible cultivars. In addition to its use in the sweet cherry breeding program despite the marker has potential to aid in the discovery of the mechanism of action of the pollen part recognition in self-incompatibility.

1340–1440

S01–P–16

PROTEOME ANALYSIS OF VACUOLAR MEMBRANE PROTEINS FROM PEAR FRUIT AND ARABIDOPSIS PLANTS

Katsuhiro Shiratake*[1], Sumie Keta[1], Takashi Sazuka[2], Daisuke Shibata[2], Shohei Yamaki[1]

[1]Graduate School of Bio-agricultural Sciences, Nagoya Univ., Nagoya 464-8601 Japan; [2]Kazusa DNA Research Institute, Kisarazu, Chiba 292-0812 Japan

The vacuole stores many compounds, which relate to the quality of horticultural crops, such as sugars, organic acids, minerals and pigments. Accumulation of these compounds mostly depends on transporters of tonoplast. However, most transporters and their genes have not been identified. Proteome, mass-analysis of proteins, is one of the most important analyses in post-genome study. In this study, we tried to identify tonoplast transporters by proteome approach in pear and Arabidopsis using two different methods. Tonoplast-enriched fractions were isolated from pear fruit and Arabidopsis plant. Our target was transporters, which are membrane spanning proteins and bind tightly to the membrane. Membrane peripheral proteins were removed from the membrane by detergent treatment and the remaining proteins, which bind tightly to the membrane, were used for further experiments. In proteome analysis, proteins are generally separated by 2D-PAGE. Thus, the proteins of pear fruit were separated by 2D-PAGE and protein spots were identified by immunoblotting or amino acid sequencing. Membrane spanning proteins did not appear as clear spots and most part of them was lost during 2D-PAGE. We concluded that 2D-PAGE is not suitable for separation of membrane spanning proteins. Therefore, the proteins from Arabidopsis plant were separated by only SDS-PAGE and digested in gel. The peptides obtained were separated by a liquid chromatography and sequenced. We succeeded in identifying more than 90 proteins and 50% of them were membrane spanning proteins. These proteins contain sugar transporters and other transporter-like proteins. We are now determining their exact subcellular localization and functions.

1340–1440

S01–P–17

IMPROVEMENT OF TOLERANCE TO *VERTICILLIUM* WILT AND CONSTRUCTION OF AN INTEGRATED GENETIC MAP BASED ON RAPD AND AFLP MARKERS IN EGGPLANT

Francesco Sunseri*[1], Nazareno Acciarri[2], Giacomo Tamietti[3], Daniela Valentino[1], Anna Sciancalepore[2], Giuseppe Martelli, Giuseppe Leonardo Rotino

[1]Dep. of Biology–Univ. of Basilicata C.da Macchia Romana 85100 Potenza; [2]Istituto Sperimentale per l'Orticoltura–Sezione di Monsampolo del Tronto (AP); [3]DI.VA.P.R.A. Univ. of Torino

Verticillium wilt is one of the most serious diseases in eggplant and one of the main goals of eggplant breeding is the achievement of *Verticillium* wilt tolerant hybrids. *Solanum sodomeum* L. showed a mechanism of partial resistance in roots against *Verticillium* wilt. Interspecific hybrids were obtained by using an accession of *S. sodomeum* L. from Sicily and the eggplant cv. Buia. The results of the screenings for eggplant genotypes tolerant/resistant to *Verticillium* wilt by using backcross are reported. In a naturally infected field, from 1998 to 2001, the resistance to *V. dahliae* of backcrossed progenies was increased by about 60%.The interspecific hybrid were both selfed and backcrossed using different types of eggplant, 48 plants of the F_2 population were utilized for mapping. An integrated linkage map of eggplant (*Solanum melongena* L.) has been obtained by using the F_2 population reported above. Starting from a genetic RAPDs map with ~100 markers mapped on 13 linkage groups, 15 AFLPs primer combinations were screened on the segregating population in order to integrate the genetic map with the aim to achieve markers linked to Verticillium tolerance. Mendelian segregation of loci was verified by chi-square tests of the expected 3:1 and 1:1 ratios. Marker order was determined and all the data were combined to construct the most likely map using the program JoinMap version 1.3. The integrated analysis of markers resulted in the construction of a map consisting of about 350 loci and 12/13 linkage groups spanning over than 1000 cm in a total distance. Linkage between different AFLP markers and the tolerance to *Verticillium* is discussed.

1340–1440

S01–P–18

RAPD MARKERS LINKAGE TO THE DISEASE RESISTANCE GENES IN CHINESE WILD VITIS

Wang Yuejin*, Zhang Jianxia, Wang Xipin, Xu Yan, Wan Yizhen

College of Horticulture Northwest Sci-Tech Univ. of Agriculture and Forestry, Yangling Shaanxi 712100, China

Randomly Amplified Polymorphic DNA (RAPD) and Bulked Segregant Analysis (BSA) were applied to detect molecular markers linked to the disease resistance genes using Chinese wild Vitis, individuals of the cross combinations between Chinese wild *Vitis* and cultivars (*V. vinifera* L.). The results were as follows: two RAPD markers, OPOV03-1380 and OPJ16-750, linkage to *Uncinula necator* resistance genes: three markers OPV02-600, OPJ13-300 and OPS03-1300, linkage to *Sphaceloma ampelinum* resistance genes; OPP09-750, linked to *Coniothyrium diplodiellas* resistance genes; OPC15-1300, linked to *Glomerella cingulata* resistance genes in Chinese wil Vitis were obtained respectively. The actual length of OPJ13-300, OPV02-600 and OPP09-750, were 305bp, 616bp, and 769bp, respectively according to sequencing. SCAR markers converted from RAPD markers, which linked to Coniothyrium diplodiella resistance genes, *Glomerella cingulata* resistance genese were also obtained from Chinese wild *vitis*. The acquired markers provide a solid basis for the molecular-assisted selection (MAS) of disease resistance and for the breeding of varieties high-resistant to disease.

1340–1440

S01–P–19

MOLECULAR MECHANISM OF GRAFT TRANSFORMATION IN RED PEPPER (*CAPSICUM ANUUM L.*)

Yutaka Hirata*, Shingo Ogata, Natsuko Maruyama, Janos Taller, Noboru Yagishita

Tokyo Univ., of Agriculture, and Technology, Fuchu, Tokyo, Japan, 183-8509

Graft-induced genetic changes have been obtained in some crops, such as pepper, eggplant, tomato, tobacco, soybean and mulberry in Japan. We have maintained graft hybrid lines, derived from the scion hot pepper grafted onto sweet pepper, for about 50 generations and characterized them by genetic and molecular analysis. In order to make clear the mechanism and applicability to breeding, we have conducted additional molecular analysis on the graft hybrid in pepper. At the first step, seventeen specific RAPD markers were obtained for the graft hybrid line and both parents. Those markers were co-dominantly controlled. One dCAPS marker was also useful for classification and selection. One graft hybrid specific RFLP marker was observed by Southern blot using atp9 probe, showing an existence of interesting cytoplasmic variation by grafting. Comparison of the one specific RAPD marker (770bp) for graft hybrid and stock which is different from that of scion (700bp) showed a high homology (97.3% in case of 770bp fragment) between graft hybrid and stock material. In another case, specific fragment for scion and graft hybrid includes retrotransposon-like sequence of tomato (69.3% homology between tomato gypsy retro and 704bp fragment), suggesting the reverse transcriptic gene introduction. The results obtained from genetic analysis and molecular evidence supports the existence of gene transfer from stock to scion. This new finding will contribute to a new gene introduction approach for distantly-related species and genera by grafting or cell-to-cell interaction.

1340–1440
S01–P–20
ISSR VARIATIONS AMONG LEMON CULTIVARS FROM A WIDE RANGE OF GEOGRAPHIC LOCATIONS

Osman Gulsen*, Mikeal L. Roose
[1]Alata Bahce Kulturleri Arastirma Enstitusu 33740 Erdemli, Icel, Turkey; [2]Dept. of Botany and Plant Sciences, Univ. of California, Riverside, CA 92507, USA

Inter-simple sequence repeats (ISSR) were used to detect genetic diversity, phylogenetic relationships among 57 lemons [*Citrus limon* (L.) Burm. f.]. The samples included many commercially or genetically important Eureka and Lisbon lemons, two main groups of lemons, from a wide range of geographic locations. Eight ISSR primers amplified a total 88 polymorphic fragments among the 57 accessions. Although 88 loci revealed very little variation, some suspected hybrids were found to have considerable degree of variation. Similarity matrices were calculated and phylogenetic trees derived using unweighted pair-group method, arithmetic average cluster analysis. Despite some phenotypic differences, Eureka and Lisbon lemons had no distinctive genetic differences from each other. Most lemons (68 %) had nearly identical marker phenotypes, having a similarity value of, approximately, 1.0, and suggesting that they originated from a single clonal parent via a series of mutations. The others had a similarity value above 0.8 and may have a origin involving inter-specific, backcross, selfing or combinations of them.

1440–1520
S01–O–21
PLANT VARIETY IDENTIFICATION AND REGISTRATION–THE ROLE FOR MOLECULAR TECHNIQUES

Michael S. Camlin*
Dept. of Agriculture and Rural Development, Plant Testing Station, Crossnacreevy, Belfast, Northern Ireland, U.K., BT6 9SH

The ever-increasing rate at which new varieties are being produced by plant breeders has meant that the registration authorities carrying out or commissioning these technical examinations face major resource problems because of the scale of such growing trials. There are also increasing technical problems in achieving distinctness from the large numbers of varieties that now make up the reference collections for many important plant species. In these circumstances, the potential of molecular systems for producing robust and reliable variety descriptions, suitable for database use and largely unaffected by the environment, has become increasingly attractive. Their potential for application in variety registration is therefore currently under careful and active consideration within UPOV (The International Union for the Protection of New Varieties and Plants). However, it is clear that the power of molecular techniques could potentially allow discrimination between varieties down to the very small differences of a few base pairs within the genome. Therefore, before their possible introduction, it has been necessary to consider how to ensure that sufficient genetic distance between varieties is maintained to be able to give the certainty of continuing protection to the breeders of existing varieties. This paper aims to explore some of the technical aspects of these issues which will have to be resolved within UPOV before the introduction of molecular techniques for variety registration.

1520–1540
S01–O–22
SEQUENCE COMPARISON AND CHARACTERIZATION OF MAPPED RGA MARKERS IN TOMATO

D.O. Nino-Liu, L.P. Zhang, M.R. Foolad*
Dept. of Horticulture, 217 Tyson Bldg, The Pennsylvania State Univ., Univ. Park, PA, USA, 16802

For several years, sequence homologies derived from active peptide domains of plant resistance genes (R-genes) have offered the opportunity to identify resistance gene analogs (RGAs) from different species by using PCR technology. Here we report the display of over one hundred PCR fragments in polyacrylamide gel electrophoresis (PAGE) after amplification of primers corresponding to the three main groups of conserved peptide domains (LRR, NBS and PtoKin). From the total, 46 fragments were polymorphic markers and mapped to 11 of the 12 tomato chromosomes in two BC1 populations of a cross between an early blight (*Alternaria solani*) susceptible tomato (*Lycopersicon esculentum*) breeding line (NC84173) and a resistant accession (PI126445) of the tomato wild species *L. hirsutum*. In both populations QTLs for EB resistance were also identified in different chromosomes. The genomic locations of several of the RGAs coincided with, or mapped closely to, the locations of various disease resistance genes (e.g. BW, Pto, Prf, I2, SW5, Mi, Cf loci) or QTLs (e.g., those for EB and LB). Polymorphic RGA markers isolated from the gel matrix were cloned, sequenced and compared to nucleotide and peptide sequences available in various databases. Sequence analyses of some of the RGAs revealed significantly different degrees of homology not only to major resistance genes or resistance gene clusters, but also to genes involved in several aspects of the plant defense response. Results from these comparisons will be discussed. Sequence information of monomorphic amplification fragments and its potential utility in genetic mapping will also be discussed.

1540–1600
S01–O–23
IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH COLD TOLERANCE IN BLUEBERRY

Lisa J. Rowland*, Smriti Mehra, Elizabeth L. Ogden
USDA/ARS, Fruit Lab, Bldg 010A, 10300 Baltimore Ave., BARC-West, Beltsville, MD, USA, 20705

A survey of blueberry research and extension scientists in the U.S. has identified lack of cold hardiness as one of the most important genetic limitations of current blueberry cultivars. Therefore, the development of more cold hardy varieties is an important need to the blueberry industry. To address this need, our laboratory has been using two distinct but related approaches to identify molecular markers/genes associated with cold tolerance in blueberry. One approach has been to map QTLs controlling cold hardiness in the cold acclimated state. Progress toward mapping QTLs will be presented including: (1) construction of initial, low density genetic linkage maps for two diploid (*V. darrowi* x *V. corymbosum*-derived) blueberry populations segregating for cold hardiness; (2) use of PCR-based markers including RAPD and more recently EST markers for mapping purposes; (3) evaluation of the mapping populations for cold hardiness; and (4) genetic analyses of the cold hardiness data. The other approach has been to identify, isolate, and characterize cold-responsive genes from blueberry and to map these genes to determine if any map to QTLs that control cold hardiness. Levels of a group of dehydrin proteins (proteins induced by dehydration stress such as freezing and drought) of 65, 60, and 14 kDa increase during cold acclimation such that they become the most abundant proteins in blueberry floral buds during the winter. Progress using a systematic molecular genetic approach will be presented including: (1) characterization of expression of the dehydrins, (2) cloning members of the dehydrin gene family, (3) mapping members of the dehydrin gene family and (4) segregation of the dehydrin genes with the cold hardiness trait. Finally, preliminary results from use of a genomic approach to characterize cold-responsive genes from blueberry will be presented.

1600–1620
S01–O–24
ANALYSIS OF ORGAN-SPECIFIC AND—PREFERENTIALLY EXPRESSED GENES FROM ANTHER AND TEPAL OF LILY

Hee-Ju Yu*, Eun-Jung Suh, Byeong Woo Yae, Bong-Hee Han, Il-Gin Mok

National Horticultural Research Institute, RDA, Top-dong, Gwon-seon-gu, suwon, kyong-gi do 441-440, Korea

We have randomly selected 2,000 cDNA clones each from an anther and tepal cDNA library of oriental lily. We isolated organ-preferentially expressed cDNA clones using differential slot blot hybridization to select 550 expressed sequence tags (ESTs) from anther and 306 ESTs from tepal. The selected clones were partially sequenced at the putative 5'-end of insert cDNAs. Among 550 anther ESTs, 498 ESTs were non-redundant and only 150 ESTs were organ-specific clones. Seventy-two anther ESTs had sequence homologies with functionally defined genes. We examined expression pattern of 150 anther specific ESTs during anther development using slot blot hybridization. Most of the genes (123 ESTs) were strongly expressed in mature anther. RNA blot analyses of 32 anther-specific genes revealed that 27 of them were also expressed at high level in mature pollen. The analyzed anther ESTs indicate that most of them are related to pollen or anther development. From 306 tepal-preferentially expressed ESTs, 79 ESTs exhibited significant similarities at the nucleotide level with functionally defined genes. The identified tepal ESTs could be classified to several groups according to their putative function. The most abundant transcripts were found to be involved in pigment production and lipid metabolite.

1620–1640
S01–O–25
POSITIONAL CLONING OF THE APPLE SCAB RESISTANCE GENE VF

Mingliang Xu, Schuyler S. Korban*

Dept. of Natural Resources & Environmental Sciences, 310 ERML, Univ. of Illinois, Urbana, IL 61801 USA

The apple scab disease resistance gene Vf, originating from Malus floribunda 821, remains an important gene locus for the development of apple scab disease resistant cultivars. A total of 11 tightly linked sequence-characterized amplified region (SCAR) markers have been tagged to the Vf locus by using the amplified fragment length polymorphism (AFLP) technique followed by successful conversion of AFLP markers into SCAR markers. These 11 SCAR markers along with three previously identified SCAR markers were used to screen two bacterial artificial chromosome (BAC) libraries constructed from M. floribunda 821 and the apple cultivar 'GoldRush' (also containing Vf), respectively. As a result, a BAC contig, consisting of five overlapping BAC clones, was constructed spanning a genetic distance of ~290 kb of the Vf region. Four receptor-like resistance gene homologues were unveiled in the Vf region after searching for encoding sequences from overlapping BAC clones. These four Vf gene homologues were designated as Vfa1, Vfa2, Vfa3, and Vfa4, and co-segregated with the Vf locus in a segregant population. The first three homologues, Vfa1, Vfa2, and Vfa3, were strongly expressed in young leaves, while the fourth homologue, Vfa4, was strongly expressed in mature leaves. Four cDNA clones corresponding to these four homologues were recovered by using rapid cDNA end amplification (RACE) method. Sequence comparison between cDNA and genomic DNA were conducted. The deduced amino acid sequences revealed that all these Vf gene homologues contain both an extracellular leucine-rich repeat domain and a trans-membrane domain. Functional complementary tests were then conducted using cDNA and genomic DNA clones for these four gene homologues.

1640–1700
S01–O–26
DEVELOPMENT OF RAPD AND SCAR MARKERS LINKED TO FLESH ADHESION GENE IN PEACH

J.H. Jun*[1], K.H. Chung[1], S.B. Jeong[1] H.J. Lee2

[1]National Horticultural Research Institute, Rural Development Administration, Suwon 440-310, Korea; [2]School of Plant Science, Seoul National Univ., Suwon 441-744, Korea

Flesh adhesion to the stone in peach (*Prunus persica* [L.] Batsch) fruits can be categorized into three groups of freestone, semi-clingstone, and clingstone. The flesh adhesion is known to be determined by a single gene pair Ff with the dominance of freestone to clingstone. Bulked segregant analysis and random amplified polymorphic DNA (RAPD) analysis were performed to detect the markers linked to the Ff gene using F_1 hybrid progenies of the clingstone ('Yumyeong') and freestone ('Baekhyang') (Ff) cross. Four dominant coupling-phase RAPD markers linked to the gene, designated as OPB05-1300, OPI07-1050, UBC439-1400, and OPD20-580, were identified from 568 10-base primers examined. The co-segregation of the four markers and the Ff gene was verified using the 95 individuals of the 'Yumyeong' and 'Baekhyang' cross. The pooled recombinant frequencies were estimated and converted into genetic distances and linkage group order scores. The resulting linkage map spaned 16.2 cm and comprised three markers located on the same side of the Ff gene and one marker located on the other side. Two RAPD markers UBC439-1400 and OPB05-1300 were developed into a dominant sequence characterized amplified region (SCAR) markers. The SCAR markers were found to be adequate to identify the Ff gene in segregating progenies and commercial varieties. These markers can reliably be used in the marker-assisted selection of peach germplasm at the early developmental stage of the trees.

Tuesday · August 13

1100–1200
S01–O–27
STRESS AND QUALITY IN IN VITRO CULTURE

Alan C. Cassells*

National Univ. of Ireland Cork, Dept. of Plant Science, Western Road, Cork, Ireland

Plant growth depends on water, light, minerals, carbon dioxide and temperature, and may suffer adversely from exposure to individual or combinations of these factors which are outside the normal range of the genotype's tolerance resulting in a stress response. Given the controlled environment in which tissue culture is carried out, the types of stresses and their range is arguably limited compared with the natural environment. In vitro stresses may include waterlogging, drought, mineral and hormonal imbalance, salinity and light, singly or in combination. Responses to stress in vitro include physiological aberrations e.g. loss of stomatal function, the hyperhydricity syndrome, establishment failure and poor post establishment performance. Changes in development are common, particularly re-invigoration (rejuvenation), while genetic change (somaclonal variation) can occur in adventitious regenerants of some genotypes. Stress is associated with changes in the metabolome, in physiology and in genomic expression, in changes in hormone balance and response to hormone balance, and in the genome. Stress adaptation is associated with genomic rearrangement and altered development programmes. On return to non-stress conditions these changes may be reversible or irreversible, the latter may be heritable. Stress markers include changes in ethylene and ABA, in reactive oxygen species, in heat shock and pathogenesis-related proteins, in DNA methylation, in DNA sequence amplification and de-amplification, in chromosome breakage and re-arrangements, in chromosome number, in transposon activation. Many stresses share common features for which the term "stress cross tolerance" is used, specifically, there is the evidence that oxidative stress is a general element underlying specific stresses. The research reported here is aimed at improving the physiological, epigenetic and genetic quality of microplants by reducing stress exposure in vitro and by enhancing abiotic and biotic stress tolerance post vitrum.

1200–1240
S01–O–28
BIOTIZATION OF MICROPLANTS FOR IMPROVED PERFORMANCE

Silvio Gianinazzi*[1], Medhat Chahbandar[2], Bachar Blal[3], Marie-Claude Lemoine[4]

[1]UMR 1088 INRA/U. Bourgogne BBCE-IPM, CMSE-INRA, BP 86510, 21065 Dijon Cedex, France; [2]FLORANIL, 1 rue Gustave Eiffel, 66440 Torreilles Cedex, France; [3]BIORIZE, 8 rue Sainte Anne, 21000 Dijon, France; [4]UME 1011 INRA/AGRI-OBTENTION UP VITRO, BP 86510, 21065 Dijon Cedex, France

In nature, beneficial soil micro-organisms play a key role in assuring satisfactory plant growth and development in microbial-rich and nutrient-poor environments. However, micropropagation technology by definition eliminates all microorganisms from plant tissues, including beneficial microorganisms. The absence of the latter, therefore, requires the use of nutrient-rich and microbial-

poor environments to guarantee growth at out planting. This is presently ensured by the use of artificial substrata and high chemical inputs (fertilizers, pesticides). However, economic factors, as well as the growing awareness about environmental problems, make it necessary to reduce the use of these chemical inputs and to develop technologies compatible with sustainable horticulture. The rhizosphere of microplants is an uninhabited niche and space where nutrients are plentiful for the introduction of beneficial microorganisms. Inoculation of microplant roots will create dynamic micro-environments where microorganisms, plant roots and soil components can interact to carry out activities known to positively influence plant fitness. Consequently, an appropriate biotization of microplants will not only ensure a preferential association with chosen beneficial microorganisms (such as mycorrhizal fungi, PGPR or antagonistic microbes), but also induce changes in plant physiology and development more compatible with their adaptation to a post vitro environment, by facilitating plant growth with low chemical inputs and decreasing risks of being colonized by harmful microbes. A case study of promoting multimicrobial plant biotization with micropropagated raspberry and artichoke will be presented. Limited knowledge about compatibility between beneficiai microorganisms in the rhizosphere considerably hampers the development of this know-how which it is reasonable to speculate will condition the full commercial exploitation of microplant technology.

1340–1440

S01–P–29

THE INFLUENCE OF THIDIAZURON ON IN VITRO SHOOT PROLIFERATION OF OAKLEAF HYDRANGEA (*HYDRANGEA QUERCIFOLIA*)

Donna I. Ledbetter*, John E. Preece

Dept. of Plant, Soil and General Agriculture, Southern Illinois Univ., Carbondale, IL 62901-4415, USA

The objective of this study was to promote adventitious shoots from leaf explants and axillary shoots from nodes in vitro. The leaf explants were excised from 12-15 month-old shoot cultures and the nodal explants were from greenhouse-grown stock plants of Hydrangea quercifolia. The leaf exlants were obtained by excising leaves and cutting them in half before placing on MS medium with thidiazuron (TDZ) at 5x10-8, 1x10-7, 5x10-7, 1x10-6, or 5x10-6 M plus 1 µM indolebutyric acid (IBA). The explants were transferred to fresh medium every 28 days and shoot number and shoot length data were taken at the end of 16 weeks. Thidiazuron significantly affected shoot number with a mean of 68.7 adventitous shoots when the medium contained 5x10-6 M TDZ. However, the majority of these shoots were shorter than 0.5 cm. As the concentation of TDZ decreased, significantly fewer adventitious shoots formed with means of 1.5, 1.8, 21.6, and 35.7 total shoots for treatments 5x10-8, 1x10-7, 5x10-7, and 1x10-6 M TDZ, respectively. Shoots that were taken from greenhouse grown plants were cut into 2 cm explants with nodes or a terminal bud. They were disinfested for 15 minutes in 0.6% NaClO and placed horizontally on MS medium with 10-10, 10-9, 10-8, 10-7, or 10-6 M TDZ plus 1 µM IBA. The nodal explants were transferred to fresh medium every 28 days and data were taken at 9 weeks. Total shoot production was greatest when the medium contained 10-6 TDZ with a mean of 5.2 shoots. At this concentration, there was a combination of both axillary and adventitious shoots. As the concentration of TDZ decreased most or all shoots were of axillary origin.

1340–1440

S01–P–30

UTILIZATION OF EMBRYOGENIC CELL CULTURES FOR THE MASS PRODUCTION OF BULBLETS IN ORIENTAL AND EASTER LILIES

S.K. Kim*, J.S. Lee, B.J. Ahn

Dankook Univ. School of Bioresource Science, Anseodong, Cheonan 330-714, Korea

To utilize a somatic embryogenic cell culture system for mass production of bulblets in Oriental and Easter lilies, methods of embryogenic callus induction, multiplication and bulblet production were investigated. The sliced bulb scales of a *Lilium longiflorum* hybrid (*longiflorum* x *fomolongi*) and Oriental hybrids ('Marco Polo', 'Casablaca', 'Le Reve' were cultured on MS agar medium (3% sucrose, 0.3% Phytagel) supplemented with 2,4-D, dicamba or picloram at the concentrations of 1, 2, 4, 6, 10 mg/L with or without 1 mg/L BA. The most embryogenic calli, determined upon their morphological characteristics and regenerability on hormone-free MS medium, were produced with 2 mg/L of dicamba in *L. longiflorum* and 6 mg/L of picloram in Oriental hybrids. They proliferated rapidly in a stationary agitation culture using MS liquid media (5% sucrose) supplemented with 2 mg/L of dicamba for *L. longiflorum* and 2 mg/L of picloram for the Oriental hybrids for the periods of half to 4 years. Adult plants of *L. longiflorum*, regenerated from the cell lines maintained less than a year, showed few somatic variants, but those cultured over 4 years produced mostly morphological variants of flower deformities or abnormal growth. For mass production, the embryogenic cell line of 'Marco Polo' Oriental hybrid was cultured in 20-L air-lift bioreactors, a continuous type using liquid MS medium (5% sucrose, pH 5.8) supplemented with 2 mg/L of picloram, and transferred to culture boxes (96x96x90 mm) containing 100 mL of hormone-free MS agar medium (0.3% Phytagel, 9% sucrose) for plant regeneration and bulblet formation. Numerous bulblets larger than 5 mm in diameter were produced in four months and planted in greenhouse after 8 weeks of 4 °C storage. They made flower bulbs in greenhouse in two years, and were found cucumber mosaic virus free by ELISA analyses

1340–1440

S01–P–31

MICROPROPAGATION OF SWEET VIBURNUM (*VIBURNUM ODORATISSIMUM*)

Gisele Martins*, Michael Kane, Thomas Yeager

Univ. of Florida Dept. of Environmental Horticulture PO, Box 110670, Gainesville, FL 32611

Sweet viburnum is a widely used shrub in Florida landscape. Because of its fast growth and prompt response to nitrogen fertilization, sweet viburnum has been used as a model plant to study root and shoot growth cycles and nitrogen nutrition. Liners grown in shade house were the source of explants for stage I. Explants were established in Murashige and Skoog inorganic salts and vitamins, supplemented with 2.2 mM of benzyladenine, 3% sugar and 7 g/L of TC agar. Explants were transferred to a medium consisting of Woody Plant Medium inorganic salts and vitamins, 3% sugar supplemented with 4.4 mM BA and solidified with 7 g/L TC agar. Shoot multiplication was successfully obtained using this medium. Because explants produced in the Stage II were short and clustered, an experiment was completed to determine the optimum growth regulators combination and concentration for shoot elongation and multiplication. Uniform clusters with about 4 shoots about 4 mm long were transferred to WPM containing BA at 5 levels (0, 0.5, 1.1, 2.2 or 4.4 µM) and GA3 at 4 levels (0, 0.35, 1.73 or 3.46 µM). Number of shoots longer than 12 mm increased as GA3 concentration increased. Shoot length was significantly enhanced by addition of low concentration of BA (0.5 µM). Medium supplemented with 2.2 µM BA and 1.73 µM GA3 resulted in higher shoot production. A BA rate higher than 2.2 µM resulted in lower number of shoots. A separated set of microcuttings maintained in WPM without growth regulators (0 BA and 0 GA3) rooted within 4 weeks. After 8 weeks, a well-developed root system was observed. These plants were transplanted to a peat-based medium under intermittent mist and after 60 days, the ex vitro survival rate was about 50%. Further experiments are needed to determine the optimum concentration of growth regulators to induce root formation increase plant survival.

1340–1440

S01–P–32

IN VITRO RESPONSE OF SOME IRANIAN PEAR (*PYRUS COMMUNIS* L.) CULTIVARS TO BASAL TISSUE CULTURE MEDIA

Seyed Zia Nosrati*, Zabihollah Zamani, Mesbah Babalar

Dept. of Horticulture, Faculty of Agriculture, The Univ. of Tehran, Karaj 31587, Iran

To evaluate the response of three famous Iranian pears to in vitro basal media, experiments were conducted. Iranian pears 'Shahmiveh', 'Natanzi' and 'Dargazi' were used for this experiment and 'Bartlett' was used as control and a factorial design was used for the experiment. Four media used in the first experiment included: 1) Murashige and Skoog medium (MS); 2) MS with half concentration of macronutrients (MS/2); 3) MS with half concentration of nitrogen compounds (MSN/2); and 4) Quoirin and Lepoivre macronutrients plus MS micronutrients and organics (MQL). In the second experiment, 10 media including the above, as well as their modified versions (doubled micronutrients or doubled nitrogen compounds) were tested. In all media, BA at 1 mg/L and IBA at 0.1 mg/L were added. Growth characters of cultures including shoot number, shoot length, leaf expansion, leaf color and callus size at shoot base were scored.

1340–1440
S01–P–33
EFFECT OF MINERAL CONCENTRATION AND VENTILATION ON ACCLIMATIZATION OF BANANA (*MUSA* SPP. CV. DWARF CAVENDISH) EXPLANTS

Mohammad Amiri*

Dept of Horticulture, Univ. of Zanjan, Zanjan, Iran

Growth and root formation of banana (*Musa* spp. Cv. Dwarf Cavendish) plants grown on the different mineral concentration (0.2X, 1X, modified MS) media significantly increased under sealed culture (RH = 100%) conditions, compared with under ventilated (RH = 70%) conditions. Under ventilated conditions, water availability and mineral availability (especially Ca^{++}, and K^{+}) are less. This resulted in decreased growth. Whereas, both the highest rate of growth and the highest percentage (80%) of ex vitro acclimatization were obtained on plants which grown on the high mineral (2X) concentration under ventilated conditions. This is due to mineral availability and mineral uptake (especially Ca^{++}, and K^{+}) by mass flow. Mass flow is significant whenever mineral condition is high (2X). Acclimatization was improved by K^{+} and Ca^{++} uptake by mass flow under ventilated conditions. Under ventilated conditions, there are both evaporation from culture surface, headspace, and transpiration from leaf surface of the explants. This resulted in higher water potential gradient between medium, plant and headspace in vitro. The rate of water flux and thus the rate of mass flow were not constant overtime. It was dependent on the water content and mineral concentration in the medium. As the medium water content decreased over time (during 16 days), the water flux and thus mass flow decreased. Similarly, as the mineral concentration decreased, the mass flow decreased. It can be concluded that a ventilation system is not only good for gas exchange, but also for mineral uptake (especially Ca^{++}) in vitro.

1340–1440
S01–P–34
MICROPROPAGATION OF CHOCOLATE COSMOS (*COSMOS ATROSANGUINEUS*) BY REPEATED DIVISION OF NODES/AXILLARY SHOOTS AND ADVENTITIOUS SHOOTS FROM MICROSHOOTS.

T. Hosoki*, H. Kobayakawa, K. Ohta

Faculty of Life and Environmental Science, Shimane Univ., 1060 Nishikawatsu-cho, Matsue, Shimane Pref., 690-8504, Japan

Chocolate cosmos is native to Mexico and the flower color and scent mimics chocolate. So, it is very popular for cut flowers and arrangements. Propagation is conducted by division of tuberous roots with eyes. This multiplication rate is extremely slow. Therefore, micropropagation is necessary to supply a large number of plants in a short period. Shoot tips, 3 mm long, were cultured at 26 °C on MS medium (pH 5.6,) containing 0, 0.1, 0.2 or 1 mg/L BA .Every three weeks, elongated shoots were horizontally cut into stem sections including nodes, and growing axillary shoots were separated. These explants were used for subcultures. A total of four subcultures were continued by this method. The results showed that three to four multiplication rates were obtained each subculture at 0.2 or 1 mg/L BA. When elongated shoots were cultured on MS medium containing 1 mg/L IBA, about 60% of shoots rooted. Ninety-two percent of rooted plants were successfully acclimatized in the pots at 20 °C after two weeks of planting. Leaves, stems, and petiole with leaves were sectioned and cultured on MS medium containing 0, 0.1 or 1 mg/L NAA combined with 1 or 4 mg/L BA. Adventitious shoots regenerated best from stem sections on the medium containing 1 or 4 mg/L BA with 0 or 0.1 mg/L NAA. A maximum regeneration rate and number were obtained on a single treatment of 1 mg/L BA (80% and three shoots per section, respectively). Thus, chocolate cosmos can be efficiently propagated by repeated shoot sectioning and division of axillary shoots. Adventitious shoot regeneration may be applied to create somaclonal variants and transformants.

1340–1440
S01–P–35
IN VITRO STUDIES FOR ARTIFICIAL SEEDS IN CHRYSANTHEMUM SP.

Pandya Himanshu*[1], O.P. Saxena[2]

[1]Smt.D.J.Shah Parivar Science College, Dholka-387810. Dist. Ahmedabad, Gujarat, India; [2]Botany Dept.,Gujarat Univ., Navarangpura, Ahmedabad-380009. Gujarat, India

Plant cells are totipotant. This means that given the appropriate nutritional and physical environment, any plant cell should be able to regenerate the phenotype of the complete and differentiated organism from which it was originally derived. This implies that it would be possible to induce cells to produce any substance characteristics of the parent plant. Chrysanthemum is probably one of the oldest among the cultivated flowering plants. Our growing season is limited up to the winter. Our growing season can be extended in to all four seasons by tissue culture. All the plant parts were incorporated for tissue culture. Callus, somatic cells, somatic tissues, somatic embryos were derived and were experimented for artificial seeds production. The most widely used method for production of synseed is hydro gel encapsulation with 4% sodium alginate. This most popular method is using a dropper to insert somatic cells, somatic tissues and somatic embryos in to the drops as they fall in to a complexing bath to form calcium alginate capsules that are 2-4 mm in diameter. A capsule hardness of breaking pressure per capsule was found to allow germination while providing sufficient integrity so that the capsules can be handled on routine basis without breakage at room temperature. For germination of synseeds, MS basal medium was used. Plantlets of Chrysanthemum could be regenerated. The regenerated plantlets looked normal and exhibited a 95% survival ratio after transferring to soil. Results of isoenzyme electrophoresis showed that there was no significant difference in the profiles of any of the tested enzymes among the regenerants, or between tissue culture and synseed-derived plants.

1340–1440
S01–P–36
IN VITRO PROLIFERATION OF *TRILLIUM GRANDIFLORUM* RHIZOMES

Susan M. Foster*[1], S.L. Kitto[2]

[1]152 Townsend Hall Dept. of Plant and Soil Sciences Univ. of Delaware Newark, Delaware 19717; [2]132 Townsend Hall Dept. of Plant & Soil Sciences Univ. of Delaware Newark, Delaware 19717

Proliferation of in vitro maintained *Trillium grandiflorum* clone 11 rhizomes were examined on liquid versus gelled media and on gelled MS vs. Cereal Crops media. In the two liquid medium experiments, a short-term, 4-week, and a long-term, 12 week, the rhizomes were transferred from gelled maintainence medium to liquid and shake-cultured at 125 rpm. In the short-term liquid medium experiment, rhizomes were cultured in either liquid medium, subcultured every 2 weeks, or gelled medium, subcultured either every 2 weeks or once a month (control). The same conditions prevailed in the long-term liquid medium experiment but the rhizomes, subcultured in the liquid medium, were subdivided at 4 weeks. Rhizomes on the gelled medium were not subdivided because they did not produce large enough buds. Short-term proliferation in liquid medium resulted in a 5-fold increase in rhizome weight and a 3.5-fold increase in number of buds per rhizome. The long-term liquid medium procedure resulted in a 12-fold increase in rhizome weight at 4 weeks and a 2-fold percent weight increase at 12 weeks. Unfortunately, 20% of the rhizomes were vitrified in the long-term liquid medium experiment. Cereal Crops medium resulted in a 4-fold increase in rhizome weight and a 20-fold increase in the number of dormant buds when compared to rhizomes cultured on the standard trillium maintainence medium. Future experiments will concentrate on delineating shaking vs. static conditions and the problems of vitrification.

1340–1440
S01–P–37
COMPARATIVE STUDY OF ZYGOTIC AND SOMATIC EMBRYOS OF *FOENICULUM VULGARE*

Irene Morone Fortunato*, C. Ruta, A. Tagarelli

Dipartimento di Scienze delle Produzioni Vegetali–Facoltà di Agraria–via Amendola 165/a, Bari Italy

A comparative study was carried out on zygotic and somatic embryos of *Foeniculum vulgare* Mill. ssp. piperitum to evaluate the effect of growth conditions on their development. Zygotic embryos, collected during one growing season were examined. In comparison, the influence of growth conditions on the development of somatic embryos in vitro was also studied. In vitro single cells divided with irregular patterns to form cell cluster of various shapes and sizes with uneven surfaces. Important were the differences between the transition of single cells to globular embryos and the transition of zygotes to globular embryos, which is caused by regular longitudinal and transverse divisions. The cell

clusters became isodiametric globular embryos with a smooth surface, and they developed to heart-shaped embryos that were bilaterally symmetrical along the apical -basal axis. From these cell clusters, globular embryos with cotyledon primordia formed, but cell clusters also developed directly to heart-shaped embryos without going through the typical globular stage. Serial observations revealed a divergent sequence of morphological stages, from cell clusters to heart-shaped embryos during somatic embryogenesis.

1340–1440

S01–P–38

EFFECT OF THIDIAZURON ON IN VITRO SHOOT PROLIFERATION OF LINGONBERRY (*VACCINIUM VITIS-IDAEA* L.)

Samir C. Debnath*

Agriculture and Agri-Food Canada, Atlantic Cool Climate Crop Research, P.O. Box 39088, St. John's, NF, Canada., A1E 5Y7

The research on the micropropagation of the lingonberry (*Vaccinium vitis-idaea* L.) began at the Atlantic Cool Climate Crop Centre of Agriculture and Agri-Food Canada in St. John's, Newfoundland in 1999 under the Small Fruit Development Program for Cool Climates, and has already yielded a considerable amount of information. In an attempt to improve the micropropagation protocol developed in the Centre, two lingonberry genotypes were compared for in vitro shoot proliferation cultured on two different media supplemented with varying levels of thidiazuron (TDZ). TDZ at 0.5 to 5 μm supported proliferation, but strongly inhibited shoot elongation. TDZ initiated cultures when transferred to medium containing 1 mM zeatin produced usable shoots after one additional subculture. Genotypes differed significantly with respect to multiplication rate. Concentrations of TDZ above 5 μm had an inhibitory effect on shoot proliferation. In both genotypes, shoot proliferation was greatly influenced by explant orientation. Proliferated shoots were rooted on a 2 peat: 1 perlite (v/v) medium, and the plantlets were acclimatized and eventually transferred to the greenhouse.

1340–1440

S01–P–39

PHOTOCONTROL OF SHOOT REGENERATION FROM TOMATO HYPOCOTYL: ANALYSIS USING PHYTOCHROME MUTANTS

Lise Bertram*, Bartolomeo Lercari

DBPA, Univ. of Pisa, Viale delle piagge, 23, Pisa, Italy, 56124

We investigated the role of phytochromes in the photocontrol of shoot regeneration from hypocotyls of tomato by comparing the regenerative capability of explants obtained from etiolated and light grown seedlings of phyA, phyB1, phyB2, phyA/phyB1, phyA/phyB2, phyB1/phyB2, phyA/phyB1/phyB2, hp-1 and au mutants and their isogenic wild types. Our results indicate that the light-dependent acquisition of competence for shoot regeneration in the tomato hypocotyl involves the action of phyA, phyB1, phyB2 and a fourth phytochrome. Distinct phytochromes have both overlapping and antagonistic action. Multiple interactions between phytochromes during the growing phase preceding the in vitro culture enhance the subsequent responsiveness of tomato hypocotyls to phytochromes (i.e. responsiveness amplification).

1340–1440

S01–P–40

ROLE OF CALCIUM IN TAMARILLO SOMATIC EMBRYO DIFFERENTIATION

Paula Barriga*[1], Maria Salomé Pais[2], Jorge Canhoto[1]

[1]Departamento de Botânica, FCTUC, Universidade de Coimbra, Arcos do Jardim, 3001-455 Coimbra, Portugal; [2]Ed. ICAT, Unidade de Biotecnologia Vegetal, FCUL, Portugal

The role of calcium in tamarillo (*Cyphomandra betacea*) somatic embryo differentiation (SED) from proembryogenic masses (PM) was studied in the presence of two calcium channel blockers: lanthanum (0.1; 1; 10 and 100 mM) and verapamil (0.1; 1; 10 and 25 mM). Three experiments were carried out: culture of the PM on media containing the inhibitors, culture on the differentiation medium (0,1 mg/l GA3) for several periods of culture (1, 4 and 8 days) followed by subculture on a medium containing the inhibitors and culture on the inhibitor medium followed by transfer to the differentiation medium. The results showed that somatic embryos could differentiate even in the presence of the inhibitors. However, under those conditions, the levels of somatic differentiation were very low (12%) when compared with the control. The culture of the PM in the presence of the inhibitors after several periods of contact with the differentiation medium showed that SED decreased with increasing concentration of the inhibitors. Similar results were obtained when the time of contact with the differentiation medium decreased. In the third kind of experiments, an increase in SED with increased levels of calcium channel blockers, was observed. It can be concluded that calcium seems to play a critical role in tamarillo somatic embryo differentiation.

1340–1440

S01–P–41

EFFICIENT PLANT REGENERATION VIA ORGANOGENESIS FROM LEAF SEGMENTS IN CUCUMBER

J.Y. Choi*, S.H. Park, Y.S. Kim, N.I. Hyung

Dept. of Horticultural Science, Sangmyung Univ., Chonan 330-720, Korea

Efficient plant regeneration system via organogenesis from leaf segment in cucumber (*Cucumis sativas* L.) was developed. Leaf explants, obtained from seedlings after 21–28 days of in vitro germination, were cultured on MS medium containing 30g/L sucrose supplemented with various combinations of zeatin (1.0–3.0mg/L) and IAA (0.05–0.3 mg/L). The explants were cultured initially in darkness for 2 weeks and then transferred to light condition. After 2 weeks in culture the initial adventitious shoots were observed. The shoots were formed mainly on the basipetal midvein region of leaf explants. Optimal concentration of plant growth regulator for shoot regeneration was 2.0 mg/L zeatin and 0.05 mg/L IAA, in which the regeneration frequency reached up to 70%. There was a tendency to increase the regeneration frequency with decreasing IAA concentration. Comparing the leaf age, the expanded young leaf showed higher regeneration frequency than old leaves. The regenerated shoots were rooted on 1/2MS medium with 0.05 mg/L IBA. The plantlets were transferred to soil and acclimated successfully. This procedure is recommended for the production and confirmation of transgenic plants in cucumber.

1340–1440

S01–P–42

OPTIMAL ROOTING SYSTEM ESTABLISHMENT FOR IN VITRO MICROCUTTING OF SIX WALNUT CULTIVARS (*JUGLANS REGIA* L.)

Pinghai Ding*[1], Dong Pei[2], Lichai Yuan[3]

[1]Dept. of Horticulture, ALS building, OSU, Corvallis, OR, 97331, USA; [2]Economic Forest Dept., Forest Academy Institute of China, Xianshan, 100000, China; [3]Dept. of Horticulture, Hebei Agricultural Univ., Baoding 071001, China

Rooting difficulty of in vitro micro-cuttings is one of the most important factors affecting rapid mass propagation of walnut cultivars by tissue culture. Within 2 years, an optimal rooting system was setup for walnut in vitro root induction. The first step was selecting enough juvenile and vigorous micro-shoots; shoots were then cultured in DKW medium containing 5 mg/L IBA and 40 g/L sucrose in darkness. After 10 to 14-day, the shoots were transferred to vermiculite medium (50 mL) containing DKW medium without IBA and incubated in the light. With this method, 70% to 90% root induction was obtained with six walnut cultivars. The developmental status of the micro-shoots was very important for root induction. Phenolic compounds and polyamines in combination with IBA induced root induction, but had no effect without IBA. IBA was more effective and stable than NAA in promoting root formation. Adding lutin to the medium increased the rooting percentage, root length and root number per micro-cutting. Endogenous IAA analysis showed that IAA synthesis induces root initiation. SDS-PAGE showed that root initiation was accompanied by the disappearance of a 81.7KD and a 61.4KD protein and the appearance of a 28.6KD protein.

1340–1440

S01–P–43

PRODUCTION OF SOMATIC EMBRYOS OF AMERICAN GINSENG IN SUSPENSION CULTURE AND REGENERATION OF PLANTLETS

Mistianne Feeney*, Zamir K. Punja

Centre for Environmental Biology, Simon Fraser Univ., 8888 Univ. Drive, Burnaby, British Columbia, Canada, V5A 1S6

American ginseng (*Panax quinquefolius* L.) is widely grown in Canada as a source of vitalizing and stimulating agents. The slow-growing nature of this plant and the 4-yr reproductive cycle make breeding efforts to select for desir-

able traits, eg. disease resistance or improved quality, difficult. A tissue culture method to clonally propagate ginseng has potential to overcome these restrictions. A range of ginseng seed sources were evaluated for their response to tissue culture conditions. Leaf and stem explants from seedlings of 11 lines were placed on Murashige and Skoog (MS) medium with 10 μM NAA and 9 μM 2,4-D, 3% sucrose, 8 g agar/L, pH 5.8. Callus developed after 3 wk in darkness and by 10 wk, embryos were observed on all lines and the cultures were transferred to light. To initiate embryogenic suspension cultures, callus (1 g) was placed in 20 mL MS liquid medium containing 2.5 μM NAA and 2.25 μM 2,4-D and shaken at 115 rpm for 8 wk. Subcultures were made every 8 wk and large numbers of somatic embryos were produced. Somatic embryo pre-treatments were assessed to enhance embryo germination and plantlet quality. Dessication for 3 days to achieve 20% to 30% water loss or placement of embryos in 50 mL half-strength MS with 1% charcoal at 130 rpm for 7 days both enhanced overall germination and germination rate. Treatment of embryos and germinants with 3 μM GA and 5 μM BA for 7 days in low light followed by incubation on half-strength MS with 1% charcoal for 15 days promoted further shoot development. Plantlets were obtained and transferred to magenta boxes containing vermiculite saturated with half-strength MS liquid containing no sucrose to acclimatize.

1340–1440

S01–P–44

SOMATIC EMBRYOGENESIS FROM IN VITRO-DERIVED EXPLANTS OF ROSE

Chang-Kil Kim*[1], David H. Byrne[1], Jung-Youl Oh[2], Jae-Dong Chung[3]

[1]Dept. of Horticultural Science, Texas A&M Univ., College Station, TX 77843-2133; [2]Dept. of Horticulture Sangju National Univ., Sangju 742-711, Korea; [3]Dept. of Horticulture Kyungpook National Univ., Taegu 702-701, Korea

Callus was initiated from in vitro-derived explants of rose (*Rosa* sp.) on Murashige and Skoog (MS) basal medium supplemented with 2,4-D, dicamba, NAA, kinetin and 6-benzyladenine (BA), alone or in combination. Somatic embryogeneic callus was obtained on MS medium containing dicamba and kinetin. Maturation of somatic embryos was achieved on MS basal salts supplemented with vitamin and 1.0% activated charcoal without growth regulators. Silver nitrate and partial desiccation of calli also enhanced production of somatic embryo. The use of MS medium containing BA and NAA facilitated embryo germination. Somatic embryo derived plantlets were hardened and successfully transferred to the greenhouse.

1340–1440

S01–P–45

REGENERATION OF INTERSPECIFIC MIXOPLOID SOMATIC HYBRIDS VIA ASYMMETRIC SOMATIC HYBRIDIZATION IN CITRUS

Jihong Liu*, Xiuxin Deng

National Key Lab of Crop, Genetic Improvement, Huazhong Agricultural Univ, Wuhan, Hubei, P.R.China, 430070

Embryogenic protoplasts of Dancy tangerin (*Citrus reticulata* Blanco), irradiated with X-rays for different times (60, 90 and 120 min) at 5mA and 80kVp, were electrically fused with embryogenic protoplasts of Page tangelo (*Citrus reticulata paradisi*) that were treated with 0.25 mM iodoacetic acid (IA) for 15 min. The IA-treated protoplasts of Page could not divide at all. Division of Dancy protoplasts irradiated for 60 min was not prevented but delayed, whereas those irradiated for 90 and 120 min did not divide. Instead, they plasmolysed or broke. The fusion-treated protoplasts could develop into embryoids for all of the fusion combinations only when the callus was transferred to MT supplemented with 2% glycerol. However, only embryoids could develop into multiple shoots derived from the fusion combination in which the donor protoplasts were irradiated for 60 min but were recalcitrant to rooting. In vitro grafting was employed to obtain complete plants. Cytological observation showed that the plants contained mainly diploid and aneuploid cells, together with very few tetraploid cells, indicating that they were mixoploids. Random amplified polymorphic DNA analyses with three 10-mer arbitrary primers confirmed the plants as true somatic hybrids. This is the first report on regeneration of mixoploid hybrid plants via protoplast asymmetric fusion in Citrus. Negative effects of ionizing irradiation on regeneration of embryoids and plantlets and possible agronomic interest of the mixoploid plants will be discussed.

1340–1440

S01–P–46

THE EFFECT OF AQUEOUS EXTRACTS OF SOME ORGANIC FERTILIZERS ON IN VITRO GROWTH AND PHOTOSYNTHETIC ACTIVITY OF M9 (*MALUS DOMESTICA* BORKH) APPLE ROOTSTOCK SHOOTS

Grazia Marino, Graziella Marcolini, Bruno Marangoni*

Dipartimento di Colture Arboree, Univ. of Bologna, Via Filippo Re 6, Bologna, Italy, 40126

In vitro shoot cultures of M9 apple rootstock were maintained through repeated subcultures (four weeks) on a modified Murashige Skoog (MS) proliferation medium (PM) enriched with 1 mg/L benzyladenine (BA). Single shoots were compared for their growth, proliferation, and photosynthetic activity on standard PM medium and on media with reduced cytokinin (0.2 mg/L BA) and salt (MS/3) concentrations enriched with aqueous extracts from the following organic fertilizers: livestock manure (LM), sugarbeet industrial waste (SW), mixed grape, poultry manure and municipal solid waste (GMW) and citrus pruning and industrial waste (CW). Aqueous extracts were prepared by mixing organic fertilizers and water at 1:10 (w/v) rate in a blender, leaving the suspension overnight at 4 °C and filtering with paper filters. The pH of the extracts was adjusted to 5.7, they were filter-sterilized (0.22 μm) and added at final concentrations of 2%, 0.2%, 0.02%, 2% and 0 (control) to previously autoclave-sterilized media. Culture conditions were 22 + 2 °C with a 16h-photoperiod, and about 20 and 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light for evaluation of growth and photosynthetic activity, respectively. Photosynthetic rates were gas chromatographically evaluated as CO2 uptake/ gram fresh weight over time inside tightly closed jars. GMW appeared to increase shoot growth when added to media with reduced BA concentration; CW treatments did not show any significant effect, regardless of the basal composition of the culture media. In contrast, LM tended to improve shoot growth and proliferation, especially when added at intermediate concentrations to standard PM and MS/3 media. Results comparable to those for LM were found for SW extracts on PM medium. Moreover, shoots grown on media with low salt levels and enriched with SW extracts tended to produce greener leaves and to show a higher photosynthetic activity than controls, in spite of their lower weight increase.

1340–1440

S01–P–47

HIGHER AMOUNTS OF COPPER AND MYO-INOSITOL IN THE CULTURE MEDIUM ENHANCE MORHOGENIC RESPONSE OF EXPLANTS CULTURED IN VITRO

Mehmet Nuri Nas*, Paul E. Read

Dept. of Agronomy and Horticulture Univ. of Nebraska- Lincoln Institute of Agriculture and Natural Resources 377 Plant Sciences Lincoln, NE 68583-0724

Increasing the amount of $CuSO_4$ $5H_2O$ up to 200-fold and that of myo-inositol up to 8-fold in the Nas and Read Medium (NRM) enhanced culture growth and morphogenic response of hazelnut explants. Such levels of Cu and myo-inositol improved mean shoot lengths of some genotypes more than two-fold. Stimulatory levels of Cu and myo-inositol led to axillary bud numbers per shoots that were greater by 1.1 to 2.7 times more compared to of control explants. At elevated levels of treatment, the number of explants developing shoots for some genotypes increased by 2.6-fold, while the amount of undesirable callus at the base of explants was reduced more than 50%. Our findings suggest that the amounts of these two medium components in many tissue culture media are sub-optimal and it might be necessary to use higher concentrations in order to enhance morphogenic potential of explants.

1340–1440

S01–P–48

DIFFERENTIATION SPECIFIC GENE EXPRESSION AND ITS REGULATION AND LOCALISATION IN LILY (*LILIUM REGALE* WIL.) CALLUS

Veli-Pekka Pelkonen*[1], Anja Hohtola[1], Kari Laine[2]

[1]Dept. of Biology/Botany Univ. of Oulu P.O. Box 3000 FIN-90014 Oulu Finland; [2]Dept. of Biology/Botanical Garden Univ. of Oulu P.O. Box 3000 FIN-90014 Oulu Finland

Differentiation processes can be divided into organogenesis and somatic embryogenesis. Several tissues that freely produce adventitious shoots, including callus, have been used for propagation of many lily species and cultivars. Still, somatic embryogenesis has not been applied in the commercial production

of lilies. Specific gene expression during somatic embryogenesis has been reported with a variety of plant species. Differential expression of various gene families has been demonstrated, and its role in differentiation processes has been discussed in many investigations. The whole picture, still, remains more or less obscure, especially as far as lilies are concerned. Besides propagation, somatic embryos are ideal tools in investigating the physiology of embryogenesis in general. Besides, understanding the physiological and genetic processes behind the differentiation helps us to develope even better mass production systems for lilies. In this study, the genetic activity in lily callus cultures was monitored in various phases of differentiation. Young seedlings of regal lily (Lilium regale Wil.) were used as a starting material for callus induction. Friable callus of several genotypes was selected for further culture and differentiation studies. Tissue samples were collected in several stages, and the activity of differential gene expression during somatic embryogenesis and organogenesis was analysed. Simultaneously, the possible role of known, embryogenesis related genes was investigated.

1340–1440

S01–P–49

MICROPROPAGATION OF NATIVE NORTH AMERICAN *LILIUM* SPECIES

Nathaniel D. Petley*[1], Mark Bridgen[2]

[1]Dept. of Plant Science, Univ. of Connecticut, Storrs, CT 06269-4067 USA; [2]Cornell Univ., Dept. of Horticulture, Long Island Horticulture Research and Extension Center 3059 Sound Avenue, Riverhead, NY 11901 USA

Three native North American *Lilium* species were studied for their ability to be micropropagated. These species were: *L. canadense* L., *L. michauxii* Poir., and *L. philadelphicum* L. Currently, there is no literature published on the in vitro culture of these native lily species. As natural habitats decline, the populations of native plants also decline. Micropropagation is a tool to produce and conserve native plants for use in the horticulture industry and to lessen the effects of collecting in the wild.

Previous in vitro research with *Lilium japonicum* Thunb. and *Lilium speciosum* Thunb. demonstrated that their bulbs respond well to tissue culture. According to their research, the addition of growth regulators to lily tissue culture medium is not required for micropropagation. However, for commercial use, the balance between low quantities of cytokinins and lower quantities of auxins has been beneficial for increased production. Protocols for the micropropagation of *L. canadense* L., *L. michauxii* Poir., and *L. philadelphicum* L. have been developed by studying factors such as the type of growth regulator used and its concentration, sucrose concentration, light intensity, and temperature regime. Individual bulb scales of uniform size from each of the species were cultured on the cytokinins 6-benzylaminopurine (BA), 6 (g,g–dimethylallylamino) purine (2iP), and kinetin at concentrations ranging from 0–20 mM. They were also tested at sucrose concentrations of 0, 3, 6, 9, and 12%. Studies with light intensity compared continuous light regimes to complete darkness and reduced light intensities. Cultures were also grown at three different temperatures: 18 °C, 21.5 °C, and 25 °C. The basal medium for all experiments was the Murashige and Skoog salts and vitamins. The data that were collected included total fresh weight, per cent fresh weight increase, callus formation, and the production rate of new bulbs. These data will be presented.

1340–1440

S01–P–50

MICROPROPAGATION OF PERENNIAL PHLOX

S.M. Lutfor Rahman*, Dennis Genovesi, Wayne Mackay

Texas A&M Univ., Texas Agr. Expt Station (TAES), 17360 Coit Road, Dallas, Texas, USA, 75252-6599

Perennial phlox (*Phlox paniculata* L.) is a widely grown perennial ornamental plant with strong stems and showy domed flowerheads, which persist for many weeks during the summer. It is an excellent plant for use in the perennial border or cutting garden. Superior selections of perennial phlox have been identified for North Texas landscapes, but availability is limited due to inefficient conventional vegetative propagation methods. The present research is an attempt to identify and establish efficient, reliable protocols for in vitro micropropagation of two superior cultivars, 'John Fanick' and 'Victoria'. Our starting point was the work previously described by Declerck and Korban (1995) J. Plant Physiol. 147:441-446. Cultures were initiated from shoot tips (2.5-7.0 mm) surface sterilized in 8% Chlorox® with 1% Tween 20 for 10 min. then rinsed (3x) in DI water. Modified Murashige and Skoog (MS) basal media supplemented with MS vitamins, plant growth regulators (2 or 5 μM BAP and 0.3 μM IAA), 4% sucrose and 0.8% agar were used for initiation and maintenance of totipotent cultures. Shoot elongation and maturation was accomplished again on modified MS basal supplemented with the same levels of BAP and IAA as before but with (or without) 1 mg/L GA added, sucrose lowered to 2% and solidified with 0.3% Phytagel (Sigma). Elongated shoots were rooted on either MS basal supplemented with 4% sucrose and 0.3% Phytagel or MS with (1/2x) ammonium nitrate and potassium nitrate supplemented with 2% sucrose and 0.3% Phytagel. In some cases, rooting media were supplemented with 11.4 μM coumarin (1, 2-benzopyrone), a secondary metabolite, for more efficient root initiation. About 80-90% of rooted plantlets were successfully transplanted to soil in the greenhouse. Cultivar differences were observed in their capacity for in vitro maintenance and differentiation.

1340–1440

S01–P–51

HIGH FREQUENCY SHOOT REGENERATION FROM LEAF EXPLANTS OF SOME CHRYSANTHEMUM CULTIVARS

So Hyun Park*, Young Hoe Kim, Byoung Ryong Jeong

Dept. of Horticulture, Division of Applied Life Science, Gyeongsang National Univ., Jinju 660-701, Korea

This study was conducted to examine differences in regeneration among chrysanthemum cultivars. Leaf explants of Sulhwa, Pyuma, Geummokseo and Sulpoong chrysanthemums were used. Explants cultured on a medium for 2 weeks formed calli at the cut surfaces. Shoots were regenerated on an MS basal medium supplemented with various combinations of NAA, IAA, and BAP at different concentrations. The explants were cultured under cool-white fluorescent lamps at a light intensity of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 h/day, at 25 °C temperature and 70-80% relative humidity. Sulhwa and Sulpoong were the most responsive cultivars in shoot regeneration. The most effective medium for Geummokseo, Pyuma and Sulpoong was a MS basal medium supplemented with 10 μM NAA and 5.0 μM BAP. Regeneration of multiple shoots was observed on a MS basal medium supplemented with 1 or 10 μM NAA and 5.0 μM BAP. High frequency regeneration of adventitious shoots from leaf explants and efficient induction of roots from these regenerated shoots were obtained.

1340–1440

S01–P–52

PHOTOAUTOTROPHIC CULTURE WITH CO_2 ENRICHMENT FOR IMPROVING MICROPROPAGATION OF *COFFEA ARABUSTA* USING SOMATIC EMBRYOSA

A. Uno*, C. Kubota, K. Ohyama, T. Kozai

Dept. of Bioproduction Science, Faculty of Horticulture, Chiba Univ., Matsudo, Chiba 271-8510, Japan

Micropropagation using somatic embryos has been investigated in many plant species. However, problems such as the low percentage of conversion of somatic embryos to plantlets limit its commercial application. *Coffea arabusta* somatic embryos were cultured photoautotrophically (without sugar in the medium) at different CO_2 concentrations, and conversion of the somatic embryos was compared with that cultured conventionally (with sugar in the medium). Torpedo-shaped and cotyledonary embryos were cultured photoautotrophically in a medium with a vermiculite-based supporting material in vessels with 0.8 h-1 air exchanges under 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux (PPF) at one of three CO_2 concentrations [C; 400 (ambient), 1500 and 5000 $\mu mol \cdot mol^{-1}$], or conventionally in an agar-gelled medium with 20 g/L sucrose in vessels with 0.1 h air exchanges under 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF at C = 400 for 61 days. When cultured photoautotrophically at C = 1500 or 5000, percent conversion of cotyledonary embryos to plantlets was 55-60%, a significantly higher percentage than that when cultured conventionally (17%). The conversion at C = 400 under photoautotrophic conditions was not significantly different from that under the conventional conditions. The percent conversion of torpedo-shaped to cotyledonary embryos was 90-100% when cultured conventionally or photoautotrophically at C = 5000, while it was 20-60% when cultured photoautotrophically at the lower C, where 15-50% torpedo-shaped embryos died. Photoautotrophic culture was applicable to Coffea arabusta somatic embryos at torpedo and cotyledonary stages provided that the CO_2 concentration was properly controlled. For torpedo-shaped embryos, a CO_2 concentration as high as 5000 $\mu mol \cdot mol^{-1}$ was critical for high

conversion to cotyledonary embryos. Once torpedo-shaped embryos converted to cotyledonary embryos, photoautotrophic culture at 1500-5000 μmol·mol^{-1} CO_2 concentrations successfully enhanced the percent conversion to plantlets.

1340–1440
S01–P–53
CLONAL PROPAGATION OF HYBRID SWEETGUM (*L. STYRACIFLUA* X *L. FORMOSANA*) VIA SOMATIC EMBRYOGENESIS

Wagner Vendrame*[1], Christopher P. Holliday[2], Scott A. Merkle[2]

[1]Tropical Research and Education Center IFAS–Univ. of Florida 18905 SW 280th St Homestead, FL 33031-3314 USA; [2]D.B. Warnell School of Forest Resources Univ. of Georgia Athens, GA 30602-2152 USA

Cultures were initiated from immature seeds derived from controlled pollinations between two sweetgum species (*Liquidambar styraciflua* and *Liquidambar formosana*) by culturing the seeds on two induction media supplemented with 2,4-D. Repetitive embryogenic cultures were obtained from which somatic embryos were selected and converted into somatic seedlings. Of the 1020 seeds cultured, representing 9 crosses between *L. styraciflua* and *L. formosana*, 2% produced repetitively embryogenic cultures capable of producing somatic seedlings. Hybrid genotypes of somatic seedlings were confirmed by RAPD analysis and leaf morphology observations. Stomatal analysis performed on leaves from hybrid somatic seedlings and parental species revealed differences in stomate size and number per unit leaf area. A protocol for cryopreservation of the hybrid cultures gave rates of regrowth near 100% for all samples. Cryopreservation will allow the long-term storage of viable embryogenic cultures while hybrid trees are field tested for identification of superior genotypes. Somatic embryogenesis appears to be a feasible approach for mass clonal propagation of hybrid sweetgum.

1340–1440
S01–P–54
ROOT AND SHOOT ANATOMY OF IN VITRO FRUIT TREE ROOTSTOCKS: INVESTIGATIONS BY LOW TEMPERATURE SCANNING ELECTRON MICROSCOPY

Luca Sebastiani, Antonio Minnocci, Claudio Vitagliano*

Scuola Superiore, Sant'Anna, Via Carducci 40, Pisa, Pisa, Italy, 56127

In vitro plant technologies are widely included in propagation and breeding programs and also as model systems in several physiological investigations. Anatomical data are one of the first steps in successful application of in vitro technologies. However, the water-saturated in vitro environment and the high water content inside the in vitro tissues, makes it impossible to maintain the original characteristics of this biological material by traditional preparation methods. Low Temperature Scanning Electron Microscopy (LTSEM) is a powerful analytical tool for anatomical investigations in plants and especially for in vitro material. In vitro samples can be fixed in a cryogen in a short time (millisecond) and maintained during all the observation in their frozen-hydrated (FH) chemically unmodified state by LTSEM; therefore, it is possible to analyze anatomical structures in their original hydrated state. In this study, root and shoot LTSEM anatomical investigations were performed on in vitro fruit tree rootstock samples. To this purpose, in vitro plants of Prunus cerasifera sel. MrS 2/5 and GF677 (*P. amygdalus* x *P. persica*) were grown in ventilated vessels and kept in a growth chambers at 25 C under a 16/8h day/night photoperiod with 45 μmol·m^{-2}·s^{-1} PAR. Leaf, stem and root samples obtained from in vitro plants were mounted on stubs and plunged frozen in liquid propane. After cryofixation, FH samples were transferred under liquid nitrogen vapour in the preparation chamber of the dedicated LTSEM Cryo Unit. FH samples were freeze-fractured, surface etched and sputter-coated with gold in an argon atmosphere before transfer into the cold stage of the LTSEM. Digital images of FH leaf, stem and root samples were processed with image analysis software. Data presented in this work showed leaf, root and stem structures close to their natural state.

1340–1440
S01–P–55
RAPID MULTIPLICATION OF VIRUS-FREE, VEGETATIVELY PROPAGATED BUNCHING ONION (*ALLIUM FISTULOSUM*)

Hung-Ying Yang*, Ting-Chin Deng

[1]No. 35-1 Dahyih Rd. Sanshing Taiwan Republic of China; [2]No. 189 Chungcheng Rd. Wufeng Taiwan Republic of China

Shallot latent virus (SLV) was detected by DTBIA, and evenly distributed in infected bunching onion (*Allium fistulosum* L.). An ELISA procedure was efficiently assigned for routine virus indexing in healthy seedling mass production system. For culture in MS medium, tissues from SLV infected bunching onion including stem-disc, apical meristem, meristem with one primordial leaf, and meristem with two primordial leaves were excised and tested. The results showed that only the plantlets derived from apical meristem are SLV virus-free. The rapid multiplication of healthy stock was also established. It was demonstrated that the number of regenerated shoots from stem-disc was reduced with TDZ and NAA combined together, but TDZ alone regenerated more shoots. The optimal number of shoots (43-62) were regenerated from stem-disc treated with 1-5 mg/L TDZ for 42 days, The TDZ had better effect on induction of shoot regeneration than BA or kinetin for plant regeneration. The stem-discs were treated with 1-5mg/L TDZ for 1.5 months, transferred to 1mg/L NAA for 3 months, until they had completely developed shoots and roots, and then transferred to hormone-free MS medium for 2 months. The plantlets were removed from the culture bottle for further growth.

1340–1440
S01–P–56
RAPID MULTIPLICATION OF BASIL, *OCIMUM BASILICUM*

Ki-Jong Lee*, Ji-Won Kim, Bu-Young Yi

Dept. of Environmental Horticulture Univ. of Seoul Seoul 130-743 Korea

Basil is one of the most important Herb plants. For the rapid multiplication method of basil, several explants were cultured in vitro on modified MS medium supplemented with several combinations of plant growth regulators. Among them, NAA and IAA were the best auxins and BAP and kinetin were the best cytokinins for inducing callus and shoot multiplication. In this study, we found the most effective combination of plant growth regulators for callus induction and shoot multiplication from basil explants in solid culture system as well as liquid culture system.

1340–1440
S01–P–57
IN VITRO MASS MICROPROPAGATION OF TEA TREE (*MELALEUCA ERICIFOLIA* SMITH)

Esssam M.A. Youssef*

Tissue Culture and Germplasm Conservation Res. Lab, P.O Box 120, Dokki-Giza,, Egypt

The effect of different medium ingredients of MS (Murashige and Skoog), WPM (Woody Plant Medium) and B5 (Gamborg) on mass micropropagation of *Melaleuca ericifolia* revealed that recycling the in vitro cultures of nodal explants on MS-medium for six times at monthly intervals increased the shoot proliferation rates to 13.9- and 11.6–fold higher than on WPM and B5 medium, respectively. The rooting response was in higher values (95%-100%) when the shoots were cultured on MS or WPM medium amended with IBA (indole-3-butyricacid) or on B5–medium provided with PG (phloroglucinol). The highest hardening–off capacity (89%–100%) was recorded for the rooted shoots from MS or WPM media supplemented with either IBA, PG or CH (charcoal). The greatest number of the ex vitro plantlets ranged from one quarter to one third of a million and was obtained with MS medium containing either IBA, PG or CH. MS medium amended with IBA gave the longest stems and increased the dry weight of plantlets, whereas MS medium supplemented with CH allowed a greater root growth. However, B5 - medium incorporated with IBA produced the maximum number of leaves and roots per plantlet

1340–1440
S01–P–58
IN VITRO SHOOT DIFFERENTIATION FROM RHIZOMES AND PLB'S OF *CYMBIDIUM* SPP. BY ETHYLENE INHIBITORS AND REDUCTION OF NITROGEN CONTENT

Y. Ogura*, H. Okubo

Faculty of Agriculture, Kyushu Univ., Hakozaki, Higashi-ku, Fukuoka, Japan, 812-8581

Most epiphytic *Cymbidium* species that are distributed in tropical regions initially form a PLB followed by subsequent shoot formation in apical meristem culture. Temperate *Cymbidiums* are, on the other hand, generally terrestrial and they form a rhizome before shoot regeneration. In this study, to clarify the mecha-

nism of shoot organogenesis, effects of ethylene inhibitors and reduction of nitrogen in MS medium on shoot regeneration from the rhizomes of *Cymbidium ensifolium*, *C. kanran* and *C. sinense* and PLB's of a hybrid of C. Sleeping Beauty (epiphytic) x *C. sinense* (terrestrial) were investigated. Approximately 5mm-long apical segments of rhizomes and 2-3 mm PLB's in diameter were used as explants. The explants were cultured on MS medium supplemented with ethylene inhibitors AVG (aminoethoxy vinyl glycine; 0.01, 0.1, 1.0 and 10mg/L) or $AgNO_3$ (0.1, 1.0 and 10 mg/L) or MS medium with 25% and 50% reduction of NH_4NO_3 and KNO_3, respectively, at 25 °C under continuous light. Ethylene inhibitors and reduction of nitrogen content promoted shoot formation both from the rhizomes and PLB's. These results indicate that the response to these treatments is common to rhizomes and PLB's, and suggest a relationship between inhibition of ethylene biosynthesis or action, or low nitrogen concentration and an increase in the endogenous ratio of cytokinins to auxins since exogenously increased cytokinin/auxin ratio promotes shoot formation from the both PLB's and rhizomes.

1340–1440

S01–P–59

INFLUENCE OF CONDITIONING ON THE REGENERATION TENDENCY OF IN VITRO GROWN APPLE LEAVES

Zabihollah Zamani*[1], Peter B. Goodwin[2]

[1]Dept. of Horticulture, Faculty of Agriculture, The Univ. of Tehran, Karaj 31587, Iran; [2]Dept. of Crop Sciences, Faculty of Agriculture, The Univ. of Sydney, N.S.W. 2006, Australia

Shoot terminals of 'Red Delicious' and 'Pink Lady' apple cultivars grown in vitro were used to compare the effect of conditioning on the regeneration of their leaves. Shoot terminals, 2-3 cm long, and containing 4-6 expanded leaves were excised from the cultures and with their attached leaves were dropped into the liquid conditioning medium. A modified basal MS medium supplemented with Staba vitamins, ascorbic acid (5 mg/L), TDZ (15 μM) and NAA at 3 or 6 μM was used for conditioning. Shoots in the conditioning medium were agitated for 4 days at 100 rpm under the cool-white light (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 20 ±1 °C. Shoots then were removed and their leaves were excised, cut in pieces perpendicular to the midrib and used for regeneration. Medium for regeneration was similar to the conditioning medium except for NAA, which was 4.5 μM and solidified with agar. Plates containing the explants were initially kept in the dark at 23-25 °C for about 6 weeks. Plates then were transferred to the cool-white low irradiance light of

1340–1440

S01–P–60

COMPARING REGENERATION CAPACITY OF THE LEAVES FROM SEVEN IN VITRO GROWN APPLE CULTIVARS

Zabihollah Zamani*[1], Peter B. Goodwin[2]

[1]Dept. of Horticulture, Faculty of Agriculture, The Univ. of Tehran, Karaj 31587, Iran; [2]Dept. of Crop Sciences, Faculty of Agriculture, The Univ. of Sydney, N.S.W. 2006, Australia

For genetic transformation experiments on plants, it is necessary to have a high incidence of regeneration. Efficiency of regeneration mostly depends on genotype as well as the culture conditions. To find out the tendency of regeneration from different apple cultivars, leaves of seven in vitro grown apples including 'Red Delicious', 'Pink Lady', 'Fuji', 'Granny Smith', 'Golden Delicious', Summer Del' and 'Hi Early' were used for this experiment. Expanded distal 4-6 leaves of in vitro grown apple shoots from proliferating cultures were collected, cut transversely through the middle, tips and petioles were removed, and the explants laid onto the regeneration medium adaxial surface in contact with the medium. Regeneration medium consisted of modified MS medium (containing Staba vitamins), plus 15 μM TDZ and 4.5 μM NAA. Plates containing the explants were kept in the dark at 23-25 °C for about 6 weeks and then transferred to the low light irradiance of about 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$, supplied by cool-white fluorescent lamps, and kept under 8-16 h dark-light periods at 24-27 °C. Numbers of regenerating explants, as well as the number of regeneration sites on each explant were scored using a low power binocular microscope. Differences between cultivars were significant for both percent of regenerating explants and the number of regeneration sites per explant. Cultivars with a higher tendency for regeneration had more regenerating explants with higher regeneration sites. 'Red Delicious', 'Pink Lady' and 'Golden Delicious' were more responsive than the others, therefore they could be divided into two main groups of high and low capacity for regeneration under this protocol.

1340–1440

S01–P–61

EFFECTS OF BENZYLADENINE (BA) CONCENTRATIONS AND AUXIN TYPE (IBA AND NAA) ON THREE IRANIAN PEAR (*PYRUS COMMUNIS* L.) CULTIVARS UNDER IN VITRO CONDITIONS

Z. Zamani*, S.Z. Nosrati, M. Babalar

Dept. of Horticulture Faculty of Agriculture The Univ. of Tehran Karaj 31587, Iran

Three Iranian pear cultivars 'Shahmiveh', 'Natanzi' and 'Dargazi' as well as the well known 'Bartlett' cultivar were evaluated for their proliferation and growth response to the three levels of BA concentrations (1, 2, and 3 mg/L). Indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) each at 0.1 mg/L were used separately in combination with BA in the media and a factorial design was used for this experiment. Murashige and Skoog medium with its macronutrients at half concentration (MS/2) was used as the basal medium. Growth characters of cultures including shoot number, shoot length, leaf expansion, leaf color and callus size at shoot base were scored for comparison. Cultivars showed differences for their response to culture conditions and various levels of BA (except for leaf color). Increasing the BA concentration resulted to higher shoot numbers, shorter shoots, bigger callus at shoot base and reduced leaf size of the shoots. Cultivars showed interaction with BA levels for shoot number and leaf size. Pears 'Dargazi' at 2 mg/L and 'Natanzi' at 3 mg/L of BA produced more shoots (with mean of 7.43 and 7.16 respectively) compared to other cultivars or BA concentrations. Shoot numbers for 'Shahmiveh' on BA at 2 or 3 mg/L did not make significant difference (average 6.31) while for 'Bartlett' it was higher at 3 mg/L (5.53). For leaf size, although increasing the BA reduced leaf size in general but 'Shahmiveh' produced larger leaves at 2 mg/L of BA. Comparison of the effects of IBA and NAA, each at 0.1 mg/L, showed that IBA resulted to longer shoots than NAA and for other cases differences in their effects were not significant. In this experiment, there was no significant interaction between auxin type and BA or auxin type and pear cultivars.

1340–1440

S01–P–62

SOMATIC EMBRYO SYNCHRONIZATION AND IN VITRO ACCLIMATIZATION OF *OENANTHE STOLONIFERA* DC.

B.Y. Lee*, H.J. Yoon

School of Plant Science, Seoul National Univ., Suwon 441-744, Korea

In an effort to establish an efficient seedling production system through somatic embryogenesis of Oenanthe stolonifera DC, factors affecting embryogenic cell production, somatic embryo synchronization, and in vitro acclimatization were investigated. Efficient cell proliferation during liquid culture occurred in a ventilated vessel capped with foil attached membrane filter (0.02 μM pore size) when subcultured with medium addition method. The ventilated system was more effective for inducing embryogenesis than the air-tight system. The culture period of 12 to 24 days was found to be effective for producing competent embryos and the maximum embryo production was obtained at 36 days after the culture. The addition of 6 mM $CaCl_2$ into the culture medium was effective for maintaining embryogenicity even with increasing the number of subculture, whereas the addition of 3 mM $CaCl_2$ was suitable for somatic embryo induction. The optimum period of in vitro acclimatization, as determined by inorganic ion uptake, appeared to be 4 weeks after the culture. The addition of 3 mM $CaCl_2$ during the first 2 weeks of culture was effective for synchronizing the somatic embryo development and enhancing their inorganic ion uptake, whereas the addition of 0.1 mg/L paclobutrazol during last 2 weeks of the culture was effective for the subsequent growth of the somatic embryo-derived plantlets. Glass bottles were more suitable for the germination of the somatic embryo-derived plantlets than urethane-sponges, but the reverse was true for their growth.

1340–1440

S01–P–63

THE USE OF THIDIAZURON IN SOMATIC EMBRYOGENESIS AND REGENERATION OF DIFFERENT GRAPEVINE ROOTSTOCKS

Robert Olah*, Andrzej Pedryc*, Janos Korbuly*

Dept. of Genetics and Horticultural Plant Breeding, Szent Istvan Univ., Budapest, Menesi ut 44, Hungary, H-1118

Grape rootstock varieties show resistance to different fungal diseases, but most of them have no resistance against crown gall and virus caused diseases.

Genetic transformation of the existing and successful rootstock varieties would be the reasonable solution of this problem. Transgenic rootstock plants have been obtained in the last decade, however reliable regeneration protocols are only known in case of some important ones. Our objective was to study the effect of thidiazuron on induction of embryogenic calli and to spread the range of those rootstock genotypes which could provide somatic embryos for the genetic transformation. Experiments with somatic embryogenesis were carried out on rootstock varieties belonging to different Vitis species and interspecific hybrids. Initiation of embryogenic calli was carried out on anthers, which were collected from flower buds closely before blooming. Excised anthers were placed on solid medium together with the filaments. The media to induce embryogenic callus development contained in addition to components of Murashige & Skoog (1962), 20 g/L saccharose, 70 mg/L Fe-EDTA and different combinations of 2,4-D and TDZ (MST media). MSE medium of the same composition with addition of 0.1 mg/l benzyladenine (BA) and 1.1 mg/L 2,4D described by Mozsár & Süle (1994) was used as a control. Callus development was checked after 30 days and the cultures were transferred to new media of the same composition. The induction of somatic embryos was attempted in hormone-free solid media containing half-strength of MS salts and vitamins plus saccharose (10 g/L) and agar (6 g/L). After one month, cultures on solid medium were exposed to light. The germinating embryos were separated and transferred to tubes containing 10 mL half-strength MS solidified medium. Five rootstock cultivars (Berl. x Rip. S.O.4, Berl. x Rip. T.5C, Börner, Richter 110, Vitis rupestris cv. St. George) produced embryogenic callus lines. Four of them conserved their embryogenic capacity after several transfers, but the embryogenic cells of Berl. x Rip. T.5C continued their development into somatic embryos on MST medium. Entire plants were successfully obtained with five varieties. Cultivars Berl. x Rip. T.5C and Vitis rupestris cv. St. George frequently showed irregular shoot development, but after several transfers, regular shoot growth was reestablished.

1340–1440

S01–P–64

THE MAIZE CRINKLY4 RECEPTOR KINASE CONTROLS A CELL-AUTONOMOUS DIFFERENTIATION RESPONSE

Philip W. Becraft[1], Suk-Hoon Kang[2], Sang-Gon Suh*[2]

[1]Zoology & Genetics/Agronomy Dept.s 2116 Molecular Biology Building Iowa State Univ. Ames, IA 50011; [2]Dept. of Horticulture Yeungnam Univ. Daedong 214-1, Kyongsan 712-749 Republic of Korea

The maize (*Zea mays*) CRINKLY4 (cr4) gene encodes a receptor-like kinase that controls a variety of cell differentiation responses, particularly in the leaf epidermis and in the aleurone of the endosperm. In situ hybridization indicated that the cr4 transcript is present throughout the shoot apical meristem and young leaf primordia. A genetic mosaic analysis was conducted to test whether cr4 signal transduction directly regulated the cellular processes associated with differentiation or whether differentiation was controlled through the production of a secondary signal. Genetic mosaics were created using r-rays to induce chromosome breakage in a cr4/ cr4 + heterozygote. The mutant cr4 allele was marked with the albino mutation, Oy-700. Breakage and loss of the chromosome arm carrying the wild type alleles created a sector of albino, cr4 mutant tissue in an otherwise normal leaf. Analysis of such sectors indicated that cr4 functions cell autonomously to regulate cell morphogenesis, implying that cr4 signal transduction regulates cell differentiation through strictly intracellular functions and not the production of secondary intercellular signals. However, several sectors altered cell patterning in wild type tissue adjacent to the sectors, suggesting that cr4 mutant cells are defective in the production of other lateral signals.

1340–1440

S01–P–65

STUDIES ON THE IN VITRO CULTURE OF *LISIANTHUS RUSSELIANTHUS* HOOK

Gabriela Popa, Viorica Balan*, Mariana Neamtu, Teodor Dan Enescu*, Miluta Negoi*

Research Station for Fruit Tree Growing–Baneasa, B-dul Ion Ionescu de la Brad, No.4, Sect.1, Code 71592, Bucharest, Romania

Lisianthus russelianus Hook, sin. *Eustoma grandiflorum* Shinn is an ornamental plant originated in Northern America and Mexico. Due to variable color of flowers, this plant is cultivated for trading as cut flowers and as plants in pots, being appreciated in a great number of countries. In Romania, it is less known, therefore our efforts have been oriented towards cultivation of this species and introducing it to the market. Starting with 1999, at the Reseaerch Station forTree Growing Baneasa, within the Laboratory of In Vitro Tissue Cultures, explants were prepareed as follows: shoot tips (10 mm), sections of stems (20 cm), leaf sections (10 x 10mm) were initiated from two varieties of Lisianthus : one with pink flowers and others with blue flowers, which have been collected from plants in glasshouse before flowering. These explants have been studied to determinate their morphogenetic capacity, and for identification of an optimum phytoformone balance for regeneration. Three variants of Murashige & Skoog basic culture media with different phytohormones were used : V1–MS + 3 mg/L BA (benzyladenine); V2–MS + 3 mg/L BA + 0.2 mg/L NAA (naphthaleneacetic acid); V3–MS + 1 mg/L BA + 5 mg/L GA3 (gibberellic acid). After some weeks, adventitious shoots were produced from each explant. Shoot tips produced the highest number of shoots on the medium supplemented with 3 mg/l BA (variant 1). The occurrence of NAA in the culture medium (V2) proved to be beneficial for explants of internodes, while leaf explants of internodes proliferated best on the MS medium to which gibberellic acid was added (V3). Thus, it was concluded that the development of explants depends on phytohormonal composition of the culture medium, and on the type of explant.

1340–1440

S01–P–66

SOMATIC EMBRYOGENESIS FROM MATURE TISSUES OF LETTUCE *LATTUCA SATIVA*

J.E.A. Seabrook*, L.K. Douglass

Agriculture and Agri-Food Canada, Potato Research Centre, P.O. Box 20280, Fredericton, New Brunswick, Canada, E3B 4Z7

The regeneration of somatic embryos (SEs) in tissue cultures derived from mature tissues of lettuce (*Latuca sativa* L.) is reported. We recently described the regeneration of somatic embryos on a wide range of genotypes, ploidy and tissues of potato (*Solanum tuberosum* L.) using a two-step protocol in vitro. Using the same protocols, SEs have been regenerated on seven cultivars (cvs.) of lettuce: Butter Crunch, Grand Rapids, Olga, Pluto, Queen Crown, Red Snails and Summertime. Seeds from head-, leaf- and romaine-type lettuce plants were germinated, grown in a greenhouse, and axillary shoots from mature plants excised, disinfested and cultured in vitro. Once the shoots had grown into plantlets, the tissues were cultured on a medium lacking growth regulators. Leaf, stem and peitole tissue was excised and used to regenerate SEs. All cultures were grown at 19 °C under cool-white fluorescent lamps with an irradiance of 20 watts m^{-2} and a photoperiod of 16 h. Somatic embryos were also regenerated from seedling tissues of five lettuce cvs. Lettuce SEs germinated without difficulty and acclimatized to greenhouse conditions. Explants (leaf, stem-internode and petiole) from seedlings of all lettuce cvs. regenerated, although stem-internode sections were generally more productive. Regeneration from mature tissues of lettuce was most efficient using leaf and petiole explants.

1340–1440

S01–P–67

THE PH-DIRECTED POLAR GROWTH AND MASS SOMATIC EMBRYO PRODUCTION IN EMBRYOGENIC CELL SUSPENSIONS OF TRIPLOID BANANAS

C.C. Lu, Chou Tou Shii*, J.P. Chung

Dept. of Horticulture, National Taiwan Univ., No 1 Sec 4 Roosevelt Road, Taipei, ROC, Taiwan, 106

The cell acid growth model suggests that the developmental regulation of proton flux or controlling external pH below the threshold (pH 3.5-4.5) stimulates cell separation and serves as cell polar axis eliminator, and results in multiplying cell propagules and apolar proliferation of preembryogenic mass (PEM). Over the threshold, the proton gradient acts as polar axis signal of preembryogenic determined cells (PEDCs), and transformed to EDC with initial unequal division and destined to proembryogenesis. According pH-triple function, it is manipulatible in cell transformation and mass production of somatic embryos (SE). It was demonstrated that the growth type of PEM in AAA and AAB genomic groups of triploid bananas exhibited higher plating efficiency than PEDCs of multiplication phase, which regenerated 1-3 x 103 SE per 0.033 mL CPV on SH3 regeneration medium, but 50% of the clustered SE were smaller than 0.5 mm. In contrast, the PEMs pretreated in TB5 maintenance medium controlling at pH 3.5-4.0, which were induced to the multiplication phase active in releasing PEDCs.

Until the achievement of homogeneous cell propagules, then, sequentially upgraded TB5 at pH 4.8-5.3, the PEDCs were rapidly transformed to EDCs with asymmetric division, and synchronizously destined to proembryogenesis. Thereafter, the homogeneous globules were transferred to SH3 medium for morphologically bipolar differentiation and maturation of advance somatic embryogenesis. There were 2.5-3.3 x 104 SE per 0.033 mL CPV with 80-90% uniformity were achieved. An estimated one mL CPV is capable of producing close to one million somatic embryos.

1340–1440
S01–P–68
THE INFLUENCE OF SOURCE TREE ON SOMATIC EMBRYOGENESIS FROM EASTERN BLACK WALNUT (*JUGLANS NIGRA*) IMMATURE COTYLEDONS

Marlene M. Steger*, John E. Preece

Dept. of Plant, Soil and General Agriculture, Southern Illinois Univ., Carbondale, IL 62901-4415, USA

The influence of stage of fruit development, media treatments and source tree (genotype) on somatic embryogenesis in *Juglans nigra* were studied over two years. Beginning at 12 weeks and continuing until 16 weeks post anthesis, 1 cm explants from cotyledons of immature seeds were excised from Eastern Black Walnut fruit during 1999 and 2000. Fruits from different trees were tested each year because of the alternate bearing habit of this species. Explants were placed on WPM, DKW, or LP medium with 1 g/L casein hydrolysate, 30 g/L sucrose, 5.0 µM thidiazuron (TDZ), 0.1 µM 2,4-D, and 7 g/L Sigma agar. The explants were under 16-hour photoperiod and 25 °C for four weeks. Explants were then transferred to basal media with 1 g/L casein hydrolysate and no plant growth regulators and incubated in darkness. Each year, the experimental design was a completely randomized design with 20 replications per treatment with factorial combinations of media salts, source tree and number of weeks post anthesis. During 1999, there was a significant interaction between genotype and media with LP being a consistently good medium for all three genotypes, with a range of 1.0 to 1.9 somatic embryos per explant and 24% to 30% of the explants producing somatic embryos depending on genotype. During 2000, there was a significant interaction between genotype and medium, and between genotype and number of weeks post anthesis. Explants from the best source tree produced 2.8 somatic embryos per explant plus 34 to 38% of the explants were embryogenic when DKW or LP salts were used. Medium had little effect on explants from the other two genotypes. Explants tended to be the most embryogenic 12 weeks post anthesis with 0.8 to 4.2 embryos per explant depending on source tree.

1340–1440
S01–P–69
MICROPROPAGATION OF FERNS THROUGH TISSUE CULTURE

Harvinder Cheema*

Apt. 902, 30 Humberline Drive, Etobicoke, Ontario, M9W 6K8, Canada

The present investigations deal with the rapid propagation of homo and heterosporous ferns viz. Marsilea and Ceratopteris employing tissue culture technology. Sexually produced diploid sporophytes of Marsilea were raised on Knop's medium supplemented with 3% sucrose. The regenerative response of the rhizome segments with and without nodal parts was different on the same medium. Segments with nodal parts were the first to sprout while the rhizome segments without nodal parts remained quiscent for about two months after which vegetative bud at the nodal end became swollen and later turned green and differentiated green cylinderical structures which indicated the appearance of first juvenile leaf. Subsequently, a normal sporophyte was formed from each of these segments on Knudson's medium. Rhizome segments regenerated multiple shoots on kinetin enriched medium. Spore to spore life cycle of Ceratopteris was completed in sterile culture in 12 weeks. Vegetative leaves both intact and excised showed competence for bud formation. The adventitious buds differentiated on the leaf lamina which further regenerated complete plants on Knop's medium. Callus in Marsilea was induced from rhizome apex, rhizome segments, leaf and petiole segments on Moores medium containing 2% sucrose and auxins (2, 4-D, IAA, NAA). Multiple shoots and complete plants were regenerated on Knop's medium containing 2% sucrose. Callus of Ceratopteris was induced from leaves, roots and shoot apex on medium containing different concentration of auxin (2,4-D and NAA). Callus induced multiple shoots on kinetin enriched medium. The results indicated that the physiological age of the explant greatly influenced its morphogenetic capacity. Furthermore, the endogenous factors besides sucrose play an equally important role for callus induction, differentiation and organogenesis.

1340–1440
S01–P–70
PROGRESS IN MICROPROPAGATION OF *ACER SACCHARUM* (MARSH)

Nicole Brassard*[1], Claude Richer[1], Jacques-André Rioux[2], Denise Tousignant[3]

[1]Agriculture et Agroalimentaire Canada 430, Boul. Gouin Saint-Jean-sur-Richelieu (Quèbec) J3B 3E6; [2]Universitè Laval, 3e ètage Pavillon Comtois Sainte-Foy (Quèbec) G1K 7P4; [3]Direction de la Recherche forestire Fort Quèbec 2700, rue Einstein Sainte-Foy (Quèbec) G1P 3W8 1340–1440

Sugar maple is an important forest crop in Quebec. A freezing rain damaged many trees in 1998 and arose the need for reliable methods of vegetative production of selected trees. Few attempts were made to tissue culture sugar maple trees. An adequate disinfestation method and control of phenol exudation was previously established. The objective of this study was to evaluate the effect of different growth regulators on bud survival, multiplication and elongation. Buds of more than 200 seedlings of two-year-old *Acer saccharum* trees were stripped of their scales and cultured on media containing 1/3 of the salts of Murashige and Skoog with Bourgin and Nitch organics, with or without growth regulator. First month survival rate was higher in media without growth regulators. First month survival rate was higher on medium without growth regulators. Stem formation and multiplication succeeded on media of the same composition but containing 0.01 and 0.025 $mg \cdot L^{-1}$ thidiazuron and 0.5 to 5 $mg \cdot L^{-1}$ 2iP, 2 to 5 $mg \cdot L^{-1}$ 2iPA, or 0.3 to 3 $mg \cdot L^{-1}$ phenylacetic acid (PAA). Stem elongation and root induction took place on media with 0.3 $mg \cdot L^{-1}$ PAA with or without 1 $mg \cdot L^{-1}$ NAA. A few shoots rooted when transferred in vermiculite.

1340–1440
S01–P–71
MICROPROPAGATION OF FRENCH HYBRID LILAC *SYRINGA VULGARIS* 'KATHERINE HAVEMEYER'

Claude Richer*[1], Denis Charlebois[2]

[1]430, boul. Gouin Saint-Jean-sur-Richelieu, Quèbec, Canada, J3B 3E6; [2]Agriculture et Agroalimentaire Canada 430, boul. Gouin Saint-Jean-sur-Richelieu (Quèbec) J3B 3E6

The micropropagation conditions for *S. vulgaris* 'Katherine Havemeyer' hybrid were studied in regards to the influence of the container (500 mL glass jar, test tube and Magenta box), the number of explants per container, the concentration of major elements, the combination of growth hormones and the source of explants. The type of container used influenced the micropropagation of lilacs. Glass jars (500 mL) gave the best results for the simultaneous development of both axillary buds (74%) when compared to the other containers (50%). A density of 4 to 5 explants per glass container gave the best growth. Other densities caused slow growth with the development of fewer axillary buds per node section. Explants reached a superior height with a normal concentration of mineral elements compared to a dilute solution (50%). Growth regulators had a strong effect on growth of the explants. Among the 12 combinations of growth hormones tested, 1.0 IBA with 2.5 $mg \cdot L^{-1}$ 2IPA; and 0.1 NAA with 2.5 $mg \cdot L^{-1}$ 2IPA produced the highest number of explants. Basal sections (one node) with callus produced bigger explants when compared to apical and nodal sections.

1440–1520
S01–O–72
LIQUID CULTURE SYSTEMS FOR PLANT PROPAGATION

Trine Hvoslef-Eide*, Anne Kathrine, Petter H. Heyerdahl, Odd Arild S. Olsen, Cristel Munster

Agricultural Univ. of Norway, Dept. of Horticulture, Plant Cell Lab. P.O. Box 5022, Aas, Norway, N1432

Micropropagation performed in the "traditional" way includes manual handling of tissue on solid medium. These methods can be time- and labour consuming. In high-cost countries where the cost of labour may represent 80-90% of the total costs, various methods of automation may be interesting to explore. Scaling-up in micropropagation can be achieved in several different ways: 1) homogenisation of plant tissue; 2) automation through use of liquid cultures and

bioreactors; and 3) robotics. This lecture will focus on the use of liquid systems, of which there are two basically different systems: 1) permanent submersion of the plant cells/tissue which requires oxygen supply (rotary shakers or bioreactors); and 2) temporary immersion systems. The presentation will more in-depth on our own automated bioreactors. In 1992, the Dept. of Agricultural Engineering of the Agricultural Univ. of Norway constructed and built six identical, computer controlled two-litre bioreactors. These were made according to specifications from the Plant Cell Laboratory and used for scaling up plant propagation in liquid cultures. We were mainly interested in somatic embryogenesis, but also shoot cultures that could be scaled up in such vessels. Strategies for large scale production of somatic embryos are based upon results with Daucus as the model plant. Bioreactor applications have been reported for several other agricultural, horticultural or forest plants. Although the idea of using bioreactors for mass propagation arose years ago, so far commercial applications have been very limited. There are many challenges in a bioreactor culture (Heyerdahl et al, 1995). An overview of the challenges will be presented. Our bioreactors have been used to cultivate carrot, birch, Norway spruce, cyclamen and begonia.

1520–1540
S01–O–73
APPLICATION OF BIOREACTOR CULTURE FOR LARGE SCALE PRODUCTION OF CHRYSANTHEMUM TRANSPLANTS

Sun-Ja Kim[1], Eun-Joo Hahn*[2], Kee-Yoeup Paek[2]

[1]Research Center for the Development of Advanced Horticultural Technology Chungbuk National Univ. Cheongju, 361-763 Republic of Korea; [2]Development of Advanced Horticultural Technology Chungbuk National Univ. Cheongju, 361-763 Republic of Korea

Chrysanthemum transplants are produced mostly by cutting of in vitro produced plantlets. In vitro propagation of disease-free chrysanthemum plantlets has been established, but problems remain. Shoot elongation in flask cultures requires 5 to 6 weeks, which takes time and labor to obtain a certain number of single cuttings. Multiplication rate is also low due to contamination and degeneration of the plantles during subculture period. In this regard, bioreactor culture can significantly increase the multiplication rate by enhancing shoot growth and shortening culture period since culture conditions such as in vitro environment (air temperature, CO_2 supply, PPF, the number of air exchanges, etc.), medium supply, and bioreactor type can be maintained at optimal levels. In this experiment, we obtained over 5,000 cuttings of chrysanthemum (Dendranthema grandiflorum Kitam 'Cheonsu') from 60 single node cuttings after 12 weeks of culture in a 20-liter column type bioreactor. Cultures were maintained at 25 °C, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, 0.1 vvm air volume, and a 1500 $mg \cdot L^{-1}$ CO_2 concentration determined as optimal culture conditions in our prior experiment. Cuttings propagated in bioreactors performed well when transferred to a greenhouse, showing 100% survival with reduction of transplant production period. The results of our experiment suggest that bioreactor culture provides quality and uniform transplants and can be employed for scale up production of a variety of horticultural plants.

1540–1600
S01–O–74
TEMPERATE FRUIT PLANT PROPAGATION THROUGH TEMPORARY IMMERSION

Carmine Damiano*, Andrea Frattarelli, Adele Gentile, Simona Monticelli, Sonia R. La Starza

Fruit Tree Research Institute Propagation Dept., Ciampino Aeroporto 00040 Rome, Italy

So far, many research activities have been reported on automation in micropropagation; recently, many liquid media systems for tissue culture have been proposed in this regard. In this work, the method of temporary immersion has been tested for in vitro propagation of strawberry, pear, apple, peach, cherry, papaya, plum, raspberry. Since the objectives of the experiments were to assay the media composition, establish the immersion-period length, define environmental parameters, only simple double bottle devices were used. The temporary immersion of the explants was achieved filling the bottle containing the explants with the medium transferred through a silicon tube from the second bottle, in which an overpressure was created by a manual air pump. The stationary liquid, and agarized substrates were compared as control. In strawberry, the shoot multiplication rate was very high and the leaves showed the typical juvenile unilobate morphology. For pear, the results changed according to the genotype, in this case the temporary immersion (T.I.) technique revealed to be highly efficient to prevent apices necrosis. Peach explants did not increase significantly, however hyperhydricity was avoided. For cherry and papaya, both multiplication and growth habit were improved. In apple and plum, the necrosis of apices and that of the leaves was drastically lower in respect to the control. In raspberry, the multiplication rate was doubled. All the species maintained the juvenile habit during the T.I. culture, while on semisolid media, papaya and strawberry occasionally did not. In general, with the exception of some pear cultivars, the best results were obtained with an immersion of 60' per circadian cycle.

1600–1620
S01–O–75
A ROLE FOR AUXIN AND CALCIUM IN SOMATIC EMBRYOGENESIS IN AFRICAN VIOLETS

Susan J. Murch*, Jerrin M.R. Victor, Praveen K. Saxena

Dept. of Plant Agriculture Univ. of Guelph Guelph, Ontario, Canada, N1G 2W1

African violet (*Saintpaulia ionantha wendl.* cv. Benjamin) is a highly regenerative species, but there have been a ew reports of somatic embryogenesis. Thidiazuron (5 or 10 $\mu mol \cdot L^{-1}$) effectively induced somatic embryogenesis on thin petiole slices and epidermal peal explants. About 75% of petiole slices and 20% of epidermal peals formed somatic embryos with an average of 7.4 and 6.2 embryos, respectively. The thidiazuron-induced somatic embryos originated from the epidermal cells around the circumference of the petiole slices and were observed only when slices were cultured with the basepetal surface in contact with the medium. A suspensor of transparent cells was clearly visible between the maternal tissue and the embryos. Somatic embryogenesis was significantly reduced by p-chlorophenoxyisobutric acid (PCIB) and completely eliminated by 2,3,5-triiodobenzoic acid (TIBA). Incorporation of the calcium channel blocker diltiazem into the TDZ-medium changed the pattern of regeneration so that embryos were observed across the cut surface of the explant. These data demonstrate somatic embryogenesis in African violet and indicate that the thidiazuron-induced response may be dependent on the movement of auxin and calcium.

1620–1640
S01–O–76
IN VITRO "REJUVENATION" OF WOODY SPECIES IS TEMPORARY

Mehmet Nuri Nas*[1], Virginia Miller-Roeter[1], Paul E. Read[1], Philip A. Rutter[2]

[1]Dept. of Agronomy and Horticulture Univ. of Nebraska- Lincoln Institute of Agriculture and Natural Resources 377 Plant Sciences Lincoln, NE 68583-0724; [2]Badgersett Research Corp. Rt. 1, Box 141, Canton, MN 55922 1640–1700

Using explants of mature plants, we have developed micropropagation protocols for hazelnut (*Corylus* sp.) and American Chestnut (*Castanea dentata*). In vitro micropropagated hazelnut plants were ex vitro acclimatized and then were transplanted in the field. These plants set normal male flowers (catkins) in one year after removal from culture. In vitro produced and ex vitro acclimatized chestnut plants were first grown in the greenhouse, and then they were cold stored for four months. Following cold storage chestnut plants exhibited re-growth and set male flowers in their second growth year (1.5 years from the culture) in the greenhouse. These results suggest that for these (and possible other) species, in vitro micropropagated plants of mature origin maintain their maturity and could bear fruit earlier then seedling plants.

1640–1700
S01–O–77
TO BE ANNOUNCED

Thursday · August 15

1100–1200
S01–O–78
TECHNOLOGIES FOR MANIPULATING QUALITY AND PRODUCTIVITY TRAITS IN HORTICULTURAL CROPS

Abhaya Dandekar*

Pomology Dept., Univ. of California, 1 Shields Ave, Davis, California, USA, 95616

Horticultural crops are recognized as an important resource of phytonutrients essential for a balanced diet and for disease prevention. Enhancing fruit quality is key to the delivery of these vital health enhancing phytonutrients. A major challenge is to characterize the network of genes that determine fruit quality and productivity. Expression of these genes occurs in fruit, a component of which determines various quality traits (color, flavor, texture, phytonutrients etc). Genome sequencing projects have dramatically altered the approach to dissect complex gene networks like those that govern quality. New tools such as, EST database development, proteomics and expression profiling can be used to create unique database resources to study/identify genes that determine fruit quality. The expression of thousands of genes can be rapidly profiled in fruit at any developmental time point using microarray technology (the biological version of parallel processing). This can facilitate quality control; validate production and help dissect physiological processes in fruit tissues. Unique fruit quality specific ESTs can be mapped and used to define the function of various chromosomal regions and to help develop high-density maps to facilitate ongoing map based cloning efforts as well as to facilitate progeny screening in a breeding program. ESTs that represent closely related gene families could be used to detect single nucleotide polymorphisms (SNPs). Microarrays can be developed to detect SNPs and these used for rapid automated cultivar/progeny identification. Established plant transformation methods and enhanced gene silencing technology can then be effectively used to evaluate and authenticate newly discovered endogenous genes to characterize their function in fruit. Plant transformation and mutational breeding could then be effectively used to genetically manipulate fruit quality and productivity.

1200–1240
S01–O–79
TECHNOLOGIES FOR IMPROVING TOLERANCE TO ENVIRONMENTAL STRESS

Ryutaro Tao

Laboratory of Pomology, Graduate School of Agriculture, Kyoto Univ., Kyoto 606-8502, Japan

Many organisms, including certain plants, accumulate water-soluble organic compounds of low molecular weight that have no inhibitory effects on metabolism. These compounds are referred to as compatible solutes and allow them to tolerate certain types of environmental stress not only by adjusting osmotic pressure in cells but also by stabilizing the quaternary structures of complex proteins. Recent studies have shown that enhancement of tolerance to environmental stresses in plants can be achieved by genetic engineering of the biosynthesis of compatible solutes. All these studies, however, deal with herbaceous plant species. We selected Japanese persimmon, which is one of the major fruit crops in Japan and susceptible to damage from salt and drought stresses, as a candidate for the first woody plant species to be genetically engineered for tolerance to environmental stress by transforming the genes involved in compatible solutes. Chimeric genes constructed from the choline oxidase gene of Arthrobacter globiformis and the cDNA for sorbitol-6-phosphate dehydrogenase of apple (*Malus* x *domestica*) were integrated into the persimmon genome to confer the ability to produce glycinebetaine and sorbitol, respectively. Tolerance to salt stress of transformed persimmon plants were determined by measuring the ratio of the variable (Fv) to the maximum of (Fm) fluorescence of chlorophyll in leaves under NaCl stress. The rate of decline in Fv/Fm under NaCl stress was lower in transgenic persimmon plants producing glycinebetaine or sorbitol than control transformed and non-transformed lines.

1340–1440
S01–P–80
EVALUATION IN CAULIFLOWER OF GENETIC ELEMENTS OF A NOVEL CRYPTIC GENE ACTIVATION SEQUENCE FROM TOBACCO

Etsuko Tsang[1], Laima Kott[1], Huaiyu Wang[2], Brian Miki[2], Daniel Brown*[2]

[1]Univ. of Guelph Dept. of Plant Agriculture Guelph, Ontaio Canada N1G 2W1; [2]Agriculture and Agri-Food Canada Southern Crop Protection and Food Research Centre London, Ontario Canada N5V 4T3

A cryptic gene activation sequence known as tCUP (tobacco constitutive promoter), isolated from tobacco by T-DNA tagging, is capable of activating gene expression in a wide variety of plants (Foster et al. Plant Mol. Biol., 41:45-55, 1999). The tCUP TATA-less core promoter region, an upstream region and an untranslated mRNA leader region were evaluated in a hybrid cauliflower cultivar (cv. Yukon) for their potential to control transgene expression. Enhancer elements were detected in the upstream region, the core promoter region was defined, and the mRNA leader sequence was found to be critical for maintaining high levels of gene expression. The addition of the tCUP mRNA leader region to the widely used minimal 35S promoter sequence also caused a significant increase in gene expression suggesting that the individual regulatory elements of tCUP may be active when combined with a wide variety of promoters. A putative cryptic Inr site and an important domain in the +30 to +40 region were discovered. The results give insight into the nature of the tCUP core promoter elements and shows that cryptic gene activation sequences are structurally and functionally similar to regulatory elements found in promoters associated with expressed genes. These results also indicate that tCUP can be engineered to modify heterologous gene expression and demonstrates that the tCUP gene expression system may be an effective modular promoter for genetic improvement in cauliflower.

1340–1440
S01–P–81
FLOWER SPECIFIC EXPRESSION OF AN ETHYLENE RECEPTOR (ETR1-1) CONFERS ETHYLENE INSENSITIVITY IN TRANSGENIC PETUNIA

D. Cobb[1], N. Schneiter[1], S. Guo[2], G.A Humiston[1], B. Harriman[1], J. Bolar*[1]

[1]The Scotts Company, 14111 Scottslawn Road, Marysville, Ohio 43041; [2]Monsanto Company, 700 Chesterfield Parkway North, St. Louis, Missouri 63198

Our overall research objective is to produce ornamental plants having extended flower life and increased color. In the present research, we are testing the tissue specificity of two different promoters to confer ethylene insensitivity in the flowers. We have developed an efficient regeneration and Agrobacterium-mediated transformation system for several ornamental crops. Leaf explants were transformed with the etr1-1 gene of Arabidopsis that encodes an ethylene-receptor unable to bind ethylene. Plasmid binary vectors used for transformation contained the etr1-1 gene under the control of either a floral binding protein (FBP1) promoter or an apetala (AP3) promoter. Over 60 transgenic petunia lines for each of the two promoters have been identified by serological and molecular assays. Several of these plants have been propagated and established in the greenhouse, and presently we are screening the plants for extended flower life. Of the plants screened so far, 73% of the FBP1-plants and 32% of the Ap3-plants have flower life two times (2x) that of the non-transgenic petunia flowers. Some of the FBP1-transgenic plants had turgid and fully opened flowers for up to 14 days compared to the non-transgenic controls (with an average flower life of 3 days). This research demonstrates the feasibility of tissue specific manipulation of the ethylene perception pathway in ornamentals to sustain more bloom, thus more color in the plants.

1340–1440
S01–P–82
AGROBACTERIUM-MEDIATED TRANSFORMATION OF HERBICIDE RESISTANCE IN BENTGRASS

Doo Hwan, Kim*[1], Ming Liang Chai[2], Jea Yeol Kim[1], Jun Woo Park[3]

[1]Dept. of Horticultural Sci. Konkuk Univ. Kwangjin Gu, Seoul, Korea [2]Dept. of Horticulture Zhejiang Univ. Hangzhou Zhejiang P.R. China; [3]Plant Genetic Engineering and Breeding Institute, KV BIO Kwangjin Gu, Seoul, Korea

Embryogenic calli were induced from the seeds of creeping bentgrass (*Agrostis palustris* Huds.) cv. Regent, Cato, Mariner and colonial bentgrass (*Agrostis tenuis* sibth. Fl. Oxen.) cv. Tiger on modified MS medium supplemented with 1.15 $g \cdot L^{-1}$ proline and 2 $mg \cdot L^{-1}$ 2,4-D. For improving transformation efficiency, the embryogenic calli were preconditioned on fresh medium for 4-7 days and then co-cultivated with *Agrobacterium tumefaciens*, LBA 4404, which contains plasmid vector-pSBGM, harboring bar coding region, sGFP coding region and matrix attachment region (MAR). After 3 days of co-cultivation, the calli were washed thoroughly and transferred to MS medium containing 2 $mg \cdot L^{-1}$ of 2,4-D, 12-15 $mg \cdot L^{-1}$ phosphinothricin (PPT) and 250 $mg \cdot L^{-1}$ of cefotaxime. Three months later, the actively growing calli of 'Regent' and 'Tiger' were transferred to MS medium with 12-15 $mg \cdot L^{-1}$ PPT and 250 $mg \cdot L^{-1}$ cefotaxime for regeneration. The transformants survived on MS medium with PPT 3 $mg \cdot L^{-1}$ for a ong period, but control plants died within one month. The transformants also showed the strong resistance to 0.4% of Basta in the pots and field but control plants died within 2 weeks. Detection of GFP with the confocal laser scanning microscope (Carl Zeiss LSM 410 CLSM) showed significant GFP expression in both young leaves and roots. PCR analysis revealed the presence of a DNA fragment of GFP gene of the

expected size (380 bp) in the transformants and its absence in the control plant. Southern bolt hybridization of DNA extracted from the regenerated plants conformed the transformation.

1340–1440
S01–P–83
PRODUCTION OF TRANSGENIC TOBACCO WITH ENGINEERED RESISTANCE TO INHIBITORS OF PLANT PEPTIDE DEFORMYLASE

Mark A. Williams*, Lynnette M.A. Dirk, Robert L. Houtz

Dept. of Horticulture, N318 Ag. Sci. Center North, Univ. of Kentucky, Lexington, KY 40546-0091

Originally thought to be restricted to prokaryotes, peptide deformylase, which catalyzes the removal of formyl groups from initiating N-formyl-Mets of nascent polypeptides, has recently been characterized from Arabidopsis thaliana. AtDEF1 and AtDEF2 are nuclear-encoded eukaryotic homologs of this essential bacterial gene. The cDNAs for the Arabidopsis thaliana DEFs have been cloned and the proteins overexpressed. Both polypeptides have peptide deformylase activity in vitro and AtDEF1 is imported into isolated chloroplasts in vitro. The naturally occurring peptide deformylase inhibitor actinonin has been shown to catalytically inactivate both AtDEFs in vitro and this compound elicited profound herbicidal effects when applied to several plant species both pre and post-emergence. Transgenic tobacco plants were engineered to overexpress AtDEF proteins. These plants are being evaluated for resistance to the herbicidal effects of actinonin and other potent peptide deformylase inhibitors. This data will elucidate the possibility of using specific inhibitors of peptide deformylase as novel broad-spectrum herbicides, and provide a means for producing herbicide selectivity by overexpresion of peptide deformylase in transgenic plants. This technology will hopefully be applicable to a wide range of horticultural crops in the future.

1340–1440
S01–P–84
APPLICATION OF POLYPLOIDY TO CRANBERRY BREEDING AND BIOTECHNOLOGY

Eric L. Zeldin*, Brent H. McCown

Dept. of Horticulture, Univ. of Wisconsin, Madison, WI, USA 53706

The development of polyploid cranberry (*Vaccinium macrocarpon*) was pursued to exploit the potential for improved fruit size and flower bud set. In addition, polyploidy may provide a method to reproductively isolate transgenic cranberry. Colchicine-induced polyploids were produced in vitro using an inverted stem technique. With a 24-hour treatment of 0.1% to 1% colchicine, up to 100% of treated microshoots yielded at least one recovered polyploid plant. Polyploid status of the resulting plants was verified by increased stem, leaf, flower and pollen tetrad size. Colchiploids of the prominent cultivar 'Stevens' lacked any useful fertility, but colchiploids of 'Pilgrim' and 'HyRed' (a recently released 'Stevens' x 'Ben Lear' selection) yielded some fertility. First and second generation inbred and outcrossed progeny of these are being evaluated both in the greenhouse and in the field. Initial greenhouse results indicate some individuals have high levels of self-fertility with large fruit size. A parallel cross between the 'HyRed' colchiploid and a colchiploid of a bar-transformed 'Pilgrim' (tolerant to glufosinate herbicides) has produced some self-fertile progeny. Greenhouse tests have found that some fruit and seeds can be produced when pollen from these plants was used to pollinate untransformed diploid plants. Even if herbicide-tolerant triploid plants are produced from diploid/tetraploid crosses, previous experience with triploids of cranberry has found no fertility when self-pollinated or pollinated with pollen from diploid plants. Verification of a lack of fertility in polyploid to diploid crosses would allow the planting of transgenic polyploid cranberry without the risk of transfer of introduced genes to either conventional cranberry crops or native cranberry populations.

1340–1440
S01–P–85
ISOLATION OF PROTOPLASTS FROM PISTACHIO (*PISTACIA VERA* L.) FOR PRODUCTION OF SOMATIC EMBRYOS

Helen M. Chan, Ali A. Almehdi*, Dan E. Parfitt

Dept. of Pomology, One Shields Ave., Univ. of California, Davis CA 95616

Propagation of pistachio (*Pistacia vera* L.) via somatic embryogenesis provides the opportunity for application of transformation technologies and introduction of novel gene constructs for crop improvement. Protoplasts serve as ideal source materials for embryo development since transformation products would not be chimeric if single cells were transformed, and a large number of single cells can be generated. Protoplasts are easier to transform than other tissues since the absence of cell walls facilitates the use of electroporation and particle bombardment technologies. Transformed cells can be plated and screened for a selectable marker as is routinely done with transformed bacteria. A procedure for effective isolation of intact pistachio mesophyll protoplasts has been developed in our lab. Varying concentrations of cellulase, hemicellulase, and pectinase were tested for cell wall removal, and a set of optimal conditions were identified. Protoplast have been successfully isolated and microcalli produced from cultivars 'Red Aleppo', 'Kerman', and 'Peters'. 'Red Aleppo' produced the greatest yield of protoplasts. Development of protocols for somatic embryogenesis and plant regeneration from protoplast cultures are continuing.

1340–1440
S01–P–86
AGROBACTERIUM-MEDIATED TRANSFORMATION OF CHINESE CABBAGE (*CAMPESTRIS L.* SSP. PEKINENSIS) WITH A PROTEIN DISULFIDE ISOMERASE GENE

Bong-Kyu Kim, Hee-Jung Cho, Young-Soo Park, Chee-Hark Harn, Seung-Gyun Yang, Byung-Whan Min*

Nong Woo Bio Co., Ltd 537-17 Jeongdan, Kanam, Yeoju, Kyonggi, Korea

An effective plant regeneration procedure and a gene transfer system via *Agrobacterim tumefaciens* were developed in the commercially important vegetable *Brassica campestris* L. ssp. Pekinensis. Cotyledonary explants from 5-day-old seedlings with 2 days pre-culture were infected with *Agrobacterium tumefaciens* strain LBA4404 harboring a binary vector Pmbp-1 containing a Ricinus communis PDI gene with full codon modification. After culture and selection on MS medium supplemented with 2 mg/L BAP, 2 mg/L Zeatin, 1 mg/L NNA and 15 m/L Kanamycin, a number of kanamycin-resistant plantlets were regenerated. Polymerase chain reaction, Southern and Northern blotting analysis were used to identify and Characterize the transgenic plants with the integrated PDI gene. Over 16 transgenic plants have been established in soil and flowered in the greenhouse. This procedure facilitates induction of another desirable gene into commercial inbred lines of Chinese cabbage.

1340–1440
S01–P–87
CELL SUSPENSION CULTURE AND GENTEIC TRANSFORMATION IN LITCHI AND LONGAN

D.C. Chen*[1], G.B. Hu[1], Q.F. Zheng[1], Z.R. Huang[1]

[1]College of Horticulture, South China Agricultural Univ., Guangzhou 510642, China; [2]Dept. of Sericulture & Habiliments, South China Agricultural Univ., Guangzhou 510642, China

In this paper, the effect of plant growth regulators including IBA, 6-BA, GA3 and CM on cell suspension culture of litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longana* Lour.), the susceptibility of cell suspension lines to kanamycin and the genetic transformation through Agrobacterium tumefaciences with API or APBD genes, respectively, and infected liquid-phase wounding of embryogenic tissues with carborundum were studied. The results showed that IBA, 6-BA, GA3 and CM significantly increased the yield of cell suspension lines, but GA3 resulted in fast growing and browning of cells. In suspension culture, cell suspension lines were more susceptible to kanamycin than that on solidified culture. More Kmr embryoids could be obtained by the way of liquid-phase wounding of embyogenic calli with carborundum.

1340–1440
S01–P–88
TRANSGENIC SWEET POTATO PLANTS FROM SOMATIC EMBRYOS/AGROBACTERIUM-MEDIATED TRANSFORMATION SYSTEM

L. Chen*, A. Bhagsari

1005 State Univ. Drive, Agricultural Research Station, Fort Valley State Univ., Fort Valley, Georgia, USA, 31030

In this study, the applicability of sweet potato (*Ipomoea batatas* L. Lam.) somatic embryos as transformation target material was evaluated using *Agrobacterium tumefaciens* strain EHA101/pPTN140 harboring beta-glucuronidase (gus) and phosphinothricin acetyl transferase (bar) genes. Some important parameters affecting *Agrobacterium*-mediated transformation such as co-cultivation duration, vacuum infiltrating, and type of target materials were also investigated through GUS transient expression. Furthermore, the novel sweet potato transformation procedure using the somatic embryo/*Agrobacterium* transformation system with glufosinate as the bar gene selective agent was conducted. GUS transient expression results indicated that sweet potato somatic embryo could be used as target material of *Agrobacterium*-mediated transformation, but the stage of the embryo development was critical. Only a late stage embryo culd be infected by Agrobacterium. Most of the experiments showed that 6-day co-cultivation resulted in higher GUS transient expression than 2-day co-cultivation. Vacuum-infiltration treatment facilitated sweet potato genetic transformation via *Agrobacterium*-mediated method compared with soaking in an *Agrobacterium* solution only. Among target materials of shoot tip, petiole, stem with one node, and somatic embryos, the petiole explant had the highest transformation frequency of GUS transient expression. But after about four months on the glufosinate selective media, putative transgenic plants regenerated only from somatic embryo as transgenic target material. Histochemical GUS expression was detected, six out of ten, in mature leaflets, stems, and roots of putative transgenic plants tested. Using somatic embryo/*Agrobacterium* transformation system avoids the callus phase, thus results in rapid and efficient plant regeneration for transgenic sweet potato.

1340–1440

S01–P–89

LEAF SENESCENCE IN TRANSGENIC PETUNIA PLANTS CONTAINING THE PSAG12-KN1 GENE

Francine Cuquel*[1], Keigo Minami[1], David G. Clark[2]

Dept. of Plant Production, Federal Univ. of Paraná, ZIP 80.035-050, Curitiba, Paraná, Brazil, 80.035-050

Delayed leaf senescence process has been engineered in transgenic *Petunia* x *hybrida* plants containing the homeobox knotted-1 gene (kn1), a mutant maize gene that is thought to be involved in cytokinin metabolism. Petunia plants were transformed with the kn1 gene fused to a senescence associated promoter (SAG12) using *Agrobacterium*. These PSAG12-kn1 lines were subjected to drought stress treatments, and leaf senescence was evaluated in the T0 and T1 generations. A wide range of delayed senescence phenotypes was observed, indicating that the promoter SAG12 did not always present complete regulation. Although expression of the Kn1 transgene delayed leaf senescence, it also caused some morphological and physiological alterations, which ranged from mild to severe in their intensity. Most of the plants with a non-senescent phenotype showed only mild phenotypic alterations, which were not considered detrimental to plant performance. Along with the trait of delayed leaf senescence, plants with mild phenotypes had a more compact architecture, while plants with a severe phenotype were stunted in growth. Our data support the concept that the PSAG12-kn1 genetic construct can be used to delay leaf senescence, but the mechanism(s) by which kn1 gene affects the senescence process remains to be elucidated.

1340–1440

S01–P–90

ANTIBIOTICS, FRIENDS AND ENEMIES OF THE REGENERATION AND GENETIC ENGINEERING BATTLES: CHRYSANTHEMUM, A CASE STUDY

Jaime A.T. da Silva*, Seiichi Fukai

Dept. Horticulture, Floriculture Lab, Kagawa Univ., Miki-cho Ikenobe, 761-0795, Japan

Despite the movement to develop and utilize non-antibiotic marker genes in the genetic transformation of plant cells and tissues, antibiotic selector markers, which code for genes that confer resistance to cells containing them by enzymatic detoxification of that antibiotic, still remain a widely popular choice. Negative associations with the use of antibiotics include slowed growth in vitro and the use of complex media to maintain transformed cells alive while selecting against non-transformed ones. Successful transformation in chrysanthemum (*Dendranthema grandiflora* (Ramat) Kitamura) is a fine balance between antibiotic selection and regeneration, especially at an early phase of both. Where the process of transformation uses an Agrobacterium vector system, the need to control the bacterium, in our case by the addition of cefotaxime, carbenicillin and/or vancomycin, also influences the capacity to regenerate transgenic chrysanthemum. The nptII gene, used as a selector marker, levels at or above 10 mg/L or 7-8 mg/L of kanamycin or G418, respectively successfully eliminate non-transformed cells when sufficiently small explants or thin cell layers are used. Both cefotaxime and vancomycin are dually positive, eliminating Agrobacterium (LBA4404 or AGL0) when used individually at high concentrations (>250 mg/L) or in combination at moderate concentrations (50-100 mg/L), while also stimulating the callusing of putative transformed or control auxotrophic cells or cell clusters. The former in turn can be positively and visibly (appearing as distinct hard, green callus) selected from non-transformed cells (typically etiolated) and used to indirectly regenerate adventitious shoots by modifying the medium and culture conditions.

1340–1440

S01–P–91

TRANSGENE EXPRESSION AND TRANSMISSION TO THE PROGENY OF STRAWBERRY PLANTS TRANSFORMED BY AGROBACTERIUM

Simona Monticelli, Carmine Damiano*

Fruit Tree Research Institute, Propagation Dept., Via di Fioranello, 52, Ciampino Aeroporto, Roma, Italia, 40

Agrobacterium-mediated transformation was used to stably introduce B-glucuronidase (gus) and neomycinphosphotransferase (nptII) marker genes into 'Egla' and 'Teodora', two cultivated octoploid strawberry (*Fragraria* x *ananassa* Duch.). Viable and fertile transgenic plants were regenerated from stipules after transformation. The transfer and the expression of the two genes was confirmed by the growth under kanamicin selection, the blue staining of the tissue after histochemical GUS-assay and the molecular confirmation by PCR and Southern blot analysis. After rooting in vitro in the presence of kanamicin, the plants were acclimated in the greenhouse. The Gus gene continued to be expressed after several months: the constitutive promoter 35S induced gus expression in different organs. The GUS reaction was detected in leaves, sepals, petals, receptacle, anthers of the mother plants and runners. Open pollinated seeds were harvested separately from each plant. GUS expression was detected in embryos and in R1 seedlings after aseptical germination. This expression in most of R1 seedlings shows that one or more functional gus gene were transferred from R0 plants to the segregating R1. However, inheritance and expression of transgenes need further investigations in relation to the environmental risk assessment.

1340–1440

S01–P–92

TISSUE CULTURE OF HEMP (*CANNABIS SATIVA* L.) AND APPLICATIONS FOR PLANT IMPROVEMENT

Mistianne Feeney*, Zamir K. Punja

Centre for Environmental Biology, Simon Fraser Univ., 8888 Univ. Drive, Burnaby, British Columbia, Canada, V5A 1S6

Hemp (*Cannabis sativa* L.) is cultivated in many parts of the world for its fiber, oil and seed. With increases in hemp acreage, pressure from insect pests and diseases is expected to increase. To develop research tools to assist with hemp improvement, we investigated the propagation of hemp in tissue culture and Agrobacterium-mediated transformation for foreign gene introduction. Four-week-old seedlings of cultivars Anka, Uniko B, Kompolti and Felina-34 were used. Stem and leaf segments were placed on Murashige and Skoog medium with Gamborg B5 vitamins (MB) supplemented with 5 μM 2,4-D and 1 μM kinetin, 3% sucrose, 8 g agar/L, pH 5.8. Dishes were placed in the dark and callus began to develop on petioles and midveins within 1 wk. Large masses of callus were produced within 4 wk for all cultivars. By 8 wk, 90-100% root formation was initiated on medium containing various concentrations of naphthalene acetic acid (NAA) or indole butyric acid (IBA) for the variety Anka. Suspension cultures were established in 20 mL MB medium containing 2.5 μM 2,4-D and shaken at 115 rpm at ambient laboratory conditions. Subcultures were made every 4 wk. To promote embryo formation, callus cultures were treated with an increased supply of organic nitrogen, hemp tissues were plasmolysed and exposed to different growth regulators and different explant ages and sources were evaluated for 2 hemp varieties. To date, no treatments have been successful in promoting somatic em-

bryogenesis. Callus lines were also evaluated for tolerance to the selectable marker phosphomannose isomerase (PMI) for use in transformation experiments. The selection conditions inhibiting callus growth was MB medium containing 1% mannose with 5 µM 2,4-D, 1 µM kinetin, with 8 g agar/L, pH 5.8. Agrobacterium-mediated transformation using strain EHA101 and PMI expression under control of the *Arabidopsis thaliana ubiquitin* promoter is being investigated.

1340–1440

S01–P–93

ISOLATION AND CHARACTERIZATION OF GAMETIC MICROPROTOPLASTS FROM DEVELOPING MICROSPORES OF *LILIUM LONGIFLORUM* FOR PARTIAL GENOME TRANSFER IN THE LILIACEOUS ORNAMENTALS

Hiroyuki Saito*, Masaru Nakano

Faculty of Agriculture, Niigata Univ., 2-8050 Ikarashi, Niigata, 950-2181, Japan

We had established a system for isolating gametic microprotoplasts from developing microspores of *Lilium longiflorum* 'Hinomoto' (2n = 2x = 24) as a first step toward the production of intergeneric hybrid plants with one or a few alien chromosomes via microprotoplast fusion in the Liliaceous ornamentals. Anthers of this cultivar containing microsporocytes at mainly diakinesis to metaphase I were cultured in a medium containing half-strength MS salts, doubled-strength MS vitamins, 1 g/L casamino acid, 100 g/L sucrose, and various spindle toxin for 3 to 4 days. Among spindle toxins examined, isopropyl N-(3-chlorophenyl)carbamate (CIPC) at concentrations of 5 or 10 µM efficiently induced micronucleation with the mean number of nuclei per meiocyte of 7.5. The CIPC treatment also efficiently induced micronucleation in other five *Lilium* genotypes, *L. regale*, *L. longiflorum* 'Georgia', *L. speciosum* 'Uchida', the Asiatic hybrid lily 'Connecticut King' and the Aurelian hybrid lily 'Golden Splendor', with the mean numbers of nuclei per meiocyte of 5.4 to 11.7. In 'Hinomoto', each nucleus in the meiocytes formed a microcell 4 to 5 days after the initiation of CIPC treatment. Following isolation of such meiocytes from the anthers and their incubation in a cell wall-digesting enzyme solution, gametic (micro)protoplasts of below 10 µM, 10-20 µM and 20-50 µM in diameter were obtained with yields of 5.5x10 4, 6.6x10 4 and 4.9x10 4 per anther, respectively. Smaller microprotoplasts with DNA contents below the 2 °C level as indicated by flow cytometric analysis were enriched through a sequential filtration with nylon sieves of decreasing pore sizes (50-, 20- and 10-µM). The relative fluorescence intensity in some of their nuclei corresponded to that of one or a few chromosomes.

1340–1440

S01–P–94

CONSTRUCTION OF AN AGROBACTERIUM-MEDIATED TRANSFORMATION SYSTEM OF NON-HEADING CHINESE CABBAGE

X.L. Hou*[1], G.P. Zhang[2], J.M. She[3]

[1]National Key Laboratory for Crop Genetics & Germplasm Enhancement, Nanjing Agricultural Univ., Nanjing, P.R. China; [2]Horticultural College, Nanjing Agricultural Univ., Nanjing, P.R. China; [3]Genetic and Physiological Institute of Jiangsu Academy of Agricultural Science, Nanjing, P.R.China

This study describes the use of cotyledons with petioles of non-heading Chinese cabbage (*B. campestris* L. ssp. *chinensis* Makino var. *communis* Tsen et Lee) as transformation recipients. Being treated for 3 days on precultivation medium with 0.1 mg/L 2,4-D and 0.1 mg/L NAA, the differentiation frequency of adventitious buds from the AB-Naihan line decreased from 60% to 44.6%; but it had no effect on the 'Duan-126' line. Kanamycin strongly inhibited the cytoledon differentiation and when added 15mg/L Kan, the explants turned white on the selection medium. As to Carb, it had no inhibitive effect but shortened the differentiation time from 20 to 14 days. The concentration of Agrobacterium had little effect on the cotyledon differentiation, but with the prolonging of the cocultivation time, the differentiation frequency was sharply reduced.

1340–1440

S01–P–95

CHARACTERIZATION OF THE 5'-FLANKING REGIONS OF S-RNASE GENES IN SWEET CHERRY (*PRUNUS AVIUM* L.)

T. Ishizaka*[1], H. Nakano[2], T. Suzuki[3], H. Kitashiba[4]

[1]Yamagata Public Corporation for the Development of Industry R&D Dept., 423 Shimaminami, Sagae, Yamagata 991-0043, Japan; [2]Yamagata Horticultural Experiment Station, 423 Shimaminami, Sagae, Yamagata 991-0043, Japan; [3]Faculty of Education, Yamagata Univ., 1-4-12 Kojirakawa-Machi, Yamagata 990-8560, Japan; [4]Yamagata Public Corporation for the Development of Industry R&D Dept., 423 Shimaminami, Sagae, Yamagata 991-0043, Japan

Sweet cheery (*Prunus avium* L.), a member of the rosaceae, has a gametophytic self-incompatibility system controlled by a single S-locus with multi-alleles. The S-locus product of pistil is the glycoprotein with ribonuclease activity, termed S-RNase. The six alleles (S1 to S6) have been identified and partial S1- to S6-RNase genes have been cloned. However, none of the promoter regions of any S-RNase genes have been analyzed. To characterize the S-RNase promoter of sweet cherry, we isolated and sequenced the 5'-flanking regions of the S3-, S4- and S6-RNase genes. A comparison of the 5'-flanking sequences among them indicated that the putative TATA box-sequences were conserved and a highly similar region (about 73%) of approximately 140-bp existed in the region just upstream of the putative TATA box. This suggests that some cis-regulatory element s may be contained within this 140-bp region. In addition, a sequence motif (named motif IB-like: CT/GCACAAT), which is similar to motif IB (CTCACACT) reported as one of the sequence motifs conserved in the 5'-flanking regions among the solanaceous S-RNase genes, was found within the 140-bp region. However, no other motifs were found in the S3-, S4-, S6-RNases and the 140-bp conserved region was only slightly similar to the 5'-flanking regions of other rosaceous (Japanese pear and apple) S-RNases.To examine the S-RNase promoter activity of sweet cherry, we fused the putative 1.46-kb promoter region of the S6-RNase gene to the GUS gene and introduced it into tobacco. Transgenic plants are currently being studied for the presence of the transgene and the temporal and spatial GUS expression patterns.

1340–1440

S01–P–96

PROMOTER ACTIVITY OF THE EL2-OMEGA AND THE UPSTREAM REGION DC-ACO1 IN LISIANTHUS (*EUSTOMA GRANDIFLORUM*)

K. Isuzugawa*[1], T. Hibi[2], S. Satoh[2], H. Nakano[1]

[1]Yamagata Horticultural Experiment Station, 423 Shimaminami, Sagae, Yamagata 991-0043, Japan; [2]Laboratory of Bio-adaptation, Graduate School of Agricultural Science, Tohoku Univ., Tsutsumidori-amamiyamachi 1-1

Lisianthus (*Eustoma grandiflorum*) is a gentian flower native to the prairies of the U.S.A. We have been applying genetic engineering to lisianthus breeding. In an attempt to develop a powerful or ethylene responsive promoter in lisianthus, we have examined the ability of the EI2-omega promoter (Plant Cell Physiol. 37(1) 49-59 1996) and the upstream region (~-247bp) of the carnation ACC oxidase gene, DC-ACO1 (J. Japan. Soc. Hort. Sci. 70(2) 215-222 2001) to drive the expression of the gusA reporter gene. The ethylene responsive promoter will be used to target the expression in cut flower senescence, because ethylene is assumed to be a major factor in the senescence of the cut flower of lisianthus. Transgenic clones were produced by Agrobacterium-mediated transformation. More than seven clones of each construct were obtained. The PCR analysis revealed the existence of each promoter in the lisianthus genome. The analysis of the GUS activity in the leaf of the transformants confirmed that the EI2-omega promoter was approximately l00 times stronger than the CaMV 35S promoter. The activity of the upstream region of DC-ACO1 was similar to the CaMV 35S promoter. In the roots, the analysis of the GUS activity also showed that the EI2-omega promoter was stronger than the other promoters. In the leaves, the upstream region of DC-ACO1 was not responsive to the ethylene treatment. Histochemical analysis confirmed the EI2-omega promoter and the upstream region of DC-ACO1 induced the gusA gene especially in vascular bundles, guard cells and mesophylls of leaves. This expression was similar to the patterns of the CaMV 35S promoter. In the roots, the upstream region of DC-ACO1 induced expression in central cylinder, compared to expression throughout induced by the EI2-omega and CaMV 35S promoters.

1340–1440

S01–P–97

GENETIC TRANSFORMATION OF 'FUJI' APPLE USING *AGROBACTERIUM TUMEFACIENS*

K.J. Song*, E.S. Seong, J.H. Hwang, Y.U. Shin, J.H. Kim

National Horticultural Research Institute, RDA, Suwon 440-706, Korea

Transgenic plants of the commercially important apple cv. Fuji (*Malus* x *domestica* Borkh.) have been obtained by *Agrobacterium tumefaciens*-mediated transformation. Some factors affecting gene transfer and regeneration including acetosyringone (AS) concentration, cocultivation periods, cefotaxime concentration, carbon sources, and explant sources on the agar-solidifying selective medium of MS salts and LS vitamins supplemented with 5 mg·L^{-1} TDZ, 0.3 mg·L^{-1} IBA, and 100 mg·L^{-1} kanamycin were investigated to develop an efficient transformation protocols of 'Fuji' apple. The putative transformants were reselected through the response of rooting activity on the half strength of MS basal medium with 0.3 mg·L^{-1} IBA and 30 mg·L^{-1} kanamycin for rooting and confirmed by PCR and southern blot analysis. The treatments of 200 μM AS or cocultivation for 4 days improved the efficiency of genetic transformation, however other factors mainly affecting regeneration had little effect on improving the efficiency of transformation. The results indicated that gene transfer was pivotal step to establish the efficient *Agrobacterium*-mediated transformation of 'Fuji' apple.

1340–1440

S01–P–98

AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION AND TRANSGENIC-PLANT REGENERATION OF ROSE (*ROSA HYBRIDA* L.)

Chang-Kil Kim*[1], David H. Byrne[1] Jae-Dng Chung[2]

[1]Dept. of Horticultural Science, Texas A&M Univ., College Station, TX 77843-2133; [2]Dept. of Horticulture, Kyungpook National Univ., Taegu 702-701, Korea

Early detection of plant transformation events and improvement of transformation efficiency are necessary for rapid establishment and optimization of transformation protocol. Modified versions of the green fluorescent protein (GFP) from *Aequorea victoria* have been introduced into *Rosa hybrida* via *Agrobacterium*-mediated gene transfer. The identification of gfp-expressing rose somatic embryogenic callus allowed for elimination of non-expressing explants and also enabled visual selection of dividing transgenic embryogenic callus in a medium without the selective agent kanamycin. The binary vector containing additional virE/virG enhanced the efficiency of *Agrobacterium*-mediated gene transfer.

1340–1440

S01–P–99

TRANSFORMATION OF GARLIC (*ALLIUM SATIVUM* L.) USING PARTICLE-BOMBARDMENT AND *AGROBACTERIUM*-MEDIATED METHODS

Do-Sun Kim*[1], Yul-Kyun Ahn[1], Ki-Taek Kim[2], Jong-Gyu Woo[1], Dae-Geun Oh

[1]Bio-Research Team, National Horticultural Research Institute, RDA, 540-41 Tapdong, Gweonseongu, Suwon 441-440, Republic of Korea; [2]Research Managemenet Bereau, RDA, Suwon 441-707, Republic of Korea

Garlic has been multiplied vegetatively due to its sexual sterility; consequently, viral diseases are a very serious problem. Genetic transformation has received much attention as an important alternative for improving garlic varieties. We have tried to establish an efficient transformation method for garlic to introduce useful foreign genes, herbicide-tolerant genes. A reporter gene, B-glucuronase (GUS) and/or selectable marker herbicide-tolerant gene, were chosen to test the protocol. For transformation, we used calli derived from meristems and foliage leaves. The calli showed very low transformation frequency in the selection medium, thus immature scapes were tried for transformation. The immature scapes were regenerated on MS medium containing 1.0 mg/L NAA and 1.0 mg/L BA, and 6% sucrose. The immature scape, inside which the floral organs and bulbils are positioned, were transformed using both particle-bombardment and *Agrobacterium*-mediated method. The explants were bombarded with tungsten particle coated with pCAMBIA3301 in various combinations of He gas pressure and vacuum chamber size. *Agrobacterium*-mediated method showed more effective transformation frequency than particle-bombardment. After preculture for 2 days, the explants were inoculated with bacterial liquid suspension (OD = 0.4) and cocultivated for 4 days showed the highest frequency of shoot regeneration on selection medium. Inoculation of *Agrobacterium* EHA101 on cocutivation medium supplemented with 50 μM acetosyringone at pH 5.2 was the most effective in transformation frequency. After 2-3 months, putative transgenic shoots were regenerated on selection medium containing kanamaycin and PPT in both methods. Among these shoots, GUS activity was observed and gene insert was confirmed by PCR analysis with a specific primer for bar gene.

1340–1440

S01–P–100

TRANSFORMATION OF POTATO PLANT WITH ISOPENTENYLTRANSFERASE GENE LEADS TO ENHANCED STOLEN DEVELOPMENT AND PRECOCIOUS TUBER SPROUTING

A. Lafta*[1], J. Wu, J. Lorenzen[2]

[1]Dept. of Plant Sciences North Dakota State Univ. Fargo, ND 58105; [2]Plant, Soil, and Entomological Science Dept. Univ. of Idaho Moscow, ID 83844

This study was conducted to show the effect of transformation of potato plants with an isopentenyltransferase (ipt) gene on plant morphology and cytokinin levels in leaves and tubers. The ipt gene, which catalyzes the rate-limiting step of the cytokinin synthesis pathway, was placed under the control of a tuber-specific patatin promoter and introduced into potato plant (cvs. Norchip, Tomasa Condemayta) via *Agrobacterium*-mediated transformation. The presence of the ipt gene in transgenic potato plants was confirmed by Southern hybridization. Shoot morphology was normal in nearly all transgenic plants prior to and after tuberization. Transgenic plants had extensive stolon growth and an increased number of tubers. Premature sprouting of tubers and an increase in the number of sprouts were also observed in transgenic plants, although this was affected by photoperiod during the tuber growth phase. Total fresh and dry weight of tubers was similar in control and transgenic plants. Expression of ipt gene was tuber specific under long photoperiods, as determined by northern hybridization and cytokinin analysis. The concentration of zeatin and zeatin riboside was increased in tubers of transformed plants, but the level in source leaves was similar in transformed and control plants. This study supports a role for cytokinin in tuber initiation and release of dormancy.

1340–1440

S01–P–101

REGENERATION OF INTERSPEFIC MIXOPLOID SOMATIC HYBRIDS VIA ASYMMETRIC SOMATIC HYBRIDIZATION IN CITRUS

Jihong Liu*, Xiuxin Deng

National Key Lab of Crop, Genetic Improvement, Huazhong Agricultural Univ, Wuhan, Hubei, P.R.China, 430070

Embryogenic protoplasts of Dancy tangerin (*Citrus reticulata* Blanco), irradiated with X-ray for different time (60, 90 and 120 min) at 5mA and 80kVp, were electrically fused with embryogenic protoplasts of Page tangelo (*Citrus reticulata* C. paradisi) that were treated with 0.25 mM iodoacetic acid (IA) for 15 min. The IA-treated protoplasts of Page could not divide at all. Division of Dancy protoplasts irradiated for 60 min was not prevented but delayed, whereas those irradiated for 90 and 120 min did not divide at all. Instead, they plasmolysed or broke. The fusion-treated protoplasts could develop into embryoids for all of the fusion combinations only when the callus was transferred to MT supplemented with 2% glycerol. However, embryoids could develop into multiple shoots derived from the fusion combination when the donor protoplasts were irradiated for 60 min was involved, which were recalcitrant to rooting. In vitro grafting was employed to obtain complete plants. Cytological observation showed that the plants contained mainly diploid and aneuploid cells, together with very few tetraploid cells, indicating that they were mixoploids. Random amplified polymorphic DNA analyses with three 10-mer arbitrary primers confirmed the plants as true somatic hybrids. This is the first report on regeneration of mixoploid hybrid plants via protoplast asymmetric fusion in Citrus. Negative effects of ionizing irradiation on regeneration of embryoids and plantlets and possible agronomic interest of the mixoploid plants will be discussed.

1340–1440

S01–P–102

PHENOTYPIC CHARACTERIZATION OF PETUNIA PLANTS EXPRESSING AN INDOLEACETIC ACID (IAA)-LYSINE SYNTHETASE TRANSGENE DRIVEN BY A SHOOT SPECIFIC PROMOTER

Song Xue[1], Maria Khodakovskaya[2], Yan Wu[2], Yi Li[2], Richard McAvoy*[3]

[1]Horticultural Sciences Dept 1143 Fifield Hall P.O. Box 110690 Univ. of Florida Gainesville, FL 32611; [2]Transgenic Plant Facility 105 Ahern Lane, U-5082 Storrs, CT 06269; [3]Agricultural Biotechnology Laboratory 1390 Storrs Road, U-4163 Storrs CT 06269-4163

Chemical plant growth regulators are used on many ornamental species to

control internode length and/or increase branching. Transgenes that reduce whole-plant auxin activity produce plants with the desirable compact, well-branched phenotype but with poor root development. In this study, petunia 'Marco Polo Odyssey' was transformed with an auxin inactivating indoleacetic acid-lysine synthetase (iaaL) gene from *Pseudomonas syringae* driven by a shoot specific promoter. Phenotypic expression of this fusion gene was characterized for several selected lines and the non-transformed wild-type by quantifying leaf shape, internode and lateral branch length, number of lateral branches, and dry weight distribution between leaves, stems, flowers, and roots.

1340–1440

S01–P–103

TRANSFORMATION OF PEAR (*PYRUS COMMUNIS* CV. 'LA FRANCE') WITH ANTISENSE OR SENSE CDNA ENCODING 1-AMINOCYCLOPROPANE-1-CARBOXYLATE (ACC) OXIDASE

M. Gao*[1], H. Murayama[2], T. Toyomasu[2], W. Mitsuhashi[2], H. Nakano[3]

[1]423 Shimaminami, Sagae, Yamagata Japan; [2]Faculty of Agriculture, Yamagata Univ.; [2]Faculty of Agriculture, Yamagata Univ.; [2]Faculty of Agriculture, Yamagata Univ.; [3]Horticultural Experiment Station of Yamagata Prefecture

The plant hormone ethylene is known to play an important role in fruit ripening especially in typical climacteric fruits including the European pear (*Pyrus communis* L.). It may be possible to extend the shelf life of pear fruits by inhibiting ethylene biosynthesis utilizing genetic engineering. We cloned a cDNA encoding 1-aminocyclopropane-1-carboxylate (ACC) oxidase (ACO), a key enzyme for ethylene biosynthesis, and introduced the cDNA into the pear (cv. 'La France') in an antisense or sense orientation using an *Agrobacterium*-mediated leaf disc transformation system. The ACO cDNA obtained from the 'La France' pear fruit, which we designated LF-ACO1is homologous to the PCACO1 of 'passe-crassane' pear (GenBank accession number X87097). The open reading frame of LF-ACO1 cDNA is 945 nucleotides long, and encodes a predicted protein of 315 amino acids. The antisense or sense sequence of LF-ACO1 cDNA was adjacent to the CaMV 35S promoter, and the gene expression cassette was inserted in the T-DNA of the vector used in pear transformation. The nptII gene cassette for kanamycin resistance driven by the CaMV 35S promoter was also contained in the T-DNA. The constructed vectors were induced into the *Agrobacterium* EHA101 strain. The transformation of pear (cv, 'La France') was performed according to *Agrobacterium*-mediated method using the Leaf discs of in vitro shoots. As a result, we have obtained 9 transgenic plant lines by the antisense construct and 5 lines by the sense construct. Both the antisense and sense transgenic pears were characterized by PCR and genomic blot analysis to confirm that the T-DNAs were integrated in their genomes. For the antisense lines, the expression of the transgene in in vitro leaves was confirmed according to the RNA blotting hybridization with the DIG-labeled sense chain RNA probe. The expression of the endogenous LF-ACO1 gene in the antisense or sense transgenic lines was investigated.

1340–1440

S01–P–104

AGROBACTERIUM-MEDIATED TRANSFORMATION OF CHINESE CABBAGE (*CAMPESTRIS L.* SSP. PEKINENSIS) WITH A PROTEIN DISULFIDE ISOMERASE GENE

Bong-Kyu Kim[1], Hee-Jung Cho[1], Young-Soo Park[1], Chee-Hark Harn[1], Seung-Gyun Yang[1], Byung-Whan Min*[2]

[1]Nong Woo Bio Co., Ltd 537-17 Jeongdan, Kanam, Yeoju, Kyonggi, Korea; [2]Kanam-Myon Jeongdan-Li 537-17, Yeoju-Gun, Kyonggi-Do, Yeoju-Gun, Kyonggi-Do, Korea, 469-880

An effective plant regeneration procedure and a gene transfer system via *Agrobacterim tumefaciens* were developed in the commercially important vegetable *Brassica campestris* L. ssp. Pekinensis. Cotyledonary explants from 5-day-old seedlings with 2 days pre-culture were infected with *Agrobacterium tumefaciens* strain LBA4404 harboring a binary vector Pmbp-1 containing a Ricinus communis PDI gene with full codon modification. After culture and selection on MS medium supplemented with 2 m/L BAP, 2 m/L Zeatin, 1 m/L NAA and 15 m/L kanamycin, a number of kanamycin-resistant plantlets were regenerated. The polymerase chain reaction, Southern and Northern blotting analysis were used to identify and characterize the transgenic plants with the integrated PDI gene. Over 16 transgenic plants have been established in soil and flowered in the greenhouse. This procedure facilitates induction of another desirable gene into commercial inbred lines of Chinese cabbage.

1340–1440

S01–P–105

TRANSIENT TRANSFORMATION OF *RHODODENDON* 'PJM HYBRID' AND *RHODODENDRON CATAWBIENSE* 'CUNNINGHAM'S WHITE' LEAF EXPLANTS VIA BIOLISTIC PARTICLE BOMBARDMENT

Christina Moore*, Robert R. Tripepi

Univ. of Idaho, 875 Perimeter Drive, Division of Plant Science, Moscow, Idaho, USA, 83844-2339

Biolistic particle bombardment is used to transfer desirable genes into difficult to transform plants, such as Rhododendron. By optimizing bombardment parameters, the number of recovered transgenic plants can be increased. Parameter effects can be determined by transient gene expression of marker genes. By optimizing parameters for transformation of Rhododendron, the number of transgenic plants recovered should be increased. Leaf explants from *Rhododendron* 'PJM Hybrid' and *Rhododendron catawbiense* 'Cunningham's White' microshoots were bombarded with 1.0 μm gold particles coated with linear or circular plasmid DNA, containing a gene that encodes for an enzyme, beta-glucuronidase (GUS). The presence of transient enzyme activity in leaf explants was histochemically analyzed 2 days after bombardment. *Dendranthema* x *grandiflora* 'Iridon' leaf explants were used as a positive control in this procedure since they exhibit high levels of transient GUS expression. Bombarding 'PJM' and 'Cunningham's White' explants with linear DNA resulted in higher transient GUS expression, 20.8% and 7.1% respectively, compared to explants bombarded with circular DNA, 16.7% and 6.3%. Fifty-one percent of 'Iridon' explants bombarded with linear DNA transiently expressed GUS compared to 33% bombarded with circular DNA. Leaf explants were also bombarded with gold particles containing 1, 2, 4, 8, or 16 μg linearized DNA per bombardment. The most 'PJM' and 'Cunningham's White' leaf explants transiently expressed GUS when 2 μg of DNA per bombardment were used, 45.8% and 47.8% respectively. In contrast, the most 'Iridon' explants, 68.8%, transiently expressed GUS when 8 μg of linear DNA per bombardment was used. Transient gene expression of GUS by 'PJM' and 'Cunningham's White' leaf explants was optimized by using 2 μg of linear DNA per bombardment.

1340–1440

S01–P–106

PRODUCTION VIA OVULE CULTURE OF INTERGENERIC HYBRID PLANTS BETWEEN *SANDERSONIA AURANTIACA* AND *GLORIOSA ROTHSCHILDIANA*

Toru Nakamura*, Hiroyuki Saito, Masaru Nakano

Faculty of Agriculture, Niigata Univ., 2-8050 Ikarashi, Niigata, Japan, 950-2181

Sandersonia aurantiaca Hook. (2n=2x=24) and *Gloriosa rothschildiana* O'Brien (2n=6x=66), both belong to the family Liliaceae, have little variations in horticultural traits, such as plant form and flower color and shape. In the present study, cross-pollination of *S. aurantiaca* x *G. rothschildiana* was carried out in order to widen their variations and to develop novel cultivars. Although growth of pollen tubes of *G. rothschildiana* was inhibited in the style of *S. aurantiaca*, some of them penetrated the style at one day after pollination. Five days after pollination, a few pollen tubes were observed in the ovary. Although a few seeds were obtained from cross-pollination of *S. aurantiaca* x *G. rothschildiana*, the failed to germinate both in soil and in vitro. Therefore, ovule culture was applied for rescuing hybrid embryos. Ovules with placental tissue prepared from ovaries 14 days after cross-pollination were cultured on a medium containing 0.01 mg/L alpha-naphthaleneacetic acid (NAA) and 0.01 mg/L 6-benzyladenine (BA), on which some ovules developed rhizome-like structures within 20 weeks. Following transfer to a medium containing 0.25 mg/L NAA and 2.5 mg/L BA, one of these structures produced multiple shoots. These shoots rooted on a plant growth regulator-free medium, and plantlets thus obtained were successfully transferred to the greenhouse. Early verification of their hybridity was accomplished by rDNA and flow-cytometric analyses. Hybrid plants had 45 chromosomes, and the parental chromosomes were identified in them using genomic in situ hybridization (GISH).

1340–1440
S01–P–107
DEVELOPMENT AND APPLICATION OF PLANT TRANSFORMATION VECTOR FOR RESCUE CLONING

Hyoung-Seok Kim, Mi-Kyung Lee, Kwi-Mi Chung, Jung-A Yu, Young-Doo Park*
Dept. of Horticulture, KyungHee Univ., YongIn 449-701, Korea

In order to apply gene technology successfully to modern agriculture, it is essential to be able to understand and control transgene expression. Recently, instability of transgene expression is frequently observed in transgenic plants, and is recognized to serious problems in molecular breeding. Rescue cloning is effective technique for research of abnormal transgene expression pattern, and it can be applied to research of insertional mutagenesis as an effective approach for functional genomics. In this study, we developed a plant transformation vector for rescue cloning (RCV2), which contained a hygromycin resistence and a gus gene, and determined an effective restriction enzyme for rescue of flanking plant DNA, which contained both side borders of the T-DNA. For effective sequencing of T-DNA region and flanking plant DNA, we also designed the primer sets. Using these primer sets, we confirmed possibility of rescue cloning from transgenic plants, by developed RCV2 vector. Finally, we applied the rescue cloning vector, RCV2, to a plant. Tobacco (*Nicotiana tabaccum* cv. Havana SR1) was transformed with *Agrobacterium tumerfaciens* LBA4404 harboring the pRCV2 construct. All transformants were confirmed by PCR and genomic Southern analysis, and flanking plant DNA sequences were obtained by the rescue cloning technique in transgenic plants.

1340–1440
S01–P–108
GENETIC TRANSFORMATION OF JAPANESE LAWN GRASS (*ZOYSIA JAPONICA* STEUD.) BY *AGROBACTERIUM TUMEFACIENS*

S.M. Lutfor Rahman*[1], Masumi Ebina[2], Hitoshi Nakagawa[2], Wayne Mackay[1]
[1]Texas A&M Univ. TAES, 17360 Coit Road, Dallas, Texas 75252, USA; [2]Laboratory of Genetic Resources, Dept. of Plant Breeding, National Grassland Research Institute, 768 Senbonmatsu, Nishinasuno, Nasugun,Tochigi 329-2793, Japan

A protocol is presented for the effective transformation of Japanese lawngrass (*Zoysia japonica*) by *Agrobacterium tumefaciens*. Factors such as infection period, co-cultivation period, use of acetosyringone (AS, as a mediator of transformation of the GUS gene from *Agrobacterium*), different *Agrobacterium* strains and hygromycin concentrations, were assessed. Japanese lawngrass (*Zoysia japonica* Steud.) cv. YK-EM-2 was attempt to *Agrobacterium* mediated transformation. Of the five *A. tumefaciens* strains tested LBA 4404 (pIG121) gave the best results (60% callus were GUS positive) in 24-hour infections. Eight days of co-cultivation with *A. tumefaciens* strain LBA 4404 (pIG121Hm) was significantly more suitable for transformation than other strains. Besides, acetosyringone (AS) actively induced the transfer of T-DNA from *Agrobacterium* to plant. Inclusion of performed the best for cocultivation and AS 50 mg/L proved to be indispensable for successful transformation of *Zoysia*. It was also observed that hygromycin had the effect of increasing b-glucuronidase (GUS)-transient activity. The results demonstrate that *A. tumefaciens* strain LBA 4404 (pIG 121) is most efficient among five others strains used in this study for GUS transient assay of a range of commercially important *Zoysia* grass and that days of infection, co-cultivation, concentration of AS and Hygromycin can influence the efficiency of transformation. These results open the way for the utilization of transgenic plants in breeding *Zoysia* spp.

1340–1440
S01–P–109
GENETIC TRANSFORMATION OF 'FUJI' APPLE USING *AGROBACTERIUM TUMEFACIENS*

K.J. Song*[1], E.S. Seong[2], J.H. Hwang[2], Y.U. Shin[2] J.H. Kim[2]
[1]Faculty of Horticultural Life Scien., Cheju National Univ., 1 Ara 1-Dong, Cheju City, Cheju, Korea, 690-756; [2]National Horticultural Research Institute, RDA, Suwon 440-706, Korea

Transgenic plants of the commercially important apple cv. Fuji (*Malus* x *domestica* Borkh.) have been obtained by *Agrobacterium tumefaciens*-mediated transformation. Some factors affecting gene transfer and regeneration including acetosyringone (AS) concentration, cocultivation periods, cefotaxime concentration, carbon sources, and explant sources on the agar-solidifying selective medium of MS salts and LS vitamins supplemented with 5 $mg \cdot L^{-1}$ TDZ, 0.3 $mg \cdot L^{-1}$ IBA, and 100 $mg \cdot L^{-1}$ kanamycin were investigated to develop an efficient transformation protocols of 'Fuji' apple. The putative transformants were reselected through the response of rooting activity on the half strength of MS basal medium with 0.3 $mg \cdot L^{-1}$ IBA and 30 $mg \cdot L^{-1}$ kanamycin for rooting and confirmed by PCR and southern blot analysis. The treatments of 200 mM AS or cocultivation for 4 days improved the efficiency of genetic transformation, however other factors mainly affecting regeneration had little effect on improving the efficiency of transformation. The results indicated that gene transfer was pivotal step to establish the efficient Agrobacterium-mediated transformation of 'Fuji' apple.

1340–1440
S01–P–110
***AGROBACTERIUM*-MEDIATED PRODUCTION OF TRANSGENIC PLANTS OF *MUSCARI ARMENIACUM* LEICHTL. EX BAK.**

Sakae Suzuki*, Masaru Nakano
Faculty of Agriculture, Niigata Univ., 2-8050 Ikarashi, Niigata 950-2181, Japan

A system for the production of transgenic plants has been developed for the Liliaceous ornamental plant *Muscari armeniacum* Leichtl. ex Bak via *Agrobacterium*-mediated transformation of embryogenic calli. Leaf-derived embryogenic calli were co-cultivated with each of 3 *A. tumefaciens* strains, EHA101/pIG121Hm, LBA4404/pIG121Hm and LBA4404/pTOK233, all of which harbored the binary vector carrying the neomycin phosphotransferase II (nptII), hygromycin phosphotransferase (hpt) and intron-containing ß-glucuronidase (gus-intron) genes in the T-DNA region. Following three days of co-cultivation, the embryogenic calli were cultured on MS medium containing 10 mg/L NAA and 500 mg/L cefotaxime for 1 week, and then transferred to the same medium but supplemented with 75 mg/L hygromycin. After 4 to 5 weeks, several hygromycin-resistant (Hyg-r) cell clusters were produced from the co-cultivated embryogenic calli. The highest efficiency of production of Hyg-r cell clusters was obtained when embryogenic calli were inoculated with *A. tumefaciens* EHA101/pIG121Hm in the presence of 100 µM acetosyringone (AS) and 0.1% (v/v) of a surfactant (Tween20) followed by co-cultivation in the presence of 100 µM AS. Following transfer to half-strength MS medium containing 500 mg/L cefotaxime and 25 mg/L hygromycin, Hyg-r calli developed into complete plants via somatic embryogenesis. Most of the plants were verified to be transgenic by GUS histochemical assay and PCR analysis. Southern blot analysis revealed the integration of 1 to 5 copies of the transgene into the genome of transgenic plants, but most of them had 1 or 2 copies.

1340–1440
S01–P–111
IMPROVED TOLERANCE TO SALINITY AND HEAT TEMPERATURE IIN TRANSGENIC TOMATO OVEREXPRESSING ASCORBATE PEROXIDASE (APX)

Yueju Wang*[1], Michael Wisniewski[2], Richard Meilan[3], Leslie Fuchigami[1], Robert Webb[2]
[1]Horticulture Dept., ALS 4017 Oregon State Univ., Corvallis, OR 97331; [2]USDA-ARS 45 Wiltshire Rd. Kearneysville, WV 25430; [3]Forest Science Dept., Oregon State Univ., Corvallis, OR 97331

The potential role of cytosolic ascorbate peroxidase (APX) in the protection against salt stress and heat stress was examined using transgenic tomato plants Transformants were selected by using Kanamycin resistance and confirmed by PCR, Southern and Northern analysis. Several independently-transformed lines were obtained and evaluated for resistance to oxidative stresses. The total leaf APX activity in the transgenic was about several folds that of the control under non-stressed conditions. After exposure of shoots cold, heat and paraquat stress, these plants showed higher survival rate and lower electrolyte leakage than untransformed controls as evaluated by either visual scoring or electrolyte leakage measurement. The transgenic seeds could germinate at 9 °C in dark. The shoot cultures could grow in the medium containing 0.20 M NaCl. The transgenic shoots could develop roots after 5 days treatment at 40 °C. We also found that the fruit are resistant the UV-B light. The results show that the presence of transgenic APX had clear effects on tolerance to the oxidative stresses.

1340–1440

S01–P–112

CELL SUSPENSION CULTURE AND GENETIC TRANSFORMATION IN *DIMOCARPUS LONGAN* LOUR. AND *LITCHI CHINENSIS* SONN.

Dacheng Chen*[1], Qifa Zheng[1], GuiBing Hu[1], FeiFei Gao[1], Ziran Huang[2], Rongwei Liu, Ming Quan Xu

Dept. of Sericulture & Habiliments, South China Agricultural Univ., Guangzhou 510642 China; [2]Tissue Culture Research Section, Research Centre of Agriculture Sciences of Shenzhen, Shenzhen 518040, China

In this paper, the effect of plant growth regulators including IBA, 6-BA, GA3 and CM on cell suspension culture of litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longana* Lour.), the susceptibility of cell suspension lines to kanamycin and the genetic transformation through Agrobacterium tumefaciences with API or APBD genes respectively infected liquid-phase woundings of embryogenic tissues with carborundum were studied. The results showed that IBA, 6-BA, GA3 and CM significantly increased the yield of cell suspension lines, but GA3 resulted in fast growing and browning of cells. In suspension culture, cell suspension lines were more susceptible to kanamycin than that on solidified culture. More Kmr embryoids could be obtained by the way of liquid-phase wounding of embryogenic calli with carborundum. Assay by Polymerase Chain Reaction (PCR) indicated that mutiple genes can be integrated in genomic DNA.

1340–1440

S01–P–113

STUDIES ON THE APPLYING OF EMBRIO CULTURE IN BREEDING NEW HYBRIDS BY CROSSING SEEDLESS GRAPE CULTIVARS

Nevin Kebeli*, Ylmaz Boz, Cengiz Özer

Tekirdag Viticultural, Research Institute, P.O. Box 7, Tekirdag, Turkey, Turkey, 59100

This study was carried to search the possibilities of propagating plants from the ovules and embryos of seedless x seedless table grape crosses. Ovules of 3A/261 x Baris and15/A-61 x Baris crosses, 42 and 52 days after the application of male pollen were cultured on E20A and ER media. It had been observed that there was no difference between the periods of time in term of living embryo number for two crosses in E20A media. In ER media, only 3/A-261 x Baris ovules which had been taken 52 days after pollination gave higher living embryo numbers than 42 days. The germination rate of living embryos of 3/A-261 x Baris cross was least in the 42th day + ER treatment. These rates were 31.52% in ER media and 80.21% in E20A media for ovules of 15/A-61x Baris that had been cultured in the 52th day.The treatment of 52th day + E20A media gave the highest rate (21.77%) of seedlings through ovules of the 15/A-61 x Baris combination. This rate was lower in both of two periods and two medias for 3/A-261 x Baris ovules.

1340–1440

S01–P–114

IN VITRO APPROACH: TARGETING THE DEVELOPMENT OF DESIRED VARIETY

Tejaswini, Prakash*

Scientist, Div. of Ornamental Crops, IIHR, Hessaraghatta Lake Post, Bangalore 560089, Karnataka, India

Breeding of floricultural crops has a different approach compared to other crop plants. Creation of variability through in vitro mutagenesis and creation of polyploids for enhanced size and vigor through in vitro polyploidisation are the strategies adopted in the present study. As a model system for the present work Dianthus caryophyllus was selected. Suitability of genotype, explant, method of treating the explant with colchicine and mutagen (MES) were worked out to finalise the most appropriate logistics for recovering the desired genotype. For initial establishment of adventitious shootlets, axillary buds were taken from three well-established genotypes grown in polyhouse. Genotype having the tendency to develop natural bud sports was compared with that of partial and complete bud sports for their feasibility and easy adaptability for generating variations. Various explants such as node, leaf, whole shootlet, and also modified structures were evaluated for tendency to generate variability. Performance varied in response to the explant and genotype. Genotype that exhibited partial bud sport in flower color was found to be the most befitting for creating variation as indicated by the frequency of variants observed. Modified structures observed under in vitro condition were found to generate further variation in higher frequency compared to any other explant. Formation of callus, appearance of pigmentation, modification in the structure of leaves was found to be dependent on their genotypic background as well as the explant used. The result suggested usage of bud sports as the most ideal genotypes and in vitro modified structures as ideal explants to enhance the probability of recovering the desired genotype.

1340–1440

S01–P–115

PLANT REGENERATION AND TEST OF KANAMYCIN CONCENTRATION THROUGH THE LEAF EXPLANTS CULTURE IN CHRYSANTHEMUM

Dong Chil Choi*, Sang Young Seo, Jeong Man Kim, Joung Sik Choi, Yeong Geun Choi

Jeollabuk-do Agricultural Research and Extention Service Iksan 570-140, Korea

These experiments were carried out to determine the best regeneration conditions and to investigate the concentration of kanamycin for transformant selection in the genetic transformation of Chrysanthemum. The leaf explants (0.7 to 1.0 mm) of *Dendranthema grandiflorum* 'Puma' and 'Subangryeok' were placed on MS basal medium supplemented with different concentrations of various plant hormones, auxin (NAA, IAA, 2,4-D) and cytokinin (BA). The concentrations of kanamycin were 0, 10, 20, 40, and 50 mg·L^{-1} in the plant regeneration medium. The results are as follows: 1) The highest number of plantlets was obtained from the leaf discs in the MS basal medium supplemented with 1.0 mg·L^{-1} BA, 0.3 mg·L^{-1} 2,4-D for 'Puma', and 2.0 mg·L^{-1} NAA, 0.5 mg·L^{-1} BA for 'Subangryeok'; 2) The rate of plantlets regeneration was 71.9% for 'Puma' and 73.3% for 'Subangryeok'; 3) The frequency of callus formation was 21.9% for 'Puma' and 26.7% for 'Subangryeok'; 4) Culture leaf discs of 'Puma' and 'Subangryeok' in the MS basal medium for 2 weeks both turned brown and plant regeneration did not occur; 5) In the high concentration of kanamycin (40 to 50 mg·L^{-1}), explants became chlorotic after 10 days and no shoots regenerated; 6) In the low concentration of kanamycin (10 to 20 mg·L^{-1}), calli were formed in the edge of explant in the early stage of cultivation but explants were discolored and plant regeneration did not occur; 7) The suitable concentration of kanamycin for selection of transformants are 20 mg·L^{-1} for 'Puma' and 50 mg·L^{-1} for 'Subangryeok'.

1340–1440

S01–P–116

PURE LINE VARIETIES IN SWEDE DERIVED FROM MICROSPORE-DERIVED EMBRYOS

Even Bratberg, Magnor Hansen

Agricultural Univ. of Norway, P.O. Box 5022, N-1432 @s Norway

Swede is an important vegetable in Norway, and production has been based up to now on open pollinated varieties. The existing swede varieties are therefore non-uniform, but because swede lacks a self-incompatibility system, it is not possible to produce F_1-hybrids. The idea of this work was to produce new varieties of swede that are self-pollinated (pure lines) based on microspore-derived homozygous lines. Most swede varieties are very responsive to microspore-culture (Hansen and Svinnset, 1993). In 1997, 350 homozygous lines were produced from the Norwegian main variety 'Vige'. After self-pollination, field experiments were conducted with 38 homozygous lines in 1998, from which 8 lines were selected as interesting for further testing. The eight selected lines were self-pollinated in winter 1998/1999, then field experiments were carried out at several locations during 1999. Based on the results of these trials, three lines were selected for official approval. Results from the field research in 2001 confirm the assumption that the two selected homozygous lines are greatly improved in many characters compared from the variety 'Vige'.

1340–1440

S01–P–117

INDUCTION OF HAPLOID CALLI WITH MORPHOGENIC CHARACTERISTICS FROM IN VITRO CULTURED ANTHERS OF *PRUNUS ARMENIACA* CV.HARCOT

Augusto Vieira Peixe*[1], Jo Barroso[1], Maria Salomè Pais Mota[2], Maria Amely Potes[1]

[1]Universidade de …vora, Dep. De Fitotecnia Ap.94 7001-554 …vora Portugal; [2]Faculdade de CiÍncias de Lisboa, ICAT-LaboratÛrio de Biotecnologia de Plantas. Ed., ICAT–Campo Grande 1700 Lisboa Portugal

In vitro induction of anthers from apricot (*Prunus armeniaca*) cv.Harcot, was tested for the production of haploid plants. Although a high number of cultured anthers (around 20000, for the 4-years period of the trials) were used and different factors influencing the androgenic response were evaluated, only haploid calli forming nodular structures with morphogenic characteristics were obtained. The best androgenic response was achieved using the basal medium and vitamin complex of Nitsch and Nitsch (1969), supplemented with 1mgl-1 of 2,4-D, 1 $mg \cdot L^{-1}$ of zeatin, 0.5 $mg \cdot L^{-1}$ of IAA and 4% of sucrose. The medium was solidified with 0.7 $g \cdot L^{-1}$ of Difco Bacto-agar and the pH was adjusted to 5.8 before autoclaving. The induction depended on the maintenance of anthers in the dark for 8 days, at 28 °C, followed by transfer to a 16-hour photoperiod, with 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light intensity and 24/22 °C day/night temperature. The best androgenic response was achieved from anthers containing microspores at the uninucleate stage. A correlation between the androgenic response and the dimensions of the floral bud, its phenologic stage and the microsporogenesic stage was established. The ploidy level of the calli, evaluated by flow cytometry, revealed that they ranged from haploid to octoploid. Histological studies showed that the calli obtained had their origin in the microspores. Microscopic observations, under ultraviolet light, allowed the identification, in certain calli, of nodular structures surrounded by an autofluorescent layer. This autofluorescence could be related to cutin deposition around the morphogenic competent cells, as reported by other researchers (Pedroso and Pais, 1997; Baptista et al., 2000).

1440–1520

S01–O–118

TARGETING OF TRANSGENE EXPRESSION IN APPLE TREE TISSUES–ROLES FOR HETEROLOGOUS AND HOMOLOGOUS PROMOTERS

David J. James*[1], John R. Gittins[2], Till K. Pellny[3], Stefano Biricolti[4], Andrew J. Passey[1]

[1]Plant Breeding & Biotechnology Dept., Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ UK; [2]Laboratory of Plant Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland; [3]Institute Arable Crops Research, Rothamsted, UK; [4]Dipartimento di Ortoflorofrutticoltura, Universitá degli Studi di Firenze, 50144 Firenze, Italy

It is desirable that the expression of transgenes in genetically improved crops is restricted to the tissues requiring the encoded activity. Using published protocols for genetic transformation, we have used qualitative (histochemical) and quantitative (biochemical) methods to examine the expression patterns of several heterologous promoters driving expression of the beta-glucuronidase (gusA) marker gene in different tissues of transgenic apple trees (*Malus pumila*, Mill.). Light-regulation and patterns of expression are recorded in various vegetative tissues of the tree for all six promoters. Of specific interest was the recording of higher transgene expression levels in vascular tissues when driven by the extA and PsMTA promoters than by the constitutive CaMV35S promoter. We have an interest in fruit specific promoters for the control of genes involved in ethylene biosynthesis. The ethylene inducible promoters beta-galactosidase and ACC synthase have been cloned from an apple genomic library using cDNA sequences as probes and sequenced to patent quality. The promoters have been ligated to a reporter and transgenic apple fruit produced. These have shown that gene expression is tightly controlled by the onset of ethylene production in the cortex of the fruit by the beta-galactosidase promoter (ABG1). Ongoing work is currently looking at gene expression driven by the ACC synthase promoter.

1520–1540

S01–O–119

IMPROVED TOLERANCE TO ENVIRONMENTALLY-INDUCED OXIDATIVE STRESSES IN TRANSGENIC TOMATO OVEREXPRESSING ASCORBATE PEROXIDASE (APX)

Yueju Wang*[1], Michael Wisniewski[2], Richard Meilan[3], Robert Webb[2], Leslie Fuchigami[1]

[1]Horticulture Dept., ALS, 4017, Oregon State Univ., Corvallis OR 97331; [2]USDA-ARS 45 Wiltshire Rd. Kearneysville, WV 25430; [3]Forest Science Dept., Oregon State Univ., Corvallis OR 97331

The effect of overexpressing a cytosolic ascorbate peroxidase (APX) gene derived from pea (*Pisum sativum*) in transgenic tomato (*Lycopersicon esculentum*) plants on oxidative stress resistance was studied. Transformants were selected using kanamycin resistance and confirmed by PCR, Southern and Northern analysis. Several independently-transformed lines were obtained and evaluated for resistance to oxidative stresses resulting from exposure to salinity and high-temperature. APX enzyme activity in leaves of the transgenic plants was several fold greater than that of the wild-type (WT) plants under non-stressed conditions. Several lines of transformants were more resistant to heat, salt, cold, and paraquat than the non-transformants. Additionally, seeds obtained from the transformed plants germinated at 9 °C in the dark in contrast to seeds from the WT plants where no germination was observed. Shoots of transformed lines grew in media containing 0.20 M NaCl and produced roots at 40 °C, whereas the WT plants grew poorly in the salt medium and produced no roots at 40 °C. A full quantitative assessment of the resistance of the transformed lines of tomato to oxidative stress is in progress.

1540–1600

S01–O–120

PROTOPLAST ISOLATION AND SOMATIC HYBRIDIZATION BETWEEN EGYPTIAN AND CHINESE GARLIC

Abdel-Hamid A. Ali*

Genetic Dept., Faculty of Agriculture, Tanta Univ., Kafr El-Sheikh, Egypt

The study was conducted to describe protoplast isolation and fusion and subsequent plant regeneration in garlic (*Allium sativum* L.). Protoplast were enzymatically isolated from the youngest etiolated leaves of both garlic cultivars (Egyptian and Chinese cultivars), using an enzyme solution (2% cellulase 'Onozuka' RS, 0.5% Driselase, 0.1% Pectolyase Y23, 0.6 M mannitol and 10 mM $CaCl_2$). Two mL of each purified protoplast cultivar suspension were mixed together. The mixture was mixed again in petri dishes with an equal volume of agarose culture medium containing B5 medium supplemented with 1 mg/L NAA, 1 mg/BAP, 0.5 M mannitol, 0.1% casin hydrolysate and 1.2% w/v agarose low melting point. The microcalli that formed after about five weeks were transferred to solid B5 medium. For plant regeneration, the derived microcalli from fused protoplasts were transferred to MS salts and B5 vitamins medium. Several organized compact green structure, appeared after about four weeks. Regeneration of complete plantlets with shoot sand roots took 4-6 weeks. Cytological analysis for the root tips of regenerants showed different levels of ploidy. Electrophoretic analysis confirmed that fusing both types of protoplasts produces some regenerants.

1600–1620

S01–O–121

THE BREAK-THROUGH IN THE REDUCTION OF JUVENILE PHASE IN APPLE USING TRANSGENIC APPROACHES

N. Kotoda*, M. Wada, T. Masuda, J. Soejima

Dept. of Apple Research, National Institute of Fruit Tree Science, National Agricultural Research Organization, Shimo-kuriyagawa, Morioka 020-0123, Japan

In contrast to herbaceous plants, fruit trees such as apple [*Malus* x *domestica* (Borkh.)] flower and set fruit only after an extended juvenile phase that lasts several years. In the course of studying juvenility in apple, we have cloned the MdTFL gene homologous to Arabidopsis TERMINAL FLOWER1 (TFL1) gene, which suppresses floral meristem identity genes, LEAFY (LFY) and APETALA1 (AP1). The expression of MdTFL (Malus x domestica TFL1) mRNA was strong in sepals and mature leaves, and highest in early July, about two weeks before flower induction, in apical buds in apple. The vector carrying MdTFL cDNA (pSMDTFL12.1.2+) was introduced into wild type Arabidopsis plants (Columbia). Thirty four independent transgenic plants that survived on kanamycin were identified. Eight of thirty four primary transformants (T1 generation) flowered later than wild-type plants and showed the similar phenotype to that of transgenic Arabidopsis overexpressing TFL1. These results suggest that MdTFL functions like TFL1 and it is an apple ortholog of TFL1. On the other hand, transgenic apples expressing antisense MdTFL gene flowered at about eight to fifteen months after grafting onto rootstocks. Non-transformed control plants have

not flowered in five years. The flower organs of the transgenic apples were normal and fertile. Based on these results, we showed that the suppression of the expression of MdTFL gene reduces juvenile phase and can induce precocious flowers in apple.

1620–1640
S01–O–122
TRANSFORMATION OF WATERMELON VIA COPPER INDUCIBLE CONSTRUCTS OF GUS AND ISOPENTENYL TRANSFERASE (IPT)

Fahrettin Goktepe*, Harrison Hughes

Dept. of Horticulture and Landscape Architecture, Colorado State Univ., Fort Collins, Colorado, USA, 80523

The constructs containing an isopentenyl transferase (ipt) gene with a copper inducible promoter (pMJM100), and a GUS gene with a copper inducible promoter (pPMB758) in *E. coli* were received from Dr. Marianne McKenzie. They were then transferred into *Agrobacterium tumefaciens* (LBA 4404) for use in transformation of watermelon. Specifically designed PCR primers were used to confirm that the modified *Agrobacterium tumefaciens* cells contained the genes of interest. Seeds, minus their seed coats, were surface sterilized with 1.0% sodium hypochlorite plus a surfactant and soaked in sterile water for 2 days at room temperature (25 °C). These seeds were then placed on sterile filter paper to remove excess water and germinated on Murashige and Skoog's basal medium (MS) under 16/8 light/dark at 25 +/– 2 °C. The transformation procedure used cotyledons of watermelon cultivar Sugar Baby as the explant. The cotyledons were cut in half (proximal and distal) and punctured 5-7 times with a sterile needle prior to preculture on solid MS media containing 1 mg/L BA for 5 days. The *Agrobacterium* was grown in liquid LB+ Kan50 at 30 °C until it reached absorbance of between 0.1 and 0.2 at 600 nm. The precultured explants were cocultured with *Agrobacterium* in liquid MS medium with 1 mg/L BA for 2 days and then transferred to solid MS medium with 1 mg/L BA, 250 mg/L carbanacilin, and 100 mg/L of kanamycin for two subcultures of 2 weeks each. Young leaves from regenerated shoots were used with specifically designed PCR to test for presense of the genes. Those testing positive for the genes were selected as transformed plants and grown to maturity. They were subsequently tested for ipt gene transcription and translation or GUS activity by after treating with copper.

1640–1700
S01–O–123
DNA METHYLATION AND SOMACLONAL VARIATION IN HIGHER PLANTS: OIL PALM AS A CASE STUDY

Estelle Jaligot, Thierry Beule, James Tregear, Jean-Luc Fox Verdeil, Alain Rival*

CIRAD-CP/IRD GeneTrop Lab., Centre IRD BP 5045, F-34032 Montpellier Cedex 01, France

In the aim of understanding molecular phenomena underlying the "mantled" somaclonal variation in oil palm, our research work has focused on the role of genomic DNA methylation. A highly significative DNA hypomethylation exists in abnormal plant material. Through a wide range of techniques, methylation studies are focussed on the only genes which are relevant with regard to the "mantled" phenotype. By studying chromatin structure and DNA-methyltransferases, we will determine whether DNA methylation is the only epigenetic regulator affected, and which regions of the genome are specifically modified in variants. By defining candidate type-specific methylation markers, and by identifying their functions, we intend to point at a few common regulation pathways. 1. Region-specific methylation studies. The isolation of the most "opened" fraction of the chromatin allow the quantification of methylation variations for the active part of the genome. Dysfunctions in the DNA-methyltransferase gene have been involved in the occurrence of genome-wide hypomethylation associated with modifications of gene-specific methylation patterns, resulting in developmental abnormalities. We have thus undertaken the isolation of an oil palm orthologue of the major DNA-methyltransferase gene (METI) through the use of both a library screening and a PCR-based method. 2. Sequence-specific methylation studies. Oil palm cDNAs were isolated and used in methylation-sensitive, restriction fragment length polymorphism (RFLP) studies involving the MspI/HpaII isoschizomeric enzymes. The Methylation-Specific Amplification Polymorphism (MSAP) approach allowed us to generate type-differential markers on the basis of their methylation polymorphism. These markers will be used as Southern blot probes and methylation patterns for their sequence will also be established by the bisulfite sequencing method.

Friday · August 16

0800–0900
S01–P–124
GENETIC IMPROVEMENT OF ROSE CULTIVARS FOR DISEASE RESISTANCE

J.P. Bolar*[1], N. Schneiter[1], F.D. Smith[1], J. Castillon[2], K. Kamo[3], D.M. Gadoury

[1]The Scotts Company, 14111 Scottslawn Road, Marysville, OH 43041; [2]USDA-ARS, U.S. National Arboretum Floral and Nursery Plant Research Unit, Beltsville, MD 20705; [3]Dept. of Plant Pathology, Cornell Univ., Geneva, NY 14456

Black spot, caused by the fungus *Diplocarpon rosae* Wolf, is a destructive disease of field-grown roses worldwide. Most popular rose cultivars are highly susceptible, and therefore control requires multiple fungicide applications. Our objective was to test the magnitude of resistance conferred by certain transgenes in the rootstock cultivar Dr. Huey. Evaluated transgenes included those encoding chitinolytic enzymes from the biocontrol fungus Trichoderma harzianum, a gene encoding the antifungal protein from Alfalfa, and synthetic genes encoding for antimicrobial peptides. We developed an efficient regeneration and microprojectile-mediated transformation system for several commercially important hybrid tea rose cultivars. So far, 55 distinct lines (cv. Dr. Huey) have been transformed as confirmed by serological and molecular assays. Several of these lines have been vegetatively propagated, increased, and established in the greenhouse. We are presently evaluating them for resistance to black spot.

0800–0900
S01–P–125
CLONING THE CP GENE OF WMV-2 AND INTRODUCING IT INTO WATERMELON

Xue Xian Peng[1], Xue Sen Huang*[2], Jun Jun Liu[1], Li Wan[3], Xin Fu[2]

[1]Beijing. The Chinese Academy of Agricultural Science, China; [2]Seed Company, No. 299, Dong Street, Hedong, Yun Chen, Shan Xi Province, China; [3]Heng Zhou Institute of Fruit Trees, Chinese Academy of Agricultural Science, China

The CP genes from watermelon mosaic virus-2 in Chinese Watermelon were PCR amplified and in the sense orientation into a plant expression vector PBI121, then into *Agrobacterium tumefaciens*. Cotyledonars of watermelon were inoculated for 10 min with *Agrobacterium tumefaciens*. The cotyledonars were cultured on MS+100 mg/L kanamycin + 5 00 mg/L carbenicillin+ 5 mg/L 6-benzyladenine. Adventions shoots formed on the explants after 4 weeks of culture. After Southern Blot testing, the CP genes were expressed in F_1, F_2 and F_3. The plants were resistant to WMV-2 in the greenhouse.

0800–0900
S01–P–126
TISSUE CULTURE OF AMERICAN GINSENG AND GENETIC ENGINEERING TO EXPRESS ANTIFUNGAL PROTEINS

Z.K. Punja*, W.P. Chen

Dept. of Biological Sciences, Simon Fraser Univ., 8888 Univ. Drive, Burnaby, British Columbia, Canada, V5A 1S6

Transformation of American ginseng (*Panax quinquefolius* L.) with Agrobacterium strain LBA 4404 containing either a rice chitinase gene or a thaumatin-like protein gene under control of the maize ubiquitin1 promoter was achieved. The phosphinothricin acetyltransferase (bar) and hygromycin phosphotransferase (hpt) genes were used as selectable markers. Epicotyl explants from 2 to 3-week-old ginseng seedlings were pre-cultured for 5-7 days on MS medium with NAA and 2,4-D at 10 μM and 9 μM, respectively (ND medium), prior to Agrobacterium infection. The explants were either immersed in a bacterial suspension for 20 min or received a 10 or 15 microl droplet of bacteria. Following this, a co-culture period of 3 or 4 days was provided on ND medium. Selection was conducted using 20 mg/L phosphinothricin or 100 mg/L hygromycin over a 10-month period. A callusing frequency of 24-27% was achieved on ND medium when explants were infected by the droplet method. Immersion of explants reduced the callusing frequency to 9.3%. Almost 90% of 32 lines that survived selection were shown to be transformed by Southern

hybridization and polymerase chain reaction (PCR) analysis. The expression of the chitinase gene and the TLP gene was demonstrated by reverse transcription PCR and Western analysis. One hundred and two ginseng plantlets were recovered from somatic embryos of 11 confirmed transgenic lines. The transgene integration in plantlets of two lines was demonstrated by Southern analysis. This is the first report of *Agrobacterium*-mediated transformation of this important medicinal plant. This technology has the potential to enhance tolerance to fungal diseases in this crop.

0800–0900

S01–P–127

IN VITRO BIOASSAY OF THE INSECTICIDAL ACTIVITY OF A CRY1A(C) TRANSGENE IN CALLUS DERIVED FROM A REGENERATION-RECALCITRANT CROP PLANT

Benjamin Steinitz*, Yedidya Gafni, Yael Cohen, Yona Tabib, Shlomit Levski, Amos Navon

Dept. of Plant Genetics, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

Crop protection against Lepidopterous pests by the introduction of *Bacillus thuringiensis* (Bt) toxin genes into plants is one of the conspicuous achievements of genetic engineering. This form of plant protection is applicable only in hosts with plant regeneration ability from transgenic cells. However, the generation of transgenic callus is possible in many crops which so far lack in vitro plant regeneration ability, thus the production of callus carrying an insect toxin gene is practicable, and an insect-transgenic callus feeding bioassay should be feasible even in plant regeneration-recalcitrant types. The present study was carried out to confirm this premise with callus of an Israeli commercial cotton (*Gossypium hirsutum* L.) variety that has no plant regeneration ability with the currently available protocols. The growth and survival of *Helicoverpa armigera*, *Pectinophora gossypiella* and *Spodoptera littoralis* was examined with aseptic neonate larvae reared on non-transgenic or transgenic callus harboring the Bt cry1A(c) toxin gene and the hygromycin B resistance (hpt) gene. Our results reflect a distinct cry1A(c) expression and a very high level of biological activity of the Bt toxin against major cotton pests. The experimental system utilized here may be advantageous also in instances where a plant regeneration protocol exists but the transgenic insecticidal activity is critical at a late developmental stage attained years after plant regeneration, for example in flowers or fruits of woody species. In such cases, the in vitro insect-transgenic callus assay provides a timesaving means to collect preliminary information long before the critical stage of host maturity has been established. In vitro co-culture assay of a transgenic callus with its pest can also circumvent the early release of transgenic plants into uncontrolled environments before initial information essential for further decision making has been gained.

0800–0900

S01–P–128

TRANSFORMATION OF WATERMELON STOCK WITH CP (COAT PROTEIN) GENE OF CGMMV

Bo Young Jeon*[1], Min Jung[1], Ki Hyun Ryu[2], Yoon Sup Shin[1], Byung Whan Min[1], Chee Hark Harn, Seung Gyun Yang*

[1]Biotechnology Center, 537-17, Jeongdan-ri, Ganam-myun, Yeoju-kun, Kyonggi-do, Korea; [2]Dept. of Horticultural Science, Seoul Woman's Univ., Seoul, Korea

For last several years in Korea, Cucurbitaceous crops including watermelon, cucumber, squash and melon have been seriously infected by CGMMV (cucumber green mottle mosaic virus). In particular, the infection has caused a great loss in total yield of the watermelon nationwide. Since CGMMV is easily transmitted by foliage contact, through seed and especially from soil contamination, an effective way to control the outbreak should be developed. The virus-resistant germplasm is not easily available by traditional breeding and if any, it requires a long-term breeding to obtain an elite resistant line. Alternative is to use viral genes such as the coat protein to be expressed ectopically inducing RNA-mediated resistance against virus infection. Watermelon stock, popularly used for grafting the commercially important watermelon varieties to avoid the virus infection from soil, is the best candidate to soften the controversial issues regarding GMO food, as the stock is only transformed and the watermelon is not. In order to develop a CGMMV-resistant watermelon stock, cotyledons of the watermelon stock (Twinser) were inoculated with Agrobacterium strain LBA4404 harboring CGMMV CP gene and cultured with kanamycin selection (500 mg/L). We have obtained stable transformation with 0.3% transformation efficiency and this is the first report of the successful transformation of the watermelon stock. Currently, we are testing the transgenic stocks to see if these are resistant against CGMMV infection.

0800–0900

S01–P–129

TRANSFORMATION OF AFRICAN VIOLET (*SAINPAULIA IONANTHA*) WITH GLUCANASE-CHITINASE GENES USING *AGROBACTERIUM TUMEFACIENS*

Narendra Ram M.S.*, Sukhada Mohandas

Dept. of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560 089 India

The African violet (*Saintpaulia ionantha* H. Wendland) is one of the most important ornamental plants used in indoor decoration, gardening and landscaping. These plants are attacked by *Fusarium oxysporum*, *Phytophthora* sp., and *Pythium ultimum,* which cause Crown Rot and by *Botrytis* sp. which causes *Botrytis* Blight. The present work was envisaged to incorporate the genes producing chitinase and glucanase which impart resistance to these diseases. Transgenic African violets were produced via *Agrobacterium tumefaciens* mediated transformation. Regeneration protocols were standardised through multiple shoot bud production from leaf and petiole explants using BAP and NAA as growth regulators. BAP at 2.5 $mg \cdot L^{-1}$ and NAA at 1 $mg \cdot L^{-1}$ gave the highest number of shoot buds (40) from leaf explants. With petiole explants, BAP at 0.5 $mg \cdot L^{-1}$ and NAA at 0.1 $mg \cdot L^{-1}$ gave the highest number of shoot buds (22). Rooting of these shoots was found to be maximum with NAA at 2 $mg \cdot L^{-1}$. Leaf explants were inoculated with the strain LBA4404 of *Agrobacterium tumefaciens* harboring the binary plasmid pBINAR carrying glucanase-chitinase genes and the nptII selectable marker. Regenerants obtained on the selection media containing kanamycin (70 $mg \cdot L^{-1}$) and cefotaxime (800 $mg \cdot L^{-1}$) were excised and rooted on media containing NAA. Integeration of the transgenes in the plant genome was confirmed by PCR analysis and Southern hybridzation. Mean glucanase activity in the transgenic plants was 44220 $\mu m \cdot L^{-1}$ while that in control plants was 27060 $\mu mg \cdot L^{-1}$. The crude protein extracts of transformed plants showed zones of inhibition when tested on *Fusarium oxysporum* and *Pythium* while control plants did not show any such inhibition antifungal activity.

0800–0900

S01–P–130

PHOSPHINOTHRICIN AND CROWN GALL RESISTANT TRANSGENIC PLANTS OF GRAPEVINE

Myroslava Rubtsova, Boris Levenko*

Institute of Plant Physiology and Genetics, Ukranian Acad.Sci. 31/17 Vasylkivska st. 03022 Kiev, Ukraine

A method for direct shoot regeneration from grapevine leaves was developed. Using this method transgenic plants cvs. Cabernet Sauvignon, Podarok Magaracha, Rubinobyj Magaracha, Krona 42 resistant to herbicide phosphinothricin (Basta) were developed. Leaves of in vitro growing plants were inoculated by *Agrobacterium tumefaciens* strain carrying the bar gene of herbicide phosphinithricin resistance. Herbicide was used as an addition to the culture medium. Plant regeneration occurred on medium with the addition of up to 20 mkl/L herbicide. The transgenic nature of herbicide resistant plants was confirmed by PCR and Southern methods. Herbicide resistant shoots were rooted and microclonally propagated. After their transfer to soil, they had no signs of inhibition after up to 10 mL/L herbicide usage, while leaves of control plants became yellowish and died after 3 mL/L herbicide implementation. In experiments where together with a plasmid carrying the bar gene a plasmid RSF 1010 carrying an antitumorigenic determinant was used, some herbicide resistant plants were inoculated under in vitro conditions by tumorigenic agrobacteria strains. Plants that did not develop crown galls were microclonally propagated and studied for their resistance to crown gall disease in vivo in the Institute of Microbiology and Virology of National Academy of Sciences of Ukraine. Two of three tested plants did not develop crown galls after agrobacteria inoculations. These plants are now propagating to study their resistance to crown gall disease under field conditions.

0800–0900
S01–P–131
EXPRESSION OF RIBOSOME-INACTIVATING PROTEIN GENE IN TRANSGENIC HOT PEPPER (*CAPSICUM ANNUUM* L.) AND THEIR PROGENY

Do-Sun Kim*, Ki-Taek Kim, Dae-Geun Oh, Il-Gin Mok

National Horticultural Research Institute, Rural Development Administration, Suwon 441-440, Republic of Korea

Plant regeneration from explants and protoplasts is feasible which has led to the development of efficient transformation procedures. Cotyledon explants of hot pepper is an excellent source for the initiation of in vitro culture, and shoot regeneration. Although a few number of studies on transformation of hot pepper from hypocotyl and cotyledon segments were reported, transformation of hot pepper was not established well. In this study, we introduced the RIP gene (for disease-resistance) by Agrobacterium-mediated transformation and analyzed expression of the introduced foreign genes. Cotyledon explants of seedlings (cv. 'Guemtap') were co-cultivated with *Agrobacterium tumefaciences* LBA4404 which contained the binary vector plasmid pGA643 harboring the RIP gene. After a preculture period of 3 days, explants co-cultivated with bacteria for 5 days showed the most efficient transformation frequency. For selecting transformed plantlets, the effective kanamycin concentration for shooting and rooting media were 100 and 50 mg/L, respectively. Putative transformed shoots were obtained and developed into morphologically normal plantlets. Transformation and stable insertion of foreign genes was confirmed by the polymerase chain reaction (PCR) and Southern blot analysis. Seeds of transgenic plants were obtained by self-pollination. To analyze expression of RIP gene and select disease resistant plants, each generation of transgenic plants were inoculated with Phytophthora capsici. The plants that exhibited a resistant reaction to *Phytophthora capsici* were selected and advanced from the T1 to T4 generation. The T4 generation of selected plants showed little segregation of expression of foreign genes and stable resistance to *Phytophthora capsici.*

0800–0900
S01–P–132
BIOTECHNOLOGICAL APPROACHES TO IMPROVE HORTICULTURAL CROP PRODUCTION IN KENYA

Daniel Chebet*[1], James Okeno[1], Patrick Mathenge[2]

[1]Dept. of Horticulture, Moi Univ., P.O. Box 1125 Eldoret, Kenya; [2]Dept. of Crop Production and Seed Technology, Moi Univ.

Horticultural production in Kenya, despite being a leading foreign exchange earner, is constrained by shortage of arable land, poor moisture availability, declining soil fertility, limited access and high cost of farm inputs, limited modern technology to farmers and pest and disease severity. Subsequently, these have often led to an increase in poverty and malnutrition, unemployment and environmental degradation. In the past biotechnology approach has been used to improve horticultural production in Kenya. The approach undertaken include the use of tissue culture for rapid propagation and disease elimination in bananas, citrus, strawberries, macadamia nuts, papaya, potatoes and ornamentals; identification and use of molecular markers and diagnostics in bananas and evolution and promotion of bio-pesticides and bio-fertilizers. However abiotic and biotic stresses continue to limit horticultural production in Kenya. Recent development of gene technology offers a new solution to these problems. Already transgenic sweet potato has been developed in Kenya, which offers resistance against feathery mottle virus. Biotechnology activities are carried out at national universities, research institutions, international organizations and the private sector. However, more rapid biotechnological advancements in Kenya are constrained by infrastructural, human resource, equipment and financial limitations.

0900–1000
S01–O–133
PLANT GENETIC ENGINEERING IS A FACT OF LIFE, IS AN EFFICIENT AND NECESSARY BREEDING TECHNIQUE, AND IS A CHALLENGE THAT WILL BE ACCEPTED BY THE PUBLIC

Arie Altman

The Hebrew Univ. of Jerusalem The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture P.O. Box 12 Rehovot 76-100 Israel

The global area of transgenic crops reached about 125 million acres towards the end of 2001, representing a 10% increase over the year 2000 and a 30-fold increase since 1996. This is while the future of commercializing GM plants seemed doomed just two years ago, as the public concerns was approaching panic, especially in Europe. The practical use of GM crops and R&D activities is likely to increase in the years to come because it is an efficient and practical breeding technique, it enables more sustainable and improved production of quality food, and is more environmental friendly. Most important, we are more aware of the potential risks and are able to take all precautionary steps. Plant biotechnology has changed the horticultural scene in three major areas: Growth and development control (vegetative, generative and propagation); Protecting plants against the increasing threats of biotic stress; Producing specialty foods, biochemicals and pharmaceuticals. The major challenges ahead include: Alleviating the hazards of abiotic stress, foremost salinity, drought and extreme temperatures; Maintenance and improvement of the environment–both large open spaces and unique ecological niches–foremost forests, grasslands and savannas; Improvement of food quality and design of "specialty food", using biochemical engineering. This includes a shift from the production of low-priced food and bulk commodities to high-priced, special plant-derived products. Biotechnology cannot solve all problems, however, it is a most powerful technique that can and will be integrated in all classical breeding programs of most horticultural plants. At the end of the day, the only criterion to evaluate the efficiency of genetic engineering will be its cost effectiveness.

1000–1020
S01–O–134
GENE EXPRESSION OF CRY 1AC AND GUS IN CABBAGE AND CAULIFLOWER

Huaiyu Wang[1], Etsuko Tsang[2], Jamie McNeil[1], Brian Miki[3], Daniel Brown*[1]

[1]Agriculture and Agri-Food Canada Southern Crop Protection and Food Research Centre 1391 Sandford Street, London, Ontario Canada N5V 4T3; [2]Univ. of Guelph Dept. of Plant Agriculture Guelph, Ontario Canada N1G2W1; [3]Agriculture and Agri-Food Canada Eastern Cereal and Oilseed Research Centre Ottawa, Ontario Canada K1A 0C6

Studies on the expression of a GUS reporter gene and a cry1Ac gene under the control of the Agriculture and Agri-Food Canada (AAFC) proprietary tCUP gene expression system have been carried out in vegetable crucifers. The tCUP gene expression system is comprised of a cryptic gene activation sequence isolated from tobacco by TDNA tagging (Foster et al. Plant Mol. Biol., 41:45-55, 1999; Wu et al. Mol. Genet. Genomics, 265: 763-770, 2001). The tCUP system has been optimized for expression in cauliflower transient and stable expression systems. This optimization and concomitant development of effective transformation protocols, allowed a cry1Ac insect resistance gene to be introduced into an inbred line of cabbage and a hybrid line of cauliflower. Here we extend the observations by evaluating the expression of a GUS reporter gene and a cry1Ac insecticidal protein gene in different parts of transgenic cauliflower and cabbage, and the expression level of the transgenes during cauliflower development from in vitro to senescence. A high level of expression was observed in all plant parts, especially in leaf tissue of vegetable crucifers and this high level of expression was consistent through out plant development to senescence. The results, using ELISA, showed that the cry1Ac gene, directed by the tCUP gene expression system, produced a high level of CRY protein. Insect bioassays of the transgenic cabbage and cauliflower lines showed no significant leaf damage after exposure to the larvae and 100% mortality of three target insect larvae (Imported cabbage worm, Diamond back moth and Cabbage looper). The results confirm the utility of the tCUP gene expression system for heterologous gene expression and the effectiveness of the use of Bt genes for Lepidopteron pest control in vegetable crucifers such as cabbage and cauliflower.

1020–1040
S01–O–134–A
TO BE ANNOUNCED

1400–1440
S01–O–135
THE CONGESTED IP LANDSCAPE AND ITS EFFECT ON THE USE OF BIOTECHNOLOGY FOR THE DEVELOPMENT OF HORTICULTURAL AND SUBSISTENCE CROPS

D.P. Delmer

Associate Director, Food Security The Rockefeller Foundation 420 Fifth Ave. New York, NY 10018-2702

In addition to concerns about public acceptance and the high costs of regulatory approval, the ability to use biotechnology as a tool for the improvement of minor commercial crops and also for subsistence crops important for the developing world is seriously impaired by difficulties in securing licensing agreements for use of the required technologies. Although much of the intellectual property (IP) needed was initially generated by public sector institutions, exclusive licensing agreements to major companies in the private sector have had a serious impact on freedom to operate. In spite of this, there are some notable cases where biotechnological approaches are being successfully applied, and this lecture will provide some examples of these and indicate ways in which agreements to allow these crops to move into the field and market place have been negotiated. Nevertheless, access to the appropriate technologies remains a continuing problem, and the Rockefeller and McKnight Foundations have initiated a dialogue to explore the possibility of forming a consortium for collective management of public-sector IP. The consortium would establish a clearinghouse to provide information about best practices for IP management, provide databases for IP, analyze emerging trends in agricultural biotechnology, and assess future needs in this area. Also being considered is the pooling of IP to create "tool-kits" that may be collectively licensed for use in improvement of specific traits or specific crops. Another initiative concerns the creation of an entity to be called the African Agricultural Technology Foundation (AATF)—an African-based and African-led initiative. It would receive licenses for new crop technologies from corporations and other research organizations. Then, while assuring proper stewardship of the technologies, the AATF would sub-license their use in specific projects by national and international research organizations in Africa. So far, the five major crop biotech corporations and the USDA have agreed to share their technologies with Africa through the AATF. All of these initiatives are based upon the belief that such partnerships and collective management of IP issues can improve our chances for obtaining access to the needed enabling technologies.

1440–1520
S01–O–136
U.S. CONSUMERS PERCEPTIONS OF AGRICULTURAL BIOTECHNOLOGY: RESULTS OF A NEW NATIONAL SURVEY

William Hallman*[1], Adesoji O. Adelaja[2], Brian J. Schilling[2]

[1]Dept. of Human Ecology, Cook College/Rutgers Univ., 55 Dudley Road, New Brunswick, NJ, 08901-8520; [2]Food Policy Institute Cook College, Rutgers Univ., New Brunswick, NJ, 08901

A survey sponsored by the Food Policy Institute at Rutgers Univ. was conducted by telephone in April, 2001 with 1203 adults randomly selected from across the U.S. The results suggest that biotechnology is not on the agenda for most Americans. Forty percent report having heard or read not much or nothing at all about genetic engineering or biotechnology while only 13 percent report having heard or read a great deal Most, (56%) do not feel that they are adequately informed about biotechnology, and more than two-thirds (68%) of Americans say that they have never discussed biotechnology with anyone. Of those who have had discussions, a third (36%) say they have only discussed the issue once or twice. No surprisingly, despite the abundance of products with genetically modified ingredients in the market today, only four_in_ten Americans (41 percent) are aware that genetically modified food products are currently for sale in supermarkets. What is surprising is that Americans tend to believe that they are generally well informed about the process of food production in the United States. When asked to rate their basic understanding of how food is grown and produced, three_quarters of the respondents indicate that their knowledge is at least as good However, Americans knowledge of food production appears to be overestimated. For example, half of those interviewed said that they had never heard about traditional crossbreeding methods even when those methods were described in simple terms. In addition, despite the fact that nearly all foods currently available are the result of traditional crossbreeding techniques, 61% of the respondents said that they had never eaten a fruit or vegetable created using these methods (another 11% indicated that they were not sure). Many Americans also seem to have a fairly romantic view of farming. For example, 57% of the respondents believe that most farmers would prefer to farm organically rather than use chemical pesticides and fertilizers. Moreover, nearly 47% of Americans incorrectly believe that most food produced in the U.S. is grown on family farms. Despite the fact that 66% of Americans reported that their knowledge of science and technology was at least good the results of a 'biotechnology quiz' suggested otherwise. A third of Americans incorrectly believe that genetically modified foods are created using radiation to create genetic mutations and a third of Americans also incorrectly believe that it is impossible to transfer animal genes to plants. One quarter of Americans incorrectly believe that ordinary tomatoes do not contain genes, while genetically modified tomatoes do. Also, 30% of Americans incorrectly believe that genetically modified animals are always larger than ordinary animals. Nearly 27% of Americans do not know that their father's genes determine whether the child is a girl and 22% of Americans incorrectly believe that tomatoes genetically modified with genes from catfish would probably taste fishy. One in five Americans incorrectly believe that if a person eats a genetically modified fruit, their genes could be modified as a result and 19% of Americans do not know that their yeast used to make beer contains living organisms. Seven of the nine questions in the quiz were also asked as part of the 1999 Eurobarometer survey of consumers attitudes toward biotechnology in the European Union (INRA Europe, 2000). The comparative results suggest that American consumers may be more knowledgeable about some basic facts related to food biotechnology than their European counterparts.

1520–1540
S01–O–137
TO BE ANNOUNCED

1540–1600
S01–O–138
Agrobacterium-Mediated Transformation of Plum (*Prunus domestica* L.) With Prune Dwarf, Prunus Necrotic Ringspot, and Tomato Ringspot Virus Coat Protein Genes

Isabel Padilla*, Kevin Webb, Ralph Scorza

USDA-ARS Appalachian Fruit Research Station 45 Wiltshire Rd. Kearneysville, WV 25430

We describe an improved method for routinely transferring genes into plum (*Prunus domestica* L.) through the use of *Agrobacterium tumefaciens*. The system is based on regeneration of shoots from hypocotyls sections extracted from mature, stored seed. Shoot regeneration medium was Murashige and Skoog salts and vitamins with 7.5 μM thidiazuron and 0.25 μM indole-butyric acid. Transferring the hypocotyl sections to regeneration medium containing 80 mg/L kanamycin and 300 mg/L timentin after 2 days of co-cultivation with *A. tumefaciens* compared with 2 weeks of culture on kanamycin-free regeneration medium following co-cultivation, reduced the number of regenerated shoots, but did not affect the final transformation rate. Thus, the number of non-transformed "escapes" was reduced using early kanamycin selection. Transformation rates using the system described were 1% to 9% of hypocotyls sections producing transgenic plants. The transgenic shoots rooted at a 90% rate on half-strength Murashige and Skoog salts with 5 μM naphthalene-acetic acid and 0.01 μM kinetin. Plantlets were transferred to the greenhouse directly from culture tubes with a 90% average survival. Using this system we have produced transgenic plants containing the prune dwarf, *Prunus* necrotic ringspot, and tomato ringspot virus coat-protein genes.