Mitteilungen aus dem Julius Kühn-Institut

JKI Mitteilungen

# First International Symposium on Biotechnology of Fruit Species

September 1-5, 2008 in Dresden, Germany

*- Program and Abstract Book -*

ISHS

**Biotechfruit2008**

416
2008

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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Bundesforschungsinstitut für Kulturpflanzen

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen (JKI)
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# *Welcome to Dresden …!*

Welcome to the ISHS First International Symposium on Biotechnology of Fruit Species.

The Institute for Breeding Research on Horticultural and Fruit Crops Dresden of the Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, will host the symposium in collaboration with the Saxon Agency for Environment, Agriculture and Geology, Department of Horticulture (LfULG) and the University of Applied Sciences Dresden, Faculty for Agriculture, Horticulture and Landscape Management (HTW).

The Scientific committee has put together what we hope will be an informative program. This symposium unites delegates from more than 30 countries of all continents, except from Antarctica. The symposium in Dresden will offer the opportunity to learn more about the progress in biotechnology being made in temperate, tropical and subtropical fruit species. This meeting will also foster interactions amongst research groups and colleagues from different countries. We are grateful to welcome excellent specialists as invited speakers. They will provide an overview of the latest developments in their field of research.

We also welcome you to the Symposium social events, including the Welcome Reception on Monday evening and the Dinner Banquet on Thursday evening.

We hope that you all have time to enjoy Dresden, the baroque city and capital of Saxony. There are also a number of nice places in the small village Pillnitz together with the Palace and the Garden. For those of you staying on after the meeting we hope that you can take advantage from beautiful sites in the Saxon Switzerland along the river Elbe or other places in Germany. On Wednesday we provide the optional Symposium Tour to learn more about the large fruit growing area in the surroundings of Dresden and to have a great view to the Sandstone area.

If you need any assistance, please do not hesitate to contact the Registration Desk.

Magda-Viola Hanke
Julius Kühn-Institut
Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst Dresden

Norbert Eichkorn
Sächsisches Landesamt für Umwelt und Geologie

Fritz-Gerald Schröder
Hochschule für Technik und Landwirtschaft Wirtschaft Dresden,
Fachbereich Landbau und Landespflege

On behalf of the Local Organizing Committee:

Nico Domurath, Frank Dunemann, Henryk Flachowsky, Christine Grafe, Conny Hättasch, Katrin Herzog, Monika Höfer, Andreas Peil, Anja Schneider, Mirko Schuster,Andrea Schwarzak, Martina Tanner, Wolf-Dietmar Wackwitz, Roswitha Wehner

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**9.00-10.00** **Opening remarks and welcome**

Jörg Wendisch
Leiter der Abteilung 5 des Bundesministeriums für Ernährung, Landwirtschaft und Verbraucherschutz

Georg F. Backhaus
Präsident des Julius Kühn-Instituts Bundesforschungsinstitut für Kulturpflanzen

Norbert Eichkorn
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Reiner Klewen
Dekan des Fachbereichs Landbau/ Landespflege der Hochschule für Technik und Wirtschaft Dresden

Rod Drew
Chairman Commission Biotechnology, ISHS

**ABSTRACTS***

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**12.00-13.00** **Lunch**

**13.00-18.00** **Cost 864 meeting wg2 and wg4**

**15.00-17.00** **Tour to the fruit genebank**

*Abstracts: Listed by session and presentation number. Presenting authors are underlined. The originally submitted were formated by the Organizing Committee.

## POSTER

# ABSTRACTS

## AO-01

### Factors affecting somatic embryogenesis induction and development in *Feijoa sellowiana* Berg

Canhoto J. M. and Correia S. I.

Centre for Pharmaceutical Studies, Department of Botany, University of Coimbra, 3001-455 Coimbra, Portugal
E-mail: jorgecan@ci.uc.pt

*Feijoa sellowiana* Berg (*Myrtaceae*) is a small tree commonly known as feijoa or pineapple guava, originating from South America (Brazil, Uruguay). In recent years, this species has gained horticultural and consumer recognition due to the qualities of its fruits which possess high levels of vitamin C and iodine. Fruit extracts have also shown bactericidal and antioxidant properties. Somatic embryogenesis induction in feijoa was first achieved by our group in 1990 from mature zygotic embryos and, since then, several improvements have been made in the protocol for somatic embryo induction and conversion of this fruit crop. However, some drawbacks still persist in the process of plant regeneration through somatic embryogenesis in feijoa such as the high number of anomalous somatic embryos, the low rates of plant somatic embryo conversion into plantlets and the reduced potential of adult explants do undergo somatic embryogenesis. Trying to overcome these problems several lines of investigation are being followed at our lab with the objective of improve the quality of the somatic embryos and, at the same time, to understand the factors responsible for this negative aspects. Recent experiments have shown that the culture of somatic embryos in abscisic acid for 4 weeks before conversion increased the levels of storage lipids and proteins reserves in the cotyledons of the somatic embryos as well as the number of somatic embryos able to develop into plantlets. Moreover, it was found that somatic embryos of feijoa usually possess a morphologically variable suspensor like-structure that, in the first stages of development, is quite similar to the suspensor of the zygotic embryos. The possible role of this structure on somatic embryo development is being analysed. Other line of investigation indicated that some cultivars (e.g. Gemini) are more responsive than others (e.g. Apollo) both in terms of somatic embryogenesis induction and plantlet conversion and that an auxinic shock instead a continuous culture in the presence of auxin reduced also the levels of anomalous embryos. The role of phenolic compounds on somatic embryo formation and development as well as some preliminary results about somatic embryogenesis induction from young leaves of adult micropropagated genotypes will be also presented.

## AO-02

### Rooting and multiplication ability of persian walnut microcuttings as influenced by mother-stock vigour and precocity

Vahdati K.[1], Rezaee R.[2] and Mirmasomi M.[3]

[1]Department of Horticultural Sciences, College of Abooraihan, University of Tehran, Tehran, Iran
E-mail: kvahdati@ut.ac.ir
[2]Department of Seed and Plant Improvement, West Azarbaijan Agricultural and natural Resources Research Center, Urmieh, Iran
[3]Department of Plant Sciences, College of Sciences, University of Tehran, Tehran, Iran

According to the frequent existence of precocious and dwarf walnut (*J. regia L.*) genotypes in Iran nurseries, different studies were conducted to collect and evaluate these valuable genotypes from various aspects including consistency of growth under *in vitro* and *in vivo* condition and impacts of seedling vigor on the walnut rooting and grafting. In the present study, the stability of seedling vigor under *in vitro* condition and multiplication and rooting ability of the microcuttings were tested. To this, nodal explants of newly grown shoots of 5- year-old seedlings from three clusters of seedling vigor were surface sterilized and cultured on DKW medium. The explants were subcultured every month up to 13 times to increase the number of microcuttings. Results of *in vitro* study showed that number of adventitious shoots raised from the dwarf and semi-dwarf genotypes was the highest in compared to the high vigor ones (3.3 *vs.* 2.3). The low vigor genotypes also showed the highest number of nodes per a given size of shoot, smaller shoot size (2.6 *vs.*4.5 cm) and lower callus formation as well as higher rooting percentage (63.5% *vs.* 37.1%) and *in vitro* flowering which are consistent with the field observations, suggesting basitonic tendency, easy rooting and dwarf stability of dwarf genotypes under *in*

*vitro* condition. In conclusion, we suggest a simultaneous recurrent selection program for both dwarfing and rooting ability (selection of dwarf/semi-dwarf as well as easy-to-root clones) to utilize their advantages in a high-density orchard system.

## AO-03

### *In vitro* rooting improvement of adult pistachio (*Pistacia vera* L. cv. "Atli")

Tilkat E.[1], Ozden-Tokatli Y. O.[2] and Onay A.[1]

[1]University of Dicle, Faculty of Science and Literature, Department of Biology, 21280, Diyarbakir, Turkey
[2]Gebze Institute of Technology, Faculty of Science, Biology Department, Istanbul Caddesi, No 101, 41400, Gebze (Kocaeli), Turkey, E-mail: ozden@gyte.edu.tr

Axenic cultures of *Pistacia vera* L. cv. "Atlı" initiated on agar solidified Murashige and Skoog (MS) medium with Gamborg vitamins supplemented with different concentration of benzylaminopurine (BA) and indole-3-asetic-acid (IAA) were proliferated and maintained on a MS medium supplemented with 4.4 µM BA. Using those proliferated cultures, factors affecting successful rooting were studied. The effects of clonned shoots, dipping of the basal-cut-ends in the different indole acetic acid (IBA) solutions, washing of the basal-cut-ends in sterile distilled water and explant sizes (1.0, 2.0, 3.0 and 4.0 cm long) were assessed for root induction. Proliferated shoots ≥ 4.0 cm grown on MS medium supplemented with 9.84 µM IBA, 3% sucrose and 0.58% agar were best rooted by subjecting to full strength MS medium with IBA (9.84 µM) for 14 days before to transfer to medium without plant growth regulator (PGR). The highest rooting frequency (92%) of microshoots was recorded at 4.0 cm long clonned and twice washed microshoots. Rooting was apparent within 14 days. The present study also aimed at reducing the occurrence of callus production. The microshoots cultured from the clonned shoots produced less calli than the mixed population, and resulted in the production of root development, resembling seedling plantlets. Those plantlets that had been rooted and exposed to sterile compost were able to acclimatize readily. Major improvement over previously protocols has been achieved for percentage of *in vitro* rooting.

## AO-04

### Micropropagation of selected trees of *Arbutus unedo* L. through axillary shoot proliferation and somatic embryogenesis

Gomes F.[1], Lopes M. L.[2], Santos T.[2], Canhoto J. M.[2]
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Email: fgomes@esac.pt
[2]Centre for Pharmaceutical Studies, Department of Botany, University of Coimbra, 3001-455 Coimbra, Portugal

*Arbutus unedo* (strawberry tree, fam. *Ericaceae*) grows spontaneously in several countries of the Mediterranean basin. Fruits can be consumed fresh or can be used to make preserves and a very good type of honey is obtained from the pollen. Considering the increasing importance that alternative crops are assuming in the scope of the agricultural policy of the European Union and the sparse information about the potential of this fruit crop to be propagated *in vitro*, a project to clone selected trees based on their fruit production was initiated a few years ago. Shoot apices and nodal segments from epicormic and coppiced shoots were used to establish *in vitro* cultures. Best results (38.7±9.8 %, survival rate) were obtained with shoot apices (< 2 mm). From the three basal medium tested (FS; AND; 1/2MS) in combination with 8.9 µM BA and 0.087 M sucrose, the FS medium gave the highest rates of multiplication. Rooting was achieved on a Knop medium containing IBA (1 week) followed by subculture (5 weeks) on the same medium without auxin and containing charcoal (1.5 %). On these conditions, best rooting frequencies (100%) were obtained with IBA at concentrations of 49.4 µM. The experiments so far carried out showed that factors as the type of basal medium, the concentration of BA, the pH of the medium, sucrose concentration as well as the genotype of the explants strongly affect the rate of multiplication. For somatic embryogenesis induction leaves from *in vitro* propagated material were used. Results have shown that a combination of BA (8.8 µM) and NAA (10.7; 26.8 µM) gave the highest frequencies of induction (94.4%). A strong association between somatic embryo formation and explant oxidation was observed. The role of the oxidation process and the type of phenolics produced during somatic embryogenesis induction is being evaluated by biochemical and histochemical methods.

Somatic embryo maturation and conversion was achieved when somatic embryos were transferred to a medium without PGRs. Plant acclimatization was successful accomplished for both types of *in vitro* propagated material and some of the produced plants are now growing in the field to be evaluated.

## AO-05

### Effects of environmental (phenotypic) factors on cryopreservation in papaya gene pools

Ashmore S., Parisi A., O'Brien C., Marton T. and Drew R.

Centre for Forestry and Horticulture Research, Griffith University, Nathan, Queensland 4111, Australia
E-mail: S.Ashmore@griffith.edu.au

This paper will present results on cryopreservation for the conservation and use of seed and clonal genetic resources of *Carica papaya* and its crop wild relatives, the *Vasconcellea* sp. Efficient germination and dormancy-breaking in fresh, desiccated and stored seeds is critical for the management of seed stocks by the industry and for long-term conservation of the genetic diversity of the crop genepool. However, seed storage is complicated by non-orthodox characteristics and dormancy, which varies with environmental (phenotypic) factors. We will report on variation in responses to germination and dormancy-breaking treatments in fresh, desiccated and cryopreserved seed. Seed stored under cryopreservation for up to 12 months gave close to 100% germination when appropriate dormancy-breaking treatments were applied, but very low germination rates were recorded from seed stored at the standard storage temperature of -20°C used at most seed banks. Cryopreservation of *in vitro* grown shoot tips of papaya genotypes allows conservation of clonal material (Ashmore *et al*, 2007) with recovery from liquid nitrogen (LN) after storage for up to one year. Investigations on the cryopreservation of shoot tips of *Vasconcellea* sp. have shown differences in responses to both sucrose and cold pre-treatments. These differences will be related to known phenotypic differences between the species. The importance of environmental factors on cryopreservation will be discussed, based on the above work, and with reference to other work in our laboratory on the effects of natural distribution on cryopreservation in wild crop relatives of citrus.

## AO-06

### Advances in the cryopreservation of fruit plant germplasm at the CNR-ivalsa institute of florence

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Cryopreservation opens today important prospects to the safeguard of fruit plant germplasm, allowing the conservation in liquid nitrogen of organs and tissues from *in vitro* culture (e.g., shoot tips, nodal segments, embryogenic callus, somatic embryos), as well as the conservation of *in vivo* collected material (seeds and buds). Explants can be stored in liquid nitrogen at low cost, for unlimited time and in absolute sanitary and genetic safety, as at the temperature of -196°C all the biochemical and physical cell processes are completely arrested. New cryogenic techniques are now available, aiming at the direct immersion in liquid nitrogen ("one-step freezing") of plant specimens, without resorting to expensive apparatus for slow cooling and with a considerable simplification of procedures. At CNR-IVALSA of Florence, several innovative techniques has been tested with fruit species and used to develop effective cryopreservation procedures. Protocols based on the "treatment with vitrification solution", the "encapsulation-dehydration", the "encapsulation-vitrification" and the "droplet-vitrification" have been developed with shoot tips, achieving promising results in terms of practical applications with plum (*Prunus domestica*) and persimmon (*Dyospirus kaki*), as well as with pear and apple germplasm, autochthonous of the Veneto region. As for these species, experiments are also in progress using buds, directly collected from the field, as explants for the conservation at -196°C ("dormant-bud technique"). The storage in liquid nitrogen of samples from embryogenic callus lines is another application of relevant scientific interest. For instance, the establishment of "cryobanks" of embryogenic cultures would allow for the safe and long-term storage of valuable morphogenetic lines, avoiding their decline due to repeated subculturing. Here, very promising results have been obtained at IVALSA with the cryostorage of an

embryogenic line of olive (*Olea europaea*). As for seed cryopreservation, an effective procedure by "dehydration/one-step freezing" has been developed for polyembryonic seeds from an ancient *Citrus* collection located in a Medicean Villa of Florence. Seed cryopreservation has been recently optimized for pistachio (*Pistacia vera*), too.

## AO-07

### A commercially feasible protocol for rooting and acclimatization of micropropagated apple rootstocks

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This work was carried out to optimize a successful protocol for shoot rooting and acclimatization of tissue culture raised apple rootstocks of Malling series. Root induction in dark in indole-3-butyric acid (IBA) containing MS medium and root elongation in medium without IBA provided the best results for *in-vitro* rooting. Among various substrates tested for root elongation, agar was found better than perlite and sand. However, maximum rooting with good shoot quality was achieved, when liquid medium in place of agar solidified medium was used for root elongation. Rooted plantlets of about 5cm long were subsequently transferred to different media for acclimatization. Plants grown in cocopeat showed maximum survival as compared to those grown in soil containing medium. On the other hand, the *in vitro* elongated shoots could also be rooted *ex vitro* successfully in coco peat after inducing root initials in liquid medium in dark for simultaneous *ex vitro* root elongation and acclimatization. Nearly 95% hardening was achieved during the months of October to March in comparison to summer and rainy months. Plants transplanted to field in March-April established more successfully as compared to rest of the period of the year. After six months, plants in the field showed satisfactory survival and growth. These plants were grafted with different varieties.

## AO-08

### Genotypic differences in shoot multiplication among five citrus rootstocks *in vitro*

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The present study deals with the manipulation of factors specifically chemical, in the tissue culture media and their interactions, which can be useful for successful tissue culture. Shoot cultures of five cultivars of Citrus viz., Rangpur lime, C35 citrange, Troyer citrange, Swingle citrumelo and *Alemow macrophylla* were established from nodal stem segments using axillary shoots. These nodal segments were surface-sterilized and cultured on Murashige and Skoog (1962) medium. The shoot cultures obtained were used as explants to investigate the effects of various media constituents necessary for growth. Among the media constituents, cytokinins played a crucial role in shoot proliferation and were a prerequisite for the shoot multiplication media in all the cultivars. All the cultivars responded positively to the shoot induction treatment. In a unique case of *Alemow macrophylla*, the synergistic effects of auxins along with cytokinins were best for the growth of axillary shoots. The concentration of the carbon source, in this case sucrose, was also genotype-dependent and therefore significantly affected the production of axillary shoots, with either 3% or 4% being the optimal concentration depending upon the rootstock. Moreover, the influence of gelling agents also varied with each cultivar. Among the five cultivars tested, shoot proliferation frequency was highest in Rangpur Lime (4.3- fold) and was the lowest in *Alemow macrophylla* (3.3- fold). The results demonstrate that genotypic differences played a significant role in the induction and growth of axillary shoots. Thus, in this study we were able to identify and optimize the most effective shoot multiplication media for each cultivar, which is a necessary step to develop an efficient micropropagation protocol.

## BO-01

### Cisgenesis in fruit trees

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Introgression of traits from wild germplasm into pip fruit cultivars by means of classical breeding is painstakingly slow. Introgression of, for example the apple scab resistance gene *Vf* from *Malus floribunda* 821 into marketable top quality apple cultivars took more than 50 years. In the mean time *Vf* resistance has been compromised by new virulent races of *Venturia inaequalis* in northern Europe. For durable resistance more than one resistance gene should be combined. However, this may take many years. This slow tempo is caused mainly by the long juvenile period and by linkage drag of hundreds of undesired alleles. The process would be much faster if only the allele of interest were inserted, without the other alleles from the wild germplasm. This process is named "cisgenesis". Cisgenesis would allow rapid accumulation of resistance genes or other desired alleles from wild sources. We have defined cisgenesis as genetic modification of plants, inserting genes of the plant species itself or from crossable relatives. The gene should contain its native introns and be flanked by its native promoter and terminator in sense orientation. A cisgenic plant does not contain genes from outside the gene pool of the conventional breeder. If the plant does contain foreign genes, the plant is named transgenic. Scientific inquiries indicate that acceptance by consumers is better for cisgenic plants than for transgenic plants. As the phenotypic traits from cisgenesis can in principle also be obtained by means of conventional breeding, induced translocation breeding or mutation breeding, cisgenic plants are at least as safe as conventionally bred plants, or plants from induced translocation breeding or mutation breeding. Therefore we propose to add cisgenesis of plants to the list of GM technologies that are exempted from the GMO regulation in the European Union (Annex 1B of Directive 2001/18/EC). The number of functionally analysed genes in the fruit trees is increasing, and will be boosted further by combining whole genome sequences with known genetic loci for interesting traits, gene expression data, and ESTs. Also technologies are available for either introduction of alleles without use of marker genes, or for later excision of marker genes, such as kanamycin resistance gene, the so called "marker-free" technologies. Cisgenesis combines the knowledge of gene sequences and their functions with marker-free technologies. Cisgenesis is an approach for utilizing the growing wealth of knowledge of plant genes to the benefit of the society in a fast, safe and acceptable way.

## BO-02

### Silencing in genetically engineered *Prunus domestica* provides durable and safe resistance to Plum pox virus (sharka disease)

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Originally identified in Bulgaria in 1915, *Plum pox virus* (PPV) is the most damaging virus of stone fruit trees including apricot, plum, peach and cherry. PPV steadily spread throughout Europe over the years since its discovery and at the turn of the century (1999-2000) it reached North America (USA and Canada). While many strategies to control the spread of PPV have been undertaken over the decades and many studies have contributed to the characterization of the virus isolates there has been relatively little progress in the development of resistant varieties. With the paucity of natural resistance, transgenic technology based on the engineering of the virus capsid gene was investigated as a useful source of resistance. This work identified the C5 plum clone as highly resistant to PPV infection. These findings were supported by detailed molecular studies indicating that post-transcriptional gene silencing (PTGS) is the resistance mechanism with resistance being mediated through the production of small interfering RNA (siRNA). The durability of PPV resistance in C5 (named 'Honey Sweet') is reflected through more than 10 years of field tests. In total, almost 16 years of research with 'Honeysweet' have demonstrated that this clone and the resistance mechanism that it represents is: i) an important tool to demonstrate the

successful deployment of biotechnology against a quarantine pest and ii) a safe product of biotechnology and iii) a useful strategy for avoiding the use of pesticides to control natural aphid vectors of PPV. The deregulation of 'Honeysweet' in the USA by USDA/APHIS (Federal Register Doc. E7-13649, July 12 2007) corroborates the utility of these findings.

## BO-03

### Systemic acquired silencing of a gusa transgene in apple

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RNA silencing is an essential, highly conserved mechanism in many eukaryotic organisms. It influences developmental processes as well as viral defence, suppression of transposon activity and silencing of transgenes. RNA silencing is based on the recognition and processing of double stranded (ds) RNA into short interfering RNAs (siRNAs) or micro-RNAs (miRNA) by DICER-like (DCL) proteins. These small RNAs guide ARGONAUTE-like (AGO) proteins to complementary RNA sequences resulting in the degradation or in prevention of the translation of these targeted RNA sequences. RNA silencing is a mobile effect, which can spread from cell to cell and over long distances. Cell to cell spreading occurs probably via transport through plasmodesmata, whereas the long-distance transport of the silencing effect is probably realized via the phloem. The hypothesis of this research is to use graft transmissible silencing signals produced by a transgenic part of the plant in order to influence the non-transgenic part. Transgenic rootstocks transformed by a silencing construct will produce small RNAs which may be transported into the scion. In the non-transgenic scion these RNAs will then reduce the gene expression of selected genes. In order to evaluate the systemic silencing system for the first time on a woody plant like apple, a vector was constructed which contained a constitutively expressed *nptII* gene and a hairpin RNA (*sigus*) homologous to a part of the coding sequence of *gusA*. Using this vector, 13 *sigusA* transgenic lines were obtained after *Agrobacterium tumefaciens*-mediated gene transfer and selection on kanamycin-containing medium. The integration and expression of *sigusA* and *nptII* in these plants were tested by PCR, RT-PCR and Southern Blot. *GusA* expressing transgenic apple shoots were grafted *in vitro* onto *sigusA* transgenic shoots used as rootstocks to investigate long-distance mRNA silencing. After four weeks of *in vitro* cultivation the *gusA* expression was analysed by histochemical GUS staining as well as by qRT-PCR. Gus staining assays revealed that the *gusA* expression in the *gusA* transgenic scion was clearly decreased compared to control shoots grafted onto non-transgenic 'Pinova'. About 20% to 70% of the total leaf blade was affected by *gusA* silencing. Silencing took place in young as well as old leaves, affecting whole leaves or different parts of leaves. Real-time PCR analyses showed that the reduction of *gusA* expression occurs on the RNA level. Furthermore, we studied the dissemination of the silencing effect in grafted one-year-old transgenic plants in the greenhouse to determine the degree, the rate and the manner of systemic silencing in woody plants such as apple.

## BO-04

### Gene stacking in one-year-cycling *Apetala1* citrus plants for a rapid evaluation of transgenic traits in reproductive tissues

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Rapid flowering is crucial for being able to perform functional genomic studies to investigate fruit and flower-related traits in fruit trees. However, long generation cycles of woody fruit plants considerably delay this evaluation. Through genetic transformation, juvenile periods can be significantly shortened by overexpression of flower meristem-identity genes. Transgenic *APETALA1(AP1)*-citrus plants behave as rapid cycling trees, since one-year-old seedlings already show precocious flowering and fruiting. By transgene stacking into these short-generation *AP1* and *nptII*/GUS-positive plants, expression of novel transgenes could be examined as quickly as one year after retransformation. The establishment of the

appropriate parameters and conditions to fulfill this objective is reported in the present work. The recovery of retransformed *AP1*-citrange (*Citrus sinensis* L. Osb. X *Poncirus trifoliata* L. Raf.) plants was accomplished by selection with the *hpt* and *bar* genes and discrimination of transgenic shoots through GFP expression and PCR amplification. Transformation efficiencies, defined as the number of transformed plants regenerated from total inoculated explants (x100), were low if compared with *nptII* selection in Carrizo citrange (around 3% with *hpt* or *bar* genes vs. 40% with *nptII* gene), but permitted effective retransformed plant recovery. Stable integration of transgenes of both rounds of transformation was confirmed by Southern blot analysis. *AP1* transgene expression was quantified by Real-Time PCR. After one year of growth in the greenhouse, GUS and GFP expression could be also examined in flower and fruit tissues of many retransformed plant lines. This system is being used now in our laboratory to test putative fruit tissue-specific promoters.

## BO-05

### Quantitative real-time PCR provides molecular evidence for regeneration of chimeras in transgenic apricot and a reliable methodology to monitor their dissociation

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When transformed *via Agrobaberium tumefaciens*, perennial fruit trees are highly prone to regenerate chimera; a mixture of transformed and non-transformed cells. This phenomenon was also observed in 'transformed' apricot cultivars after PCR amplification of the *nptII* transgen, from DNA extracted at various cycles of sub-culture in the absence of selective pressure. The intensity of the amplicon decreased at each sub-culture, until been undetectable.

Although southern blot analysis has been widely used to confirm the integration of the transgen, we demonstrated here that it can not be considered as a sufficient proof for the uniformity of T-DNA insertion. In this study, we developed a more reliable method, which allowed us to quantify this phenomenon and to monitor its dissociation. This was based on quantitative real time PCR amplification of the *nptII* as well as of an internal control (*actin*), used to normalize the quantity of the *nptII* transgen at each reaction. This method allowed us to detect chimeras both, analyzing several shoots of the same line or even in different DNA extractions from the same shoot. In addition, the quantity of the transgen assessed from leaves was dependent on their localization; high in the basal leaves, while in the upper ones the transgen remained lower. A gradual increase in selection pressure during sub-culture of some chimerical lines enhanced the quantity of the transgen until saturation, which may be a good indicator of their reversion to fully homogeneous transgenic shoots.

## BO-06

### Improving tissue culture, micropropagation and biotechnological applications in *Rosaceae* crops

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Presently *Rosaceae* tissue culture and micropropagation practices frequently rely on traditional and often under optimized media compositions and procedures. This situation often impedes biotechnological advances in these crops. In order to address this, an effort must be made to develop efficient and universal tissue culture methodologies within the Rosaceae family. One of the approaches in our program focuses on the integration of a temporary immersion system for the rapid in vitro growth of rosaceae crops. We are also studying the effect of light quality treatments on rosaceae plant growth and development in tissue culture. Additionally, various treatments of hormones and physiologically active bio-molecules are being investigated to optimize the production of directed organogenic differentiation from cultured explants. With further focus directed to the development of robust tissue culture and micropropagation methods, there promises to be an increase in the quality and speed of *in vitro* generated horticultural crops for scientific and commercial purposes. Our long-term goal is to utilize the *in vitro* platform for testing novel gene function in rosaceae via nuclear and chloroplast genome modifications. Particularly in apple we are using the non-juvenile line (Flachowsky et al., 2007) for optimizing

parameters for recovery of transgenic plants. Nuclear transformation is being performed with established protocols using Agrobacterium as the transgene delivery vehicle. In order to insert genes in the chloroplast genome, particle bombardment-mediated transformation is being standardized. In contrast to nuclear transformation vectors, chloroplast transformation vectors integrate transgenes via homologous recombination and the process of recovering homoplastomic lines involves repeated cycles of regeneration. Different chloroplast transformation vectors that target the transgenes to different regions of the apple plastid genome are currently being tested. We expect to extend the tissue culture and transformation methodologies standardized in apple to other members of *Rosaceae*.

## CO-01

### Isolation and functional analysis of genes encoding flavonoid 3'-hydroxylase in apple

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Flavonoids are important secondary metabolites in fruits, and contribute in a number of ways to fruit quality, including color, flavor, bitterness or texture. Apples are one of the main contributors of flavonoids in human diet. To gain insight into the mechanism responsible for the hydroxylation of flavonoids in apples, we have recently isolated three genes encoding flavonoid 3'-hydroxylase (F3'H) from apple; these genes have been designated *MdF3'H*-1, *MdF3'H*-2, and *MdF3'H*-3. All three genes are composed of 3 exons and 2 introns, and contain an open reading frame of 1,536 bp encoding a putative protein of 511 amino acids. *MdF3'H*-1 shows ~60% and 91% nucleotide sequence identities in genomic sequence and coding region sequence, respectively, with both *MdF3'H*-2 and *MdF3'H*-3. *MdF3'H*-2 and *MdF3'H*-3 share an overall identity of 94% in genomic DNA sequence and 99% nucleotide sequence identity in the coding region. In addition, *MdF3'H* genes are expressed in leaves, flowers, and fruits. Transgenic *Arabidopsis* tt7 mutant lines expressing these apple F3'H genes exhibit similar pigment accumulation as wild-type Arabidopsis, thus suggesting that functionality of F3'H genes are conserved among higher plants.

## CO-02

### Using functional genomics to identify molecular markers for fire blight resistance (*Erwinia amylovora*) in apple (*Malus*)

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Fire blight, caused by *Erwinia amylovora* (*Ea*), is a destructive disease of apple (*Malus*), pear (*Pyrus*) and some woody ornamentals in the rose family (Rosaceae). The goal of this project is to use a functional genomics approach to develop tools to breed fire blight resistant apples. Six hundred fifty expressed sequence tags (ESTs) associated with fire blight were identified from *Ea*-challenged apple leaf tissue by suppression subtractive hybridization (SSH) and cDNA-AFLP analysis. ESTs were ranked for their potential impact on resistance based on bioinformatics and inferences drawn from model systems. Simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers derived from highly ranked fire blight-associated ESTs were mapped in a 'M.9' x 'Robusta 5' population in which a major QTL for fire blight resistance has been located on Linkage Group 03. Highly ranked fire blight-associated ESTs were mapped to this QTL, as well as to the positions corresponding to the location of at least two QTLs reported in other populations (Calenge et al. 2005, Khan et al. 2006). Markers for heat shock protein 90 (Hsp81-2), a secretory class III peroxidase and a serine/threonine-protein kinase

mapped to the LG03 fire blight resistance QTL and reduced its size from 12cM to 4cM. Markers for a "putative disease resistance protein" (NCBI AY347778) and Skp1 (SCF-type E3 ubiquitin ligase) mapped to positions corresponding to the location of two known QTLs on LG07 and LG12, respectively (Calenge et al. 2005, Khan et al. 2006). To date, of 28 candidate fire blight resistance gene markers that have been mapped, 6 have co-located to or near known fire blight resistance QTLs. This research will facilitate new methods of marker-assisted selection to efficiently breed superior apple cultivars with fire blight resistance.

## CO-03

### Possible role of sams gene in fe uptake mechanism of *Malus xiaojinensis*

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Iron is one of essential elements for plant including tree apple. Iron deficiency chlorosis causes, particularly on calcareous soils, a serious impact on growth and production of apple worldwide. By practice and experiment, it has been proved that use of Fe-efficient rootstock is a fundamental way to alleviate the disorder. Used the Fe-efficient genotype of *Malus xiaojinensis* we had screened out, this experiment studied changes of protein expression of the genotype under Fe deficiency stress. More than 700 of protein spots with high repetition were obtained by 2-DE (two-dimensional electrophoresis) method, in which 13 of up-regulated protein spots being classed into 11 kinds of proteins were identified by use of MALDI TOF (matrix-assisted laser desorption/ionization-time of flight), PMF (peptide mass fingerprinting) analysis and database. Combined with results of Fe deficiency subtracted library of *M. xiaojinensis*, total length of both cDNA and DNA of MxSAMS was cloned by RACE, a SAMS (S-adenolyl-L-methionine synthetase enzyme) gene from the Fe-efficient *M. xiaojinensis*. The results by RT-PCR showed that expression of MxSAMS in roots or leaves of *M. xiaojinensis* was induced by Fe deficiency stress. Under Fe deficiency stress, either roots or leaves of Arabidopsis plants with overexpression MxSAMS had higher Fe contents than the wild type, with higher resistance of the plants to the Fe deficiency.

## CO-04

### The genomics of fruit quality

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Consumers of whole foods, such as fruits, demand consistent high quality and the development of new varieties with enhanced health, convenience, novel taste, and reduced impact on the environment. Conventional breeding of temperate fruit crops such as apples and kiwifruit, the focus of HortResearch, exploits both existing cultivars and the extensive germplasm collections of related species and novel accessions. Our genomics research is focused on the key producer and consumer traits. We achieve this by defining the biology of our key fruit traits and developing an understanding of the processes in model plants. Our translation genomics research then transfers this molecular information to our target crops. To do this, we have developed an extensive fruit EST sequence database and are in the process, through collaboration, of developing a Whole Genome Sequence for these crops. In our work on fruit colour, we have described both the metabolic and regulatory genes involved in anthocyanin accumulation. In addition, we have analysed novel germplasm of apples with red flesh and shown that this colour is due to the ectopic expression of an MYB regulatory gene. A simple rearrangement in the promoter DNA is sufficient to account for this desirable phenotype, and we will discuss the molecular mechanism responsible for this altered phenotype, and how this information is being used to develop novel red-fleshed cultivars that retain the flavour, texture and long-term storage of cultivated apples. This, along with our work on carotenoids, chlorophyll, flavonols and vitamin C, provide compelling evidence that genomic research on temperate fruit can accelerate the development of novel cultivars with improved quality and consumer appeal.

## CO-05

### Next-gen sequencing technologies: drawbacks and opportunities

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The next-gen sequencing technologies, embodied by the Roche GS FLX, the Illumina Genome Analyzer and the ABI SOLiD System, generate huge amounts of sequence information. The analysis and visualisation of these data are key challenges to the successful application and development of the next-gen sequencing technologies. GATC presents data of genomes that has been sequenced using all next-gen technologies.The long reads of the 454 technology allow a de novo assembly of smaller genomes.The GS De Novo Assembler Software provides a format which can be impor-ted into other programs (e.g. SeqMan of the Lasergene Suite; DNASTAR) and assembled with Sanger or short reads data. The high coverage of the Genome Analyzer and SOLiD technology is ideal for resequencing to find SNPs and other differences. With sequencing of mate pairs, especially with libraries of large inserts, structural variations can be identified. The mapping of reads from these technologies allows a visualization and analysis of SNPs between the genome of interest and the reference genome using a standard genome browser web interface. With the combination of different sequencing technologies, the drawbacks of one system can be overcome by another:

- Some regions ("hard stops") can only be sequenced with difficulty using the Sanger method. The next-gen technologies can solve these problems.
- Sequencing quality in homopolymer regions is low using the GS FLX system. Neither sequences from the Genomes Analyzer nor from the SOLiD show this effect.
- GATC has developed a proprietary cross-platform tagging system for parallel sequencing with a virtually unlimited increase in the number of samples. The technique is highly efficient, resulting in 99.9% of sequences successfully tagged. The tagging system can be used e.g. for a BAC approach to sequence large eukaryotic genomes.
- For the bioinfomatic analysis of datasets of different technologies, e.g., DNASTAR SeqMan Genome Assembler (SMGA) can be used. SMGA assemble sequence data from Sanger and next generation sequencing technologies. The SeqMan Pro module can show the assembly from SMGA and display electropherograms as well as flowgrams.

## CO-06

### Genomics technologies for development ff genetic markers for breeding in apple (*Malus*)

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Now that plentiful sequence information is available for *Malus*, efficient methods are needed to use it to develop robust, easily used genetic markers for a range of traits critical for agronomic performance and consumer appeal of new cultivars. Once such genetic markers are available, the application of marker assisted selection (MAS) in apple breeding programmes will increase rapidly. We discuss the use of bioinformatics and functional analysis to identify candidate genes for disease resistances, fruit flesh colour, and flavour from a database containing >250,000 *Malus* sequences. These expressed sequence tags (ESTs) have been derived from a variety of developmental stages, tissues and cell lines of a range of cultivars subjected to biotic and abiotic stresses. Single nucleotide polymorphism (SNP) or simple

sequence repeat (SSR) markers developed from candidate genes identified by bioinformatic searches, are subjected to the type of analysis best suited to their function, to narrow down a list of typically several hundred candidates, to a smaller number that can be more readily screened for co-segregation with the trait of interest. Options for functional analysis available at HortResearch include transcript profiling using oligonucleotide microarrays, transient expression in *Nicotiana benthiama*, stable transformation of *Arabidopsis* and *Malus*, and gene silencing. Suppression subtractive hybridisation analysis (SSH) and cDNA-AFLP analysis have also been used in a collaboration with the USDA. Screening of the most likely candidates identified by functional analysis is generally performed by a whole genome scan of previously unmapped, phenotyped populations segregating for the target trait, to identify markers co-segregating with the phenotype. Such markers are then located on one of two framework genetic maps constructed in 'M.9' x 'Robusta 5' or 'Royal Gala' x A689-24 populations, respectively, to identify approximate map positions. Markers in the framework map in this region are then mapped in the specialized population segregating for the trait of interest, in order to increase the density of markers around the trait locus. Markers co-segregating with, or closely flanking the trait, are then modified to increase robustness and ease of use if required, prior to application for MAS in cultivar and rootstock breeding programmes.

## CO-07

### A genomics approach to fruit softening in apples

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Fruit softening is a key factor in fruit quality which directly affects commercial returns to growers. Softening occurs in mature apple fruit after the detection of the ripening hormone ethylene. We have used a 16,000 feature microarray hybridised with labelled RNA from a number of ripening treatments[1,2], to identify changes in gene expression that occur with apple fruit softening. Genes that showed a large increase in expression with ethylene included a number of cell wall-related enzymes such as polygalacturonase (PG), xyloglucan endotransglucosylase/ hydrolase and B-galactosidase. As the level of PG expression seemed to correlate with softening, the transcription factors that activate this gene were further investigated. Members of the AP2 transcription factor family of genes were initially targeted as they have been implicated in the first steps of ethylene signal transduction as well as the transcription factors that were up regulated during ripening. Using high throughput qPCR we identified 18 ethylene-induced transcription factors 4 of which were AP2 like genes. To establish which transcription factors directly activated the PG gene, a previously published 2.8 kb PG promoter sequence was cloned[3] and screened against the HortResearch transcription factor library using a transient assay system[4]. Combining the results from these two screens we have identified candidate transcription factors that may be involved in the regulation of fruit softening.

## CO-08

### An upstream minisatellite causes red apple flesh colour

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Mutations in the genes of the anthocyanin pathway or its regulators in plants have been linked to colour phenotypes. Generally, this is a loss of function with a reduction of anthocyanin or a change in patterning. Here we describe an insertion in the upstream regulatory region of the apple anthocyanin-regulating transcription factor MdMYB10. This modification results in a gain of function, producing an increase in anthocyanins throughout the plant and a striking phenotype that includes red foliage and red fruit flesh. The mutation comprises a 23 base pair sequence duplicated in five tandem repeats to form a minisatellite repeat unit. We show the association between the MdMYB10 minisatellite duplication and

the red foliage and red fruit flesh phenotype found in all apple varieties tested. Our results show that the repeat-containing promoter can act in a way that is sufficient to account for the increased MYB10 transcript levels and subsequent ectopic accumulation of anthocyanin.

## CO-09

### Molecular characterization and analysis of geographical differentiation of indian mango (*Mangifera indica* L.) germplasm

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India has a long history of mango cultivation and centuries of selection exercised by farmers have lead to a panorama of cultivars and heirloom landraces. Majority of the mango landraces are unique in nature and have specific geographical origin. In order to determine the extent of geographical differentiations, a set of 241 mango landraces and commercial cultivars from 15 different regions from India were analyzed for microsatellite variability using 18 polymorphic SSR primer pairs. A total of 103 alleles with an average of 5.78 alleles per primer pair were observed in the cultivars analyzed. The number of alleles per primer pair ranged from 3 (for LMMA14) to 9 (for MiSHRS-18). The pair wise similarity coefficients for the cultivars ranged from 0.024 to 0.808 with an average of 0.258 indicating presence of high genetic diversity among the germplasm analyzed. High observed heterozygosity (0.6513), average heterozygosity (0.5214) and Shannon's information index (1.2959) for the loci analyzed also substantiated the above observations. Partitioning of total molecular variance among germplasm from 15 regions representing eight groups based on geographic contiguity showed that among groups difference accounted for 1.91%, among populations within groups for 3.80%, among cultivars within population 57.87% and differences within individual cultivars for 36.42% of the total variations. The $F_{ST}$ value derived from these estimates was 0.019 indicating that the cultivars in the 8 major geographic groups were not strongly differentiated. The average pair-wise $F_{ST}$ value between the cultivar groups ranged from 0.077 for cultivars from Lucknow to 0.341 for cultivars from Manipur region indicating that the germplasm from north-eastern India was greatly differentiated from the rest. Further, the cultivars from Tamil Nadu, Karnataka, Goa and Andaman and Nicobar islands were also observed to be equally well differentiated from other regions. Significance of synonyms and homonyms for cultivar names were investigated by analyzing cultivars bearing the same names but under cultivation in different regions. More than one collection per variety was studied in 24 cultivars. Results indicated presence of large differences in molecular profiles for all the cultivars analyzed except in case of 'Banganapalli' and 'Mallika' where all accessions exhibited comparable SSR profiles. Hence, traditional cultivars from different geographical regions should also be given importance in genetic resources collections. Analysis of molecular variance, cluster analysis and high values for estimates of Nm indicated presence of substantial gene flow between the cultivars from different regions, possibly due to cultivar exchange followed by gene introgression by crossing and selection.

## CO-10

### The use of *Heterologous oligonucleotide* microarrays for transcriptomics in a non-model species; application to the study of drought stress in musa

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The diversity of physiological, biochemical and molecular strategies adopted by plants during adaptation to (a) biotic stress conditions creates particular difficulties for the scientist wishing to study them. The availability of non-biased, 'systems-wide' approaches such as transcriptomics and microarray RNA-profiling are however well-suited to this type of analysis. However commercial microarrays are currently only available for a small number of species and microarray development costs are so substantial as to be generally prohibitive for most research groups. Recently it has been demonstrated that oligonucleotide

microarrays from closely-related, heterologous species can be used to probe the transcriptomes of non-model plants. Here, we present results from the use of Affymetrix high-density oligonucleotide GeneChip® microarrays to profile the response of the banana (*Musa* spp) leaf transcriptome to drought stress using a genomic DNA (gDNA)-based probe-selection strategy to improve the efficiency of detection of differentially expressed *Musa* transcripts. Using this approach, probes from over 15,000 individual gene-specific probe sets from the Rice GeneChip® microarray hybridized with a suitably high efficiency to *Musa* gDNA and were retained for subsequent transcriptomic analyses. Cross hybridization of RNA isolated from leaves of drought-stressed and control *Musa* cv. 'Cachaco' plants to the Rice GeneChip® array resulted in the identification of 2910 genes with a greater than 2-fold difference in expression level. Of these differentially-regulated genes, 1671 were up-regulated and 1239 down-regulated. Gene ontology classifications based on both rice and *Arabidopsis* annotations indicated that the functional gene categories over-represented (relative to the genome) included many classes associated with plant biotic and abiotic stress responses, including pathogen response, cold, salt, osmotic stress and water deprivation. In addition, a range of regulatory genes including transcription factors known to be involved in coordinating plant abiotic stress responses were identified, including members of the ERF, DREB, MYB, bZIP and bHLH families. Our results demonstrate that despite the lack of extensive genome sequence data in *Musa*, heterologous cross-hybridisation studies using commercial oligonucleotide GeneChips® is a highly promising strategy to study complex plant-environment interactions in non-model species and outlines the potential applications of such strategies for the breeding and development of new stress-resistant varieties.

## CO-11

### The golden delicious apple genome sequencing project: progress and perspectives

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Apple is one on the most diffuse fruit crop over the temperate climates, and one of the most important representatives of the Rosaceae large family. In our region of Italy, Trentino Alto-Adige, apple represents the most important agricultural resource. We have therefore concentrated our efforts on apple genome sequencing, selecting the elite cultivar Golden Delicious, grown worldwide and representing over 80% of apple in Trentino. Our research has the multiple goals of genome assembly, gene identification and annotation, and identification of a maximum number of polymorphisms. Golden Delicious is highly polymorphic with two clearly distinguishable haplotypes, expecting to reveal several million SNPs and small indels. They represent a substantial resource for molecular breeding programs, as well as trait and QTL marker association. Based on our previous experience on the large heterozygous grapevine genome, we have used novel algorithms to address this challenge, which will be applied also to the apple genome. A total coverage of 4 genome equivalents of libraries of ascending size sequenced by the Sanger method, coupled with 10 genome equivalents of 454 Life ScienceTM sequences, will allow us to create an effective genome sequence. Assembly will be based on adding sequences of a BAC libraries and a fosmid library, end-sequenced to assemble large meta-contigs. Contigs will be oriented and ordered on appropriate chromosomes by high throughput marker development and genotyping in an F1 cross of Golden delicious x Scarlet. Currently, over 3 billions of nucleotides and 1,2 billions of pyrosequencing, Sanger and 454 technique, have been produced. Further 6 billions of nucleotides will be developed to the final goal of 14 genome equivalents of apple (4x Sanger and 10x pyrosequencing, respectively). Following ESTs clustering in tentative consensus (TCs) and TC blast against the genome

sequence, 2,000 SNPs of Golden Delicious are currently under development. Sequencing and mapping data will be public available at IASMA, NCBI and GDR databases.

## CO-12

### How bent is the future of bananas? – A genomics perspective

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Despite being the No. 1 fruit crop on Earth both in terms of economical importance as well as production, relatively little information is available about the genome structure and functional genomics in banana. In this presentation, present knowledge is summarised and updated on (i) genome sequencing and annotation, (ii) transcriptome analysis, and (iii) gene tagging in banana. Current genome sequencing efforts are focused on the analysis of randomly selected or mapped BACs, which have revealed that, in an evolutionary aspect, bananas are positioned between dicots and grasses. A recent initiative to sequence the banana genome during 2009 undoubtly will have a huge impact on progress in genomic research of this crop. Transcriptomic data available to date are based on EST sequencing as well as SuperSAGE analysis. Novel vectors have also been developed for tagging large numbers of genes and promoters, including tissue-specific genes or those active during the regeneration process *in vitro*. Finally, an overview will be given on transgenic applications of candidate genes for disease resistance and on expertise gained in performing transgenic field trials in the tropics. This expertise can be further extended to other traits as a means to address new challenges emerging during banana production.

## CO-13

### Biotechnology for cocoa improvement in venezuela

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Venezuela is the center of origin and diversity of cocoa *(Theobroma cacao)*, being during colonial times an important producer. Venezuelan cocoa is considered the finest of the world, due its unique flavors and aromas. However, the introduction of imported materials and the fact that cocoa is a cross pollinated species has had a negative impact in the quality of the cocoa production; the age of plantations has a negative effect in productivity. To improve production and quality a network of research labs has been established, including institutions from all around the country. The ongoing projects include the characterization of the genetic diversity in germplasm banks using molecular markers (ISSR, ISTR) for fingerprinting purposes and for resistance genes. The conserved NBS domain was used to amplified a resistance gene analogous (RGA) by PCR, to generate resistance gene analogous polymorphism (RGAPs). The selection of fine materials and its propagation through somatic embryogenesis is in process and until now 58 different varieties have been propagated, including Criollos, Trinitarios and Forasteros.The culture, characterization and molecular identification of pathogens (fungi) and its antagonists will permit the establishment of a program for the correct integrated management of diseases. Due the existence of beneficial compounds, the metabolomic of natural products important to health it is being analyzed in *in vitro* cultivated cells, starting with phenolic compounds. A mathematical model for this purpose is in construction.

## CO-14

### Functional genomics in *Fragaria*

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Strawberry (*Fragaria x ananassa*) is one of the most popular fruit crops worldwide and is grown in all temperate regions of the world. Much of the popularity of this fruit is due to the attractive flavor and the deep red color. In addition to traditional nutrients such as carbohydrates, vitamins and minerals, strawberries are also rich in phenolic compounds such as flavonoids e.g. epiafzelechin, which is the focus of intense study due to its proliferative effects on osteoblastic cells and selective inhibitory activities against cyclooxygenase-1 (COX-1) over COX-2. The majority of flavonoids in strawberries are anthocyanins, the compounds responsible for the blue, red and purple hues of berries, grapes and other fruits. To confirm the *in vivo* function of a recently cloned strawberry UDP-glucose:anthocyanidin glucosyltransferase (*FaGT1*) gene we downregulated its expression in strawberry fruit by injection of *Agrobacterium tumefaciens* cells harboring an intron-hairpin construct of a partial *FaGT1* sequence. In about one third of the injected fruits this led to a significant downregulation of *FaGT1* transcript levels that corresponded to reduced concentrations of anthocyanin pigments in ripe fruits. In contrast, significant levels of epiafzelechin - formed by anthocyanidin reductase (ANR) from pelargonidin - were identified in *FaGT1* silenced fruits. Thus, the redirection of the metabolic flux towards the flavan-3-ol through downregulation of FaGT1 offers a new method to increase the levels of this bioactive metabolite in fruit crops. In addition a dormant biosynthetic pathway of strawberry volatiles was uncovered by using the transient RNA-inference system. Silencing of the flavonoid pathway by downregulation of the chalcone synthase gene (*FaCHS*) provided phenylpropenoids for the biosynthesis of anol, chavicol and eugenol in the fruits. These studies serve as foundation for metabolic engineering of strawberry flavor.

## CO-15

### Identification and characterization of a grapevine (*Vitis vinifera* ) DREB2a gene, belonging to the AP2/EREBP family

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The fruit trees are exposed to many types of abiotic stresses during their life cycle. Water deficit derived from drought, is one of the most common environmental stresses that affects growth and development of fruit crops through alterations in metabolism and gene expression. Adaptation to drought conditions may involve passive tolerance or active homeostatic mechanisms for maintaining water balance. It is well established that abscisic acid (ABA) is a major physiological signal that induces the drought responses and mediates adaptation to drought by activation of bZIP proteins which then bind to so-called ABA-responsive regulatory elements (ABREs) in target genes and induce their transcription, recently studies have been described another ABA-independent pathway that leads to rapid responses to drought stress and functions through members of the AP2/EREBP family of transcription factors, which recognize the drought-responsive elements (DREs) in target promoters. Although these different pathways are usually considered to function independently from each other it is certainly possible that some cross-talk exists between them. However, recent studies have been reported that DREB proteins are important transcription factors with conserved AP2/EREBP domain, widely existing in woody perennial fruit crops. On this account, we started to examin, and identify the ABA-independent DREBs involved in drought tolerance in the commercially used Iraninan grapevine cultivar ‘Askari’. In this regard, a DREB gene, designated as DREB2A, was isolated from leaves of grapevine under drought stress conditions using mRNA capture screening technique. the full length cDNA was isolated, cloned and sequenced. The sequence homology was analyzed using BLAST program at the NCBI. Multiple alignments of amino acids were carried out using the Clustal X 2.0 program and the putative molecular weights of the deduced proteins were analyzed by bioinformatics tools. To our knowledge, this is the first report on

isolation of DREB2A gene in grapevine (*Vitis vinifera* cv. 'Askari') and the study indicated that DREB2A may play a regulatory role in drought stress tolerance.

## CO-16

### Identification of candidate genes involved in *Phylloxera* resistance in grapevine rootstocks

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Phylloxera (*Daktulosphaira vitifoliae*) is one of the major pests in viticulture causing increasing damage in ever-growing infestations worldwide. The rootstock cultivar 'Börner' (*Vitis riparia* x *Vitis cinerea*) and two of its siblings are the only commercially available rootstocks that are completely resistant against Phylloxera. Several years ago a project was initiated at the Geisenheim Research Center to investigate the underlying resistance mechanism. The chronological and spatial events leading to the formation of necroses on leaves and roots in 'Börner' were analysed in detail by histological and biochemical analyses, and light and electron microscopic studies demonstrated that the formation of those necroses is the result of a hypersensitivity reaction (HR) including programmed cell death (PCD). Using cDNA subtraction and GeneFishing in 'Börner' roots inducted by IAA and analysed at different times post induction, differential expression of several genes putatively involved in phylloxera resistance was established. These genes include stilbene synthase, quinone reductase, elongation factor, resveratrol synthase, zinc-finger protein, auxin- and ethylene responsive GH3-like protein genes and genes involved in the oxidative burst. Furthermore, DNA microarray analysis of subtracted cDNA populations using a heterologous system (*Arabidopsis thaliana*) yielded more than 600 genes differentially expressed at various times after induction. These included signal transduction genes (14%), DNA/RNA transcription associated proteins (18-19 %), transport proteins (8-10 %), defence, pathogene response and senescence related proteins (9 %), while others were of unknown function (9-10%). Recently, we began the quantitative analysis of differentially expressed genes by advanced microarray techniques using 'Geniom one' (febit AG) oligo-DNA custom chips harbouring more than 450 relevant entries of the Vitis UniGen databank and a number of pathogen response-related genes of *A. thaliana*. Using this method, 25 up- and 7 down-regulated genes could be identified. Differential expression of several of these candidate genes has been validated by quantitative RT-PCR. The functional analysis of candidate genes by silencing (antisense) and overexpression is in progress.

## CO-17

### A mapping approach to defining the sex chromosomes in the dioecious species *Actinidia chinensis*

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All species recorded in the genus *Actinidia* display XX/XY female/male sex chromosome dimorphism. The sex chromosomes, however, have not been amenable to characterization because of the high chromosome number, 2x = 58, and their very small, and similar, size. Two species, *A. deliciosa* and *A. chinensis* produce the green and gold kiwifruit that are major horticultural crops in New Zealand. Dioecy in the genera not only has implications for orchard management, but also in breeding programmes aimed at producing new and novel cultivars for the commercial market. Toward a greater understanding of sex determination, we have developed a genetic linkage map in a population from an intraspecific cross of diploid *A. chinensis*. Dinucleotide microsatellites from expressed genes were the marker type of choice being co-dominant, highly polymorphic and frequently occurring in *Actinidia*, all attributes that would be expected to increase marker utility within and across species. Flower phenotype was mapped, together with sequence-characterised amplified region (SCAR) sex-linked markers, to identify the linkage group corresponding to the sex chromosomes. A number of EST-derived microsatellite markers, indicative of functional genes, mapped in the same vicinity, but, because of a lack of recombination in this region, marker order was difficult to determine. BACs were selected from a library constructed from the female

parent of the mapping population with the sex-linked markers. These were used to confirm the position of the sex-determining locus by Fluorescent in situ hybridisation (FISH). BAC contigs of this region are being assembled so that the order of markers, and their distance apart, is defined in a physical map. This map will be a tool for cloning and sequencing, and for studies of gene function, as BLAST searches classified several of the genes mapping to the sex-determining region as of 'unknown function'. Additional allelic information is being obtained for markers in the region from the use of judicious backcrosses with the parents, progeny, and close relatives of the original mapping population.

## CO-18

### Adventitious shoot regeneration in almond: a stressful condition seen from the molecular aspect

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The use of genetic engineering in woody fruiting species is often hampered by their reduced regeneration plasticity. In almond (*Prunus dulcis* Mill.) protocols for regeneration of transgenic adventitious shoots were already established, but the efficiency of the regeneration system is still the limiting step. In this system we can define two main stages of organogenesis, the 'early stage' (deddiferentiation) and the 'late stage' (redifferentiation). The fact that developmental processes occur in specialized tissues or cell types makes gene expression studies very appellative. Aiming to identify key genes involved in these two stages of organogenesis, we have used induced leaves of almond micropropagated shoots and a suppression-subtractive hybridization strategy to build cDNA libraries from each of the two time-frames. cDNA clones from both libraries were randomly picked, amplified by PCR and arrayed on glass slides. A total of 128 cDNA clones were found to be differentially expressed with (over 2-fold), being 58 expressed in the early stage and 70 expressed in the late stage. The sequenced cDNA clones represent 92 unique contigs. Expression profiling and bioinformatic analysis revealed, in the early stage, differential expression of genes encoding proteins involved in transcription or related to protein synthesis and fate, nitrogen and carbon metabolism. In contrast, in the late stage, we found a differential expression of genes coding for stress-induced proteins. The putative involvement of these genes in almond adventitious shoot development, points for a possible link between stress and adventitious organogenesis. Quantitative RT-PCR (*Q*RT-PCR) experiments confirmed the reliability of the micro-array data and allowed quantitative expression studies along the whole process of adventitious shoot induction.

## DO-01

### Recent progress in use of biotechnology to improve important traits in temperate fruit crops

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Many successes have been reported of experiments in the laboratory, growth chamber and greenhouse, in which many traits of temperate fruit crops have been improved using genetic engineering (GE). However there are fewer reports of these improvements being carried through to field trials, and none of progress to commercial utilization. As yet our temperate fruit crops have not benefited from GE to the same extent as the tropical fruit papaya and the temperate vegetable squash, of which 50% and 25%, respectively, of the U.S. crop are GE for resistance to virus diseases. Nevertheless, a GE plum pox-resistant plum cultivar has partial approval for commercial use in the U.S. Among fruits, the most traits altered by GE and confirmed in a field trial have been reported for apple, and include: self-compatability, delayed softening and sorbitol content of fruits, non-browning of cut fruit, resistance to fire blight, and increased rooting of rootstocks. Coat protein-gene transgenic virus resistance is the trait most frequently reported for other GE fruit crops, including cantaloupe, grapefruit, grapevine, lime, orange, raspberry, strawberry, walnut and watermelon. The *Bacillus thuringiensis* cry1Ac protein has been used to improve insect resistance of walnut, strawberry and apple, which has also been field trialed as a trap crop to protect

adjacent walnut trees from codling moth damage. Heterologous transgenes have been trialed for resistance to fungal or oomycete diseases in apple, grapevine, kiwi, lemon, olive, orange, papaya, plum, raspberry, and strawberry, and to nematodes and bacterial leaf blight in walnut. Delayed fruit softening by suppression of ethylene level has been field trialed in apple, papaya and strawberry. Other traits including parthenocarpy in watermelon, *Xylella* resistance in grape, herbicide resistance and altered flowering time in strawberry and orange, modified fruit aroma in orange, and compact growth in citrange have been field trialed. Information about the results of many of the field trials have not yet been reported in the literature, and it is difficult to determine how successfully some of the targeted traits have been improved. However some traits have clearly been improved by GE and probably will be commercialized in the next decade.

## DO-02

### Performance and long-term stability of the barley hordothionin gene in transgenic apple cultivars: scab resistance monitored in a 4-year field trial

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Transgenic apple lines were produced by *Agrobacterium*-mediated transformation of the cultivars Gala, Golden Delicious and Elstar. The transgenic lines were characterized by PCR analysis and lines confirmed in containing the barley hth-gene were rooted and transferred to soil in the greenhouse. Scab bioassays were performed on primary transgenic lines and the year after on a selection of individual clones that was propagated by grafting on rootstocks. Seven lines (2 Elstar and 5 Gala) showed a highly significant reduction in susceptibility compared to the controls. Subsequent Southern analysis confirmed integration of 2-7 copies of T-DNA and RT-PCR and Northern analysis showed expression of the hth-gene. One Elstar line was found to contain vector backbone sequences and could not be incorporated in the field trial. The remaining six transgenic lines were propagated and grafted on rootstocks as were the controls, i.e. non-transgenic Elstar and Gala, transgenic Gala without the hth-gene and resistant Santana. Fourty individuals of each of the 10 lines were planted in a field plot in 2003. Scoring scab incidence in four consecutive years from 2004-2007 demonstrated that four out of six GM lines showed a significant reduction in scab symptom development, 60% in the best performing line. This observation was consistent over the entire four years. A few lines showed morphological aberrations. In conclusion, the barley hordothionin gene can be used to alleviate scab disease pressure. Combination with apple scab resistance genes might produce more durable resistance.

## DO-03

### Functional characterisation of the native promoter of the apple scab resistance gene *HcrVf2*

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Apple scab, caused by the fungus *Venturia inaequalis*, is the most damaging disease of commercial apple orchards. Recently the first scab resistance gene derived from the *Vf*-region of the wild apple *Malus floribunda* 821, *HcrVf2*, was cloned. The gene encodes a receptor like protein and has been shown to confer scab resistance in a transgenic susceptible cultivar, when controlled by the CaMV35S promoter. In order to minimize non-plant DNA in genetically modified apple and to go a step forward to the development of cisgenic apples, we have used the *HcrVf2* gene along with sequences from its own promoter. Three gene-constructs containing progressive 5`deletions of the promoter region (115, 288, and 779 bp) and the *HcrVf2* gene were used to transform the scab susceptible apple cultivars ‘Gala’ and ‘Elstar’. Several transgenic lines were regenerated and the influence of the lengths of the promoter region on resistance to *Venturia inaequalis* was analysed. Highly scab resistant 'Elstar' and 'Gala' plants were obtained, indicating that the *HcrVf2* gene controlled by its native promoter is effective in conferring

resistance to *Venturia inaequalis*. Comparisons of promotor length, strength of expression and resulting resistance to *Venturia inaequalis* will be presented.

## DO-04

### Stilbene synthase gene transfer resulted in down regulation of endogenous chalcone synthase in strawberry (*Fragaria* x *Ananassa*) and led to the identification of novel phenyl-propanoid glucosides

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Modification of plants with the stilbene synthse (*STS*) gene has often resulted in novel production of the phytoalexin resveratrol, and provided enhanced resistance against several pathogenic fungi. In the present study, a grapevine *STS* expressing strawberry (*Fragaria* × *ananassa*) was investigated for the effect of the transgene by techniques including quantitative real-time PCR, UPLC-qTOF-MS profiling and NMR analysis. The results indicated that the introduced *STS* gene caused down regulation of the strawberry endogenous chalcone synthase (*CHS*), which was shown as diminished transcript levels. The perturbation of the gene expression was reflected at the metabolite content, as the CHS enzyme downsteam products, mainly flavonols, were found at reduced levels concomitantly with the accumulation of the precursor molecules like phenolic acid derivatives. In addition, several previously unidentified metabolites were accumulating at the leaves of the transgenic strawberry, and were subjected to structural elucidation by NMR analysis accompanied by UPLC-qTOF-MS/MS. The analysis revealed a metabolite group, phenylpropanoid glucosides, that has not been reported previously in strawberry. A detailed metabolite analysis was pertinent for the detection of unintended consequences of the gene transfer, and eventually provided deeper insight in the phenolic compound metabolism of strawberry.

## DO-05

### The development of genetic resistance to plum pox virus in transgenic *Nicotiana benthamiana* and *Prunus domestica*

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Plum pox virus (PPV) is a member of the genus *Potyvirus* in the family *Potyviridae*. It is the causal agent of the most devastating viral disease on many stone-fruit spp., such as plum, peach, nectarine, apricot and cherry. The viral disease, known as Sharka, was found in Europe over a hundred years ago. Recently, it has been found in the United States and Canada. Genetic resistance is considered the most effective means to control PPV. Despite over 60 years of extensive screening for germplasms resistant to PPV, few resistant resources have been identified. Here we reported engineering genetic resistance against PPV through the hairpin-mediated RNA silencing (RNAi) approach. Two highly conserved regions of the PPV genome corresponding to portions of viral RNA coding for P1 and CP, respectively, were cloned into a plant transformation vector under the control of the double *Cauliflower mosaic virus 35S* promoter as inverted repeats spanned by an intron from the peach *endo-polygalactuoranase* genomic DNA. These two constructs (pAWp1 and pAWcp) were initially tested in *Nicotiana benthamiana*. Over 50 transgenic lines transformed with pAWp1, pAWcp and an empty plasmid pAWck (as a control) were generated. The transgenic *N. benthamiana* plants were mechanically inoculated with PPV. ELISA and RT-PCR analysis revealed that 49.1% of pAWp1-derived lines and 41.2% of pAWcp-derived lines were PPV-negative and 100% of control lines (transformed with pAWck) showed PPV-positive. The pAWp1 plasmid was used to transform plum (*Prunus domestica* L.). Transgenic plum plants were challenged

with PPV by chip bud inoculation. PPV was detected in all the pAWck-derived plum lines but was not detectable in 50% of pAWp1-derived transgenic lines. Two species of PPV-specific small interfering RNAs, a hallmark of posttranscriptional gene silencing (PTGS), were present in the resistant plum lines, suggesting PTGS is responsible for resistance. The PPV-resistant transgenic plum generated in this study provides an alternative resistant source for the control of PPV.

## DO-06

### Genetic transformation of somatic tissues and molecular biology approach for improving resistance to sharka disease of plum (*Prunus domestica* L.) varieties

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Plum Pox Virus (PPV), causal agent of Sharka disease, currently is classified by US and EC plant quarantine agencies as the most injection pathogen in apricots, plums and peaches. However there are no sources for PPV resistance among the species sexually compatible to plum cultivars. The transformation of plants with viral genes, such as coat protein, can provide novel virus resistant varieties or gene resources for breeding new resistant varieties. The most limiting factor in the plum genetic transformation is the very pour regeneration and transformation efficiency of plum somatic tissues. The efficient protocol for plum plant regeneration and genetic transformation has been developed. Plum (*P. domestica* L.) scion cv."Startovaya" was transformed using *A. tumefaciens* strain CBE21:pCamGFP carrying the hygromycin phosphotransferase gene (*hpt*) *gfp* gene; pBin-mGFP5-ER carrying neomycin phosphotransferase gene (*npt*II) and *gfp* gene; pNOV35S-GFP contains *gfp* gene driven by the 35S promoter and *pmi* gene (phosphomannose isomerase gene derived from *E.coli*) under the CMPS promoter from Cestrium Yellow Leaf Curling Virus. Transformation experiments resulted in ten *pmi,* five *npt* and thirty five *hpt* transgenic lines. Hygromycin as a selective antibiotic was more effective for plum transformation (2.2%) than "antibiotic-free" selection method with the *pmi* gene. Kanamycin based selection demonstrated worse transformation efficiency.To improve plum cultivars resistance to PPV we used coat protein gene expression and RNAi phenomena. Using the developed protocols leaves of cv."Startovaya" were transformed using *A. tumefaciens* strains AGL0:pCamPPVcp with gene of PPV coat protein in sense orientation and *hpt* selective marker and AGL0:pCamPPVRNAi carrying *hpt* and *gus* genes and self-complementary sequences of fragment of PPV-CP gene separated by an intron to produce a "hairpin" RNA (hp-RNA) structure. Regenerated plums on hygromycin media were analyzed by GUS assays and by PCR detection of the PPV CP gene.

## DO-07

### Transcriptomic and proteomic response of fruit trees to low temperature and drought stress

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Together, temperature and water availability are the primary determinants of the global distribution of major vegetation biomes and as such have a major impact on the cultivation of temperate fruit trees. The regulation of both low temperature and water deficit stress has been widely studied in herbaceous plants using transcriptomics, proteomics, and transformation technologies. These studies have revealed stress signaling pathways, specific stress-tolerance genes, and transcriptional regulators. Using direct data or empirical approaches, biotechnology has been utilized to produce transgenic plants that have greater stress tolerance. For example, plants overexpressing the transcription factor CBF (under the control of a low-temperature-inducible promoter) have increased freezing tolerance. However, only recently, have these same approaches been used to study stress tolerance in woody plants and more specifically fruit trees. Evidence suggests that although there is a high level of conservation in mechanisms of stress tolerance between annual herbaceous plants and perennial woody plants, the perennial habit has also

resulted in additional mechanisms that are specific to perennial plants. We have utilized several different global approaches to study stress tolerance in apple and peach. These include subtractive hybridization (SSH), bioinformatics analysis of ESTs derived from stress-induced cDNA libraries, and 2D Difference in-Gel Electrophoresis (DiGE) for proteomic analyses. These approaches are beginning to reveal the complexity of stress response in fruit trees and helping us develop a comprehensive understanding of stress tolerance in fruit trees. An overview of our results will be presented including: a comparative EST analysis of stress and drought response in apple xylem, bark, leaf, and root tissues in response to low temperature and water deficit, comparative expression analysis of CBF and dehydrin gene expression in bark and leaf tissues of apple and peach, and a proteomic analysis of drought response in apple and peach. Additionally, data on increased stress tolerance in transgenic apple overexpressing a cytosolic, superoxide dismutase (SOD) gene will also be presented.

## DO-08

### Transgene technology for developing salt tolerance in tomato

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Salt tolerance is a complex trait involving response to cellular osmotic and ionic stresses and their consequent secondary stress and whole plant coordination. We have attempted to develop abiotic stress tolerance in tomato in order to grow the crop in drought prone areas. Tomato (*Lycopersicon esculentum* Mill.) is a highly nutritive vegetable crop. It ranges 3$^{rd}$ among the vegetable crops. Tomato is rich in vitamins A & C and fiber and is also cholesterol free. Tomato contains approximately 20-50 mg of lycopene / 100 gm of fruit weight which is the most powerful antioxidant in the carotenoid family and it protects humans from free radicals. Though it has importance in daily food, as vegetable and medicine, the crop can not be grown in drought prone areas. In view of the importance of tomato, molecular cloning was done by using antiporter gene (AtNHX1) with stress inducible promoter (SIP) and constitutive promoter CaMV35s with bar gene as a selectable marker gene by replacing hpt gene in the p $^{cambia}$ 1300/1301 cassettes. Two different vectors ($p^{MS}$ & $p^{MEX}$) were developed to use in the genetic transformation experiments for engineering abiotic stress resistance. Genetic transformation studies have been carried out by using *Agrobacterium tumifaciens* LBA 4404 harboring cloned $p^{MS}$ (with SIP 1314 SWAP2 + Antiporter Gene) and $p^{MEX}$ (with CaMV 35s +Antiporter gene) separately in tomato cv PKM-1 to find out and compare the abiotic stress tolerance based on the cloned vectors. The PPT resistant / transformed shoots were rooted on MS +0.1 mg/I NAA. The putative transfomants / transgenic plants were identified by using different types of molecular techniques viz. PCR, RT-PCR, Southern and Northern blot. Two transgenic lines were identified (AtNHX1 with 35s & AtNHX1 with SIP 1314 promoter). Expression of these two promoters was studied in relation to different physiological parameters. They are: total proline content, $Na^+$, $Cl^-$ ion concentration in old and young leaves, roots, fruits, Sugar levels and also $K^+$ ion content. Thus, various aspects of these promoters in relation to abiotic stress in tomato will be presented.

## EO-01

### Fruit set regulating genes are differently expressed after cross pollination, self pollination and treatment with plant growth regulators in *Malus* x *domestica* cv. jonagold

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Currently, the processes involved in fruit set are not well understood. To investigate this, we have studied the expression of genes differentially regulated following either self-pollination, self-pollination in combination with plant growth regulators (PGRs) or cross-pollination in the apple cv ‘Jonagold’.

Jonagold is a triploid apple cv with an irregular bearing pattern due to inadequate pollination and fruit set. The different types of pollination in our experiment lead to differences both in initial fruit set and fruit numbers at harvest. We randomly sampled lateral flowers/fruitlets from terminal buds at several time points during the fruit set process and these samples were used for determination of fruitlet cell number and cell size by SEM. These parameters were chosen because they are related to sink strength which is one of the main driving factors behind fruit set. In parallel we studied the expression of genes involved in auxin and cytokinin production and signaling by RT-PCR, and in addition measured auxin, cytokinin and gibberellin concentrations of the tissue sampled. Results revealed differences in the expression levels and timing of auxin and cytokinin genes as well as genes related to cell division and growth. Higher expression levels of auxin and gibberellin genes were found following cross-pollination compared to self pollination, while self-pollination combined with the application of PGRs led to an intermediate situation. These molecular differences were also supported by the results from the hormone analyses, although patterns were sometimes complex due to the presence of multiple forms (precursors, inactive and active forms). The molecular data were also supported by the observations on cell number and cell size, which increased more in time in cross-pollination compared to self-pollination. This early fruit development took predominantly place in the hypanthium. Based on the results, we argue that cell growth and hormone gene expression markers offer reliable molecular tools to evaluate the success of the fruit set process.

## EO-02

### Expression analysis of apple *Floricaul/Leafy* genes

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The apple *AFL* (apple *FLORICAULA/LEAFY*) gene promoter (the 5' upstream region, about 2.5 kb) linked β-glucuronidase (GUS), *AFL1*p:GUS or *AFL2*p:GUS, was performed in apple root stock JM2. The JM2 was constantly transformed with high ratio and could be manageably propagated. Both the *AFL1* and *AFL2* promoter GUS clearly displayed staining at the meristems of the shoot apexes, lateral axils and leaf primordia on vegetative culture shoots. The culture shoots were induced root and potted up for two years. The potted transformants with *AFL2*p:GUS showed same tissues staining at vegetative meristem. This suggested that the regulation of *AFL2* was not affected by the growth condition. The expression of them were quantified by real-time PCR method, it revealed that the expression of *AFL2* was much higher than that of *AFL1* at all organs. But, the expression of *AFL1* showed drastic induction on floral buds at the transition of vegetative to reproductive phase compared with *AFL2*. Both expressions of floral buds showed much higher than vegetative shoot (seedlings of three month old and watersprouts from a trunk of 30 year old). *In situ* hybridization showed slight complex results. The *AFL1* and *AFL2* expressions were detected at the meristem of the shoot apexes and leaf primordia in watersprouts and culture shoots, these pattern was almost same as the GUS staining of the *AFL* promoter. But in the seedlings *AFL2* expression was detected only at the leaf primordia. These results demonstrated that each approach make up for the analysis of *AFL* gene expressions. Then it suggested that the *AFL* genes involved in the transition of flowering and vegetative growth including leaf morphogenesis in apple.

## EO-03

### The switch to flowering: genes involved in flower induction of the apple cultivar 'pinova' and the role of the flowering gene *MdFT*

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Meristems in apple have to pass two developmental phases before they can produce flowers: the vegetative and the reproductive phase change. Meristems of seedlings finish the juvenility and reach the adult stage during the vegetative phase change. Then flower induction activates the reproductive phase change and vegetative meristems change to reproductive meristems. These processes are of high economical importance in fruit production as well as in fruit tree breeding. Accelerated phase changes result in precocious flowering which is an important breeding goal. In the previous ten years several genes of apple were identified which are homologous/orthologous to flowering genes of *Arabidopsis thaliana*. In relation to results obtained in *A. thaliana* we realized mRNA expression analyses for selected apple genes which are potential canditates to be involed in the vegetative and/or reproductive phase change.

The flowering genes *MdCOL1*, *MdCOL2*, *MdFT*, *AFL1*, *AFL2*, *MdMADS5*, *MdTFL1-1* and *MdTFL1-2* were isolated from genomic DNA of the apple cv. 'Pinova'. Based on the obtained sequences, Real-time PCR protocols were established for these genes as well as for *MdMADS2* and *MdSOC1*. Using these protocols we found that the mRNA expression of *MdFT*, *AFL2* and *MdTFL1* in one-year-old apple seedlings of 'Braeburn' x 'Pisaxa' was low in old leaves and drastically increased in younger leaves next to the shoot apex. Furthermore, we showed that in adult apple trees meristems of terminal buds chanced to the reproductive phase at the end of May. It was shown that *AFL1*, *AFL2*, *MdMADS2*, *MdFT*, *MdSOC1* and *MdTFL1* were involved in this process.

Based on the recently published results that *FT* from *A. thaliana* seems to be the long searched florigen, we examined the function of the apple homologous gene *MdFT*. We produced transgenic *A. thaliana* and apple lines which over-expressed *MdFT* under the control of *CaMV 35S* and the phloem specific promoter *Suc2*, respectively. Preliminary results indicate that the transgenic A. thaliana showed an accelerated flowering. The effect of transgenic over-expressed *MdFT* on other flowering genes of apple was examined using Real-time PCR.

## EO-04

### A new addition to the buffet

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Today, we are seeing more fresh-cut fruit and vegetables being consumed as people seek to replace unhealthy snacks foods with healthier fruits and vegetables. Fresh-cut produce has been available since the 1980's with fresh-cut carrots leading the way; but thus far, fresh-cut fruit has been limited to melons and berries and other fruits that did not suffer enzymatic browning, with the quintessential apple lagging behind in the marketplace. Fresh-cut apple products cannot be prepared without antioxidant treatments that contribute significantly to the cost of the end products and can result in some off-flavoring. Okanagan Specialty Fruits has pioneered the development of the first truly non-browning apple varieties. Down regulation of the multi-gene PPO family through expression of a single chimeric PPO suppression gene results in marked decrease in total PPO Activity and confers a non-browning phenotype. Four years of field testing has confirmed the stability and function of the non-browning trait. Now, Okanagan Specialty Fruits in conjunction with commercial and academic collaborators are developing an improved commercial platform for delivery of minimally modified non-browning apple varieties. Future apple varieties will incorporate all the benefits of the non-browning trait and will be delivered on a platform

carefully designed to ensure consumer acceptance and freedom to operate. Our apple varieties are indistinguishable from the established commercial varieties they were derived from and can be immediately substituted to the benefit of all stakeholders. Consumers will be drawn to a non-browning apple for its appeal and convenience. Producers, packers and retailers will benefit from less superficial bruising which translates into less shrinkage and enhanced display appeal. Fresh-cut apple producers realize dramatically reduced production costs associated with antioxidants treatments. Traditional processors will be able to produce tastier, truer colored apple juices and sauces.

## EO-05

### Identification and characterization of flavonoid biosynthesis related transcription factors in strawberry

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Strawberry fruits contain a high level of flavonoids, which are known for their health beneficial properties. In ripe strawberry fruits the red colored anthocyanin is the most abundant flavonoid, while in unripe green fruits mainly so-called proanthocyanidins (PA's) (or condensed tannins) are found. Both anthocyanins and PA's are end-products of the flavonoid biosynthesis pathway and in strawberry most of the genes encoding the enzymatic steps in this pathway have been identified. However, almost no information is present on the regulation of the expression of these structural genes. In plants specific Myb and bHLH transcription factors (TF's) are known to modulate the expression of flavonoid biosynthesis genes. In *Arabidopsis*, such TF's have been identified which specifically modulate the expression of genes important for PA synthesis in seeds. Overexpression of these *Arabidopsis* transcription factors in transgenic strawbrry resulted in a change in flux in the flavonoid pathway in ripe fruits, leading to increased levels of PA's and reduced levels of anthocyanin. Using these *Arabidopsis* TF's as bait in a yeast-2-hybrid screening, and using the *Arabidopsis* TF gene sequence information for degenerate PCR, we isolated different Myb, bHLH and WD40-repeat TF's from green and red strawberry fruit cDNA libraries. These strawberry TF genes show homology to flavonoid biosynthesis related TF genes found in amongst others grape and apple and show a gene expression pattern that coincides with flavonoid accumulation during strawberry fruit development. Further characterization of the strawberry TF's involved TF-TF interaction studies using a yeast-2-hybrid assay and a mutant complementation analysis using *Arabidopsis* lines mutated in the PA-related transcription factors. Next to this, TF activity analysis by transient transformation of *Nicotiana tabacum* leaves was performed. The possible role of the identified strawberry TF's in flavonoid and in particular in PA biosynthesis will be discussed.

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## EO-06

### Understanding the molecular basis for stone formation in *Prunus* species.

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A unique aspect of *Prunus* fruit is the presence of a hard wood-like carapace called the stone surrounding the seed. The stone represents a somewhat unique biological adaptation that presumably protects the seed from stress and/or pathogens. We have begun biochemical and functional genomic studies on stone formation to elucidate its biological function and identify gene targets useful for engineering pitless fruit. Phloroglucinol-HCL staining revealed that the endocarp layer accumulates lignin starting from the blossom end of the fruit around 7-10 days prior to hardening. Derivatization followed by reductive cleavage (the so-called "DFRC" method) showed that peach stones contain nearly 50% lignin, more than any other woody material examined to date. To identify genes and pathways associated with stone development we conducted expression profiling studies using a developing peach fruit series prior to ripening. Total RNA was extracted from each sample, labeled, and hybridized to 2 different microarray

platforms: 1) labeled cDNA from 4 developmental time points was hybridized to a custom printed peach fruit 5K oligoarray and 2) labeled cDNA from 7 time points was hybridized to a 15K apple oligoarray. Results showed that the phenylpropanoid (PP), lignin, and flavonoid pathways are strongly induced in peach fruit prior to ripening. A subset of PP and lignin pathway genes was induced specifically during stone hardening. qPCR analysis of dissected fruit revealed that these genes are endocarp specific. In contrast, a number of lignin and flavonoid genes were induced throughout the fruit and showed evidence of co-regulation. Comparison of these expression profiles to apple and *Arabidopsis* expression data revealed that flavonoid pathway induction in early fruit is highly conserved while induction of specific PP and lignin pathway genes appears to be specific to *Prunus* species.

## EO-07

### Plant size control of apple through genetic transformation

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Dwarfing trees are commonly used in modern apple (*Malus domestica*) production for achieving high production efficiency. The most commonly used dwarfing method is grafting cultivars on dwarfing rootstocks. Production of new cultivars or rootstocks with dwarf characteristics has been an important goal for apple breeding. Genetic improvement through traditional breeding is inefficient due to a long life span and heterogeneous genetic background of apple trees. Genetic engineering is an attractive complement method to conventional breeding. Plant height can be regulated by introduction of different genes into the plant genome. The *rol* genes, isolated from *Agrobacterium rhizogenes*, especially the *rolC* gene, have been tested in dwarfing plants in some species including apple. The mechanism underlying the function of rol genes is still largely unknown. Since plant height is essentially regulated by endogenous phytohormone gibberellins (GAs), the alternation of GA biosynthesis pathways is a direct way to control plant size. A number of genes involved in GA biosynthesis and deactivation enzymes have been cloned and have shown to reduce the plant size in some plant species by either overexpression or down-regulation of these genes. The mutant *Arabidopsis gai* (gibberellic acid insensitive) has been shown to reduce the plant height in some species. The wild type *GAI* gene encodes a protein (GAI) containing features that are characteristic of transcription factors. The *gai* allele encodes a mutant protein, lacking 17 amino acids near the amino terminus, which is thought to confer the altered gibberellin responses characteristic of the *gai* mutant. Genetic analysis indicates that the GAI protein is a repressor of GA responses and endogenous or exogenous GAs can release this repression. Aiming at reducing the plant size and obtaining compact phenotypes, we have introduced the *rolB*, *rolC* and *gai* genes into apple cultivars and rootstocks by *Agrobacterium*-mediated transformation method. The transgenic plants have been evaluated in the greenhouse. The dwarf effect of the three genes on apple is very obvious in some transgenic clones. The use of other GA genes and effects of promoter on transformation efficiency are discussed.

## EO-08

### Biotechnological approaches for fruit crop improvement in korea

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Korean fruit industry has been sustained by the diversity for species and cultivars originating temperate and subtropical fruit crops. Apple has been the most important cultivated fruit species in Korea. For that reason, 'Fuji' apple has been centralized for biotechnology research of fruit trees by a public sector, National Horticultural Research Institute, since early 1990s. The major achievement in the research on genetic engineering for fruit trees especially in apple in Korea include: (1) Development of efficient

regeneration systems, (2) Genetic transformation, (3) Identification and characterization of genes. To facilitate the breeding of new cultivars in fruit trees, full understanding of genetic information is preliminary. Development of molecular markers and genetic map construction for fruit trees are still at early stage in Korea. Targets of genomic study for fruit trees are focused on increasing selection efficiency (MAS) and understanding genetic events. Most of results from fruit tree genomics in Korea are restricted to qualitative traits in Rosaceous family. Development molecular markers to use for early selection were linked to flesh adhesion gene *F*, pollen sterility gene *ps*, fruit acidity gene *D*, columnar growth habit gene *Co* in peach and apple. Molecular maps were also constructed using inter-specific cross populations between domestic and wild species in pear and apple.

## FO-01

### Environmental risk assessment of gm plants at the European level

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The following topics will be included in the talk: (i) GMO Regulation in the EU, (ii) Role of the European Food Safety Authority (EFSA), (iii) Environmental Risk Assessment, (iv) Future Developments and (v) Biosafety Research. The European Food Safety Authority (EFSA) is the keystone of EU risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and clear communication on existing and emerging risks. The EFSA Panel on genetically modified organisms provides independent scientific advice on the safety of (i) GMOs such as plants, animals and micro-organisms, on the basis of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and (ii) genetically modified food and feed, on the basis of Regulation (EC) No 1829/2003 on genetically modified food and feed. The GMO Panel carries out risk assessments in order to produce scientific opinions and advice for risk managers. Its risk assessment work is based on reviewing scientific information and data in order to evaluate the safety of a given GMO. This helps to provide a sound foundation for European policies and legislation and supports risk managers in taking effective and timely decisions. In the public debate on the biosafety of genetically modified organisms, the results and even the existence of GMO biosafety research are often ignored. As a consequence, the already established stable basis for a science-based discussion on GMO biosafety is not fully explored. Therefore, a main prerequisite to improve the science-based risk assessment and the science-based decision-making for placing GMOs on the market is to focus and strengthen the voice of GMO biosafety research. The International Society for Biosafety Research (ISBR) plays a key role in this process.

## FO-02

### Combining early flowering gmo's and application of molecular markers is effective to speed up the breeding cycle in apple

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Recently, it was several times reported that the juvenile stage of apple *Malus domestica* BORKH. could be efficiently reduced by genetic engineering. Overexpression of a MADS-box gene from silver birch induced early-flowering in apple (Flachowsky et al., 2007). The T0 generation of apple plants developed first flowers *in vitro* within a few weeks after transformation and continued flower production in greenhouse. Such a dramatic reduction of the juvenile stage is impossible by using alternative floral inducing techniques like training of shoots, optimized environmental conditions or grafting onto rootstocks. Transgenic early- flowering trees seem to be a powerful tool to speed up the breeding cycle in early stages of a breeding program. In later stages, the flower inducing transgene can be removed by out-crossing. A genetically improved, but still non-transgenic plant will be obtained as a result of this technique. We started a proof of concept to demonstrate the feasibility of a breeding program based on the combination of transgenic early-flowering apple trees and the use of molecular markers for selection in progenies. Transgenic early-flowering greenhouse plants were pollinated with pollen of the fire blight

resistant wild species *Malus fusca* under greenhouse conditions. Fruits were harvested in fall of the same year when plants were transferred from the *in vitro* culture to the greenhouse. Seeds were sown in spring next year after vernalization. All seedlings were screened by PCR analysis on the presence of the transgene. Transgenic early-flowering seedlings of the T1 generation were selected, grown up and they developed first flowers within a few weeks after seeding. These flowers were directly pollinated with pollen of the scab resistant cultivars 'Topaz' and 'Antonovka', respectively. The fruits of these crosses were harvested in fall of the second year in greenhouse. The seeds were sown in spring and seedlings of the T2 generation were screened on the presence of the flower-inducing transgene and the natural scab resistance genes *Vf* and *Va*, respectively by PCR analysis. The results of this prototype for a breeding program will be presented and problems as well as strategies for future research will be discussed.

## FO-03

### The use of genetic engineering for the improvement of stone fruit virus resistance as a model case for the success and challenges of this technology in fruit tree species

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Sharka disease caused by Plum pox virus (PPV) is the most important virus disease of stone fruits (peaches, nectarines, apricots, cherries). Since the first report of PPV from Bulgaria in the early 20$^{th}$ century the virus has invaded virtually the entire European continent and has been spreading world-wide to countries including the USA, Canada, China, Egypt, Chile, and many others. Millions of trees have been destroyed. Tree removal is the only means of controlling the disease. Resistant varieties are a critical need but there are few sources of resistance in stone fruit genetic resources and few resistant varieties have been produced nearly a century after discovering this disease. Recent advances in molecular biology and genetic engineering have allowed for the development of a genetically engineered (GE) plum highly resistant to PPV with almost 20 years of research including a 10 year plus history of field tests documenting the effectiveness and safety of this new variety, 'HoneySweet'. Despite these results and the record of success and safety of GE virus resistant papaya and squash, regulatory and acceptance challenges to the approval and deployment of this plum remain. A timeline of research progress, regulatory submissions and approvals and the road ahead will be presented. This will serve as an example to highlight the challenges facing the use of biotechnological approaches for the improvement of specialty crops at a time when these technologies are needed to face important problems such as climate change, population growth, environmental degradation, and the need for improved nutrition.

## POSTER

### AP-01

**Cryopreservation of strawberry cultivars by droplet vitrification**

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Droplet-vitrification method was applied to shoot tips of micropropagated strawberry cultures *Fragaria ×ananassa* DUCH. cv. 'Senga Sengana', 'Korona' and 'Aroma'. Shoot tips of 2 -3 mm in size were precultured in sucrose enriched media for 24 and 48h. Subsequently, shoot tips were transferred into 6µl droplets of PVS2 vitrification solution for 20 min and plunged into liquid nitrogen. The effect of sucrose concentration (0.1, 0.25, 0.5, 0.75, 1.0M) used in preculture of the shoot tips of strawberries regarding their regrowth, histological changes and the morpholocical stability of the regenerants in the field was analyzed. After freezing, the highest recovering rate in all cultivars (60%) was achieved after a preculture with 0.25 M sucrose for 24h. Starch has been accumulated in meristematic cells after preculture in sucrose. Sucrose concentration of 0.75 M, however, caused already plasmolyses, damaging the cells before PVS2 incubation and liquid nitrogen treatment. From the recovered shoot tips only up to 60% continued with shoot development. However, these shoots started to multiply considerably enhancing the number of plantlets ready to transfer in the acclimatisation. For field evaluation 15 to 165 plants per sucrose concentration were evaluated. The number of off-types was affected by the sucrose concentration used in preculture.

### AP-02

**Cryopreservation of Lily (*Lilium ledebourii* baker bioss.) germplasm by encapsulation dehydration**

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*In vitro* conservation of germplasm is one of the best methods for long-term storage of valuable genetic resources of many crop and forest species. Over the past decades, plant cryopreservation technologies have been evolving rapidly. It is estimated that up to 100,000 plants are currently threatened or face extinction in the wild. Cryopreservation at ultra-low temperature (-196ºC) is a perfect method for the long-term conservation of plant genetic resources, since under these conditions, biochemical and physical processes are completely arrested. Cryopreservation of tissues can be successful only if intra-cellular ice crystal formation is avoided. The best method for reduction of cellular water is vitrification. Also encapsulation within alginate beads was shown to be beneficial to the technique. Lilium ledebourii (Baker) Bioss., is distributed in the Damash of Ammarloo and Kalchooleh of Dorfak areas (ca. 2100) of Guilan province in the north of Iran. This species is the rarest lily and very attractive. It is under careful surveillance. Seeds of lily were isolated from the ripe capsules and disinfected. For encapsulation seeds were suspended in MS medium supplemented with 3% (w/v) sodium alginate and 0.6 M sucrose. After 30 min seeds were picked individually and dropped into MS medium containing 100 mM CaCl2 (w/v) and 0.6 M sucrose for 30 min. Encapsulated and non- encapsulated seeds were desiccated in the air current of a laminar flow chamber for 1 h. For cryopreservation after dehydration samples were transferred into sterile polypropylene tubes which were directly immersed in liquid nitrogen (LN) for 1 h. Then frozen samples were thawed rapidly by placing the tubes into a water bath at 37ºC for 2 min and transferred directly to MS culture medium. Control seeds do not survive after LN treatment. The rate of viability in non- encapsulated seeds, pretreated with sucrose and dehydration was 25%. But if seeds are encapsulated in complement to sucrose and dehydration the survey is increased until 50%. To conclude,

encapsulation may be suitable to reinforce the tolerance of tissues. Also the encapsulation-dehydration technique has been continuously improved and applied to an increasing number of species.

## AP-03

### Application of cryopreservation technology for Pistachio germplasm conservation

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Pistachio (*Pistacia vera* L.) is a drought and salt resistant species that can be grown without irrigation in conditions where other crops can not be cultivated. So with other few species, pistachio opens this marginal lands to cultivation and provides significant income not only to the local breeders, but also to the country's economy. Although pistachio has an important economical value especially for Mediterranean countries where its largest cultivation is found, today it is under the threat of genetic erosion due to the destruction of natural habitats by severe anthropogenic pressures, such as land clearance, charcoal burning, over-grazing, and abandonment of local varieties with the specialization of pistachio orchards on a few commercial varieties. Therefore, it is very important to develop efficient biotechnological strategies for the safeguard conservation of pistachio biodiversity. Up to date, storage of plant germplasm in liquid nitrogen (LN) at -196ºC, which is termed as "cryopreservation", is the only viable technique that enables to maintain plant germplasm for a long time as it preserves the cells in ultra low temperatures that almost all metabolic and most physical and chemical processes are arrested. Hence in this study, experiments were conducted to develop and optimize efficient cryogenic protocols for the preservation of not only the seeds, but also the axillary buds of pistachio. By using dehydration-one step freezing technique, 90% germination was obtained with decreasing the moisture content of the seeds to 1l.7% (FW basis) via 8 hours drying in silica gel prior to the direct immersion in LN. As regards the long-term preservation of axillary buds of *in vitro*-grown pistachio shoots, vitrification-one step freezing technique was also tested with incubation of buds in PVS2 solution for various period of time (0, 30, 60, 90, 120 min) and different temperatures (0 and 25ºC) before immersion in LN. Following the optimization of the vitrification protocols, trials are now in progress for the development of effective cryopreservation protocols for pistachio axillary buds both by using the encapsulation-dehydration and the encapsulation-vitrification techniques.

## AP-04

### Researches regarding the behaviour of the romanian quince cultivar "aurii" and specific rootstocks "bn 70" and "a type" at *in vitro* propagation

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The researches made at cells and tissue culture open big and large perspectives regarding the handling of genetic material and it's leading through the propagation, growing and development of the plants to the aim of a performing agriculture. Having as a reason the production of the quince trees (*Cydonia oblonga*), that doesn't satisfy the request of the fruit growers in the last years, the present work paper proposes to establish the technology of "in vitro" propagation of "BN 70" and "A Type" rootstocks and of "AURII" quince cultivar. In this study we introduce the elements as: the culture media with all the specific elements, the harvesting of the branches and preservation of the meristems. Thus, to establish the culture and to gain the best results for the growing of the explants, good results were obtained when we used the culture media *Murashige & Skoog*, with adding of phytohormons as: Giberelic acid (GA3) 1 mg/l, Indolilbutiric acid (IBA) – 0,1 mg/l. For the multiplication media was used the combination macroelements - microelements-vitamins and Giberelic acid (GA3) 0, 1 mg/l, Benzilaminopurine (6-BAP) – 1mg/l, Naftalen acetic acid (ANA) - 0, 2 mg/l that assured the biggest multiplication rate. In this phase was identifying the vitrification of the plants on the Lepoivre media.

## AP-05

### Developing of ppv virus free plants by *"in vitro"* culture

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Our investigations were focused on developing of PPV virus free plants by meristems culture. The plum cultivars of various susceptibility levels were involved: Tuleu dulce, which seems to be the most susceptible cultivar, it is used as indicator as well; Anna Späth with a light tolerance and Centenar with mid symptoms on leave and fruit. The cultivars were selected by biological and serological tests. The experiments have had in view the size of the explant utilized knowing that the virus purification level is conversely proportional with the explant size. Therefore, the meristems were excised as: 0,1mm; 0,2 – 0,3 mm and over 0,3 m. We had 3 replications for each variant. Besides the explant size, the composition of the nutrient media and number of "in vitro" subcultures were considered. Our results have proved the major importance of these factors in virus purification on the above mentioned cultivars. It is noticed that a diminish of the viral protein in plants is related to the explant size mm explants, we recorded the highest percentage (72%) of "in vitro" virus free plants, this percentage increasing with the number of subcultures. The composition of the nutrient medium enriched with hormones leads to a lower viral infection and hastens the development of axilary buds (multiplication). Those hormones plays also the role of inhibitions in virus multiplication. Combining these techniques it is possible to eliminate the viral infections even in case of 0,2 - 0,3 mm explants.

## AP-06

### The influence of imidazole fungicides on multiplication *in vitro* of low vigorous pear and cherry rootstocks

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The application of fungicides in *in vitro* culture has shown that some of these substances may exhibit organogenic and morphogenic effects on *in vitro* plants. Similarly, it has been discovered that the imidazole fungicides, such as imazalyl, prochloraz, triflumizole, and triazole retardant paclobutrazol either intensify the effect of exogenous cytokinins or inhibit biosynthesis of the gibberellic acid. Low vigorous pear and cherry rootstocks, Pyrodwarf and Gisela 6, were used as model plants in these investigations. Under *in vitro* conditions, successful micropropagation of Pyrodwarf is largely based on the nodal transplantation of shoots due to low potential of lateral shoots to form. Therefore, the objective of these investigations was to study the influence of imidazole fungicides on multiplication of these two genotypes. The commercial chemical SPORTAK 45–E–450, which contains active substance N-propyl-N-[2-(2,4,6-trichlorphenoxy)-ethyl]imidazole-1-carboxamide (prochloraz), was used as the source of the imidazole fungicide. The experiment was performed during the multiplication phase. It involved the study of 19 types of media containing macro and micro salts according to Murashige and Skoog (1962) (MS), different prochloraz concentrations, BAP, IBA and $GA_3$. Prochloraz involved 3 concentrations (1, 5 and 10 µM), either individually or combined with BAP (4.5 µM), IBA (4.9 µM) and $GA_3$ (0.3 µM). Upon the second subculture, shoot multiplication parameters, multiplication index, and the length and number of leaves on axial and lateral shoots were deterimined. Fresh and dry shoot weight, i.e. callus, stem and leaves were also checked. Individual or $GA_3$-combined application of fungicides gave no effect on the multiplication of both rootstocks, but had influence on shoot rooting (from 5.6% Gisela 6 to 72.2% Pyrodwarf). The highest multiplication index and the quality of shoots were obtained on media containing prochloraz combined with BAP and $GA_3$. The obtained results suggest that prochloraz not only induces shoot induction but also intensifies the effect of exogenous added BAP on *in vitro* multiplication of the Pyrodwarf and Gisela 6 and could be recommended for micropropagation of these rootstocks.

## AP-07

### Effect of carbohydrate source and polyethy-lenglycol on maturation and germination of somatic embryos in Walnut (*Juglans regia* L.)

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Low efficiency of somatic embryo maturation, germination and conversion to plantlets is a major problem in many species including J. regia L. Germination efficiency of somatic embryos is very low in walnut. In this study, effects of two carbohydrate sources, sucrose and maltose (each at two levels of 3 % and 6 %), and two kinds of PEG (4000 and 6000 each at four levels of 1.5 %, 3 %, 5 % and 7.5 %) on maturation and germination of walnut somatic embryos were tested. The number of somatic embryos increased on medium containing PEG. The higher levels of PEG 4000 (7.5 %) could remarkably enhance the morphogenesis of somatic embryos and the number of embryos produced. PEG 4000 stimulated somatic embryo maturation of walnut. This stimulatory effect was dependent on the carbohydrate source used. Sucrose in combination with PEG 4000 produced 50% of cotyledonary and normal somatic embryos while PEG 6000 and maltose both at applied levels caused an unfavorable effect and increased the frequency of abnormal shaped somatic embryos. Treatment with PEG 6000 and maltose (each at 3%) produced the highest number, 25%, abnormal somatic embryos.

## AP-08

### The effects of colchicine and oryzalin treatments on growth, multiplication rate and ploidy level in *Musa acuminata* cv williams

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Banana is the third most important tropical fruit in the world after citrus (FAO statistics: www.fao.org) where diseases are seen as the greatest difficulty in the crop. Global production is composed of the growth of a small number of mostly sterile polyploid varieties which are vegetatively propagated and attempts to breed new commercial varieties are hampered by the lack of resistance in these traded cultivars and by their triploid genome thus making improvement through conventional breeding extremely difficult. Polyploidization through chromosome doubling is a useful option for breeders. This research investigates the efficacy of colchicine and oryzalin in inducing polyploidy in the commercial banana variety 'Williams'. Ploidy level was determined using flow cytometry and stomatal measurements. The effect of the treatments on multiplication rate and plant development will also be discussed.

## AP-09

### In vitro techniques to study the shoot-tip grafting of cherry *(Prunus avium* L.*)* var. seeyahe mashad

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The union grafting and feasibility of mass propagation of disease free of (*Prunus avium* L.) Var. Seeyahe Mashad by new technique of shoot tip grafting (STG) was studied. Seeds of sour cherry (*Prunus cerasus* var. Albaloo Telkhehe), were stratified, scarified and inoculated on gelled hormone-free medium. *In vitro* germinated seedling which emerged 3-4 weeks after inoculation, were decapitated and used as rootstock. Five to six week *in vitro*-cultured meristematic apices and bud apices (axenic shoot-tip and nodal cultures) established from cherry with length 3-15 mm was also used as microscions. The technique of grafting, and the effect of scion size and its origin on grafting success, was studied. Grafting success was significantly dependent on the method of grafting and size of the scion. The highest percentage (65.5%) of successful grafts was obtained with apex graft (shoot-tip), with apical bud scion length greater than 6 mm. Success of side bud apices (wedge) grafts was 43%. Graft union, as graft compatibility was satisfactory, although, it formed very slowly during the first month. Contamination

and necroses were occurred more often *in vitro* conditions. Nutrient disorder and mineral deficiency symptom was observed frequently when physiologically active meristematic apices were used. This technique has a good potential for mass propagation of diseases-free for cherry var. Seeyahe Mashad.

## AP-10

### Photoautotrophic micropropagation for cost-effective and successful clonal multiplication of woody fruit crops

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Micropropagation for woody plant species has become one of the most effective ways for clonal multiplication. However, its widespread use is still limited mainly due to high production costs, low growth rate, losses due to microbial contamination and physiological and morphological disorders. Photoautotrophic (sugar free) culture system possesses many advantages offsetting drawbacks of conventional hetertrophic micropropgation system. Present study was focused on twin objectives to achieve cost-effective, high frequency clonal regeneration with successful acclimatization by introducing photoautotrophy at subsequent stages in cultures, and to investigate clonal fidelity of plantlets for Japanese plum (*Prunus salicina* Lindl.) cv. Methley in comparison with heterotrophic mode of conventional micropropagation. Nodal segments collected in July from mature (25 year old) mother plants and established in photoautotrophic MS liquid medium supplemented with tapioca, BA (0.5 mg/l) and IBA (0.05 mg/l IBA) showed maximum survival (68.0%) with minimum contamination in cultures. During initial shoot proliferation, however, heterotrophic liquid MS medium supplemented with tapioca was found to be the best with quick and early shoot proliferation showing as high as 1:19.83 multiplication ratio after 150 days of implantation and photoautotrophic medium exhibited least multiplication and deficiency symptoms as well. Thereafter, subsequent sub-culturings were done in photoautotrophic media. Rooting in proliferated shoots could be achieved within a week and photoautotrophically pre-hardened plants showed 100% survival with quick growth response within 2 days of transplanting in soil. There was 2.17 times increase in cuticular wax content and 1.08 times increase in total chlorophyll content after 21 days of acclimatization of PAM-grown plantlets in comparison to heterotrophically grown plantlets. Protein banding pattern of SDS-PAGE revealed them to be true-to-the type.

## AP-11

### Propagation of some date palm varieties by using tissue culture methods

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Date palm (*Phoenix dactylifera* L.) is one of the fruit crop in the Middle East with high economically importance. In the near future a big number of young date palms are necessary for Syrian farmers. The traditional propagation by using offshoots is limited by the few number of offshoots produced per plant in its whole life. Propagation of date palm by using tissue culture techniques is very necessary to produce plants with high quality in a large scale. In our experiments, somatic embryogenesis protocol to propagate Syrian date palm cultivars (cv. Zahdi, Khistawi, Deglet Nour, Barban) by using zygotic embryos and shoot tips cultured on modified MS media with different concentration of auxins (2.4-D, Picloram) was developed. The role of activated coal on callus formation was studied. The effect of several plant growth regulators in various concentrations on proliferation of the embryogenic callus, on elongation and multiplication of the shoots were studied.

## AP-12

### Micropropagation of primocane-fruiting raspberry cultivars

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Raspberry micropropagation via tissue culture is useful not only for large-scale production of new cultivars and high phytosanitary status of received plants material, but also because of it considerably decrease the period before cultivars will be passed to the State Inspection for Testing and Protection of Plant Cultivars and included in the State Register of Cultivars and Trees and Shrubs Races. The purpose of our work was to optimize the technique of primocane-fruiting raspberry in vitro propagation. It was necessary to study an influence of physiologically active substances on plants morphological parameters at the multiplication stage, to establish the optimum concentration of hormones providing high multiplication coefficient without plantlets quality decrease. In vitro propagated raspberry cultivars ′Heracl′ and ′Babye leto-2′ were used for the experiments. The researches were carried out using Murashige and Skoog (1962) medium supplemented with various concentrations of growth regulators: 6-BA and IBA. As a result it has been established optimal hormones ratio for raspberry plantlets morphogenesis during micropropagation. Rooting was induced on $^{1}/_{2}$ MS medium with IBA (0-0.5 mg $l^{-1}$). Optimum IBA concentration for producing maximum quantity of plants with well developed roots was determined. Regenerants acclimatization to unsterile conditions is a final and important stage of plants micropropagation. Several substrates were tested: a mix of peat and sand (3:1), perlite, ion exchange substrate BIONA-112 and a mix of BIONA-112 and perlite (1:2). The results of our experiment showed high efficiency of ion exchange substrate application for raspberry plants adaptation without previous in vitro rooting. The post-influence effect of ion exchange substrate on successful adaptation was note. It might save time and/or resources in raspberry plants commercial micropropagation. Micropropagated plants were planted in a greenhouse and used as a nuclear stock after their testing for virus; another part of healthy material was passed for Primary Cultivar Trials.

## AP-13

### Rapid micropropagation of *Aloe vera* L. via shoot Multiplication

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In this research, shoot tip explants of *Aloe vera* L. were used for rapid proliferation. The present study indicates the importance of biotechnology investigations in order to overcome limitations such as slow growth, to be expensive and low income practice in this plant. This study can be utilized to extend a manner to rapid micropropagation of plants that may provide a proper source for medical and cosmetic industries. Explant used for the in vitro culture was shoot tip. The shoot tip explants was disinfected with 2% NaOCl and washed throughly with sterile water. Then, explants were placed on soild MS medium with the addition of various concentrations of benzyladenine and α-naphthaleneacetic acid. After 8 weeks the best proliferation of shoot per explant (9.67) and the best rooting was shown on the medium supplemented with 0.5 mg/l BA + 0.5 mg/l NAA. The rooted plantlets were gradually acclimatized in plastic pots containing a mixture of cocopeat and perlite (1:1) covered with transparent plastic. About 95% of the transplanted plantles survived.

## AP-14

### Micropropagation, rooting and acclimatization of blackberry cv. agavam

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Plant micropropagation of blackberry (*Rubus idaeus* L.) was carried out in late summer – second half of august. Meristem tip explants from the axillary buds were cut in three sizes - <0.5 mm; between 0.5 and 1mm and >1 mm. Two media compositions were used for establishment of culture: Murashige and

Skoog mineral salts and vitamins media supplemented with different proportions of BAP, IAA, IBA and $GA_3$. As well as two types of proliferation media were used: 1) one strength M&S mineral salt and vitamins media and 2) M&S media with half strength nitrogen compounds both with different composition of BAP (0,5 and 1 mg/L correspondingly) and IAA (0,25 mg/L and without). Rooting was performed by using two methods: *in vitro* and in peat substrate. Media of two compositions were used for *in vitro* rooting: one strength M&S mineral salts and vitamins supplemented with 0.5 mg/L IBA in one variant and 2 mg/L IAA in other. Rooting in peat substrate was performed by direct planting microplants in unsterilized substrate and kept under high relative air humidity conditions. Culture initiation was started late therefore establishment of alive explants was quite weak. Higher percentage of alive microplants was obtained from 0.5 – 1 mm size explants initiated in the media of the 1st composition (M&S mineral salt and vitamins media with BAP 1 mg/L, IAA 0.3 mg/L and $GA_3$ 0.2 mg/L). Further proliferation coefficient differed in both media compositions, but differences were not stated as significant. Rooting performed in peat substrate yielded significantly higher outcome of rooted plants than *in vitro* rooting.

## AP-15

### Micropropagation of *Malus sieboldii* hybrids resistant to apple proliferation disease

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Natural resistance to apple proliferation (AP) disease was found in apomictic *Malus sieboldii*-derived genotypes which can be used as rootstocks for apple. Whereas their agronomic value is currently improved in ongoing breeding programs they are recalcitrant to propagation by standard procedures. Therefore, a micropropagation protocol was developed for *in vitro* establishment, multiplication and rooting of eleven interesting AP-resistant genotypes. Four different macro and micro element formulations were tested: MS, QL, WPM and DKW. Phytohormones (0.25 µM IBA, 4.44 µM BAP and 0.28 µM $GA_3$) and vitamins (MS modified for thiamine at 2.96 µM), established for the propagation of *M. domestica*, were also suitable for the propagation of *M. sieboldii*-genotypes. The MS medium yielded in general the highest proliferation rates and the best shoot growth. A significant improvement of the growth was obtained by replacing Fe-EDTA by Fe-EDDHA as iron source. By comparing four different rooting treatments a significantly higher percentage of rooting was observed when the induction was carried out in the dark with 25 µM IBA either in liquid or agarised medium. Three classes of genotypes with low, medium and high rooting efficiency were found. The acclimatisation method used yielded survival rates between 90-100% for most of the genotypes.

## AP-16

### *Citrus limon* micropropagation: effect of different phytohormones on multiplication and rooting

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Factors affecting *in vitro* propagation from nodal explants of mature trees of lemon cultivars were studied. Although the basal medium did not affect any variables, explants on DKW medium were greener than MS and this medium was used in the following experiments. High concentrations of BA (1-3 mg/l) and GA (1 and 2 mg/l) were necessary for optimal multiplication. When BAP was the only phytohormone used, proliferation was not observed and the shoots were very short with great leaves. Several combinations of BA and GA were carried out for to optimize the proliferation phase, and important differences between cultivars were observed. The number of shoots was independent of the BA concentration and best results were obtained with 2 mg/l of GA. Explant length was shorter with the highest BA concentration and, in all genotypes, shoot length was maximum with 2 mg/l GA. When the GA concentration was 1 mg/l the productivity (number of shoots x the average shoot length) was independent of BA and, as in the other variables, was higher with 2 mg/l GA. The best results in productivity were obtained with 1.5 mg/l BA and 2 mg/l GA. Transferring in vitro shoots to rooting

media, containing different concentrations of IBA (1 and 3 mg/l) or different combinations of IBA and IAA (0 and 1 mg/l), produced complete plantlets. Lemon shoots rooted well in all rooting combinations. The highest rooting percentage was obtained on a medium containing 3 mg/l IBA or IBA in combination with 1 mg/l IAA, whereas the highest number of roots was produced on a treatment containing 1 mg/l of IAA and was independent of IBA concentration. The average root length was significantly affected by IBA and IAA concentration. Root length was higher when 3 mg/l IBA and 0 mg/l IAA were used and in this rooting medium explants showed a better appearance with leaves greener and higher. Plantlets that survived acclimatization exhibited normal growth in soil under greenhouse conditions.

## AP-17

### Micropropagation of troyer citrange - *Poncirus trifoliata* (L.) Rat. x *C. sinensis* (L.) Osbeck

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In citrus, the success of the scion greatly depends upon the rootstock on which it has been grafted. Troyer citrange is an important rootstock used worldwide for high density planting. It is also one of the most promising rootstock for several Indian scion varieties such as Nagpur mandarin, Kinnow mandarin, grapefruits, acid lime, Satsuma sweet orange and Mosambi sweet orange. In addition, it has high resistance to tristeza virus, which causes citrus decline and has good adaptability for acidic soil. Commercial rootstock cultivars are commonly propagated by growing seeds. Seeds of citrus species are moderately recalcitrant and thus conservation gets limited by the loss in viability within a short period. Moreover, the plants grown from seeds also exhibit extended juvenility. Besides this, Troyer citrange is a slow growing rootstock. Considering the lack of reports on axillary branching of this rootstock, the present study focuses on developing efficient micropropagation protocol for the production of clonally uniform plants through axillary branching method. Multiple shoots can be obtained from single nodes of field grown trees cultured *in vitro* on Murashige and Skoog's (MS) medium containing BA (1.11 µM), Kinetin (1.1625 µM), and 3% sucrose. A shoot multiplication fold of 3.86 was achieved. Proliferated shoots were rooted in half strength MS medium with NAA or IAA (0.1 µM) and transferred to a mixture of soil and agropeat. 100% survival was observed in hardening of the rooted plantlets. The comparative analyses of the DNA fingerprinting profiles confirmed the genetic stability of the regenerated plantlets. The results of this study have enormous commercial application for the propagation of clean and healthy disease-free citrus rootstocks in the coming future to meet the ever-growing demand for superior quality planting material.

## AP-18

### Development of a suitable protocol to overcome hyper-hydricity in apple (*Malus sp*.) during *in vitro* regeneration

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The occurrence of hyperhydricity in plant tissue cultures of apple (*Malus sp*.) is one of the key problems in tissue culture as well as in the utilisation of gene transfer technologies. Hyperhydricity, or vitrification, is a physiological malformation affecting tissue culture-generated plants. This deformity is associated with excessive hydration and poor lignification and results in poor regeneration of new shoots from leaf explants.

We have assessed hyperhydricity prevention in different apple rootstock genotypes (M.9/T337, M9/29 and M.26) by several approaches. Among these the influence of the gelling agent type (agar vs. gelrite) and concentration the carbon source (different sugar types) and the cytokinin type (BAP vs. TDZ) were tested. Leaf segments cut from *in vitro* propagated plant material were incubated on regeneration medium containing the above mentioned ingredients. First results indicated that the application of agar, fructose and BAP during shoot regeneration resulted in a significant lower incidence of hyperhydric shoots.

## AP-19

### *In vitro* propagation of wild european plum (*Prunus domestica* L.), a rare and endangered species

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Wild European plum (*Prunus domestica* L.) is a rare and endangered species in China. It was only found and distributed in Xinyuan County and Gongliu County of Ili region in Xinjiang. Because of the biotic and abiotic reasons, the distribution sites of wild European plum in Ili region decreased significantly, compared with the time it was found 20 years ago. The objective of this study was to develop an efficient *in vitro* propagation protocol for wild European plum. Nodal segments were used as the explants in our study. For the initiation of culture, B5 medium was better than Murashige and Skoog (MS) medium, which were shown by the rapid growth of axillary bud within 3days. Shoot organogenesis could be induced from both 6-benzylaminopurine (BAP) with α-naphthalene acetic acid (NAA) and BAP with indole-3-butyric acid (IBA) in B5 medium, but the regeneration efficiency depended on the ratio and concentration used. The higher regeneration efficiency existed in BAP:NAA=3 5:1 and BAP:IBA=1 3:1, when BAP concentration was in 0.5-1.0mg/l. Rooting efficiency could be greatly increased with the addition of 0.4mg/L IBA and no callus was observed on shoots in rooting media. Rooted plantlets were successfully acclimatized and grown in different transplanting medium. Plantlets in sawdust show higher survival rate than in soil and in vermiculite. This new system proved to be a practical way for germplasm conservation and for further investigation of wild European plum.

## AP-20

### *In vitro* propagation of some grape rootstocks

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The objectives of this study were: a) to establish tissue culture protocols for micropropagation of some new introduced grape rootstocks and cultivars. This included the effect of benzylaminopurine and medium type on plantlet vegetative and chemical aspects and b) to study *in vitro* tolerance of such rootstocks and cultivars to *in vitro* salt and drought stresses. Murashige and Skoog medium induced larger average plant height followed by Woody and Gamborg media. While using Woody Plant medium recorded the significantly highest value of roots number and average root length followed by Murashige and Skoog and Gamborg. Rootstocks (1103 Paulsen, Ru 140, Freedom and So4) generally had better values than cultivars in plantlet height, produced leaves, survival percentage and number of roots. BAP at 2.0 mg/l is highly recommended for mass production of grape rootstocks and cultivars compared to other concentration. On the other hand rootstocks (1103 Paulsen, Ru 140, Freedom and SO4) generally had higher value compared to cultivars. SO4 rootstock recorded the highest value compared to other rootstocks. Rootstocks used in this investigation showed tolerance to salinity more than cultivars. Rootstocks recorded the highest values in plant height, leaf area, internode length, fresh weight, N, P, K, Ca, Na, Mg, Proline contents and survival percentage (71.11 vs. 66.87 %). Drought treatments as 5 and 10g/l PEG *in vitro* indicated that Rootstocks were more tolerant than cultivars based on Proline contents. 1103 Paulsen rootstock exhibited the highest Proline content (more tolerant) in response to drought stress.

## AP-21

### In vitro propagation of *Zizyphus spina-christi*

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Hozaien is a mutation in *Zizyphus spina-christi* (Indian type jujube) was originated in the early 1950's in a private orchard in Assiut, Egypt. Results of asexual propagation were inconstant and were influenced by species, means and environmental conditions which result in difficulties in routinely vegetative propagation as stem cutting propagation. Therefore, micropropagation through tissue culture technique was investigated. To establish a tissue culture protocol for micropropagation of *Zizyphus spina-christ*i the effect of benzylaminopurin and medium type on plantlet vegetative and chemical aspects in addition to *in vitro* tolerance to drought and salt stresses were studied. BAP at 0.5 mg/L was found highly recommended for to induce proliferation which would result in suitable number of produced plantlets of mass production of Hozaien. Using M&S medium without supplements would fulfil the objective of having mass production of rare explants (mutation) meanwhile to obtain better vegetative propagules one shoud use Nitsch & Nitsch medium. Salinity treatments at 8, 12 and 16 g NaCl/L produced same *in vitro* vegetative characteristics but at different degrees. Plantlets grown on media containing NaCl were found to have higher values of macroelements, N, P and Fe and lower values of microelements Mn and Z. Though, Hozaien nabk would be considered tolerant to salinity at the levels used in this experiment. Drought treatments at 10% and 20% PEG reduced some *in vitro* vegetative characteristics. Nitrogen, phosphorus and iron were found to be higher in the presence of drought agent compared to control meanwhile microelements (manganese and zinc) were found to greatly decreased by drought treatments.

## AP-22

### Improving the in vitro multiplication of guava (*Psidium guajava* L.) in hot arid region

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The *Psidium guajava* L. is the member of family myrtaceae was domesticated more than 2000 year ago and introduced in India by the Portuguese during early 17$^{th}$ century. Guava is a rich source of Vitamin-C and pectin and is available throughout the year except during summer season. Owing to its hardy nature, guava is grown successfully in tropical and subtropical region and is now fetching the attention of orchardists. But the non-availability of good quality and true to type planting material is emerging as a major bottleneck in the cultivation of this crop. A Study was conducted at CCS Haryana Agricultural University, Hissar in collaboration with Center For Plant Biotechnology, CCS HAU campus, Hissar, Haryana, India to improve the *in vitro* multiplication of guava. The *in vitro* studies revealed that surface sterilization was best when explant was treated with ethanol (70%) for 30 sec. followed by $HgCl_2$ (0.1%) for 6-8 min. The nodal segment explant of size 3 cm with second and third node from shoot apex showed regeneration of *in vitro* cultures. Treating the explants with antioxidant solution [ascorbic acid (0.2%) + citric acid (0.4%) for 10 min. and then drying under laminar air flow for 75 min, followed by subculturing of explants, twice, once after the first day and second after third day of inoculation led to phenolic exudation almost controlled. MS medium supplemented with 2 mg/l BAP + 0.25 mg/l kinetin was the best media for culture establishment and maximum shoot regeneration (76.1%). Shoot multiplication was the best (12.80 shoots/explant) in MS medium supplemented with 1 mg/l BAP + 0.2 mg/l kinetin + 0.1 mg/l IAA + 25 mg/l adenine sulphate]. The best rooting of *in vitro* derived micro cuttings from the regenerated shoots resulted with ½ MS medium supplemented with 2 mg/l IBA + 200 mg/l activated charcoal. The potting mixture of sand + soil + FYM (1:1:1 v/v) proved best for the maximum survival of plantlets in pots under green house conditions. Based on these studies, tissue culturist can develop guava plants with varying degree of success. Owing to the specific needs and growing demand in horticulture sector and keeping in view, the preference of growers, the improvement in *in vitro* multiplication of guava is the need of hour.

## AP-23

### *In vitro* proliferation of newly bred czech pear cultivars

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The objective of this study was to develop a method for rapid in vitro shoot multiplication of four newly bred Czech pear cultivars (´Bohemica´, ´Dicolor´, ´Elektra´ and ´Erika´). Six proliferation MS media containing different concentrations of BAP (6-benzylaminopurine), TDZ (thidiazuron) or 2iP (6-(g,g-dimethylallylamino) purine) were tested. For the four cultivars, the effect of three growth regulators on proliferation, callus formation and shoot morphology is shown. Selected pear cultivars were successfully established in vitro. Proliferation rates varied depending on the genotype and medium used. The highest proliferation rate was obtained for pear cultivar ´Elektra´ that produced 4.1 ± 0.1 shoots (longer than 10 mm) on MS medium containing 1 mg $l^{-1}$ TDZ. The lowest proliferation rate was obtained for cultivar ´Dicolor´, which did not multiply at all on MS medium containing the lowest concentration of BAP 1 mg $l^{-1}$. Abundant callus formation at the base of explants was observed on the media containing TDZ.

## AP-24

### Effect of light and sucrose on proliferation, *ex vitro* rooting and acclimatization of kiwifruit cv. hayward

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Simultaneous *ex vitro* rooting and acclimatization is an important goal for a large scale micropropagation of kiwifruit cv. Hayward *Actinidia deliciosa* (A. Chev) C. F. Liang et. A. R. Ferguson var. *deliciosa*. Many papers described *in vitro* rooting for kiwifruit however there are no many information about *ex vitro* rooting and subsequent acclimatization of kiwifruit. Many factors affect rhizogenesis and acclimatization of micropropagated *in vitro* shoots. Light intensity and sucrose during *in vitro* culture are two of the most critical factors related with a high production of vigorous plantlets prepared to field conditions. The aim of this work was to examine the effect of different light intensities and sucrose on proliferation stage, and during *ex vitro* rooting and acclimatization. Three different light intensities were used: high light (HL, 200 µmol $m^{-2}$ $s^{-1}$), medium light (ML, 100 µmol $m^{-2}$ $s^{-1}$) and low light (LL, 60 µmol $m^{-2}$ $s^{-1}$). Shoots ranged to 2-3 cm were transferred to Ch medium, with 0, 1, 1.5, 2, 2.5 and 3% of sucrose and supplemented with 1 mg/L of BAP (6-bencilaminopurine) and 1 mg/L of $GA_3$ (gibberelic acid). Shoots were maintained two months under these conditions. After this period, shoots were dipped in a concentrated and sterilized auxin solution 25 mM IAA (indole-3-acetic acid) and set on minipots with planting mixture (perlite:compost 1:1 (v:v)). They were placed in a growth room under 16 h-photoperiod. The initial value of relative humidity was set at 100% and decreased gradually over 42 days to 70%. Histological studies were realized to observe anatomical changes on root formation corresponding to the emergence of root primordia, vascular conexions between the shoot and the roots, root development and leaf structure. Preliminary results showed that sucrose increases response index, proliferation and length of the shoots in the three light intensities tested during the proliferation stage. However, during *ex vitro* stage HL treatment influences positively on higher survival percentages and growth parameters of the plantlets.

## AP-25

### Use of Cassava starch as a cheap substitute of agar in tissue culture media for in vitro growth of strawberry (*Fragaria* x *ananassa* duch. cv. chandler)

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One of the most expensive ingredients in tissue culture is the gelling agent. The price of agar in developing countries is very high, since it has to be imported, usually from the US. The search for cheap substitutes is a high priority in order to significantly lower the cost of tissue culture. The use of cassava starch as a cheap substitute for agar in the solidification of tissue culture media for strawberry micropropagation was the objective of this research. The experiment was carried out in the Biotechnology Laboratory of the Universidad de Oriente, located in Maturín, Monagas state, Venezuela, using a randomized statistical block design with four treatments (agar and three cassava starches: AIM TF-351, AIM TF-352 and AIM-TP-212), with five repetitions, ten test tubes per experimental unit and one explant per tube. Explants were taken from crown shoots of strawberry plants grown in the field. The adequate concentration for tissue culture media solidification was determined to be 15, 17 and 16% (w/v) for cassava starches AIM TF-351, AIM TF-352 and AIM-TP-212 respectively. According to our results, cassava starch AIM TF-351 can substitute agar in the initiation phase, since its effects on the explants are very similar to those of agar and it allows a higher survival rate. In the multiplication phase, explants grown on cassava starch AIM TF-351 had a higher dry weight. Agar has advantages over the cassava starches because is traslucid, very easy to manipulate and allows a higher multiplication rate of the explants. On the other hand, cassava starches are produced locally and are fifteen times cheaper than agar. Cassava starches are a feasible and economical alternative for agar substitution in strawberry micropropagation.

## AP-26

### Impact of exogenous saccharose, raffinose and proline on cold acclimation of strawberry *in vitro*

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Despite climatic changes, winter and cold hardiness remain among most important traits for strawberry and other fruit crops. Biotechnological, *in vitro* methods makes it possible to investigate separate factors of those complex traits in detail and give recommendations for improving cold hardiness by genetic engineering, marker assisted selection or breeding. Effect of saccharose, raffinose and proline additions to growing media for cold acclimation efficiency, duration and cold hardiness were investigated *in vitro*. Plants of strawberry cultivars ‘Elsanta’, ‘Melody’, ‘Holiday’ and ‘Venta’ were planted on MS media with addition of different variants of 3, 6% saccharose, 1% raffinose and 0.001% proline. After one week plants were transferred for acclimation at +2°C temperature for 7 and 14 days, frozen at -9°C temperature for 15 hours. After thaw in +2°C temperature for 12 hours plants were grown in growing chamber at +22°C temperature. Survival rate and cold injury score (0-5) were evaluated 3 weeks after freezing. It was shown direct correlation between acclimation duration, saccharose, raffinose concentration and cold hardiness of investigated strawberry plants. Possible reasons of data inconsistency of proline addition and explants condition impact to strawberry cold hardiness *in vitro* were discussed.

## AP-27

### Effect of mineral concentration according to seed concen-tration and organic medium component on micropropagation of walnut (*Juglans regia* L.)

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Tissue culture success is strongly dependent on chemical composition of the culture medium. Micropropagation of walnut has been partially limited to the lack of an appropriate culture medium. We hypothesized that the minerals in proportions similar to those found in the walnut seeds could provide an optimum medium for micropropagation of walnut. Therefore, the mineral composition of seeds were analyzed by ICP-OES in five walnut varieties (’Serr‘, ’Pedro‘, ’Lara‘, ’Hartley‘ and ’Rond e Montignac’). Results showed that the mineral concentration of walnut seeds were 2-26 times of those in DKW as specific walnut medium. Two new media (as ×1.5 and ×2 strength of macro and micro nutrients of DKW) were formulated using minimum concentration of the minerals in seeds. The growth of explants on these two new media was compared to DKW medium. Explants cultured on ×2DKW and ×1.5DKW formed shoots having green-colored leaves but produced calluses similar to those cultured on DKW medium. The better growth was observed on ×1.5DKW medium although the potential multiplication rate was different among the varieties so that ‘Sundland’ had the maximum stem length (4.70 cm) and number of auxiliary buds (21 buds/explant) in ×1.5DKW.

## AP-28

### Sterilization of non-autoclavable vessels by sodium hypochlorite for plant tissue and *Agrobacterium* culture

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Experiments in biotechnology, particularly in plant tissue culture, need a huge amount of culture vessels. They are normally disinfected by autoclaving. However, this sterilization procedure is costly and not applicable for destructible materials with high temperature. Disinfection protocol by sodium hypochlorite (NaOCl) in substitution for autoclaving was then developed. Reused polystyrene 6-well plates (Corning) were used as non-autoclavable vessels, containing a modified liquid MS medium for plant tissue culture and a liquid MYA medium for Agrobacterium culture. The vessels were first disinfected with 0.002% NaOCl and poured autoclaved liquid MS medium. No microbial contamination appeared after one month of incubation in dark at 28 °C. However, when a bacterium culture medium (MYA) was used instead of the MS medium, bacterial contamination was detected. The 0.01% NaOCl was needed to have efficient disinfection of the polystyrene plates. No toxic effect was detected neither banana plant tissue nor *Agrobacterium tumefaciens* cultivated in the NaOCl-treated vessels. Phytotoxic effect was observed only on banana suspension cell culture but not on shoot tip culture, when the 0.002% NaOCl was also added to the culture media for disinfection instead of autoclaving. The results show the 0.002% NaOCl acts as bacteriostasis but not bactericide. In nutrient rich medium such as MYA medium, contaminant bacteria can take re-growth after the 0.002%-NaOCl disinfection. Disinfection by this concentration may be applicable to vessel sterilization for plant tissue culture, but the 0.01% NaOCl should be used to vessel sterilization for Agrobacterium culture.

## AP-29

### Methods to reduce the level of latent bacterial contamination in banana micropropagation

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Banana is a vegetatively propagated crop and although commercial *in vitro* micropropagation is well established, latent contamination is a major problem in the industry. The presence of endogenous latent bacteria contamination in tissue culture can affect the performance of the material and ultimately lead to losses in production. In this case commerically purchased banana microplants when transferred to multiplication medium with high BAP, exhibited signs of latent contamination. An inspection (ie. 100% inspection) of the plant material revealed that 75% of the original stock was contaminated. Subsequent subcultures of the remaining 'clean' stock led to further expression of the contaminants. Two gram negative bacteria, namely *Burholderia cepacea* and *Empedobacter brevis* were identified as the main contaminants consistently found in all of the infected microplants. This research examines the effects of media and antibiotics to control the level of contamination in banana.

## AP-30

### Use of *in vitro* propagation methodologies for the production of disease free stock plants of avocado

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Root rot caused by *Phytophthora cinnamomi* (PC) and the sunblotch viroid are diseases of avocado (*Persea americana Miller*) that are often distributed with planting material and cause the infestation of plantations. In order to produce tested, disease free stock plants, we are adapting *in vitro* propagation metodologies for elite cultivars and PC-resistant rootstocks. Explants for initiating *in vitro* cultures were young shoots of 2 to 3 nodes taken from adult field growing trees. A critical step in starting *in vitro* cultures from these explants has been their disinfection. This has been achieved with efficiencies as high as 80% with an improved disinfection methodology which includes the treatment in 70% ethanol and 1% sodium hypochlorite solutions under vacuum (500 mm Hg), and the use of a solution of silver nitrate (250 mg/l) and copper sulphate (100 mg/l) as a liquid layer over the solidified culture medium (1/8th strength B5 medium with 1 mg/l BAP) during the first four weeks of culture. The meristems present in the leaf axils of successfully disinfected explants developed buds after three to six weeks. However, they showed a slow growth rate producing less than one node a month. Higher growth rates were obtained after micro-grafting of the developed buds onto seedlings obtained from *in vitro* cultured seeds. Avocado lines successfully established *in vitro* will be tested for the absence of the above-mentioned diseases, and used for implementing certification programs by distributing them to local commercial nurseries.

## AP-31

### *In vitro* thermotherapy of apricot cultivars

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Placing the plants on hot air conditions is commonly used for the elimination of viruses. This method is based on the effects of a decreasing concentration of viruses towards the apex on the one hand and of inhibition of viral propagation and diffusion due to increased temperatures on the other. Presented work deals with thermotherapy of *in vitro* plants (in test tubes) and *in vivo* plants (in pots) during elimination of PPV (Plum pox virus), PDV (Prunus dwarf virus) and PNRSV (Prunus necrotic ringspot virus) and with comparison of these two methods for viruses elimination. For experiments the following varieties were used: ´Bergeron´, ´Leskora´ and ´Marlen´. For thermotherapy were selected plants, which – when tested by means of RT-PCR method – showed a positive reaction to presence of viruses described above. *In vitro* cultures were established using nodal segments. Plants used for experiments were transferred to a

fresh medium every four weeks. After four months, young plants approximately 10 mm long were placed in thermotherapy chamber with temperature 37°C (light) and 35°C (dark) and with photoperiod 16/8 (light/dark). During *in vitro* therapy every plant in separate test tube was placed. A total of 30 plants from each variety were treated. In case of *in vivo* therapy 2 years old plants were putted on thermotherapy chamber under the same conditions. In this case air humidity was adjusted. After both treatments, the apical segments (approximately 3 mm long) were taken from *in vitro* and *in vivo* plants and cultivated in vitro conditions.

## AP-32

### Preliminary results of *in vitro* thermotherapy of pear cultivars

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Pear cultivars 'Alexander Lucas' and 'Elektra' were selected for application of *in vitro* thermotherapy at 39 ± 0.5 °C. The presence of viruses in selected initial trees was detected by ELISA and RT-PCR before the beginning of thermotherapy. The results of testing proved the presence of *Apple stem pitting virus* (ASPV) in two pear cultivars. Cultivars were successfully multiplied in in vitro cultures on MS medium with 1.5 mg $l^{-1}$ BAP. After the end of thermotherapy, eight clones of pear cultivar 'Elektra' and seven clones of pear cultivar 'Alexander Lucas' were free of all tested viruses after repeated RT – PCR. Achieved results are preliminary. Other sanitation and testing of pear cultivars used in the project will be carried out in the following years.

## AP-33

### Scenario and diversity analysis of Prunus necrotic ring spot virus infecting stone and pome fruits in India

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Prunus necrotic ring spot virus is a member of Ilarvirus genera family bromoviridae. Genus Ilarvirus comprises large group of plant viruses infecting primarily woody hosts especially *Prunus* spp. (stone fruits). Stone fruits (plum, peach, cherry, apricot and almond) and pome fruits (apple and pear) are the major fruits grown in the North western Himalayan region of India on commercial scale. These fruits are mainly commercially grown in the states of Himachal, Uttarakhand and Kashmir. Threat to their production is due to many fungal and viral pathogens, which lowers their quality and quantity. In the present study several surveys were conducted in the various stone and pome fruit growing areas of Himachal Pradesh, Uttarakhand, Punjab and Jammu & Kashmir. Samples wee collected from trees showing virus like symptoms such as shot holes and necrotic spots and analyzed by Enzyme linked immunosorbant assay (ELISA) using PNRSV specific antibodies (Agdia, USA). Positive results were obtained from plum, peach, nectarine, cherry and almond including Himalayan wild cherry (*Prunus cerosoides*) and in apple. To confirm PNRSV, Reverse Transcription Polymerase Chain (RT-PCR) was performed using specific primers for the coat protein gene. Fragment of about 675bp was amplified as reported earlier. Sequencing results confirmed the presence of PNRSV in the plum, peach, nectarine, wild cherry, almond, cherry and apple (accession numbers: AM494934, AM408910, AM408909, AM493717, AM712614, AM920668, AM4198141, AM491772). These were compared with 58 isolates from other countries using ClustalW programme. The isolates showed showed 87% to 100 % homology among each other at nucleotide and amino acid level. The cloned fragment has been used as probe for PNRSV during nursery certification and disease monitoring. This will be first step towards effective management of PNRSV through virus free propagative material.

## AP-34

### Apple chlorotic leaf spot virus: genomic diversity and strategies for disease management

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The importance of Apple chlorotic leaf spot virus (ACLSV), a type species of the genus Trichovirus, is due to its worldwide occurrence and large host range in pome (apple, quince, pear) and stone (plum, peach, cherry, apricot, almond) fruit crops which are of great economic value. Infections are normally symptomless but severe graft incompatibilities in some Prunus combinations in nurseries have been reported. The severity of symptoms elicited by ACLSV depends largely on plant species and virus strains. Incidence of diseases reduces the quality and quantity of these fruits. Viral diseases cause economic losses through lower yields and reduced quality of plant products. In India the states of Jammu & Kashmir, Himachal Pradesh and Uttrakhand falling in the north-west Himalayan belt are major pome and stone fruit growing area of the country. Extensive surveys were conducted for understanding the scenario of ACLSV infection in the country. Presence of some other economically important viruses *viz*. Apple mosaic virus (ApMV), *Apple stem grooving virus* (ASGV) and *Prunus necrotic ring spot virus* (PNRSV) were also confirmed by enzyme linked immunosorbant assay (ELISA) using virus specific antibodies (Agdia, USA). Though some samples showed mixed infection of two of the tested viruses, ACLSV came across as a major virus infecting apples plantations. To confirm ACLSV, Reverse Transcription Polymerase Chain (RT-PCR) was standardized using primers designed for full coat protein amplification (Accession numbers AM490253 and AM490254). Fragment of about 800bp was obtained from apples (12 different locations). Among other hosts Himalayan wild cherry, pear, plum, peach, almond, wild/cultivated apricot and quince also gave the desired amplification in RT-PCR. The virus spreads due to mechanical inoculation and different horticultural practices like grafting and pruning. The virus has also been detected by DAS-ELISA in clonal rootstocks of apple (M7, MM111, MM106) and commonly used seedling rootstocks of stone fruits (wild Himalayan cherry, wild apricot). All the CP-ACLSV sequences from the present study show percent identity ranging from 91-100 and 83-100 at amino acid and nucleotide level respectively. Phylogenetic relationship in comparison to sequences available show that though values of bootstrap separation in some of the ACLSV isolates from India are very high, most of the Indian isolates fall in group A though in different sub-groups. The infected scion and rootstocks present a dangerous proposition to virus spread. Identification of healthy mother plants by proper indexing, establishment of virus-free budwood banks and use of clean horticultural practices and exploitation of pathogen derived resistance strategies would be worthwhile to prevent grave losses that ACLSV has been reported to cause. There would also be need of proper and strict quarantine measures to check the import of virus infected stone or pome fruit budwoods and their rootstocks.

## AP-35

### The status of sub-tropical fruit genetic resources of Iran

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Various geographical regions of Iran allow many subtropical fruits such as: citrus, chico, date, fig, loquat, mulberry, persimmon, pistachio, pomegranate, pecan, quince, and guava produced. Iran is considered one of the unique zone of rich and natural genetic resources of some subtropical fruit species namely: *Citrus medica* L., *Crataegus* spp., *Cydonia oblonga* L., *Diospyros lotus* L., *Ficus* spp., *Morus* spp., *Olea europea* L., *Phonexia dectylei* L., *Pistacia* spp., *Punica granatum* L., and *Vitis vinifera*. May be the highest number of *Cydonia* spp. (50 accessions), *Pistacia* spp. (350 accessions), *Punica* spp. (850 accessions), *Phonexia* spp. (650 accessions), and *Vitis* spp (800 accessions) germplasm resources are reported in Iran. Some important fruit species such as: *V. vinifera; C. medica, C. citrange, P. dectylei, P. vera*, and *P. granate* were transferred to other countries from Iran, on the other hand, other fruit species such as banana, chico, citrus, mango, papaya, pecan, pineapple and so on have been introduced from India, Pakistan, North Africa, so on. Since, populations of some wild *spp.* appeared to suffer from genetic erosion and losses, it is very important to collect and conserve of wild *spp.* and local varieties

related to major subtropical fruit trees remains an urgent priority. The conservation of genetic resources had been undertaken traditionally by gardeners from long time ago and later by several National Research Institutes of date and subtropical fruits, which conservation of the local cultivators *in situ* and *ex situ*. In this chapter, the matters regarding their distribution within the provinces, nomenclature, characteristics, ethnobotanical aspects and uses are discussed.

## AP-36

### Formation of grape intergeneric genomes by means of biotechnology

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In our Institute, we have attempted research to achieve grape intergeneric hybrids from crosses between the genus *Vitis* L. (the species *Vitis vinifera* L. as the initial female parent, 2n = 38 chromosomes) and the genera *Ampelopsis* Michaux (the species *Ampelopsis acontifola* Lavallée, 2n = 40) and *Parthenocissus* Planch (the species *Parthenocissus quinquefolia* Planch, 2n = 40). It is known that fertile intergeneric hybrids may be raised by fusion of diploid gametes. That is why the tetraploid variety Chasselas Gros Coulard (2n = 76), the mixoploid Kharti pro Livie and Yakhei whose meristems contain both tetraploid and diploid cells and Yantarnyi Magaracha (2n = 38, colchicine-treated) were used as female parents. In spring, buds broken from dormancy of the genotypes Yantarnyi Magaracha, *Ampelopsis acontifolia* and *Parthenocissus quinquefolia* were treated with 0.5% colchicine aqueous solution to induce mixoploid shoots and diploid egg cells and pollen. Several buds produced morphologically altered thick shoots with approximate internodes, which may be formation of diploid egg cells and pollen. The berries had seeds which considerably differed in shape, size and color within each cross, while the majority of the seeds lacked the embryo and the endosperm. The seeds were disinfected and cut transversally, and the parts where the embryos were expected were established in liquid medium supplemented with 6-benzylaminopurine at 0.5 mg/l and 1 mg/l and seedlings was obtained.

## AP-37

### Somatic embryogenesis and rapid plant regeneragtion from leaf derived callus of african daisy

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African daisy (*Osteospermum sp*) is a beautiful garden plant, tolerant to heat and drought stress of Asteraceae family. The flowers are available in a variety of shades such as orange, cream, yellow etc. Although they are native of Africa, they are growing throughout the Mediterranean region, Asia and Europe. The plant mainly propagate vegetatively by cutting and the propagation from seed is time consuming and difficult. To overcome this difficulty, tissue culture technique was developed to regenerate a large number of plants rapidly. Young leaves were collected from 3-week old young seedlings developed *in vitro* on MS (Murashige and Skoog, 1962) media. Leaf explant was cultured on modified MS media with B5 vitamins (Gamborg et al. 1968) in presence of different concentrations and combinations of 6-benzylamino purine (BAP), kinetin (KIN), indole acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). Friable, nodular and highly embryogenic calli were induced in MSB5 media supplemented with 2.2 µM BAP, 2.3 µM KIN, 1µM 2,4-D and 1.2 µM IAA. The calli were subcultured in MS media supplemented with 0.9-1.8 µM 2,4-D and 1.1- 4.6 µM KIN. The somatic embryos of embryogenic callus were differentiated and regenerated a large number of plants in MS media supplemented with 2.2-4.4 µM BAP and 1.1-2.4 µM IAA. The developed protocol established a highly efficient method of mass propagation of plantlets through somatic embryogenesis from leaf explant.

## AP-38

**Somatic embryogenesis and plant regeneration of monastrell (*Vitis vinifera* L.), a red wine variety**

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Grapevine is an economically important fruit crop world-wide. Although improvement of this crop is possible by conventional breeding, it is rather difficult because of the high levels of heterozygosity, inbreeding depression and is time-consuming because of the 2-3 year generation cycle. An alternative to overcome this situation is the use of genetic transformation. A successful application of gene technology requires, as a first step, an efficient regeneration method that permits both transformation and regeneration into plantlets. One red wine variety of grapes, Monastrell, has been used to induce somatic embryogenesis and plant regeneration. As explants, immature anthers and ovaries from inflorescences collected in the vineyard 10-12 days before anthesis were used. The effect of chilling of the inflorescences for 0, 24 h or 27 h at 4ºC before explant culture was studied. The explants were cultured on MS medium with 7 μM 2,4-D and 1.3 μM BAP and maintained in the dark at 25ºC. Calli were transferred onto a MS medium without growth regulators and 2.5 g/l activated charcoal, for embryo differentiation. The somatic embryos were cultured in MS medium for plant regeneration. Somatic embryogenesis and normal plant regeneration have been obtained from ovaries and anthers. Best results were obtained when inflorescences were maintained for 24 h at 4ºC: embryogenic calli induction efficiency was up to 3.4 % in anthers and 42.2 % in ovaries.

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## AP-39

***In vitro* germination of stoneless and isolated embryos of 14 sicilian cultivars of *olea europaea* L.**

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Olive seeds are characterized by an orthodox behaviour and exhibit a state of dormancy caused by different factors such as the hard covering structures and the inhibitory germination substances contained both in the teguments than in the endosperm and within the embryo. For these reasons, olive seed germination rates are usually low and germination is slow. To speed up olive seedling development can be very advantageous in breeding programs as well as in rootstocks production for grafting of hard–to–root cultivars. In this study, the *in vitro* germination potential of stoneless seeds (seeds without the sclerified endocarp) without teguments and of excised embryos of fourteen Sicilian olive cultivars was evaluated. For the *in vitro* germination of embryos, three different media (presence/absence of plant growth regulators, different carbon source concentrations) have been evaluated. Embryo germination started after 9 days and its percentage was higher than that one of stoneless seeds in eleven cultivars (it reached 100% in six genotypes). *In vitro* stoneless seed germination varied from 6,7 to 76,7 % according to the cultivar, while isolated embryo germination varied from 0 to 100%. Differences in the isolated embryo germination percentages due to culture media were recorded. The growth of plantlets derived from *in vitro* germinated embryos in the greenhouse was normal. *In vitro* germination of isolated embryos could be a useful method for increasing the efficiency of germination. According to these results, embryo culture can increase the efficiency and shorten the time for starting initial progeny evaluation of olive breeding programmes.

## AP-40

### *In vitro* germination and seedling development of caper (*Capparis spinosa* L.) mature seeds

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*Capparis spinosa* L. (Capparidaceae) is a perennial tropical and subtropical shrub plant worldwide known. In the Mediterranean basin, where it grows both wild and cultivated, and particularly in the southestern Iberian peninsula and in Sicily and its islands (Pantelleria and Salina), caper dues its importance other than its medicinal proprieties well known by the ancient Greek and Romans, also to aromatic properties of the young flower buds that processed, (brined or fermented), have increasing importance in the food industry because of their employ in the Mediterranean cooking. Despite the increasing demand and economic importance of capers, little information is available regarding the propagation of this shrub. In fact, it is usually propagated by seed, but their percentage of germination is very low. In this report in vitro seeds germination and seedling development of *Capparis spinosa* L. were studied and many treatments were evaluated to determinate the best factor increasing the germination percentage. High variability was observed among the germination percentages of the different treatments (3%-81%) due to their different efficiency to break dormancy. The scarification through seed coat rupture was determinant to improve the germination percentage. In vitro obtained seedlings are valuable to establish micropropagation protocols for caper.

## AP-41

### Somatic embryogenesis in tamarillo (*Cyphomandra betacea*): recent advances

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*Cyphomandra betacea* (Cav.) Sendtn. is a woody plant of the Solanaceae family commonly known as tamarillo or tree tomato. This species is native of the Andean (Peru, Chile) regions where it has been under cultivation for a long time. Tamarillo is used mainly for its edible fruits and, to a lesser extent, as an ornamental. Fruits have a high nutritional value and contain relatively high amounts of vitamins B6 and E and provitamin A. New Zealand is the main producer of this crop but an increasing of its culture is arising in some other countries such as Portugal. In recent years, different aspects related with somatic embryogenesis induction and somatic embryo development of tamarillo have been studied at our lab making it a suitable model to understand the cytological and molecular mechanisms involved on somatic embryo formation and development, a morphogenic process with important applications both for plant cloning and genetic transformation. Several explants of tamarillo have the potential to initiate embryogenic cultures including, mature zygotic embryos, young leaves, cotyledons and hypocotyls. Attempts are being made to induce somatic embryogenesis from adult plants with the objective of cloning selected genotypes. This goal was already achieved through the induction of somatic embryogenesis from adult material propagated *in vitro*. However, a more direct approach is necessary to reduce the costs and the time of the process. To achieve this goal assays with the pith stem are being performed. The ability of different genotypes to undergo somatic embryogenesis is also being tested with preliminary results showing that some cultivars are more susceptible than others for somatic embryo formation. Tamarillo embryogenic calli can be maintained *in vitro* in an auxinic containing medium for several years. However, some lines of these long-term embryogenic calli are quite unstable in culture and variations in chromosome number and in the amount of DNA have been detected. Following the identification of a protein associated with non-embryogenic calli of tamarillo somatic embryogenesis induction is under investigation in mutant lines of *Arabidopsis thaliana* that do not express a similar protein. A resume of these different lines of investigation will be presented during the symposium.

## AP-42

### Regeneration and field evaluation of banana (*Musa acuminata*, aaa group) plants from proliferating inflorescence-derived embryogenic suspension cultures

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Large scale commercial propagation of banana plants is currently based on in vitro shoot-tip cultures. This technique offers a number of advantages over conventional propagation methods to produce high quality planting material, but still requires extensive manual labor. Although not routinely used for multiplication, somatic embryogenesis has been considered of great promise for the mass propagation of banana. However, the possible occurrence of somaclonal variation in suspension-derived plants, together with the limited availability of embryogenic competent tissues, has restricted its applicability to basic laboratory research. In this work, in vitro proliferating florescence cultures are proposed as a new source of embryogenic responsive tissues for an intensive and recurrent production of competent explants. The potential of its use for the establishment of embryogenic cell suspensions is discussed and the whole plant regeneration schedule is described. This new methodology was used for the regeneration of 'Dwarf Cavendish' banana plants of the local selection 'Gruesa'. A set of regenerated plants was established in the field and evaluated for phenological, morphological and productive parameters during a first production cycle under commercial greenhouse conditions in the Canary Islands.

## AP-43

### Introducing new genes into thompson seedless grapes

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Thompson Seedless grape cultivar is the major common in Egypt in which it is adapted to local environmental condition in addition to its widely acceptance to consumers. New introduce colored Seedless cultivars such as Beauty Seedless, Flame Seedless and others have a good appearance but with less acceptable taste. Therefore, it was of great importance to introduce genes responsible to color and flavor of the new cultivars into Thompson Seedless cultivar. Hybridization of seedless x seedless will result in seedless hybrid that needs a tool such as embryo rescue to germinate the aborted embryo. The objectives of this study were to a) rescue embryos of Thompson and Beauty Seedless grape cultivars, b) define the optimal age and size to isolate the embryo as well as the best media for planting under *in vitro* conditions and determine the anatomical stage of embryo development of the two seedless cultivars to illustrate the embryo abortion and rescue ages. Embryo survival was affected mainly with embryo age; number of survived embryo started mainly five weeks after fruit set and reached its maximum at the seventh and eighth weeks after fruit set for both Thompson and Beauty Seedless grape cultivar. Embryo rescue technique succeeded to rescue embryos of used cultivars for six weeks starting from the 5$^{th}$ week after fruit and at different degrees of success meanwhile, embryo abortion reached its maximum after the 9$^{th}$ weeks after fruit set. MS, WP and B5 plant media produced the highest percentage comparing to WH and NN plant media on the period from the third week till the 9$^{th}$ week after fruit set. Correlation study was made between embryo length and embryo survival and germination for both Thompson and Beauty Seedless grape cultivars .Embryo formation appeared two weeks after fruit set and developed normally until it reached its maximum length at 7 and 8 weeks after fruit set. Embryo abortion clearly occurred 9 and 10 weeks after fruit set when embryo length dropped until it completely degraded. Vascular bundles through mesocarp and endocarp reached to the embryo appeared two to four weeks after fruit set, while it started to degrade early than the embryo at the 7$^{th}$ and 8$^{th}$ week after fruit set.

## AP-44

### Protoplast isolation from leaves of three cultivars of peach

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Peach *(Prunus persica)* is one of the important fruits in moderate regions. In deciduous fruit trees, such as peach, owing to their heterozygosity in genotype and long juvenile period ‡classical methods of breeding is difficult and time-consuming. Therfore, parasexual breeding methods by using their protoplasts will be very beneficial. In this study, protoplasts were isolated from four different size of leaf (4-10 mm, 10-16mm, 16-22, and 22-30 mm in lenght) of three cultivars (Elberta, Red Haven, and Mashhadi) of peach under 5 different digestion time (10, 14, 18, 22, and 26 h). The results showed that effect of cultivar on viability and quantity of isolated protoplasts was not significant. The yields of 106 – 109 peach protoplasts/ gfw-1 were obtained depending on factors such as digesting time, and leaf size. For protoplasts isolation, leaf segments were incubated on enzyme solution that consists of cellulase onozuka R-10 (2%) and macerozyme (1%). In respect of leaf size, small leaves, 4-10 mm in lenght, were a best source for protoplast isolation than medium or big expanded leaves, 22-30 mm in lenght. Furthermore, the high yields of protoplasts were obtained when the leaf segments were incubated on digestion enzymes for 18-22 h. By this time of incubation, viability of isolated protoplasts was 85%-90% for all cultivars.

## AP-45

### The effect of plasmolysing time, enzyme concentra-tion, and digestion time on protoplasts isolation from leave mesophylll of apricot

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Apricot *(Prunus armeniacana)* is one of the important fruits in moderate regions. Progress in ‡classical breeding of apricot is impeded by its complex genome and the long breeding cycle. Therefore, its improvement by ‡classical methods of breeding is difficult and time-consuming. Protoplast transformation and culture is one of the suitable tools for breeding of this fruit. Different factors such as source of explants, plasmolysing pretreatment, digesting enzyme solution and digestion time affect the quantity and quality of isolated protoplast. The purpose of this study was to determine the effect of plasmolysing pretreatment and digestion time on frequency and viability of isolated protoplasts from leave mesophyll of apricot. In order to isolate the protoplasts, the leaves were plasmolised in sorbitol 13% under 3 time treatment (6, 9 and 12 h). Then, the leave mesophyll were incubated in enzyme solution that consists of cellulase R-10 (1%), Pectolyase Y-23 (0.1%, 0.2% and 3%) and macerozyme (0.5%, 1% and 2%) under five digestion times (10, 12, 14, 16 and 18). The results showed that quantity and viability of isolated protoplasts was significantly affected by plasmolysing, enzyme concentration, and digestion time. Plasmolysis of leaves for 90 min in a 13% sorbitol solution greatly increased the number of protoplasts obtained. The best of enzyme solution was consists of cellulase R-10 (1%), Pectolyase Y-23 (0.1%) and macerozyme (0.5%). After 16 h. enzyme treatment, $10^8$ protoplasts with 85% viability was obtained, that this time is the best time-term treatment for protoplast isolation from mesophyll cells of apricot.

## AP-46

### The reaction of raspberry and blackberry cultivars to drought stress simulated *in vitro* by polyethylene glycol (PEG) 6000

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The goal of this study was to evaluate whether *Rubus* genotypes could be selected in vitro for tolerance to drought stress. Drought conditions were simulated by introducing polyethylene glycol 6000 (PEG) into the proliferation medium. The content of proline and soluble sugars were used as markers of stress reaction. The proline content increased in all the tested cultivars as a result of culturing the shoots on a PEG-containing medium. The highest content of proline was found after the first week of culture. After the second and third weeks, the content dropped, and after the fourth week, it increased in three of the six cultivars. The increase in proline content was usually proportional to the PEG concentration. In the control explants (grown on the medium without PEG), the proline content in red raspberry amounted to 100-400 µg/g F.W. depending on the cultivar. In blackberry, it was about 1000 µg/g F.W. The maximal content was 900 µg/g F.W. for raspberry and 2500 µg/g F.W. for blackberry. The highest stressor concentration (2.0 g PEG) seemed to be the most usable, as it yielded a stable and equivocal increase in proline content. The higher PEG concentrations (up to 40 g/l) arrested shoots growth, which made the determination of proline content impossible, due to insufficient tissue. In this experiment the proline content was determined after a further four weeks, when the explants formerly exposed to PEG were cultured on a medium without PEG and growth regulators. The proline content increased with PEG concentration in the past medium, although the quantities were different to those obtained on the medium containing PEG. The proline content seems to be an applicable marker in raspberry selection, because all the cultivars showed an increase in proline content in reaction to the drought stressor. The reaction differed in strength depending on the concentration of the stressor in the medium and the genotype. To prove a practical value for breeding, the results obtained in vitro will be compared with results from in vivo experiments which are in preparation.

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## AP-47

### Effect of salt stress on the growth and physio-biochemical attributes of ten *Brassica juncea* L. Czern. & Coss. genotypes

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$Na^+$ is toxic to plant species, making soil salinity is an increasing threat for agriculture and is a major factor in reducing plant productivity; therefore, it is necessary to obtain salt-tolerant genotypes. India is a paradise of oilseed crops. *Brassica juncea* L. Czern & Coss. is cultivated as a major oilseed crop in India. The effect of NaCl stress on shoot length, leaf area, leaf area index (LAI), fresh (FW) and dry weight (DW), sensitivity rate index (SRI), net photosynthetic rate ($P_{N)}$, stomatal conductance ($g_{s)}$, total chlorophyll content, malondialdehyde (MDA) content, NR and ATP-sulphurylase (EC:2.7.7.4) activities, leaf- N, K and Na content, K/Na ratio, GB and proline content were investigated in ten *Brassica juncea* L. genotypes at 55 and 65 days after sowing (DAS). NaCl treatments decreased all above parameters except GB and proline, MDA, Na content and SRI at both stages. Salt stress resulted in accumulation of GB and proline, in all genotypes. The magnitude of increase in both osmolytes was higher in genotype $V_8$ than the other genotypes. Salt stress induced Na and MDA accumulation while it decreased K and N and K/Na accumulation, total chlorophyll synthesis and activity of NR and ATP-sulphurylase in all genotypes. But the magnitude of increase in Na and MDA accumulation and decreased in shot length, leaf area, leaf area index, $P_N$, gs, total chlorophyll synthesis and activity of NR and ATP-sulphurylase in genotype $V_8$ in comparison of other genotypes. These results suggest that salt-tolerant genotype may

have better osmotic adjustment and protection from free radicals by increasing the accumulation of proline and GB with overproduction of K, N, K/Na and enzyme activity of NR and ATP-sulphurylase under salinity stress.

## AP-48

### Magnetic field-induced modification of DNA content in date palm (*Phoenix dactylifera* L.)

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Plant stress caused by exposure to magnetic fields (MF) induces modifications at molecular level, particularly DNA synthesis, structure, and function. The objective of this study was to determine the effect of various doses of MF on date palm (*Phoenix dactylifera* L.) based on DNA content. Date palm seedlings (cv. Khalas) were established for 2 weeks on filter paper after which they were subjected to static magnetic field using electromagnetic circuit at 10, 50, and 100 mT for 30, 60, 120, 180, 240 and 360 min. Following exposure, the seedlings were grown in potting soil for 8 wk after which DNA was extracted from leaves and its content was determined. Generally, the exposure to magnetic field caused reduction in the content of DNA. The lowest exposure time tested, 30 min, was sufficient to induce reduction in DNA content. This was true even at the lowest intensity, 10 mT. This dosage caused the DNA content to decrease from 49 µg/g to 45 µg/g. Further increase of the exposure duration to 60 min caused further significant reduction in the DNA content, 36 µg/g; however. At intensities higher than 10 mT, DNA content decreased significantly even at the shortest exposure of 30 min. At 50 and 100 mT, significant decrease in DNA content was also noticed in response to 30 min exposure; however, longer durations caused no further decrease in the DNA content. These observations indicate that magnetic fields interact with DNA processes, probably by inhibiting synthesis or stimulating degradation of DNA. This response merits further exploration as a mutational agent for date palm genetic manipulation.

## AP-49

### Tree canopy characterization, modelling and effect on olive (*Olea europaea* l.) production

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The tree canopy of fruit species is one of the major factors that affect their cultivation regardless to the plantation density, cutting, vegetative growth and production. For olive tree specie, the canopy parameters vary widely among varieties. In Tunisia, a large number of varieties and local types was identified and characterized in the last two decades. Recently, we have planted olive orchards in several regions in the centre and the south in order to examine their performances. Orchards were installed in Tataouine (2002, intensive and irrigated by wastewater, 19 varieties), Zarzis (2002, rainfed conditions, 20 varieties), Hicha (2001, intensive and irrigated by salty water, 15 varieties) and Ettaous (2001, intensive and irrigated, 11 varieties). Since 2005, we have followed the olive production per tree and the canopy measurements which are the vegetative height, the two vegetative diameters and the trunk diameter. Theses measurements were taken for three trees per variety and for three successive years and from them we calculated the canopy parameters: volume and area and trunk section area. In Ettaous orchard, we have followed monthly in 2006 the canopy parameters. Multiple regression analysis was performed to determine the canopy parameters that play a major rule in olive production. Also, cluster analysis was performed to understand how the olive canopy behaviour was expressed in relation to variety, region or year. A canopy growth evolution and modelling by variety was done for Ettaous orchard. The main objective of this study is to know how to manage canopy for highest olive production.

## AP-50

### Mineral composition of some walnut cultivars (*J. regia* L.) for evalution of ionome and ionomics in stress condition

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Walnut is one of the very sensitive plants to abiotic stresses and finding genetic resources to drought tolerance is important in this species. Effects of salt stress on germination of seeds was studied in seven cultivars ('Serr', 'Lara', 'Pedro', 'Chandler', 'Hartely', 'Vina', 'Roundemontig') of J. regia L. on germination and early seedling growth. Stress treatments were 50, 100, 150, 200 and 250 mM obtained using NaCl. The objective was to determine genotypic differences among walnut cultivars in terms of salt stress and evalution of mineral composition in cultivars to achieve the mechanisms that involve in stress tolerance in walnut. The results of mineral composition measurement showed that Ca and K accumulation was increased by increasing in osmotic stress level especially in shoots of most tolerant and tolerant varieties. However, in the semi tolerant varieties the range of K in root was more than shoot. It is proposed that this reflects differences in membrane transport properties of cell in different stress tolerant groups. Osmotic stress increased K uptake from root to shoot, which is likely to be a regulation of osmotic adjustment. The range of variation of Mg in the root and shoot samples were not significant for all stress levels and were not dependent to varieties. Range of Na was minimum in compare with the other minerals in different stress leveles. Na level in root was more than shoot for more varieties especially in the most tolerant and tolerant varieties. K+ efflux following severity stress level may be a suitable screening indicator for drought tolerance in walnut.

## AP-51

### Timing of 1-methylcyclopropene exposure influences shelf life of cavendish bananas in relation to ethylene application

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We examined the response of shelf life and fruit quality of banana (cv. Williams) from the middle of the bunch to the application of 1-MCP and ethylene simultaneously as well as the effect of pre- and early-climacteric application of 1-MCP (multiple applications) treatment of bananas harvested from the top or bottom of bunches. Fruit were treated with ethylene at 100 µL L-1 for two consecutive days as a control or simultaneously with 1-MCP at different concentrations (30, 100 or 300 nL L-1) on the first day or second day, or treated with 1-MCP alone on the third day. To examine the effect of low concentrations of 1-MCP in the pre-climacteric stage fruit were treated with 1-MCP at (0, 2, 4, 5, 6 or 10 nL L-1) for 6 h at 22 C and followed by ethylene at 100 µL L-1 for two consecutive days (control) or ethylene followed by early-climacteric 1-MCP application at 300 nL L-1. Shelf life increased significantly compared to the control when 1-MCP was applied coincidently with ethylene in the second day and reapplied alone in the third day or applied only in the third day. The highest increase in shelf life (120%) was obtained when 1-MCP was applied on the second day at 30 nL L-1 simultaneously with ethylene and at 300 nL L-1 alone on the third day compared to the control. Application of 1-MCP at the lower concentrations at the pre-climacteric stage in combination with reapplication of 1-MCP in the early-climacteric stage increased shelf life significantly in both fruit from the top and bottom of the bunch. The highest increase in shelf life was obtained in fruit from the bottom of the bunch when exposed to 2 nL L-1 1-MCP (43%) and in fruit from the top of the bunch when exposed to 5 nL L-1 1-MCP (39%) prior to ethylene treatment and

subsequent 1-MCP treatment. Higher concentrations of applied 1-MCP in both experiments sometimes caused fruit ripening not to occur, in other treatments 1-MCP had no negative impact on shelf life and quality parameters such as firmness, discoloration index, weight loss, total soluble solids. These observations suggest that the efficacy of 1-MCP to improve shelf life and quality of bananas is reliant on not only the concentration of applied 1-MCP but also the timing of 1-MCP application in relation to ethylene application. We conclude that simultaneous application of 1-MCP is more effective than the more common method of extending banana shelf life through application of 1-MCP after ethylene treatment.

## AP-52

### Breeding for early maturity and seedlessness in grapevine by means of biotechnology

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In our research into breeding for early maturity and seedlessness in grapevine, methods relying on biotechnology enabled viable progeny. To this end, a protocol was developed which envisaged establishment of the embryo having a fragment of the seed coat with adjacent tissues in culture under in-vitro conditions on nutrient media supplemented with growth regulators. A number of general peculiarities pertaining to growth and development of plants were revealed. Those included long germination time, high proportion of anomalous plantlets in the progeny, occurrence of polyembryony and high mortal rates of plants in early developmental stages. Normal development was generally observed in plantlets with expressed hypocotyl and cotyledonary leaves though the proportion of such individuals in the progeny was low. Single tropisms were registered, and complete of cotyledonary leaves in the testa also occurred, which resulted in poor development and death of the plant. A considerable proportion of plantlets had anomalous axial organs. They were susceptible to BA levels in the medium. By adjusting BA concentrations, the formation of the plant was regulated for such directions as indirect development of the plant, proliferation of numerous shoots with subsequent rooting, shoot formation by hemogenesis, direct and indirect embryogenesis. Thus, such a differentiated approach to the realization of a new genotype's morphogenetic potential will reduce death rates of plants in early developmental stages.

## AP-53

### Study on softening of vinagrillo (*Averrhoa bilimbi*) fruits during ripening

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Vinagrillo is a climacteric fruit and ripening occurs in a few days after harvest. Ripening mainly involves a series of changes characterized by softening and changes of color by the combined action of ethylene and some hidrolases enzymes such as pectinmethylesterase (Pme), polygalacturonase (Pg) and celulase (Ce). Some physical and chemical characteristics of the fruits during ripening were determined, the kinetic activity of enzymes previously mentioned and the activity of these enzymes with fruits softening were a relation. The fruits were harvested in different states from maturation: green, yellow green, ripened and overripened. The activity of Pme determined by the method of Hagerman and Austin (1986). The activity of enzymes Pg and Ce were determined according to the methodology of Durbin and Lewis (1988). Pme displayed greater activity at pH between 5 and 11 and performed a greater activity to 30 degrees Celsius. Pg showed greater activity in the green state and showed an optimal temperature of 30 degrees Celsius and the maximum activity to pH 7. Kinetic characterization of Pme and Pg, was not made since they did not show a kinetic one of Michaelis-Menten. In the case of detectable activity of CE in any of the ripening states was not demonstrated. Péctic substances in cellular wall undergo modifications due to the action of enzymes Pme and Pg. Statistical analysis were variance analysis with Tukey test and Kruskal and Wallis test with a significance level of 5%

## AP-54

### Influence of rootstock on mineral compositin of apple fruits and leaves

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Significant differences were evident in levels of minerals (N, K, Ca, Fe, Mn) uptakes by apple (*Malus domestica* Borkh.) trees grafted on 5 various rootstocks (East Malling = EM.27, EM.9; Malling Merton = MM.106, MM.111; seedling = *M. domestica* Borkh.). Mineral concentrations (DW%) in plant tissues were compared on 4 various apple (Gala, Fuji, Red and Golden) grafted trees in a Latin Square Trial Design in the province of Zanjan, Iran, in 2005-2007. All treated trees were similarly fertigated with essential minerals in accordance with traditional local standard (soil mineral nutrient analyses). Concentrations (DW%) of minerals in plant tissues, when sampled over a 3-year period, were significantly (P = 0.05) affected by the kind of rootstock, and the fruit mineral concentrations, rather than the kind of scions (Gala, Fuji, Golden, and Red). Leaf and fruit tissue analysis showed that different rootstocks had different N uptake efficiencies throughout the season. M.27, M.9 RN29 and M.9 EMLA were more efficient at N. Trees on seedling rootstock were among the highest in leaf Ca, and Mg, whereas, M.27 and M.9 were the most efficient rootstocks in N, and Fe uptakes. Consequently, M.9 was most efficient in microelement deficiencies (Fe, and Mn). MM.106, and MM.111 were the least efficient rootstocks in Fe, and Mn uptakes, but the most efficient rootstocks in K and Mg uptakes. Consequently, trees on MM.106 rootstock had the lowest (DW%) minerals (N, Fe, Mn) throughout the season regardless of the amounts of minerals in their fruits. Rootstock also significantly affected the N/K ratio of fruit, which it was the highest in dwarf rootstocks (M.27 and M.9), and the lowest in MM.106, and MM.111.

## AP-55

### Bioactive compounds in black currants (*Ribes nigrum* L.) and their potential health-promoting properties

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Considerable amount of recent epidemiological data suggest that a high intake of fruits and vegetables offers a number of health benefits against degenerative diseases and can promote longevity. The potential health benefits of berries and fruits as well as industrial products (juices, jams, yogurts) may be greatly increased if crop cultivars with high contents of the health-promoting compounds are used as such or as raw materials. Black currants (*Ribes nigrum* L.) contain a diverse range of phenolics and possess a high antioxidant activity, which makes them an interesting target for functional food industry. A very high content of anthocyanins (250 mg/100g of fresh fruit) have been detected from berries, and at least part of the orally administered anthocyanins are absorbed by humans and are found as intact anthocyanin glycosides in the blood. A high level of several flavonols including myricetin, quercetin, kaempferol and isorhamnetin as well as polymeric anthocyanidins, has also been detected. Among hydroxycinnamic acids, caffeic acid, m-coumaric acid, p-coumaric acid, ferulic acid and sinapic acid, are the most abundant. The potential health benefits of flavonols, anthocyanins and other phenolics such as reducing risk of having cancer, cardiovascular and type II diabetes have been suggested, but specific health benefits remains to be waiting for extensive clinical trials. We outline of our new European research project (BrainHealthFood), where neuroprotective fractions will be extracted and bioactive compounds determined from black currants. The neuroprotective activity will be analyzed in neuroblastoma cells and in a transgenic mice model of Alzheimer`s disease. Bioavailability of phenolics from black currants will be demonstrated by human trials. Understanding of the neuroprotection activity of the black currants and

their bioactive compounds will provide essential information for the development of specific products for elderly people.

## AP-56

### Physicochemical characteristics of *Detarium microcarpum* fruits

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The fruits of *Detarium microcarpum* are very much appreciated for consumption during the hot dry season by Malian populations. Therefore, we tried to assess their food value and physicochemical properties according to the pedoclimatic diversity in Mali. An ethnobotanical survey on food values was done with bobofing, senoufo and minianka ethnic groups in the south of Mali. Thirty samples of fruits per group were analyzed in the laboratory for taste, Brix degree, proteins, dry matter, vitamin C and pH. Some correlations were established between these parameters and fruit dimensions. Results show that the fruits of *D. microcarpum* are very much used in traditional food. They also show that these fruits can constitute a source of plant proteins and sugar. The local knowledge and the physicochemical parameters of *D. microcarpum* fruits can justify the valorization of this plant.

## AP-57

### Phenotypic characterization of a large-fruited mutant in pear

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Fruit size is an economically important trait in various fruits including pear. Especially in Japan, consumers prefer large fruits for gifts, and large fruits are sold in high price. Therefore, growers thin flowers and young fruits to increase the fruit size. In spite of its economical importance, molecular bases of the fruit size control are poorly understood. Few studies have been conducted except for FW2.2, which controls the fruit size of tomato by regulating cell division. To gain insights into the molecular and cellular mechanisms of fruit size control, we have characterized a large-fruited mutant of pear, which was found in grower's orchard. Observations for two years indicated that fruit weight of the mutant was 1.6-2.1 times larger than that of wild type cultivar 'La France'. Differences in receptacle size were already observed at full bloom. Significant increase in fruit diameter was observed throughout fruit development. Microscopic analysis indicated that an increase of fruit size was due to cell size, but not cell number, of the cortical cells of the mutant. Interestingly, flow cytometry analysis showed 2C, 4C and 8C peaks in receptacle and pedicel cells at full bloom, and 4C peaks in fleshy cortex cells at harvest, whereas only 2C peaks were observed in leaf cells. This phenomenon was similar to endoreduplication, which had been reported in pericarp of tomato fruit. The unique fruit traits, such as high acidity, low starch content and low firmness were also observed. Surprisingly, the soluble solid content of the mutant fruits was similar to that of wild type, indicating high sink strength. Further analyses of this mutant will clarify the mechanism of the fruit size control through cell enlargement in pear.

## AP-58

### Evaluation of means to increase the content of bioactive phenolic compounds in soft fruits

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Phenolic compounds form a large group of plant secondary metabolites with many functions related to the acclimation and adaptation of plants to changing environment and to the interaction with other organisms. Interestingly, numerous studies have shown the positive influence of phenolic compounds on human health, and a higher intake can be considered beneficial. Thus, phenolic compounds can be considers as an important quality factor in soft fruits. In this study potential of different cultivation practices to enhance the content of phenolic compounds in red raspberry (*Rubus idaeus* L.), strawberry (*Fragaria* x *ananassa* Duch.), and black currant (*Ribes nigrum* L.) was evaluated. Plant genotype is known to affect the phenolic content, which was shown also in this study with red raspberries and strawberries. However, the phenolic content was strongly affected by the environment. Thus the range of natural variation of the phenolic profile needs to be established for each genotype. Fertilization influences plant metabolism, and higher fertilization levels were shown here to lower the phenolic content in strawberries. Mulch colour also affected the phenolic content in strawberries, the white mulch increasing the content of total phenolics compared to the brown one. This is apparently due to enhanced photosynthesis caused by the increase in light and temperature. Interestingly, white mulch also led to decreased fruit yield. The fruit order had a significant effect on strawberry fruit phenolics. The phenolic content increased from primary to tertiary fruits. Furthermore, later planting date augmented the difference, which might be due to higher amount of light. The effect of fruit order on the contents of ascorbic acid and sugars in the fruits was opposite to that of phenolic compounds. Organically produced food is generally considered by the consumers as being healthier than conventional food. In this study, the production system was not found to be the major factor in determining the content of measured phenolic compounds in strawberry or in black currant fruits. Several possible ways exist to enhance the content of phenolic compounds in crop plants. However, as different methods have different shortcomings, they should be thoroughly evaluated before their application in practice. Interactions between different factors make it though difficult to apply techniques in the field conditions, whereas in the more controlled greenhouse conditions techniques could be more easily introduced.

## AP-59

### Effect of hexanal treatment on postharvest quality of 'darselect' strawberry (*Fragaria ananassa* Duch.) fruit

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Strawberry (*Fragaria ananassa* Duch.) is one of the highly valuable and important fruit in the world. Strawberry fruits are highly perishable and vulnerable to tissue damage during postharvest storage. Besides, strawberry fruits can be easily infected with *Botrytis cinerea* during storage. Loss of strawberry fruit quality is mainly due to the onset of rots, which is often caused by *Botrytis cinerea*. While as a natural volatile, hexanal (C6 aldehydes) acts as residueless antifungal agent in fruits and vegetables. Therefore, the effect of hexanal treatment on physiological and biochemical responses in strawberry fruit (*Fragaria ananassa* Duch, cv. Darselect) was studied during storage at 4. Fruits treated with hexanal showed lower fungal decay and better overall quality than control confirming the reports about hexanal on its antifungal activity. Hexanal treatment did not change the soluble solid contents of strawberry fruits with the relatively higher titratable acidity content than control. Lowered respiration rate and reduced superoxide radical production were observed in both 0.05% and 0.1% hexanal treatments as compared with the control. Moreover, 0.1% hexanal treatment could effectively reduced lipoxygenase activity during storage. However, hexanal treatment increased phospholipase D activity of strawberry fruits. According to the results we got, we might suggest that hexanal treatment effectively maintains

postharvest quality of strawberry fruit by controlling fruit fungal decay and decreasing respiration rate with less direct impact on the membrane deterioration process.

## AP-60

### Preliminary results on the characterization of fruit and oil quality of some unknown olive genotypes in iran

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The aim of this work was to identify and select olive varieties with desirable characteristics for fatty acid composition among 15 unknown genotypes in Iran. The study was carried out on 15 unknown 8-year-old genotypes in an olive collection in north part of Iran (Zanjan, Tarom). All the selected genotypes were originally planted in Gorgan olive collection (North-East of Iran) in 1975. Fruit samples were collected from 15 genotypes, during September to November 2007. Three replications from each genotype were selected and morphological descriptors of fruits and endocarps were studied. P-NMR (Minipec.Mq-20) was used to measure the total lipid component from dried fruit samples. Fatty acid compositions of the oils were measured in different olive genotypes by using Gas Chromatography (GC). Analysis of the percentage of saturated and unsaturated fatty acids of olive fruits showed the variety of genetic makeup between them, based on the percentage of main fatty acids "palmitic acid, oleic acid and linoleic acid". Interestingly, some of the studied genotypes consisted of a high range of oleic acid. These genotypes seem to be remarkable varieties and could be used for olive propagation program. Further investigation on these varieties is in progress.

## AP-61

### Researches regarding the quality and flavor of some of some apple species and hybrids cultivated in romania

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The surface of apple culture is on all continents and in temperate zone, north and in the south hemisphere. In fruits crops, apple represents the 4th place on the globe with 56 millions tones annual (FAO, 1998). Nutritive value is well known and represents the variable content of sugar, proteins, ascorbic acid, and mineral substances. Consume of fresh fruits or juice, alimentary pastes, jellies, gems assure on entire year the vitamins for a better life. Apples are a part in all food diets and therapeutically value is well known in different illness (determine the absorption of gastric secretions, the elimination of toxins, diuretically effect). In Romania apple culture, have good pedoclimatical conditions in obtaining better crops with high fruit quality (Dejeu et al., 1992). Therefore, the apple tastes from Romania is different and equilibrate because of the accumulation of nutritive substances. Research was made in the University of Agronomic Sciences and Veterinary Medicine Bucharest on some apple - tree species and hybrids which were cultivated in Romania at Voinesti Tree Society: Iris, Irisem, H 1/26, H 3/23, H 4/50, H 4/103, H 5/56, H 5/79, H 6/42, H9/11, H 9/98. Research regarding the apple quality consists in biochemical determinations of sugar, vitamin C, acidity and determinations of mineral elements calcium, iron and toxic compounds nitrates, zinc, copper, lead and cadmium.

## AP-62

### The functional properties of sweet cherry as a new criterion in the breeding program

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The agronomics requirements (chilling unit, flowering, fruit set, harvest date, productivity…) and fruit quality (weight, color, firmness, sweetness…) are traditionally the main aspects considered in a plant breeding program. However, the content of functional properties could be taken into account when parental are chosen for a traditional or biotechnological breeding program. In this work some functional properties as the contents of anthocyanins, polyphenols and antioxidant activity were studied in 11 sweet cherry cultivars harvested at commercial ripening stage. The results indicated that two cultivars ('Cristalina' and 'Sonata') had the highest levels in total anthocyanins (≈200 mg 100g-1) while 'Brooks' and 'Sommerset' showed the lowest (<70 mg 100g-1). On the other hand, 'Sonata', 'Cristalina', 'New Star' and 'Sunburst' had a total phenolic content over 90 mg 100g-1, while in 'Prime Giant' and 'Brooks' this content was lower than 70 mg 100g-1. The total antioxidant activity was determined in both hydrophilic and lipophilic fractions, with 'Sonata' and 'Cristalina' being the cultivars with the highest values, while 'Brooks' showed the lowest one. Considering these results, some sweet cherry cultivars with very good fruit quality parameters, like 'Brooks', do not reach the maxima functional properties. Other cultivars with high functional properties (like 'Sonata') could be considered as a good parental for a sweet cherry breeding program with the aim to improve other characteristics.

## AP-63

### Enrichment, conservation and regeneration of fruit trees ex-situ

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In Albania different climatic zones and microzones as well as human selection have created a comparably large biodiversity in fruit crops such as apple, pear, cherry, peach, apricot, fig, pomegranate, mediar-tree, jujube, cornel-bush, arbutus tree, nut, almond, blueberry, gossbery, date-tree, strawberry, hazelnut, chestnut, olive, citrus, and grapes. The main objectives of this study are enrichment, conservation, and regeneration of fruit trees. Different genetic materials have been selected locally for propagation. Genetic variability is identified, characterized, conserved, evaluated, utilizated and saved for future generations. In Albania country, fruit trees species collections are included in the national project of plant germplasm conservation. An overview will be given for the strategy to collect and conserve fruit tree biodiversity in Albania.

## AP-64

### Evaluation of dry matter in sour cherry (*Prunus cerasus* L.)

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Dry matter of fruits may be an essential parameter in fruit processing. Especially in the very energy-intensive freeze drying process high values of dry matter in the freshly harvested fruits contribute to a lower requirement of energy and to lower costs. In sour cherry often processed by freeze drying, little is known about the dry matter content of various cultivars. For that reason, the parameter was evaluated in 33 cultivars during the years 2005-2007. Dependent on cultivar and year values between 10,7 % ('Montmorency' in 2007) and 28,2 % ('Stevnsbaer Viki' in 2006) could be observed. In the average over the years, 'Montmorency' was characterized by the lowest dry matter content (13,1 %). Maximum values very different from all other cultivars reached 'Stevnsbaer Viki' (23,9 %) and 'Stevnsbaer Birgitte' (24,1

%). In spite of clear variations between the years, cultivars differed significantly. The results show that cultivars with high dry matter content are available for using in freeze drying. The content of dry matter was highly correlated to the content of soluble solids (correlation coefficient 0,91). Therefore the investigation of soluble solids can be used for rapid testing of dry matter of a wide range of genotypes.

## AP-65

### Physiological bases and biotechnological methods for induction of the entry of grape seedlings into bearing

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Grape seedlings normally need four to five years to come into bearing under field conditions. Direction of our research is to induce accelerated entry of grape seedlings into bearing for selection of promising individuals. The selection is based on yield quality, berry resistance to mildew, oidium and gray rot and the number of cluster per shoot during the first to second year of their maintenance in the glasshouse. Optimum levels of cytokinins, biologically active substances and wetting agents for treatment of seedlings have already been adjusted. Cultural practices which induce branching of shoots and development of lateral suckers as well as formation of inflorescences (tipping and pruning of green and mature wood) have been determined. We also attempt to induce formation of inflorescences during the first year of the maintenance of seedlings in the glasshouse with subsequent crossing between candidates based on the information already available about their resistance to biotic and abiotic factors as well as yield quality in the second year. The protocols to be developed will enable the breeder to select seedlings resistant to frost, phylloxera, mildew and oidium from a large sampling of seeds over a two-year period. They will also enable yield quality of these seedlings to be assessed by inducing their accelerated entry into bearing during the first year of their maintenance in the glasshouse, which will allow their use as sources of desired traits in breeding programs as soon as during the second year.

## AP 66

### Efficient shoot regeneration system from pear rootstock OHF – 333 (*Pyrus communis* L.) leaves

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The pear rootstock OH x F (P. communis L.), selected in USA, shows 10% more vigorous grouth comparing to BA29, but has slower growth than seedlings of "Williams". The rootstock is characterized with high yield efficiency and resistance to fire blight (Erwinia amylovora). Some clones of OH x F are difficult to propagate, that's way they are propagated in vitro. The aim of the present research is to develop an efficient system for shoot regeneration from pear rootstock OHF – 333 leaf explants and to investigate the effect of plant growth regulators on the regeneration capacity. Studies were carried out with leaf segments of in vitro propagated plants of OH x F (P. communis L.) cultivar. Leaf segments of the source plants were cultivated on eight nutrient media for regeneration based on MS with added TDZ (7.5 and 9μM), 2.5 μM IBA, sucrose 10 g/l, sorbitol 30g/l and different IAA content – 5, 10, 15 and 20 μM. The explants were cultivated in darkness for 15 days, after which – at a photoperiod of 16/8 hours (40 μmol m-2 s-1 PAR) at a temperature of 22±20C for 25 days. The best efficiency of somatic organogenesis (over 80% regeneration and more than 5 regenerants from explant) were achieved on two nutrient media - with 7.5 μM TDZ, 2.5 μM IBA and 20 μM IAA or with 9 μM TDZ, 2.5 μM IBA and 10 μM IAA. All regenerants obtained are cloned, propagated and after acclimatization will be tested for resistance to fire blight (Erwinia amylovora).

## AP 67

### Effect of sucrose level on the photosynthetic ability of *in vitro* cultured apple rootstock MM 106

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The aim of the present research is to observe the effect of sucrose level in the nutrient media on the photosynthetic ability of in vitro cultivated apple rootstock MM 106. The plantlets of stage elongation (hormone-free medium) were grown at different sucrose concentrations (0, 1, 2 and 4% w/v) in the medium and in two types of vessel closure - tightly closed and gas-permeable (gas exchange rate 3 volumes/hour). The net photosynthetic rate (light and CO2 curves), chlorophyll fluorescence (Fv/Fm ratio, effective Yield – Genty- parameter, ETR, qP, qN and NPQ) and content of light-harvesting pigments are observed. There is a positive correlation between sucrose concentration and accumulation of dry matter and light-harvesting pigments. But increased sucrose concentration leads to decrease in the net photosynthetic rate (Pn) of the plantlets. Fv/Fm ratio was in the range 0.813 – 0.791 – typical for non-stressed plants. Plantlets cultivated with gas-permeable closure without sucrose showed highest value of Genty- parameter, ETR and qP. The highest increasing of leaf area is achieved in plantlets cultivated on medium with 1% sucrose and gas-permeable closure. The results from this investigation as well as the recent experiments show that plantlets photosynthetic ability and growth in vitro are restricted mainly because of the environmental factors in the cultural vessels.

## AP 68

### Response to different carbohydrate concentrations in the medium during long-term cold storage of fruit shoot cultures

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The maintenance of stock cultures at above-freezing temperatures (generally 4-5°C for temperate species) is a common storage technique, imposing a long-term slowdown of cell metabolism and permitting culture regrowth when standard culture conditions are restored. The technique, also termed “slow growth storage”, is commonly applied by commercial micropropagation laboratories in dark cold rooms; this way, the periodic subculture shifts from the normal 3-4 weeks to a much longer interval (generally 6-12 months, depending on the species), hence reducing the costs due to manual labour and the risks of culture contamination. The present study investigated the effect of the type and concentration of carbohydrates, supplemented in the storage medium, on the long-term *in vitro* conservation of shoot cultures from three fruit rootstocks (‘Gisela 5®’, ‘Myrabolan 29C’, and ‘GF677’) and one kiwi cultivar (‘Hayward’). The shoot cultures, coming from the proliferation stage of the standard micropropagation process, were stored at 4°C in darkness, inside 500-ml glass jars, containing 140 ml of gelified media. Sucrose at concentrations of 30, 45, or 60 gr/l, or a combination of sucrose and mannitol (at 30 gr/l and 15 gr/l, respectively) were used in the storage medium, in order to test their influence on shoot quality and maximum storage time. $CO_2$ and ethylene accumulation were gas-chromatoghraphically determined during conservation. The study was prolonged up to 16 months and the storage characteristics were evaluated on the basis of the following parameters: (i) the slowdown of shoot “relative growth rate” (RGR) during conservation, (ii) the percentage of culture units (=jars) lost in the time, (iii) the appearance and gravity of shoot abnormalities (hyperhydricity, decay, etiolation of shoots), and (iv) the RGR of shoot cultures, 4 weeks after their return to standard culture conditions. In general, all the cultures showed a satisfactory reduction of the RGR during conservation, while best conditions of conservation and regrowth were obtained when they were stored in sucrose-enriched media (45 or 60 gr/l). For all the cultures, such conditions made available the maintenance of an high shoot quality for more than 12 months of cold storage. In kiwi, in particular, the conservation at 4°C in 60 gr/l sucrose-

containing medium made it possible the maintenance of shoot cultures in excellent conditions up to 18 months.

**AP 69**

**Optimization of temporary immersion system for *in vitro* propagation of peanut**

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Semi-automatic "Temporary Immersion System" (TIS, also known with the commercial name of RITA®), developed in the '90s, combines the advantages of both classic micropropagation on solidified medium (i.e., sufficient oxygen availability for the explants) and liquid culture (i.e., full contact of the explants with the nutrient medium) by means of periodic immersions in liquid medium followed by dry periods. Accumulation of culture gases (such as ethylene and $CO_2$) inside air-tight containers is also avoided by periodic air replacement made available by pneumatic transfer. Thus, the growth of shoots can be improved in terms of both quality and quantity. To date, the system has been tested with several fruit and crop species and often produced an enhanced proliferation rates and higher quality of the cultures. Present study is the first example of the use of TIS for *in vitro* production of peanut, a plant species that is still considered relatively recalcitrant to tissue culture applications. Several immersion periods (16 min immersion in every 16 hours; 8 min immersion in every 16 hours; 4 min immersion in every 2 hours) and medium compositions (liquid MS medium containing 27.5; 55; 110 µM BA; 5; 7.5; 10 µM TDZ) were tested to optimize the efficiency of the system for the induction of organogenesis on de-embryonated cotyledon explants of Virginia type peanut plants. Optimized protocols were then tested for the applicability to three valuable Turkish local cultivars. The effectiveness of the technique was evaluated on the basis of the following parameters: (i) the percentage of shoot regeneration, (ii) the number of developing shoots, and (iii) the length of developing shoots. The system was proved to be very effective in inducing multiplication, with a shoot forming capacity of about 10-fold (when BA was used) and 7-fold (when TDZ was used) in comparison to the solidified medium.

**BP-01**

**A multi-vector transformation approach for high-efficiency generation of rnai mutants of apple**

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A high-efficiency system of transformation was used to select apple RNAi mutants for determination of function of candidate genes in resistance of apple to Erwinia amylovora (fire blight). M.26 apple was transformed with a mixture of five RNAi EST-silencing vectors in each transformation experiment to allow selection of up to five types of mutants from a single experiment. RNAi-silencing constructs were created using ESTs associated with response to E. amylovora which were identified by bioinformatics analysis. These constructs were transferred to Agrobacterium tumefaciens strain EHA 105pCH32. The five silencing constructs were mixed, and the mixture used to transform leaf-slice explants. Regenerants were selected on M.26 regeneration medium with 100 mg/L kanamycin and screened by PCR using universal primers for the presence of a silencing construct. In almost all lines PCR showed only single genes had been inserted. Because amplicons from some transgenics co migrated, to better determine the identity of the ESTs contained in the silencing-insertion, the PCR fragments were cut with 4-cutter restriction enzymes. Thus far ESTs from genes in six functional categories, general metabolism (1), photosynthesis (2), nucleic acid metabolism (1), protein metabolism (3), signaling (1), and defense/stress(4), have been subjected to this protocol. To assay their resistance phenotype, young plantlets will be inoculated with E. amylovora, and bacterial populations and reaction symptoms

determined. This project is supported by a National Research Initiative Competitive Grant 2005-35300-15462 from the USDA Cooperative State Research, Education, and Extension Service.

**BP-02**

**Development of cisgenic apples having durable resistance to apple scab**

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Apple is one of the important fruit crops of the world. Most of the apples growers are facing serious disease problems with apple scab which is caused by the fungus *Venturia ineaqualis*. It is important to develop new varieties resistant to scab as many of the present day varieties are susceptible. Developing a resistant variety in apple through classical breeding takes nearly half a century and it is difficult to eliminate the linkage drag as unwanted alleles other than the scab resistance will also be introgressed. So we are working on improving existing apple varieties through a new concept called "cisgenesis" which can be defined as the genetic modification of a recipient plant with a natural gene from a crossable or sexually compatible species. The gene includes its native promoter and terminator in the normal sense orientation as we see in the natural situation. Malus floribunda 821 is a source of the *Vf* resistance, and the underlying genes have been named *HcrVf1* and *HcrVf2* (abbreviated here as *Vf1* and *Vf2*). We isolated the *Vf1* and *Vf2* genes with their native promoter and terminator as one stretch per gene from a BAC library of a Vf resistant apple variety. These fragments were cloned into the cloning vector pGEM-T easy for sequencing. Subcloning was done into the binary vector pMF-1 which can be used to obtain marker-free plants. Production of marker-free plants involves recombinase-based excision of a fragment carrying undesired gene sequences, such as antibiotic-selection marker genes, leaving behind only the gene(s)-of-interest and one recombination site. Using this vector it is possible to stack several genes. Apple transformations will be done with variety Gala introducing *Vf1* and *Vf2*, separately or in combination or combined with other resistance genes. Combinations will be made by stacking or by introducing them in one T-DNA. Performance of all different types of cisgenics will be tested by monitoring scab resistance levels phenotypically and by determining expression profiles through real time qPCR. We intend to add other isolated resistance genes from Malus to obtain durable resistance. First results will be presented.

**BP-03**

***Agrobacterium*-mediated transformation of olive (*Olea europaea* L.) embryogenic cultures**

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Olive (*Olea europaea* L.) breeding programs are hampered by the long juvenile period shown by this species. Genetic transformation would allow incorporation of interesting agronomical traits in much shorter generation times. Our group has developed an *Agrobacterium-mediated* transformation protocol for this species using embryogenic cultures as explants. In this protocol, somatic embryos derived from radicle explants are cocultured with *A. tumefaciens* strain AGL1 containing the binary vector pBINUbiGUSint with the *nptII* gene for kanamycin selection and *uidA* gene as reporter. After 12 weeks of selection in the presence of 200 mg/l paromomycin, an average transformation rate of 17.5±5.2 was obtained, based on the number of infected explants proliferating in the selection medium. Almost 33% of the paramomycin resistant calli showed GUS activity. Somatic embryos from different transgenic lines were maturated and germinated under a constant selection pressure of 200 mg/l paramomycin, and the shoots obtained proliferated in DKW medium. Putative transgenic shoots were challenged to proliferate in the presence of increasing concentrations of paramomycin. Control shoots did not grow at low antibiotic concentration (25 mg/l). However, transgenic shoots showed good proliferation rates at 25 and 50 mg/l paramomycin, and the shoot length was only reduced by 60% at 100 mg/l paramomycin. Transgenic olive plants have been acclimated in the greenhouse and are currently being analyzed at the molecular level.

**BP-04**

**Development of an *Agrobacterium* ransformation ystem for 'Bramley's'seedling apple (*Malus* x *domestica* borkh.)**

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Following the development of a successful leaf disc regeneration system for the cultivar Bramly's apple seedling, the 'Greensleeves' agrobacterium transformation protocol was applied to leaf discs. The GUS contining plasmid used was pRT99gus, containing a chimaeric beta-glucuronidase (GUS) gene, controlled by the CaMV 35S promoter and terminator and the Npt II gene to allow selection of transformants driven by a CaMV35S promoter and terminated by the CaMV35S terminator. A second plasmid - pZC35, containing the CH5B chitinase gene as part of a 4.7kb fragment of bean genomic DNA, again with the NptII gene was also used. For both plasmids, transformed callus of Bramley was generated, but shoot regeneration proved to be very difficult. A wide range of conditions known to effect shoot regeneration was tested without success. Finally, when transformed callus was transferred to Light (after 48 wks in the dark) Bramley shoots were formed which were sufficiently robust to survive transferring into a standard tissue culture system. The shoots were tested and found to have been successfully transformed for both GUS and CH5B genes. It was not possible to evaluate the transformed Bramley material in the field and so at the end of the project all the cultures were destroyed.

**BP-05**

**Efficient Ethylene Control for *Agrobacterium* mediated transformation in 'Fuji' apple**

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The ethylene controlling effects on transformation efficiency of gene transfer mediated by *Agrobacterium tumefaciens* (AGL1/pCAMBIA3301) were investigated in apple (*Malus domestica* cv. 'Fuji'). During three days of *Agrobacterium* inoculation, leaf explants excised from apple shoots produced ethylene, the production of which was increased, and inhibited by the addition of 0.001, 0.01, 0.1, or 0.5 mg/L aminoethoxyvinylglycin (AVG). However, the treatments of Silver nitrate ($AgNO_3$) (0.1, 0.5, 1.0 or 3.0 mg/L) increased the production of ethylene. After three days of co-cultivation with *Agrobacterium*, gene transfer into the explants was assessed by transient GUS-expression assay. Either treatments of AVG or $AgNO_3$ increased *Agrobacterium* infection efficiency by about 60%. However, there is no significant difference in the *Agrobacterium* infection efficiency at the different concentration of AVG or $AgNO_3$. Reapplication of AVG and $AgNO_3$ on selection media resulted in ethylene production and regeneration efficiency differently. The production of ethylene was increased with the addition of $AgNO_3$ and inhibited with AVG during four weeks in general. AVG treatments on selection media resulted in regeneration efficiency increasing (AVG 0.001 mg/L) or similar (AVG 0.01, 0.1, or 0.05 mg/L) to the control. However, $AgNO_3$ inhibited the regeneration efficiency about 30%. These results suggest that the efficiency of *Agrobacterium* infection to be significantly increased by both AVG and $AgNO_3$ treatments. However, the shoot regeneration rate was increased with 0.001 AVG, but inhibited with $AgNO_3$. Among three steps (1. gene transfer to explant cells, 2. selection of transformed cells, and 3. plant regeneration from transformed cells) of 'Fuji' apple transformation mediated by *Agrobacterium*, ethylene synthesis inhibitor, AVG was positively working on step 1 and step 3.

**BP-06**

### Effect of antibiotics on regeneration and to eliminate bacteria during gene transfer in apple

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A few antibiotics were tested to determine their effect on morphogenesis, during chitinase gene transfer in apple rootstock MM106. Hygromycin was used to select transgenic plants whereas a range of antibiotics carbenicillin, cefotaxime, augmentin, genticin were taken into consideration to eliminate *Agrobacterium tumefaciens* from leaf explants. Hygromycin concentration as low as 5 mg/l was found toxic to leaf explants and considered for selection of putative shoots. Cefotaxime sensitivity showed remarkable difference in growth behaviour of leaves. 500 mg/l cefotaxime was found the best to control Agrobacterium growth in cocultivation experiments whereas 200-300 mg/l cefotaxime enhanced leaf regeneration capacity as well as favoured shoot elongation, after checking the growth of the bacteria. Carbenicillin strongly inhibited regeneration at 400-500 mg/l, and at lower concentrations, regeneration frequency was reduced. Exposure of leaf explants to regeneration medium containing augmentin & genticin singly or in combination failed to eliminate *Agrobacterium tumefaciens* from the explants even after 6 weeks of culture. Transformed shoots were selected on medium supplemented with 5-mg/l hygromycin and 500 mg/l cefotaxime & maintrained on same medium with reduced cefotaxime (250 mg/l).

**BP-07**

### A high-throughput transformation system in plum (*Prunus domestica* L.) provides a powerful tool for functional genomics in rosaceae

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We have developed an improved *Agrobacterium*-mediated protocol in plum (*Prunus domestica* L.) through the addition of 2,4-D to the regeneration media. This method has increased the regeneration efficiency of independent transgenic plants up to 10 fold over previous reports. DNA blot analysis of putative transgenic shoots revealed transformation efficiencies of up to 42% with an average of 25% over all trials. The timing in each step of the regeneration/transformation process has been optimized for producing self-rooted transgenic plants in approximately 6 months. The high transformation rates coupled with the rapid plant establishment methodology make it possible to utilize plum transformation not only for the introduction of agronomically useful genes into this species, but as model plant for functional genomics studies in Prunus spp., Rosaceaous species, and woody plants in general. In addition, transformation with genes that promote early flowering such as leafy, apetala1, or ptft1 are being explored to reduce the time to flowering, enabling rapid evaluation of flower and fruit specific gene functions.

**BP-08**

### A genotype-independent *Agrobacterium*-mediated transformation method to obtain genetically engineered apricot plants

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In most woody fruit species, transformation and regeneration of cloned commercial cultivars are not routine, generally being limited to a few genotypes or to seedlings. This is the most serious biological obstacle to the application of genetic engineering to fruit trees. Transformation of apricot seeds was

reported previously and more recently our group has succeeded in the transformation of leaves from a cloned apricot cultivar. However, the published transformation procedure is dependent on the genotype and need further optimisation to be used for transformation, if feasible, of other genotypes. Transformation of meristematic cells with high regeneration potential would allow skipping the adventitious regeneration process, the bottle neck of most transformation methodologies in fruit trees. However, the high organisation of the meristem has made very difficult to control Agrobacterium overgrowth or to avoid the production of chimerical plants. Here, we described a transformation procedure that has allowed transforming four different apricot cultivars with relatively high efficiencies. We have taken advantage of the special characteristics of the meristematic cells which, when the meristem is removed, are still able to reorganise a new "organising centre" and then a new meristem. By transforming the remaining cells after removing axillary buds, we have been able to produce transformed shoots. The method seems to be very consistent and important modifications in the regeneration media do not significantly affect regeneration percentages. Histological examination of nodal explants, after removing the axillary buds, demonstrated that although a certain percentage of regeneration are secondary axillary buds, which remain after removing the main one, most of the regeneration corresponds to the new formed meristems. These are clearly distinguishable, at the microscope, between 5 and 10 days in regeneration medium. Secondary axillary buds can be eliminated by using a binocular but they can be easily distinguished because of their fast growth that allows seeing them with the naked eye one week after starting the experiment. Transformed plants could be obtained with frequencies (based in PCR evaluation) ranging from 0.8 to 7.6% depending on the cultivar and selection pressure.

**BP-09**

### Regeneration and transformation via *Agrobacterium tumefaciens* in strawberry *cvs* that adapted with weather of kurdistan province of iran

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Strawberry (*Fragaria ananassa* Duch.) is one of the most important fruit crops cultivated worldwide. Many cultivars of strawberry have already been bred, thereby allowing this crop to be cultivated under various conditions. Kurdistan province located in western part of Iran and because of suitable weather, consumption staple of strawberry in Iran is produced in this province. Some cultivars of strawberry are more adapted for cultivation in Kurdistan, such as *Fragaria ananassa* Duch. *cv.* Selva, Camarosa, Victoria and Gaviota. However, there are still many factors which limit plant yield significantly, such as susceptibility to drought, fungi, bacteria, virus, temperature, etc. Thus, creation strawberry plants without culture limitations have been pursued in breeding programs. Genetic transformation has opened a new era for greater creativity in strawberry breeding. Due to the unavailability of breeding material with resistance against some cultivate limitations in cultivars which are adapted with Kurdistan weather, we set up tissue culture and Agrobacterium mediated transformation in the cultivars which mentioned above, for next improvement goals and achieving better quality. Competent tissues for regeneration and transformation obtained for micropropagated plants of the strawberry *cvs* Selva, Camarosa, Victoria and Gaviota. In this experiment we used The Agrobacterium tumefaciens strain LBA 4404 containing binary vector pBI121, containing nptII gene which confers kanamycin resistance as a selectable marker, and gus gene which controls the expression of β–glucuronidase as the reporter gene. Leaves and meristems from strawberry plants, cultivated in vitro in MS, B5 and NN media containing different amount of BA and BAP as cytokinin and NAA, IBA and 2,4-D as Auxin hormones. infected explants in liquid bacterial suspension cultivated on basal medium without plant growth regulators and antibiotics for 3 days. Explants were then transferred to the regeneration and selection medium developed for strawberry plants. MS basal medium supplemented with cytokinin and Auxin hormones, 50 mg/l kanamycin and 500 mg/l cefotaxim. Co-cultivation of inoculated explants and shoot and root regeneration took place in the optimum condition. GUS assay was detected in leaves and other explants. Molecular analysis on plant material coming from in vitro and in vivo to confirm gene integration are underway. New experiments were designed to beter regeneration and introduce genes with high performance.

## BP-10

### Analysis of transgenic shoots regenerated *via* axillary organogenesis of *Vitis vinifera* L. cv. Albariño

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Actually, woody plants are still considered recalcitrant for genetic transformation. Commonly, the most typical organogenesis pathway used for regeneration of transgenic explants in *Vitis* sp. is somatic embryogenesis. The use of citokinins to promote the formation of adventitious shoots *via* axillary organogenesis appears like an alternative to these methods. In this work, we have examined the sonication-assisted Agrobacterium-mediated transformation (SAAT) system for genetic transformation of grapevine. Microshoots of proliferation stage were used as a source of explants. The hypervirulent strain AGL1 of *Agrobacterium tumefaciens* was used. The vector contains the *npt*II gene that provides resistance to antibiotic kanamycin, and the reporter genes *uidA* and *gfp* under control of *EgCCR*, a specific promoter of vascular tissues from the *Eucalyptus gunnii* cinnamoyl CoA reductase gene. Explants were exposed to bacteria in the cocultivation liquid media MS supplemented with 1 mg/L BAP, acetosyringone (100 µM), and proline (1mM) for 30 minutes. SAAT was performed during liquid coculture, with sonication for 60 s followed by vacuum infiltration during 5 minutes. Explants were dried in a sterilised paper and placed in the cocultivation solid media MS with the same supplements as described for liquid media on darkness at 23ºC for 6 d. Selection and regeneration of the putative transgenic microshoots were performed on MS solid media with 1 mg/L BAP, 50 mg/L kanamycin and 500 mg/L Augmentine. Data of survival and proliferation of the shoots in the selection media were recorded. Regenerated putative transgenic shoots were analyzed by GUS histochemical detection and visual screening of GFP by epifluorescent microscopy. Stable integration of the *npt*II gene into the explants was evidenced by PCR. The transgenic state of PCR positive lines was verified by Southern blot analysis to investigate the integration patterns of the *npt*II gene. Two out of six transgenic lines showed one insertion into their genomes. Actually, more transgenic lines are being analyzed by Southern blot to investigate the integration of the T-DNA. This system opens interesting perspectives for genetic transformation of grapevine.

## BP-11

### *Agrobacterium*–mediated transformation of a walnut cultivar

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A protocol for *Agrobacterium*-mediated transformation has been developed for shoots explants of the Paradox (*Junglans hindsii x Junglans regia*) walnut rootstock (*cv*. Vlach), which is one of the most used rootstocks for walnut. The effect of different concentrations of kanamycin on regeneration was evaluated and complete inhibition from control explants was observed when 70 mg/L kanamycin was applied. Also, the influence of antibiotic on the growth of the regenerated buds, micropropagated shoots and rooting was tested. Limiting concentration of kanamycin for growth of buds and shoots was 100 mg/L, where all of them died. Rooting decrease from 58% rooted shoots, without selection, to 1.7% when 30 mg/L of kanamycin was applied. Kanamycin at different concentrations from 30 to 150 mg/L was assayed in transformation experiments. After inoculation of explants with the strain EHA105/p35SGusint, buds that were able to regenerate in selective medium were rescued and transferred to medium with 75 mg/L of kanamycin for development of elongated shoots. Transformation efficiency, based on PCR analysis of individual putative transformed shoots, ranged from 0.7 to 2.7% when antibiotic concentration was between 75 to 65 mg/L. In order to avoid escapes or chimeras, the selection pressure in the proliferation medium was gradually increased and healthy shoots from some transgenic lines were able to growth in medium with 100 mg/L of kanamycin. To our knowledge, this is the first report of transformation of a cloned walnut cultivar.

**CP-01**

### Genetic diversity in cashew germplasm assessed by rapd markers

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Cashew *Anacardium occidentale* a tropical tree crop grown for its delicious nuts, is a cross pollinated crop with good load of genetic variability due to natural cross-pollination and segregation. Molecular marker RAPD have been proven as an efficient tool to assess genetic diversity in many crops. This study aims to analyze the genetic diversity of 33 cashew accessions which included Indian accessions and varieties developed from them, Brazilian accessions, and Panama accessions maintained in the germplasm using RAPD markers. RAPD analysis was conducted using seventeen oligo nucleotide primers. Genetic distance from the RAPD scores was subjected to cluster analysis employing UPGMA using SAHN module of NTSYS pc. The robustness of clusters was estimated performing bootstrap analysis using Winboot. Principal component analysis was conducted using the Eigen procedure of NTSYS pc. RAPD analysis of 33 varieties of cashew using 17 Operon primers produced 266 amplification products between 5.0 and 0.125Kb.Out of the 266 amplicons, 114 were monomorphic and shared by all the individuals and 152 were polymorphic, showing an overall 57% polymorphism. Out of the seventeen primers used for the study,OPP-5 and OPP-16 produced maximum polymorphism. The UPGMA clustering and dendogram constructed showed that the extent of similarity ranged from 57% to 91% and it gave three major clusters.All the major clusters had bootstrap values above 50. RAPD analysis shows that good genetic diversity exists among the thirty-three cashew accessions at the molecular level. The observed clustering and association of individuals deviated from the expectations based on known geographical origin, morphological traits and parental relationship. RAPDs proved to be potential tool for assessing genetic diversity in cashew germplasm. The dendrogram constructed based on the RAPDs helps to locate the divergent types and hence to decide the suitable combinations to provide maximum heterosis for gentic improvement in cashew.

**CP-02**

### Sweet cherry cultivars of iranian and foreign origin clustered by rapd markers representing relations between some of them and good accordance with pollination incompatibility groups

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Randomly amplified polymorphic DNA (RAPD) markers were used for categorizing and grouping of 39 sweet cherry accessions of Iranian (28) and foreign (11) origin. Of 100 decamer random primers, 23 produced 153 polymorphic bands with profile consistency. RAPD data were analyzed for estimation of genetic similarities with Jaccard's coefficient and used for clustering by UPGMA method. Divided genotypes into subclusters were showing in some to contain Iranian and foreign cultivars close to each other, representing their genetic associations. The phenotypic clustering of genotypes mainly based on fruit characteristics such as color, shape and size were not in accordance with the RAPD genotyping. However, when the subclusters were concerned with the available data for pollination incompatibility (S alleles), good accordance were found between them. Therefore, RAPD technique, besides its application for genetic diversity estimation, seems to be also a good approach for rapid preliminary examination of the S alleles for unknown genotypes, being more feasible than the standard time needed and season dependent pollination experiments.

## CP-03

### Genetic diversity among cyclamen genotypes revealed by morphological characters and rapid markers

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The genetic diversity of 26 cyclamen genotypes including *Cyclamen persicum* and *C. com* was evaluated with morphological and molecular (RAPD) markers. Total of 23 qualitative and quantitative characters and a set of 84 arbitrary 10-mer oligonucleotide primers were examined.The results showed the positive and negative correlations between traits. According to cluster analysis genotypes divided to 5 groups that differ in the color and longevity of flowers. Nine primers showed reliable polymorphic patterns and together yielded 104 polymorphic markers. The highest similarity detected among wild genotypes and the lowest diversity was detected intra species with the value of 0.99 and 0.08, respectively. At a similarity of 73 percent, genotypes were divided into five sub-clusters. Cophenetic correlation coefficient between similarity matrix and cophenetic matrix of dendrogram was relatively high (r=0.9) showing the goodness of fit of the dendrogram. The RAPD analysis offered a rapid and reliable tool for the estimation of variability between both inters and intra species of cyclamens. The RAPD markers also let distinguish labeling mistakes and identification of genuine cultivar in question.

## CP-04

### Rapd as useful markers for charcterization of *Annona squamosa* L. genotypes

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Naturally a large inter and intra specific variability exists in *Annonaceae* family. Annona plantations owe their origin to vast populations of seedlings that have originated in nature from scattering of seed. As a result of this they exhibit great diversity in fruit physico-chemical qualities and bearing tendency. This has offered ample scope for studying the genetic variation in Annona. To expedite the crop improvement programme it is necessary to trap the natural variability through surveys and the variability should be conserved ex situ and in situ to utilize it for further hybridization programmes. For primary screening, the morphological characterization is effective and suitable for confirming this variability and to trace true genetic variation, molecular characterization is essential. DNA fingerprinting techniques are quick and accurate which excludes the environmental and managemental undesirable affects. In the present study morphologically differing 11 genotypes (including one released variety) of sugar apple (*Annona squamosa* L.) were studied for diversity at molecular level using random amplified polymorphic DNA (RAPD) markers to confirm the diversity as revelled by morphology. Of the seven primers used, five random primers yielded total of 31 scorable markers, of which 19 markers were polymorphic. RAPD analysis revealed that three genotypes viz., AKCa 05, AKCa 07 and AKCa 10 were divergent and the remaining eight genotypes were almost similar to each other. These 11 genotypes were grouped into three distinct clusters/groups based on the dendrogram prepared using NTSys PC.

## CP-05

### Construction of a reliable pcr marker for selecting pollination constant and nonastrin-gent (PCNA) type offspring among breeding population of persimmon (*Diospyros kaki* Thunb.)

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Pollination constant and nonastringent (PCNA) type persimmons lose their astringency naturally on the tree with their fruit development and can be edible at maturation without any artificial treatments for astringency-removal after harvest. This natural astringency-loss is the most desirable trait for fresh fruit consumption and is controlled by a single locus, called as AST locus. PCNA type cultivars are originated in very recent era and have very narrow diversity with limited numbers of the cultivars. Currently, the breeding project for yielding new PCNA type cultivars with good characteristics is facing to inbreeding depression by repeating the crossing among very limited cultivars and/or selections of PCNA type. One of the solutions to overcome this inbreeding depression is to include non-PCNA cultivars in the breeding project, which will extend genetic pools for PCNA breeding due to wide diversities among non-PCNA cultivars. However, so far, $F_1$ generation of the crossing between PCNA and non-PCNA type cultivars or selections yield only non-PCNA offspring. To obtain PCNA offspring, this offspring have to be backcrossed to PCNA type cultivar or selection. As persimmon is hexaploid, the obtainable rate of PCNA offspring in backcross population is only 10-15%. Until now, we found very tightly linked AFLP and RFLP markers for the trait of natural astringency-loss in persimmon. In addition, we have demonstrated a possibility that the sequences of seed clone of fosmid library of *D. lotus*, diploid relatives of *D. kaki*, screened by AFLP marker as a probe, can apply to *D. kaki* as PCR primers for distinguishing between PCNA and non-PCNA types. We have sequenced this seed clone (ca. 40 kb) and could find a PCR marker for selecting PCNA type among backcross population of persimmon by constructing a primer pair from a part of these sequences. This PCR marker was very promising and will be beneficial for the future breeding programs of new PCNA cultivars.

## CP-06

### Aflp-derived methods as a tool for study of genomic, epigenomic and transkriptomic changes in stressed grapevine plants

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Many studies describing phenotypic and genetic variation among standard and stressed plants were already published. A possibility to estimate stress-induced changes in genome, epigenome and transcriptome (standard AFLP, methylation-sensitive AFLP and cDNA-AFLP) arose by establishing three AFLP-based methods within our laboratory. Three variants of grapevine plants after different stress actions (stress by in vitro cultivation, thermotherapy and virus infection) were prepared for this study. In case of standard AFLP application, comparison of obtained spectra in form of 0/1 binary matrix is usually performed. Within our group of variants the results show insignificant changes in its genomes. MS-AFLP spectra evaluation needs more detailed comparison of results generated both by two used izoschizomers. On the other hand, more precise information about DNA methylation characters and impact of individual stress factors on epigenetic changes was possible to evaluate. From the point of view of functional methodology, the cDNA-AFLP appeared as most difficult to establish. During first experiments only short PCR amplicons (at most 150 bp) were generated. Further, calculated similarity coefficients showed surprisingly low values. These facts signalised certain unspecific RNA degradation before its transformation onto ds cDNA. The significant improvement of obtained spectra was recorded by using SpectrumTM Plant Total RNA Kit, RNA later reagent, RNase Our enzyme and MINT cDNA synthesis kit in cDNA-AFLP protocol. In case of cDNA-AFLP spectra evaluation, there is possible either to prepare 0,1 binary matrix or differences can be estimated on the base of ratio between intensities of individual peaks. Of course, finally used approach will affect resulted values of similarity coefficients.

This fact could be taken into account before comparison of cDNA-AFLP results with the results obtained by other methods like microarrays.

## CP-07

### Functional diversity for response to water deficit among different strawberry genotypes compared to their genetic structure as assessed by AFLP markers

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Strawberry (*Fragaria x ananassa* Duch.) belongs to the rose family (*Rosaceae*, subfam. *Rosoideae*) and is mainly cultivated in the northern hemisphere. Drought stress is one of the most important environmental factors that limit plant growth and development, thus reducing yield. The objective of present research was to determine the effect of genetic diversity on plant responses to drought stress among different strawberry species and cultivars. Experiments were performed in a growth chamber under constant temperature (17°C), RH (60 %), and light intensity (around 100-105 µmol m-2 s-1). Youngest fully expanded triplet leaves were cut placed on filter paper in the growth chamber and the decline of fresh weight was recorded over time. Water losing rate (WLR, calculated as the leaf weight loss over time), was compared for 21 cultivars, one diploid species *F. vesca* and one octaploid species *F. chiloensis*. WLR could discriminate genotypes showing a contrasting response to water stress. This response allowed clustering strawberry genotypes in susceptible and drought-tolerant groups. Secondly, the genetic diversity within and among these groups was determined using Amplified fragment length polymorphism (AFLP) genetic marker as a first attempt to evaluate the genetic basis for the observed variation in WLR among strawberry genotypes. Cluster analysis of the AFLP data, starting from the genetic distance among different genotypes allowed evaluating if drought resistance in the set of characterised plant genotypes is related to genetic origin or cultivar pedigree. Drought resistance can be an important element in the "functional diversity" of a strawberry genotype, selecting adapted genotypes for survival under stringent environmental conditions. In further genetic research, we will also apply EST-markers for candidate genes in strawberry to assess better differences in drought stress responses and to pinpoint possible genetic factors of control.

## CP-08

### Isolaton and characterization of ltr retrotrans-posons from strawberry genome

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Strawberry (*Fragaria* spp.) is a kind of herbaceous perennial plant that propagates vegetatively. The conserved domains of reverse transcriptase (RT) genes of *Ty1-copia* and *Ty3-gypsy* groups of LTR retrotransposons were amplified from the cultivated and wild strawberry genomes. In the cultivated strawberry, sequence analysis of clones demonstrated that 5 of 19 *Ty1-copia* group unique sequences and 2 of 10 *Ty3-gypsy* unique sequences possessed either stop codon or frameshift. *Ty1-copia* group sequences are highly heterogeneous (divergence ranged from 1 to 69.8%), but the *Ty3-gypsy* group sequences are less (divergence ranged from 1 to 10%). In wild strawberry, sequence analysis of clones demonstrated that 11 of 34 *Ty1-copia* group unique sequences and 6 of 14 *Ty3-gypsy* unique sequences possessed either stop codon or frameshift. Southern dot blot hybridization result suggested that both of the LTR retrotransposons are present in the genome of cultivated strawberry (*F.* × *ananassa*) with high copy number (*Ty1-copia* group 2,875 *Ty3-gypsy* group 348). Both types of LTR retrotransposons contributed to 15.8% of the cultivated strawberry genome of which the contribution of *Ty1-copia* retrotransposons being higher (13.5%) than that of *Ty3-gypsy* (2.3%). RT-PCR amplification from total RNA, which was extracted from calli induced from strawberry leaves cultured on MS medium supplemented with 2,4-D, yield the RT fragments of *Ty1-copia* retrotransposons.

## CP-09

### Optimalisation of the preparation of ds cDNA from flower buds of apricot as a means of determination of signal genes for exit from endogenous dormancy using the cDNA-AFLP method

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The trascriptom of flower buds from four apricot variants has been analysed in this project by the cDNA-AFLP method. The aim of this project was the preparation of ds cDNA so that it would be as suitable as possible for the cDNA-AFLP analysis. The flower buds were sampled weekly during the estimated time of exit from endogenous dormancy. The apricot variants 'Sundrop', 'Stark Early Orange', 'Vestar' and 'Betinka' were chosen to achieve significant differences in the times of exit from endogenous dormancy. The sampled tissues were preserved using a commercial agent RNAlater® Soln. (Ambion). Four samples, closest to the date of exit from endogenous dormancy, were chosen for further analysis, from the entire set of samples collected. The dates of exit from endogenous dormancy were estimated using results from several methods determining the time of exit from endogenous dormancy. The RNA from the chosen samples was isolated using a commercial isolation kit supplied by Sigma (Spectrum™ Plant Total RNA Kit), which helped the isolation of sufficient amounts of high quality RNA for further analyses from these highly complicated tissues. Transcription from the entire RNA onto the ds cDNA was achieved using the MINT cDNA synthesis kit from Evrogen. The cDNA-AFPL analysis was carried out according to an amended procedure by Bachem et al. (1998) and the samples obtained from selective amplification were analysed on genetic analysis machine ABI-PRISM 310. The results were evaluated graphically. Suitable commercial agent, kits and procedures for the preparation of ds cDNA of sufficient quality and quantity for further analysis by cDNA-AFLP method have been identified and are recommended for similar type of work.

## CP-10

### Proteins characterization in lemon fruit (*Citrus limon*, L. Burm. f. var. femminello, rocca imperiale)

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Lemon is a traditional crop of the Mediterranean area. Its fruit juice is used to make refreshing drinks, while essential oils are extracted from lemon peel and used as scents. The effects of lemon fruit products on human health have been also described, mainly due to their high content of antioxidant molecules, such as vitamin C, monoterpenes, carotenoids and flavonoids. In Italy (Campania, Calabria and Sicily) are the most important regions devoted to lemon crops, where a large number of genotypes is present. We have started to characterize the fruits of lemon trees grown in Rocca Imperiale, a nice locality in Calabria (Southern Italy), where the selected genotype (var. Femminello) is cultivated under optimal conditions of climate, thus producing some high quality fruits. In particular the fruit epicarp is characterized by a large number of oil glands containing a high amount of monoterpens, among which lemonen is the major component (up to 70%) in respect to other genotypes of the same grove. These observations suggest that some genetic trait might be responsible of the organolectic qualities of var. Femminello fruits; thus we have applied a proteomic approach in epicarp tissue with the aim of obtaining a genomic characterization at protein level. We have used different protein extraction methods (Spadafora et al., 2008; Saravanan and Rose, 2004; Wang et al., 2003), two-dimensional electrophoresis (2-DE) of protein extracts, mass spectroscopy analyses and bio-informatic tools for protein identification. Adapting the extraction method based on TCA (trichloroacetic-acid) proteins precipitation with subsequent purification in a phenol phase, we have obtained 2-DE protein maps from epicarp tissues with more than 200 proteins, 60 of which were present in all experimental replicates. Each protein was characterized in terms of molecular weight (MW), isoelectric point (pI), relative abundance (NV) in protein extracts, among which four proteins represented more than 15% of extracted proteins. The identification of the proteins has been made by software BLAST, while the matching of the proteins in

the databases has been searched by the software MASCOT. For some spot has been attributed more than one protein. The spots identified with reliability are 24.

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## CP-11

### Proteomic analysis of β-aminobutyric acid-primed drought resistance in crabapple seedlings

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In a variety of annual crops and some model plant species, the non-protein, amino acid, DL-β-aminobutyric acid (BABA), has been shown to enhance disease resistance and increase salt and drought tolerance, through sensitization, and not direct induction of defense genes. This process is referred to as chemical priming. Primed plants do not suffer from costly defense investments (such as inhibition of photosynthesis) since their defense arsenal is not activated before stress exposure. However, there are no reports on BABA-induced resistance in woody species. Additionally, the metabolic pathways through which BABA mediates both abiotic and biotic stress resistance are still being elucidated. In the present study, drought tolerance of four-week-old crabapple (*Malus prunifolia*) seedlings was significantly increased ($P \leq 0.05$) following a soil drench treatment with 500 µM BABA. On the tenth day after cessation of watering, the level of water loss in BABA-primed seedlings was 2-3 fold less than that of untreated plants, clearly indicating the ability of BABA to induce tolerance to drought stress in perennial plants. 2-D Difference in-Gel Electrophoresis (DiGE) was employed to characterize and compare differences in protein expression in leaf tissue sampled from control, BABA-primed and ABA-treated seedlings exposed to drought stress. A comparison of the different treatment combinations on the third and tenth day of dehydration revealed that 102 and 202 proteins, respectively, were differently expressed ($P<0.05$), in at least one condition. Among those, there were proteins that showed almost identical patterns of upregulation (57) or down regulation (34) in BABA and ABA treated seedling that supports the general concept suggesting that BABA-induced resistance in plants is achieved by potentiating ABA-regulated pathway. However, some differentially expressed classes of proteins were uniquely up-regulated (54) and down-regulated (38) only in BABA-primed plants, indicating that BABA may also mediate resistance via some ABA-independent pathways. MALDI-TOF MS/MS is being utilized to identify the proteins of interest. A quantitative analysis of the proteomes of control, ABA and BABA-treated tissue will be presented together with the discussion of possible mechanisms of BABA-mediated resistance in woody plants.

## CP-12

### Parent identification of hungarian apple cultivars using ssr markers

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In a classic apple-breeding program and to characterize fruit cultivars it is important to know the origin of our common cultivars. Molecular markers are highly useful to distinguish genotypes and codomoninant markers like microsatellites can be further used to verify the parenthood of apple cultivars. This way the supposed parents could be confirmed or dismissed. 13 simple sequence repeat markers (SSRs or microsatellites) are applied to verify the parentage of twelve cultivars, four cultivar candidates, two breeding clones and their supposed parents. 'Jonager', 'Kovauguszt', 'Kovelit', 'Kovmulti', 'Kovsztár' and 'Nyári zamatos' cultivars were originated from crosses between the cultivars 'Jonathan' and 'Egri piros'. 'Fertődi téli' is known as the hybrid of 'Jonathan' and 'Török Bálint'. The female or male parent of cultivar candidates and breeding clones was 'Prima', except one case. The male parent of cultivar candidate MR-03 and MR-10 were unknown because the seed resulted from open pollination. The male parent of cultivar candidate MR-11 and some old cultivars (e.g. 'Éva', 'Mizsei', 'Pirtóspur Delicious') is uncertain. In case of MR-11, based upon several fruit characteristics we thought that the pollen was mistaken, so we try to find the exact parents. The results of the marker analysis will be shown on the poster.

## CP-13

### Genomic characterization of the us apple germplasm collection

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The United States Department of Agriculture (USDA) Agricultural Research Service (ARS) coordinates a system of about 30 germplasm repositories that each focus on specific crop collections. The collection of apple (*Malus*) germplasm is maintained in Geneva, NY and includes over 8500 accessions representing at least 50 species. Of these, some 2600 accessions are clonally propagated cultivars, 3100 are seedlings mostly representing species collections, 1600 are in the form of seed, and 1250 are wild by elite hybrids that were generated specifically for genetic studies. Our core mission for this collection includes the acquisition, maintenance, characterization and distribution of this diversity of *Malus*. Until fairly recently, the collection was primarily characterized with 154 categories of descriptors which include pomological, pathological, anatomical, and phenological characteristics, resulting in over 95,000 independent observations recorded in the Germplasm Resources Information Network (GRIN: www.ars-grin.gov) database. We have recently extended our characterization efforts to include a set of seven (or eight) microsatellite markers. The markers were selected since they amplify stably and widely across diverse *Malus* germplasm and that their localization in the *Malus* genome is not clustered. Use of additional microsatellite markers is under investigation. Ideally, future selected molecular markers will be associated with traits of interest. Initially, all accessions will be genotyped using at least seven microsatellite markers and data will be made available and downloadable in the newly expanded molecular tables within the GRIN database. At this time, microsatellite data for *M. sieversii* and *M. orientalis* accessions are publicly available at the following website:

http://www.ars-grin.gov/cgi-bin/npgs/crop/evaluation.pl?493453

http://www.ars-grin.gov/cgi-bin/npgs/crop/evaluation.pl?493599.

## CP-14

### Using SSR-markers for identification of apple varieties in belarus

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A collection of 108 apple accessions was screened with SSR markers previously identified in *Malus domestica* Borkh. to investigate the level of genetic differentiation among them. A collection included old, modern, introduced varieties grown in Belarus and *Malus* species. In total, 334 polymorphic alleles were detected at the 20 SSR markers with an average number of alleles of 16.7 per marker. Polymorphism information content (PIC) varied from 0.68 to 0.94 with an average of 0.84. Cluster analysis was conducted in order to determine the phylogenetic similarity and relationships among cultivars. Apple accessions were grouped according to their pedigree and their geographic origin. Many modern cultivars of Belarusian breeding are close for genetic aspect to old varieties traditionally grown in this area and presented in this pedigree. The species of *Malus* formed an individual cluster. In common, apple varieties have a high level of polymorphism. Three markers pairs covering various genome regions CH01C06 and CH02C02b, CH02B12 and CH04H02, CH03D12 and SdSSR were selected and used in multiplex PCR for certification of apple-tree genotypes at the molecular level. Totality 108 apple-tree varieties and species were identified by means of these markers.

## CP-15

### The genetic finger printing of the irish apple collection using single sequence repeats in multiplex systems

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The vast majority of apples in the Irish Heritage collection are of unknown origin and have only local names. It was therefore decided to fingerprint the collection for comparison with other data bases. Following initial attempts at using SSR's for finger printing the Irish apple collection, it became evident that whilst the methodology worked, it was of limited value unless all the major collections used the same SSR's. Consequently a series of SSR markers were grouped into three sets of four makers each (at East Malling Research Station) where each set of markers had similar requirements for PCR. There multiplexes were then applied to the Irish Heritage Collection so that the data could be compared to that generated for the UK apple collection at Brogdale. This paper reports on the results to date.

## CP-16

### Transferability of *Malus* x *domestica* micro-satellite markers to other species and genera of the *Maloideae* subfamily

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Wild, cultivated and ornamental *Maloideae* species and genera can comprise potential genetic resourses to improve the cultivated apple *Malus* x *domestica*. Therefore we have carried out interspecific and intergeneric hybridizations and checked crossing barriers and (true) hybrid character of the resulting seedlings. For the latter we have chosen for macrosatellites (SSRs) as molecular markers since SSR loci are highly conserved among species and genera and because SSR primer information is available in cultivated apple. To study the transferability of apple SSR to other species or genera of potential breeding parents of the *Maloideae*, a prerequisite to check hybrid of progenies, we screened 147 accessions of the *Maloideae*. These 147 accessions were divided over 10 genera, with several

representative species within each genus: *Amelanchier* (9 accessions), *Aronia* (5), *Chaenomeles* (8), *Cotoneaster* (11), *Crataegus* (9), *Cydonia* (11), *Malus* (20), *Mespilus* (10), *Pyrus* (47) and *Sorbus* (16). In total we tested on each accession 31 *Malus x domestica* SSR loci and primers combinations were initially developed by Guilford et al. (1997), Gianfrancheschi et al. (1998) and Liebhard et al. (2002). We considered a marker as transferable when a successful amplification was obtained of 75 %. The transferability from cultivated apple to other genera and *Malus* species varied between 58 % (*Pyrus*) and 94 % (*Malus*). The mean number of alleles per (transferable) locus ranged from 1.9 (*Cydonia*) and 2.2 (*Mespilus*) to 11.5 (*Malus*) and 12.5 (*Pyrus*). Accessions of the first two genera belong almost to the same species. The low allele diversity of a locus in these species reflects most likely their apomictic behavior. Considering the transferability of the primer combinations from *M. domestica* to other genera substantial differences were found among microsatellites: from 0 % to 100 %, but for most of the primer combinations the transferability of a microsatellite marker was higher than 70%. The highest number of alleles over all species and genera (100% transferability) of a specific locus was 62, the lowest only 5. In the latter case the primer combination of this locus amplified in most species just one allele. Most primer combinations can be used to generate molecular markers to test hybrid character of intergeneric seedlings and to align genetic linkage maps within *Maloideae*.

## CP-17

### Preliminary molecular marker maps constructed by using an interspecific cross between *Pyrus communis* and *P. ussuriensis*

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A preliminary linkage maps of European pear ('Doyenne du Comice') and Chinese pear (*P. ussuriensis*) were constructed based on AFLPs, AFLP-RGAs and SSRs from pear and apple using their $F_1$ progeny. The map of the female parent 'Doyenne du Comice' consisted of 39 loci including 15 AFLPs, 23 SSRs and one AFLP-RGA on 8 linkage groups over a total length of 194 cM, while that for *P. ussuriensis* contained 40 loci including 20 AFLPs, 19 SSRs and one AFLP-RGA on 8 linkage groups encompassing a genetic distance of 298 cM. These maps were partially aligned using 23 codominant markers which showed segregating alleles in both parents. The position of 13 SSRs originating from apple could be succesfully determined in pear maps, which enabled us to compare the two maps. Syntenic relationships between pear and apple maps have been considered for the chromosomes carrying two or more SSR markers. The alignment among the two maps supports the colinearity of the two genomes with respect both to identification and to orientation of the linkage groups.

## CP-18

### Microsatellite markers (SSR) as a tool to assist in idenfication of sweet (*Prunus avium*) and sour cherry (*Prunus cerasus*) cultivars

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Sweet (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.) are two economically important species in Europe. The capability to distinguish among cherry cultivars in breeding, cultivation and germplasm collection is extremely important for scientific as well as for economic reasons. There is a demand for a rapid and reliable method of cultivar identification for cultivar registration, protection, cultivation and management. Normally morphological traits are used to identify cultivars but these traits are often differently expressed in different environments and production practices.

DNA-based markers are useful for germplasm identification, diversity analysis and verification of rootstock identity. Among molecular markers, microsatellites, or SSRs, are known for being highly polymorphic, codominantly inherited, abundant and evenly distributed in the genome. The most

widespread technique for SSR detection is PCR with specific primers, which is simple, reproducible and suitable for automation. SSRs provide a more reliable method for DNA fingerprinting compared with RAPDs, which can not fully match the advantages of SSRs to differentiate the cultivars. SSR markers have been used widely for cultivar identification, genetic mapping and phylogenetic studies. Our objective was, parallel to the usual morphological identifying methods, to fingerprint a subset of 43 sweet- and 30 sour cherry cultivars with 15 SSR primers, to determine if there was sufficient polymorphism to differentiate the cultivars. And furthermore, to establish one routine method for cultivar identification for cherries in KOB, and then use this new method as a practical technique to differentiate two unknown samples from each other, to exam the authenticity and purity of cultivars for the nursery, import and export chains and to support the quality inspection in the region of food trades. 15 SSRs revealed polymorphism. The polymorphism information content (PIC), frequencies of 15 SSR loci distributed on 73 cultivars are described and discussed.

## CP-19

### Assessment of genetic variation in citrus germplasm of south of iran using SSR markers

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The long history of citrus cultivation, their wide dispersion in the world, possibility of fertilization among genus and species, apomixis and a great deal of mutation have been caused abundant genetic variation within and between the citrus germplasm. Identification of these variations is a challenge in citrus industry and breeding. Identification of genotypes and assessment of their genetic distance is important for genetic researches and breeding programs. In this study, 8 SSR markers 15 morphological characters were used to assess the genetic variation among 48 citrus genotypes (12 orange genotypes, 6 grapefruit genotypes, 20 tangelo genotypes and 10 lime genotypes) from the collection of the Agricultural Research Institute of South of Iran. From each accession a full-grown tree was selected and genomic DNA was extracted from young leaves using CTAB procedure. DNA amplification was performed in a thermocycle and SSR banding pattern was detected in an acrylamide gel. The bands were transformed into binary data of presence–absence and resulting matrices were processed by NTSYS software. In total, 32 alleles were detected using these 8 primer pairs. The number of putative alleles per primer pair ranged from 3 to 7 with an average of 4. Polymorphic information content (PIC) values varied from 0.31 to 0.82. Cluster analysis was done using Complete Linkage and UPGMA methods, and the similarity measures of simple matching and Dice. Comparison of the above mentioned methods showed that the best dendrogram without chaining effect was that of the Complete Linkage using Simple Matching. This method divided the studied genotypes into two groups. This study detected genetic similarities and differences between citrus germplasm of south of Iran.

## CP-20

### Clonal selection of of askari grapevine cultivar by molecular markers

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Askari cultivar is one of the most important seedless(stenospermocarpy) table grapes in Iran. It has been growing from 5000 years ago and has been propagated by cutting, so accessions of that in all of part of Iran must be clone, but morphological variations within varierty show differences in the some accessions, e.g. some accessions have 4 large empty seeds and some accessions don't have observable seeds that may be because of mutation. In this research Askari accessions were gathered from all of parts of Iran and survey by genetic molecular marker to confirm that differences are genetic, not enviremental effects. SSR marker identified differences within variety Askari.

## CP-21

### Genetic diversity among clones of cv. Keshmeshi (*Vitis vinifera*) by molecular markers

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Ten accessions of cv. Keshmeshi from a clonal selection vineyard in the West Azerbaijan area (Iran) were screened by SSR and AFLPs markers in order to assess their uniformity. Results showed SSR analyses didn't able to distinguish variability between clones but the 7 primer sets of AFLP generated a total of 499 scorable fragments from all the analyzed accessions, of which 8 were polymorphic. On an average, each primer set generated 71.3 fragments. Primer set E31-M32 generated the highest 97 scorable fragments whereas E34-M34 had the lowest 44 fragments. Results of cluster analyzing showed two separate group which all clones of Keshmeshi Sefid (white color skin) stay at on and Keshmeshi Qermez (red color skin) formed the last group. Genetic similarity between two groups was 98.5. Based on SSR analyses results, Red Keshmeshi is one member of or one clone of Keshmeshi group with only red color of berry difference. It may be obtained from somatic mutation or bud sport that changed the color trait only. Results of AFLP analyses could separate this accession from other clones. About the ability of AFLP technique to determine the genetic variability between clones there are many conflicted report.

## CP-22

### Evaluation of genetic relationships among pistachios using microsatellite markers developed from *Pistacia khinjuk*

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For phylogenetic inferences of genus *Pistacia* and the analysis of genetic variation among Iranian pistachio genotypes, two DNA libraries enriched for dinucleotide (AG)n and trinucleotide (ATG)n microsatellite motifs were developed from *Pistacia khinjuk* genome. Following screening of clones by colony PCR technique, 44 clones were sequenced and 27 pairs of primers designed from flanking regions of the repeats. After optimization of their PCR amplifications, the primer sets were used to assay the polymorphism of *Pistacia* species and Iranian accessions of *P. vera*. Examination of primer pairs designed from *P. khinjuk* sequences showed extensive cross-species amplification (92%) within the genus *Pistacia*. A dendrogram constructed on the basis of the Unweighted Pair Group Method with Arithmetic Average (UPGMA) clustering algorithm revealed an interesting evolutionary trend from *P. vera* to *P. Khinjuk*, followed by *P. integerrima*, *P. palestina* and then to *P. atlantica*. The dendrogram further separated the genotypes of Iranian pistachios into three different groups and three independent genotypes of wild Sarakhs, Ghazvini Zoodras and Italiaei Zoodras. According to cluster analysis, it could be postulated that the domesticated genotypes of *P. vera* are evolved from Sarakhs and then this wild genotype likely develops to Ghazvini Zoodras, Italiai Zoodras and other local pistachios. Hence, it seems that the wild Sarakhs pistachio plays an important role in evolutionary trend of the genus *Pistacia*. The results indicated that microsatellites developed in *P. khinjuk* are distribute in the genome of other pistachio species including *P. vera* genotypes and therefore they are highly useful in characterization of pistachio species and genotypes.

## CP-23

### Molecular characterization of iranian olive cultivars

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The old commercial olive orchards are located mainly in the North of Iran and more than 85% of olive production belongs to these regions. In the last ten years, olive plantation has grown in several provinces and currently, olive cultivation covers more than 100000 hectares in Iran. In this study we aims at characterizing – by the use of microsatellite markers- main Iranian olive cultivars in three North provinces which are considered as the most important regions of olive cultivation in Iran. The plant specimens were collected randomly from 600 adult trees of 10 traditional cultivars in 13 regions in the north. These olive cultivars were screened by morphological descriptors and microsatellite markers. Based on the analysis carried out so far:

- 84% of olive accessions analyzed were discriminated.
- Genetic variation among and between cultivars was revealed by using thirteen SSR markers.
- Synonymy and homonymy cases were identified among the cultivar studied,
- genetic relationship among these olive cultivars was studied.

In general, the present study showed the utility of SSR markers in identification and discrimination of the olive cultivars under study.

## CP-24

### Development of microsatellite markers and evaluation of genetic diversity in fig (*Ficus carica* L.) germplasm

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The fig (*Ficus carica* L.) is one of the oldest fruit crops that is well adapted to different environmental areas of the Mediterranean zone. The fig germplasm is characterized by a great diversity and a high number of accessions has been inventoried in different plant germplasm collection in Mediterranean countries. In Italy, the major area of fig cultivation is the Southern part of the country, where most fig germplasm has been collected. Despite the importance of the preservation of genetic diversity, there is few information on fig biodiversity and on the genetic structure of the germplasm. In this contest, there is a need for highly standardized methods of identification to evaluate the genetic resource and to tackle problems regarding confusion in plant material conservation due to the occurrence of cases of synonymy, homonymy and misnaming. For these reasons, microsatellite markers have been developed from a genomic library of *F. carica* enriched for GA/GT repeats. Eighteen selected loci were used to evaluate cultivars and clones from local germplasm in Apulia. All primers pairs produced an amplification product of expected size and detected high polymorphisms among the analysed samples. Microsatellite markers are expected to be a very effective tool for evaluating genetic diversity in fig germplasm. Cross-transferability in different species and genera is evaluated.

## CP-25

### Identification and stability of QTL for fruit antioxidant contents in apple

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There is currently much interest in the breeding of crops for enhanced nutritional value, including increased antioxidant contents. However for many of these phytonutrients, little is currently understood about the genetic factors underlying the homeostasis of tissue levels in reproductive organs. To help identify the genetic determinants underlying fruit antioxidant contents we have carried out a comprehensive QTL analysis for fruit vitamin C, glutathione and total antioxidant contents in apple over 2 years. Using an $F_1$ progeny derived from a cross between the cultivars 'Telamon' and 'Braeburn' we identified major (% significance >20%), highly significant (LOD > 3.0) quantitative trait loci (QTL) associated with fruit vitamin C (L-ascorbate, L-AA) contents in both peel and flesh tissue of apples. Despite substantial differences in the climactic conditions, several of these major, QTLs for fruit vitamin C contents proved to be stable, mapping to the same regions of homologous linkage groups in the two parental cultivars over 2 production years. However, other major vitamin C QTLs however were not year-stable, illustrating the importance of environmental effects int this type of analysis. Interestingly, distinct, minor QTLs for fruit glutathione and total antioxidant activities were also identified. Results are discussed in the context of the development of molecular breeding tools for the breeding of biofortified foods and crops and the impact of environment on QTL stability.

## CP-26

### Mapping Plum pox virus resistance in apricot

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One of the most economically important diseases of stone fruit crop in Europe is Sharka disease that is caused by the *Plum pox virus* (PPV). The introduction of resistant cultivars of stone fruit into orchards is the best long-term solution to virus control. The resistance to PPV was found only in some North American apricot cultivars. The identification of genomic regions involved in PPV resistance could facilitate breeding process. The F1 progeny derived from a cross between 'Harlayne' (resistant to PPV) and 'Vestar' (susceptible) was used to identify PPV resistance loci. The descendants were inoculated with PPV strain M by chip-budding. Symptoms of PPV infection on leaves were scored over 5 consecutive growth periods. The Kruskal-Wallis non-parametric test and composite interval mapping were used to identify regions involved in PPV resistance. Both methods located the regions controlling PPV resistance in the upper part of linkage group 1.

## CP-27

### Almond genetic linkage map and quantitative trait loci for flowering time in a "Nonpareil x Lauranne" population

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Almond (*Prunus dulcis*) is the only nut crop of the *Rosaceae*. As one of the earliest species to bloom in spring, almond trees can experience severe crop loss due to late frosts and unfavourable climatic conditions. The Australian almond breeding program is one of the world's major almond breeding programs and has developed a genetic linkage map. One of the objectives of the project is to investigate the inheritance of flowering time. The flowering time of a "Nonpareil x Lauranne" population was evaluated for two years. The proportion of buds and flowers including seven stages of bud and flower development recorded for each tree using same procedures in each of two years. A proportional odds logistic model was used to analyse the data, considering the timing of development of buds and flowers as a latent variable that was estimated for each tree on each occasion recorded. These estimates of flower timing were used in QTL analysis of flowering time. It is hoped that our work will lead to the identification of genetic markers useful in breeding for altered flowering time.

## CP-28

### QTL and candidate gene mapping for aroma compounds in the apple progeny 'Discovery' x 'Prima'

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Improving fruit quality of apple varieties is an important but complex breeding goal. Flavour is among the key factors of apple fruit quality but in spite of the analytical and biochemical knowledge about volatiles little is known about the genetic and molecular bases of apple aroma. The aim of this study was to use a saturated molecular linkage map of apple to identify QTLs for aroma compounds such as alcohols, esters and terpenes, but also for a variety of unidentified putative aroma compounds. Two parental genetic maps were constructed for the apple cultivars 'Discovery' and 'Prima' by using mainly AFLP and SSR markers. 'Discovery' and 'Prima' showed very different volatile patterns, and 'Discovery' mostly had the higher volatile concentrations in comparison with the *Vf*- scab resistant 'Prima' which has its origin in the small-fruited apple species *M. floribunda.* About 50 putative QTLs for a total of 27 different apple fruit volatiles were detected through interval mapping by using genotypic data of 150 $F_1$ individuals of the mapping population 'C3' together with phenotypic data obtained by **HS-SPME (**head-space solid phase micro-extraction) **gas chromatography.** QTLs for volatile compounds putatively involved in apple aroma were found on 12 out of the 17 apple chromosomes. These are the linkage groups 1, 2, 3, 5, 6, 7, 9, 11, 12, 15, 16 and 17. By far the largest QTL cluster was detected on the lower half of LG 3 of 'Discovery'. Four out of the six alcohols analysed as well as esters showed pronounced QTL effects in this genomic region of the *Malus* genome. Other important QTL clusters were located on linkage groups 2 and 9. In a first attempt to map candidate genes putatively involved in volatile metabolism a LOX (lipoxygenase) candidate gene was mapped on LG 9 which seems to be genetically associated with a QTL cluster for ester-type volatiles. The position of additional candidate genes putatively involved in biosythesis of aroma-related volatiles will be presented. Implications for aroma breeding in apple are discussed.

## CP-29

### Intron flanking PCR as a tool to find new molecular markers for mealiness in *Prunus persica* and its use in candidate gene positioning using BIN mapping approach

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Peach (*Prunus persica)* is a commercially important species for Chile. Unfortunately this fruit presents serious problems associated with the long distances of shipment when exported to the northern hemisphere. These problems are related to a long exposure to low temperatures that triggers chilling injury in the ripe fruit, producing a final product that has a mealy mesocarp. Information from four different cDNA libraries was gathered to search for new candidate genes differentially controlled under chill injury conditions. Since the integrity of the cell wall is supposed to be directly related to carbohydrate metabolism and stress response genes could be involved in the triggering of mealiness related genes we selected two groups of unigenes annotated as these biological functions. Intron Flanking EST-PCR markers were developed with the goal of position these markers over the TxE Prunus reference map through Binmapping. The idea is to associate a novel cluster of unigenes to a particular region in the Prunus genetic map. The development of these new markers based on EST sequences is projected to be useful for evaluating the segregating populations of peaches for the mealiness phenotype.

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## CP-30

### Linkage and association mapping in citrus: developments and potentials

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Although Citrus fruits are the most produced in the world, they have been relatively less studied by the means of biotechnological tools compared to the other major crops. Genetic mechanisms of many agronomic traits are unknown. Conventional breeding programs face serious problems caused by high level of apomixis, extended juvenility, and high level of heterozygosity when traditional methods are employed. The objectives of this study are to review recent developments and potentials in linkage mapping (LM) and association mapping (AM) in Citrus. Traits to be mapped are listed based on current studies. Both approaches differ in several aspects: nature of mapping population, statistical tools, limitation, and resolution. Examples are given where data is available. Few computer softwares for statistical analyses in LM and AM are introduced.

## CP-31

### Towards identification of the co (columnar growth) gene in *Malus*

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The spontaneous mutation causing columnar growth (co) in apple, originally found in the 1950th in the 'McIntosh' variety, has increasingly been used in breeding programs, as the erect phenotype and the almost complete absence of branching confer a tremendous economical advantage. A joint project between the Geisenheim Research Center, the Institute of Molecular Genetics Mainz University and GENterprise GENOMICS GmbH has been initiated to identify and characterize the co gene for the systematic application in apple breeding. Existing molecular markers for the co phenotype are not completely linked (74-97%) and, thus, their usefulness in breeding is limited. Those markers will,

nonetheless, serve as starting points for the fine mapping of the co gene. A BAC library is being constructed from high molecular weight DNA of in vitro plantlets of Procats 28 (co phenotype), a selection from the Geisenheim breeding program. A BAC walking will be conducted to identify the co region. The end sequences of the BACs will be used to design markers to be tested for practical usage in a seggregating population of 600 trees and, eventually, in our co phenotype evaluation (appr. 450 entries). The BAC carrying the co region will be "skimmed" and candidate genes will be identified. These candidate genes will be cloned for transcriptional and functional analysis. The sequence information of these genes will allow for the construction of robust and completely linked SCAR markers for the co gene and the phenotype it causes. The identification and characterization of the gene(s) involved in the formation of the co phenotype will increase our understanding in shoot development and its regulation. It may well pave the way to better regulate the growth habit of apple trees and other woody species for economical purposes.

## CP-32

### The polymorphism of the genes involved in ethylene biosynthesis and perception in apple

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Apple (*Malus* x *domestica* Borkh.) is a climacteric fruit whose ripening is associated with a burst of autocatalytic production of hormone ethylene. Ethylene burst is required for normal apple ripening, however its high production negatively affects shelf life and quality of apple fruits. Although the biosynthesis pathway of ethylene is well characterised, the understanding of the molecular mechanism underlying ethylene perception and signal transduction pathway is limited. 1-Aminocyclopropane-1-carboxylate (ACC) synthase (EC4.1.1.14; *ACS1* gene) and ACC oxidase (EC1.4.3; *ACO1* gene) are enzymes which catalyze the final two steps in the ethylene biosynthetic pathway. Ethylene receptor in apple is encoded by *ETR1* gene. Little information is available about the functional relevance of allelic polymorphism of the genes involved in ethylene biosynthesis and perception. The study describes allelic polymorphism of *ACS1*, *ACO1* and *ETR1* genes in apple cultivars characterised by different ripening time and storage capability. The polymorphism was analyzed by the PCR, restriction analysis and cloning. Two alleles of *ACS1* (*ACS1-1* and *ACS1-2*), five alleles of *ACO1* (*a*, *b*, *c*, *d* and *n*) and five alleles of *ETR1* gene (*a*, *b*, *c*, *d* and *e*) were detected. The position of all three genes on the apple genetic map was determined.

## CP-33

### Comparative genomics of the evergrowing locus among apricot, peach and poplar

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The evergrowing (evg) mutant of peach [*Prunus persica* (L.) Batsch] fails to cease growth and enter dormancy under dormancy-inducing conditions. The EVG locus has been mapped and sequenced in wild-type peach. Six dormancy associated MADS-box (DAM) genes have been found in this locus and proposed as candidates for the regulation of growth cessation and terminal bud formation in peach in response to dormancy-inducing conditions. To study the duplication history of the DAM genes, we identified two BACs corresponding to the EVG locus in apricot (*P. armeniaca* L.) by southern hybridization. We sequenced and annotated a 70 kb region that contains five predicted DAM genes in apricot. We compared the peach EVG locus by global pair-wise alignment with the 70 kb apricot sequence, and the *Arabidopsis* [*Arabidopsis thaliana* (L.) Heynh.] and poplar (*Populus trichocarpa* Torr. & A. Gray) sequenced genomes. The phylogenetic relationships among DAM genes were also studied. As expected, there was a strong similarity between peach and apricot genomic sequence with a conserved order and orientation of orthologous genes. The peach EVG locus had sequence similarity to three loci in poplar reflecting a different duplication history. Greater synteny between the peach EVG locus and the

poplar loci was found, than between peach and Arabidopsis. DAM gene phylogeny and locus distribution in the respective genomes indicates that duplication of the DAM genes occurred after *Prunus* and poplar divergence. Our data suggest that poplar is a better model than *Arabidopsis* for predicting gene presence and order with *Prunus* although the large scale duplication history of poplar will complicate the comparison.

## CP-34

### Identification and mapping of a new apple scab resistance gene

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Here we report the identification of a new resistance gene (*Vd3*) against the apple scab (*Venturia inaequalis*) from the apple resistant selection 1980-015-25 of the breeding program at Plant Research International. This accession also contains the *Vf* gene. We mapped *Vd3*, using SSR and DArT markers, on linkage group 1, at a distance of about 4 cM from *Vf* gene, but in repulsion phase to *Vf*. Based on pedigree analysis and resistance tests, it could be deduced that 1980-015-25 had inherited *Vd3* from the founder D3. This gene provides resistance to the highly virulent EU-NL-24 strain of the race 7 of *V. inaequalis*. This strain has overcome the resistance from both *Vf* and *Vg*. However, *Vd3* has been not effective against the majority of other *V. inaequalis* strains we used in our disease tests.

## CP-35

### Definition of apple scab resistance (*Vf*-gene) from russian apple cultivars and seedlings by PCR

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Apple scab (*Venturia inaequalis* (Cke.) Wint.) is the most harmful disease of apple in cool and humid climates. Control of apple scab is expensive and may require more than ten sprayings of fungicides per season. The overcome of monogenic *Vf*-resistance by new races of apple scab can be possibly hindered by digenic breeding strategy and by using donors from several monogenic loci. The screening of resistant cultivars and seedlings can be done with inoculation experiments, but the best way to test for the presence of heterozygous loci or several monogenic genes (*Vf, Vm, Vr*) is to use targeted PCR analysis. In this study, the presence and homozygosity of *Vf*-gene were analysed by PCR from Russian apple cultivars Afrodita, Bolotovskoje, Imrus, Jubiley Moskvy, Kandil Orlovski, Orlovski Pioneer, Solnysko, Tshistotel, and Svezhest and from 16 open pollinated seedlings of Kandil Orlovskyi, Kurnakovskoje and Stroveskoje. The *Vf*-gene was found in all cultivars except Orlovski Pioneer and Tshistotel, which are the donors of *Vm*-gene originated from SR0523. The *Vf*-gene was found as homozygous in cv. Svezhest and two seedlings of Kandil Orlovski. The PCR method used enabled the identification of homozygous and heterozygous plants. The identification of homozygotic donor plants at early stage of breeding program will help in the digenic breeding of scab resistant apple cultivars.

## CP-36

### Resistance gene analogues of the *HCRVF2* gene are present on linkage group 1 and 6 of the apple

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The *HcrVf2* gene, isolated from the *Vf* locus, is the first apple scab resistance gene cloned from apple. The R-gene belongs to the family of receptor-like proteins sharing structural homology to members of the *Hcr9* resistance gene family of tomato. Its function against the causal agent of apple scab has been demonstrated as well as its race specificity. During the cloning of *HcrVf2* it was found that the R-gene

belongs to a cluster of four genes and that *HcrVf2*-like genes are present in other (unknown) loci of the apple genome. The genomic library of the apple cv. 'Florina' (*Vf*) used to identify *HcrVf2* has been screened by hybridization using the whole *HcrVf2* sequence as probe to identify all the BAC clones carrying sequences homologous to this gene. Eleven BAC clones not belonging to the contigs assembled to clone *HcrVf2* were found. The relative overlapping of the 11 BACs was studied. Seven BACs were assembled in three different contigs, while four BAC clones remained ungrouped. SSRs markers were developed from one BAC clone of each contig and from the ungrouped BAC clones and were mapped in the cross 'Fiesta' x 'Discovery'. The study of seven out of the 11 newly identified BAC clones was sufficient to identify all the *HcrVf2*-like sequences present on the 11 BACs. In addition to these BACs, other 7 clones, belonging to the contigs assembled to clone *HcrVf2* and known to carrying (unstudied) *HcrVf*-like sequences, were also investigated. All the 14 BAC clones have been subcloned, subclones carrying *HcrVf*-like sequences identified, sequenced and for each *HcrVf*-like sequence a consensus sequence has been generated. Of these 14 BAC clones, 22 sequences showing homologies to *HcrVf2* were retrieved. Eight sequences encoded ORFs showing all seven domains found in *HcrVf2*, and five of them have been shown to be expressed constitutively in young apple leaves by RT-PCR. The mapping position of the currently known major genes conferring apple scab resistance has been compared with the mapping position of the *HcrVf* paralogs. As no match has been found, we anticipate that these do not share sequence homology to the *HcrVf2* gene.

## CP-37

### First evidence of the d-hplc efficiency for an authomated cDNA-AFLP in the apple scab resistance model

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The cDNA-AFLP analysis (Bachem et al., 1996; Breyne et al., 2003) has been successfully applied to study plant-microbe interactions (Cooper et al., 2001; Escalettes et al., 2006). In this work, this approach has been chosen to study the *Malus – Venturia inaequalis* interaction. After the identification of the resistance gene *HcrVf2*, capable of conferring scab resistance to the susceptible cv. 'Gala' (Belfanti et al., 2004), it is necessary to identify and characterize the genes that are differentially expressed after pathogen challenge. At this aim, we first set up an optimized and highly reproducible cDNA-AFLP protocol on PAGE, starting with RNA extraction from apple leaves until gel band elution from polyacrylamide gels, fragment re-amplification, cloning and sequencing. Nonetheless, as electrophoretic analyses are labor-intensive with only limited potential for automation and the recovery of DNA fragments from gels is cumbersome, we verify the possibility of separating cDNA fragments by dHPLC (denaturing high performance liquid chromatography), collecting fragments of interest by fraction collection using the Transgenomics WAVE® System (Hecker et al., 2000). The feasibility of cDNA-AFLP by the dHPLC for fragment separation in order to automate all band elution steps will be discussed and preliminary sequencing results will be reported.

## CP-38

### Targeted isolation of nontir NBS LRR resistance gene analogs in apricot

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Numerous R genes have been cloned from a wide variety of plant species. The largest class encodes a nucleotid-binding site (NBS) and a leucin rich repeats (LRR) domain. These genes are contain several highly conserved and strictly ordered motifs like p-loop, kinase-2, kinase-3a and a hidrophobic domain. Large number of NBS-LRR-encodig sequences, the so called R gene homologues or analogs (RGHs or RGAs) have been isolated from different plant species. The NBS-LRR RGHs are classified into two

distinct groups: the TIR and nonTIR subfamilies. Both types of the NBS-LRR RGA's can be found in dicots, while in monocots only nonTIRs were found. In the genome-wide RGA isolation of apricot, only members of the TIR family were previously found. The authors explained this phenomenon with the possibility that there is an unequal distribution of TIR/nonTIR sequences also described in other species or that there is a bias in the PCR strategy. In the present study, we aimed to find nonTIR RGAs in apricot. Based on the amino acid aligment of already described NBS-LRR genes, degenerated primers were designed to anneal whithin the p-loop and GLPL hidrophobic domain.The PCR products were cloned. The clones were screened for nonTIR NBS-LRRs with semi-nested PCR using a further degenerate primer, which was designed for the kinase-2 domain. This motif contains a unique trypthophan on the C terminus highly specific to nonTIR NBS-LRRs. From the putative NBS-LRR PCR products tested so far, 20 percent were positive for the nonTIR-specific motif. Sequence analyses are required to confirm the existence of nonTIR RGAs in the apricot genome. Based on these preliminary results, this approach seems feasible to isolate and characterize nonTIR NBS-LRR RGAs from apricot.

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## CP-39

### Study of genetic variability induced by artificial hybridization in apple on the basis of mas selection

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Phenotypic selection in apple seedling populations (F1), derived from semidiallel hybridization between genitors with different peculiarities (eg. Florina and Liberty, possessing *Vf* gene; Starkrimson and Goldenspur, with spur ideotype) was completed with marker-assisted selection (MAS), proceeding from BSA technique (Bulk Segregant Analysis). The quality and quantity of the selected DNA samples vary among genitors and hybrid combinations; the DNA content for genitors alternate from 229.1 ng/µl dsDNA (Goldenspur) up to 694.3 ng/µl dsDNA (Liberty), and from 302.8 ng/µl dsDNA (Starkrimson x Liberty - spur ideotype) to 1984.3 ng/µl dsDNA (Goldenspur x Liberty - apple scab sensitive) for hybrid combinations. Due to DNA amplification and electrophoresis migration it was possible to emphasize the polymorphism within genitors, hybrid combinations and their offspring for resistance to apple scab, powdery mildew and architectural ideotype of the trees. By analyzing the migration of the reaction products into the agarose gel (Bulk Segregant Analysis technique) the plants genotype, which determines the resistance or sensitivity to apple scab attack, to powdery mildew attack and architectural ideotype was directly appreciated by the existence or lack of the stripe specific to the analyzed characteristic feature.

## CP-40

### The CBF and R genes as key elements to study the molecular response to abiotic and biotic stress in almond

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Almond (*Prunus dulcis* [Mill.] D.A.Webb), as a woody perennial needs to cope with cyclical environmental changes and several biotic constrains that can affect plant growth and development. Given its high genetic variability, almond has been considered to be tolerant to diverse biotic and abiotic factors, but little is known concerning the molecular mechanisms involved in the response to stress. In this study we have focused on the isolation of almond homologues to the CBF/DREB1 transcription factors (TFs), known to play an important role in abiotic stress response, and on the search for candidate resistance genes (*R*) involved in host-pathogen recognition. The CBF/DREB1 is a family of TFs involved in the abiotic stress signalling pathway. These TFs are upstream regulators of stress responsive genes

encoding enzymes or structural components important for direct protection of cells. Almond CBF/DREB1 homologous sequences were isolated from cDNA samples obtained from *in vitro* plantlets exposed to either cold or drought conditions. Preliminary RT-PCR analysis showed that almond CBFs are expressed during cold and drought treatments, few minutes after stress induction. The full-lengh cDNA sequences were obtained and accurate characterization of the functional CBFs is underway. Different almond genetic backgrounds will be included in these analyses, namely almond cultivars and wild relatives, in order to search for natural variations in stress response. Additionally, a set of resistance-gene candidate sequences (RGCs) were isolated from the genomic DNA of one almond cultivar, described as resistant to several diseases, and two wild almond ecotypes (*P. webbii*). Phylogenetic analysis revealed that RGCs, from both cultivar and wild ecotypes, clustered together in 5 different groups. This observation suggested a great similarity of the genetic backgrounds from both species, regarding their biotic resistances. The isolated RGCs are being mapped in the Texas x Earlygold *Prunus* genetic map. This will provide valuable information for development of molecular markers that can be used in breeding programs.

## CP-41

### Transcriptomic analysis of ethylene-induced tolerance to non-chilling peel pitting in citrus fruit

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Non-chilling peel pitting often occurs during postharvest storage in fruit of different citrus cultivars and affects both the inner (albedo) and outer coloured part of the peel (flavedo). Conditioning mature 'Navelate' oranges for 4 days with 10 µL $L^{-1}$ ethylene reduced the loss of peel integrity during storage at 22ºC and 90-95% RH, while peel breakdown was enhanced by inhibiting ethylene action with 1-methylcyclopropene (1-MCP). To have a general insight of the mechanism underlying the ethylene-induced tolerance of citrus fruit to this disorder, we have evaluated global changes in gene expression occurring in the flavedo and the albedo of 'Navelate' fruit stored under the above conditions after being treated with ethylene or 1-MCP by using a cDNA microarray containing 12000 unigenes generated by the Spanish 'Citrus Functional Genomics Project'. Gene ontology analysis of differentially expressed genes revealed that ethylene induced the metabolism of amino acid derivatives, including phenylpropanoids, and electron transport in both tissues. Biological processes related to hormone signalling, such as ethylene and jasmonic acid, and to the biosynthesis of aromatic amino acid were also induced in the flavedo, whereas an over-representation of genes involved in the metabolism of sulfur-containing amino acids were found in the albedo. On the other hand, the severe peel damage in the 1-MCP-treated fruit was accompanied by marked changes in the expression of genes related to programmed cell death belonging to the ubiquitination and proteosome pathways, resembling the plant's defence response in an incompatible interaction.

## CP-42

### The inheritance of the anthocyanin and flavonol profile in *Vitis vinifera* intraspecific hybrids

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It is common that innovation in agriculture is based on the development of new varieties. However, in winegrape viticulture, innovation has been based on the improvement of agronomy techniques and on the development of new enological technologies, with a lack of genetic breeding programs, even when it has been demonstrated very recently that the origin of two of the more known varieties all over the world (Cabernet Sauvignon and Chardonnay) is from intraspecific crosses. Anthocyanins and flavonols are final products arising from the flavonoid biosynthetic pathway. Anthocyanins are the responsible for the color of red grape varieties and the wines produced from them. Flavonols are also important products

since these compounds participate both in stabilizing anthocyanins in young red wines through copigmentation and increasing healthiness of this drink.. *Vitis vinifera* varieties are characterized by the usual presence of 3-O-glucosides of delphinidin, peonidin, petunidin, cyanidin and malvidin, together with their acylated derivatives. The hydroxylation pattern of B-ring is one of the main structural features of flavonoids and is an important determinant of their coloration, stability and antioxidant capacity. As hydroxylation of the B-ring is carried out by F3'H and F3'5'H, the composition of anthocyanins in grape skins will be determined by the relative activities of these enzymes as well as the ratio dihydroxylated/trihydroxylated anthocyanins. At the same time, methyl transferase activity will determine the different methoxylation pattern of B ring and acyl transferase, the presence of acyl derivatives. Most of these enzymes also regulate the synthesis of flavonols. Previous studies regarding the anthocyanin profile in different grape varieties have shown that the anthocyanin and flavonol profiles were different among varieties and therefore these flavonoids could be a very useful chemotaxonomical tool. Monastrell grapes (also known as Mourvedre or Mataro) are very well adapted to the agro-ecological conditions of Southeast of Spain, and a program to obtain intraspecific hybrids crossing Monastrell with internationally prestigious varieties (Cabernet Sauvignon, Syrah, Barbera) has started. The study of the flavonoid profile of the different hybrids allow us to study how the flavonoid profile is inherited among the hybrids and to reach conclusions on which profiles are dominant among the studied grapes. The objective is to obtain new varieties showing a good adaptation to our agro-ecological conditions, as Monastrell does and with a high anthocyanin and flavonol content as other premium varieties.

## CP-43

### Functionality of a class I beta-1,3-glucanase and chitinase from skin of table grapes berries

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We have analyzed gene expression of a class I β-1,3-glucanase (*Vcgns1*) and chitinase (*Vcchit1b*) in the skin of red table grapes (*Vitis vinifera* cv. Cardinal) as markers for changes in response to low temperature, and also to assess how high $CO_2$ levels (20% $CO_2$ plus 20% $O_2$) modulated its transcript accumulation at 0 $^oC$. To elucidate the possible physiological role of class I β-1,3-glucanase and chitinase in table grapes as cryoprotectant, and/or antifreeze, we also report on recombinant expression of *Vcgns1* and *Vcchit1b*. The results indicate that storage at 0 ºC for 3 days increased *Vcgns1* and *Vcchti1b* mRNA levels in the skin of non-treated grapes. However, the accumulation of the transcripts were lower in the skin of grapes after 3 days of $CO_2$ treatment, as well as when treated fruit were transferred to air. By using heterologous expression of the cDNAs in *Escherichia coli*, we showed that VcGNS1 encoded a protein with glucanase activity with an optimum pH and temperature of 6 and 45 ºC, respectively, being active at 0 ºC. Since the VcCHT1b protein was produced as insoluble inclusion bodies, the protein was solubilized and refolded showing chitinase activity. Furthermore, the purified proteins exhibited *in vitro* cryoprotective activity for the freeze labile L-lactate dehydrogenase enzyme. The recombinant VcGNS1 was as cryoprotectant as BSA; while the purified VcCHIT1b was 2.5 times more cryoprotectant than BSA. In contrast, when the thermal hysteresis activity of the recombinant VcGNS1 and VcCHIT1b was measured, using differential scanning calorimetry, the results indicated that they did not show antifreeze activity. The high activity and stability of the recombinant β-1,3-glucanase at 0 ºC, and the cryoprotective activity shown in this work suggest that VcGNS1 and VcCHIT1b may participate in the response of table grape to combat low temperature conditions.

## CP-44

### Expression analysis of an endodormancy-related MADS-box gene in winter buds of fruit tree species of *Prunus*

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Winter bud dormancy in temperate woody perennial plants is a complex process necessary for plant survival in the unfavorable environment. In contrast to the extensive study for physiology of dormancy break, the internal factors controlling endodormancy are poorly understood. Endodormant buds require a certain amount of cold temperatures for the transition to an ecodormant state capable to resume growth in a favorable environment. By suppression subtractive hybridization (SSH) with mirror orientation selection (MOS) approach, we searched for the genes of which expressions were up-regulated by endodormancy induction and down-regulated by endodormancy release in the dormant buds of Japanese apricot. Differential screening and sequencing indicated that genes involved in plant hormone (abscisic acid and gibberellins) responses, stress resistance, cell wall modification, and signal transduction such as transcription factors, are up-regulated in endodormant buds. Thus, an integrated complex signaling system is likely involved in the regulation of endodormancy after receipt of an environmental signal. After a further expression survey, we found that a gene similar to the SVP/AGL24-type MADS-box transcription factor showed endodormancy-specific expression. RT-PCR and northern blot analysis indicated that MADS6 shows endodormancy-specific expression and its expression was suppressed by chilling accumulation in lateral buds of several fruit tree species of *Prunus*. Possible involvement of MADS6 in endodormancy release of lateral buds of *Prunus* species is discussed.

## CP-45

### Microsatellite (SSR) and Retrotransposon markers detecting clonal polymorphism in apple (*Malus* x *domestica*)

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Clonal polymorphism causing sport mutations in apple have been reported numerous times. However, identification of sport mutations with the help of DNA markers has proved to be complicated. In a diversity study of the Finnish apples, 300 samples analysed with 10 microsatellite loci produced 220 different –profiles. The profiles revealed 33 groups, in which the same genotype was associated with two to five different cultivar names. Many of these synonymous names were obviously resulting from mislabelling or renaming old cultivars, but also in some cases representing phenotypically clearly distinguishable sport mutations. Only four out of the ten well-known sports could be identified with microsatellites: 'Atlas Red' (sport of 'Atlas'), 'Åkerö Hassel' (sport of 'Åkerö'), 'Olli' (sport of 'Korobovka') and Lohjan Kirkas' (sport of 'Gyllenkrok's Astrakan'). The detected difference was usually a two base pare shift in one allele (out of 18-30 alleles/sample detected in this study). In one sport a shift was observed in two alleles, and in another case a null allele was detected in the sport. All these differences were stabile in separate repetitions. Furthermore, microsatellite instability was also discovered in phenotypically uniform clonal material. Samples of two cultivars, Borgovskoje ja Antonovka Safrannoje, were obtained from four different locations representing two clonal collections and two specialist plant nurseries. For both two cultivars one sample, out of eight and four respectively, microsatellite analysis demonstrated a repeatable two base pare shift. All the sports that had produced microsatellite profiles identical to the mother cultivar were further analysed with retrotransposon-based markers. In the present study, we used our previously cloned and published TRIM (terminal-repeat retrotransposon in miniature) – group retrotransposon elements cloned from cv. Antonovka. These apple specific TRIM primers were first employed in IRAP (inter-retrotransposon amplified polymorphism) or REMAP (retrotransposon-microsatellite amplified polymorphism) analyses. The standard apple cultivars were well distinguished, but all the six tested sport mutations pairs remained identical in IRAP/REMAP profiles. On the other hand, the same primers in a SSAP (sequence-specific amplified polymorphism)

analysis revealed several differences, suggesting that this method is at present the most appropriate marker method for detecting clonal polymorphism in apple.

## CP-46

### Development and mapping of molecular markers derived from genes expressed during fruit ripening in apple

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Understanding the genetic mechanisms underlying fruit quality traits is one of the most important steps to improve marker-assisted selection and breeding of apple cultivars. cDNA microarray technology was used to identify genes showing a fruit modulated expression on which to develop functional molecular markers. Microarray hybridizations were always performed to compare mRNA from fruits of the cultivar 'Prima', collected 46 days after full bloom (DAFB), with mRNA samples isolated from the same cultivar at later developmental stages up to maturity. Expression analysis suggests that the transcription level of about 10% of the genes present on the slides changes during fruit development. It was possible to assign a function to 75% of the sequences and the most represented functional categories are: primary metabolism (15%) and aminoacid and protein metabolism (11%). Gene Ontology annotation was employed to univocally describe the differentially expressed genes, while clustering analysis was used to group genes according to their expression profile. Seventy-six and 104 DAFB fruits show only minor changes in gene expression levels, while 133 and 165 DAFB fruits are characterized by a high number of genes up or down regulated when they are compared to the 46 DAFB stage. Quantitative RT PCR was employed to validate microarray results and 70% of the data could be confirmed, proving that microarrays are indeed a good tool to identify genes potentially correlated with fruit quality. The most interesting transcripts showing modulated expression during fruit development (about 70 out of 189) were screened for the presence of polymorphisms in the parental genotypes of 2 segregating populations ('Fiesta', 'Discovery' and 'Prima'). Twenty-four CAPS, 4 SSCP, 8 length polymorphisms (6 SSRs and 2 INDELs) were developed. Thirty-six polymorphic markers were placed either on the 'Fiesta' x 'Discovery' or on the 'Prima x Fiesta' reference linkage maps.

The work is part of the European project named HiDRAS (High-quality Disease Resistant Apples for a Sustainable Agriculture).

## CP-47

### Understanding the molecular basis for stone formation in *Prunus* species

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A unique aspect of *Prunus* fruit is the presence of a hard wood-like carapace called the stone surrounding the seed. The stone represents a somewhat unique biological adaptation that presumably protects the seed from stress and/or pathogens. We have begun biochemical and functional genomic studies on stone formation to elucidate its biological function and identify gene targets useful for engineering pitless fruit. Phloroglucinol-HCL staining revealed that the endocarp layer accumulates lignin starting from the blossom end of the fruit around 7-10 days prior to hardening. Derivatization followed by reductive cleavage (the so-called "DFRC" method) showed that peach stones contain nearly 50% lignin, more than any other woody material examined to date. To identify genes and pathways associated with stone development we conducted expression profiling studies using a developing peach fruit series prior to ripening. Total RNA was extracted from each sample, labeled, and hybridized to 2 different microarray platforms: 1) labeled cDNA from 4 developmental time points was hybridized to a custom printed peach

fruit 5K oligoarray and 2) labeled cDNA from 7 time points was hybridized to a 15K apple oligoarray. Results showed that the phenylpropanoid (PP), lignin, and flavonoid pathways are strongly induced in peach fruit prior to ripening. A subset of PP and lignin pathway genes was induced specifically during stone hardening. qPCR analysis of dissected fruit revealed that these genes are endocarp specific. In contrast, a number of lignin and flavonoid genes were induced throughout the fruit and showed evidence of co-regulation. Comparison of these expression profiles to apple and *Arabidopsis* expression data revealed that flavonoid pathway induction in early fruit is highly conserved while induction of specific PP and lignin pathway genes appears to be specific to *Prunus* species.

## DP-01

### Transgenic plant regeneration of grapevine over-expressing the stilbene synthase 1 gene from *V. vinifera*

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The use of genetic engineering for grapevine improvement appears to be a powerful tool to introduce new traits without any alteration of the essential characters and identity of the cultivar. Fungal diseases are one of the most serious problems in this species. Grapevine produces resveratrol as defence reaction when attacked by fungi. The production of this phytoalexin is controlled by the enzyme stilbene synthase (StSy). Transgenic tobacco, barley and wheat plants expressing the StSy grapevine gene showed increased resistance to *Botrytis cinerea*. In the present work, embryogenic calli of two grapevine (*V. vinifera* L.) cultivars, Sugraone (Superior Seedless) and Monastrell were co-cultured with *Agrobacterium tumefaciens* strain EHA105 carrying the binary over-expression vector pKESTS706. This plasmid, that includes the complete coding region of the StSy cDNA (*Vvst1* gene) under the CaMV 35S promoter terminator, was constructed using the Gateway cloning system (Invitrogen). Briefly, a PCR with specific primers with attB1 and attB2 flanking sequences and *in vitro* BP clonase recombination reactions were carried out to obtain a PCR product of the full StSy cDNA. The product of the BP recombination reaction was used to transform competent cells of *Escherichia coli* strain DH10B by electroporation. Next, after the LR clonase reaction, the cDNA was transferred from the entry clone to the destination vector pK7WG2D. This vector includes a kanamycin resistance gene under the control of the nopaline synthetase gene promoter and terminator control, as well as a GFP expression module provided by the E-*gfpER* gene. Somatic embryos expressing GFP were isolated from the selection medium, ½MSAC plus 20 mg/L kanamycin, and cultured on germination medium without kanamycin. Sugraone regenerated plants have been analysed by PCR with s*gfp*-, *nptII*- and *StSy*-specific primers and all were positive. The analyses for Southern blot confirm the integration of the genes. Concentration of resveratrol in control and transgenic plants were analysed.

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## DP-02

### Cloning and characterization of *VCHI III* gene related to grapevine powdery mildew resistance

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A class III chitinase gene (*VChi III*) was isolated from six *Vitis vinifera* cultivars and six accessions from NPGS, UC Davis, California, *V. rupestris* and *V. labrusca*. The open reading frame of gene comprised

893 bp with no introns and encoded 297 amino acids. Pair wise alignment through BLAST showed *VChi III* is settled on chromosome 16. The *VChi III* sequence showed high similarity to the class III chitinase of *V.vinifera cv*. Koshu and acidic chitinase groups. All *VChi III* sequences showed similarity to each other; however there were single nucleotide polymorphisms among them.

## DP-03

### Characterization of transgenic apples expressing the HRP N gene

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Fire blight is the major bacterial disease of *Maloideae* (apple, pear, and other members of the *Rosaceae*) caused by the necrogenic bacterium *Erwinia amylovora*, which pathogenicity requires a functional type III protein secretion system (TTSS). The protein harpin $N_{Ea}$ is secreted through the TTSS and targeted to the intercellular spaces of plant tissues where it acts as an elicitor of plant defense reactions. In order to create novel mechanisms for fire blight resistance in apple, we have generated transgenic apples expressing the *hrpN* gene from *Erwinia amylovora* under the control of the constitutive *CaMV35S* or the pathogen-inducible tobacco *Str246C* promoters, as well as with or without the tobacco *PR1* gene secretion signal peptide. The characterization of these harpin $N_{Ea}$ transgenic apples was carried out by: (1) evaluation of fire blight resistance after greenhouse inoculation to determine the more resistant clones; (2) quantification of the constitutive and induced expression of the transgene (by Q-RT-PCR) to determine the highest expressing clones and to analyze the correlation between transgene expression and resistance levels. Further characterization is underway by: (1) in planta localization of the harpin $N_{Ea}$ protein; (2) targeted transcriptomic analysis to identify transduction pathways involved in the harpin $N_{Ea}$ induced resistance.

## DP-04

### Transgenic expression of *Erwinia amylovora* (fire blight) effector proteins in *Malus* (apple)

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*Erwinia amylovora* (*Ea*), the causal agent of fire blight, uses a type three secretion system (TTSS) to deliver effector proteins into plant host cells. Once inside the host cell, effector proteins are thought to function as elicitors and suppressors of host defense, however the exact mechanisms by which Ea effectors regulate these responses is not clearly defined. To investigate the role of individual bacterial effector proteins, M.26 apple was engineered to transgenically express the *Ea* effector proteins Eop1, HopCEa or AvrRpt2Ea under the control of an inducible promoter. Each bacterial effector was directionally cloned from *E. amylovora* strain 273 into a Gateway compatible entry vector using gene specific primers that were modified to incorporate a Kozak sequence in the 5' end of the effector gene to facilitate proper translation in a eukaryotic system and a His tag at the 3' end for protein detection. The cloned bacterial effector genes were subsequently cloned through Gateway technology into a binary vector, pBinPlusARS.XVE. This binary vector incorporates the regulatory elements of the estradiol-induced XVE gene expression system developed by Zuo et al. (2000) and was used in the *Agrobacterium*-mediated transformation of apple. Transgenic apple lines were confirmed through PCR analysis with effector specific primers and evaluated for Agrobacteria contamination with *virG* specific primers. Inducible expression of *eop1* and *hopCEa* in the presence of 25 uM estradiol was confirmed by RT-PCR. Callose deposition is a cellular marker of host basal defense. When non-induced and induced leaf tissue of T98, a non-leaky *Eop1* apple transgenic, was challenged with an *Ea* TTSS- mutant a significant reduction in callose deposition was observed in induced tissue, suggesting that transgenic expression of *Eop1* suppresses host basal defense mechanisms. These transgenic lines will enable us to investigate the role of *Ea* effector proteins in regulating host defense mechanisms, as well as determining their effects on host gene expression in the future.

## DP-05

### Abiotic stress resistance in young apple trees is enhanced by overexpression of a cytosolic superoxide dismutase

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Reactive oxygen species (ROS) are induced during both biotic and abiotic stress, either as signaling molecules or as a response to stress injury. ROS are highly destructive to cell components and the injury resulting from these compounds is referred to as oxidative stress. Antioxidant enzymes, such as superoxide dismutase (SOD), scavenge oxygen radicals preventing the injury resulting from oxidative stress. The objective of the present research was to produce transgenic apple plants (*Malus* x *domestica* 'Royal Gala') with enhanced production of a cytosolic SOD. A full-length SOD cDNA was isolated from pea by a combination of RT-PCR and conventional plaque lift screening of a pea cDNA library. The SOD gene was mobilized into a binary vector consisting of pBINPLUSARS and pRTL2 for *Agrobacterium*-mediated transformation of apple. The resulting SOD-overexpression (SOD-OX), blank-cassette, and un-transformed lines were evaluated for resistance to acute and prolonged exposure to high temperature, and freezing injury in non-acclimated and acclimated plants, by ion leakage assays of leaves and bark from 1 year-old trees. Results indicated that SOD-OX leaves exhibited improved resistance to both acute (30 min) and longer-term exposure (2h to 24h) to elevated temperatures compared to the non SOD-OX lines. Cold tolerance of non-cold-acclimated SOD-OX tissues did not differ from the control plants. Cold acclimated (2 weeks exposure to a short day photoperiod at 4ºC) leaves of SOD-OX trees, however, were more cold tolerant compared to the other lines, while bark was not. The overexpression of antioxidant enzymes is believed to help cells recover from post-injury ROS rather than directly increase stress tolerance. Therefore, the differences observed in increased stress tolerance in the transgenic apple plants may be a reflection of the type and extent of injury caused by heat vs. freezing stress.

## DP-06

### Development of transgenic mexican lime plants for resistance to Citrus tristeza virus using post-transcriptional gene silencing

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Two obstacles which may hinder the development of citrus resistant to Citrus tristeza virus (CTV) via post-transcriptional gene silencing (PTGS) are the genetic diversity of CTV and the presence of multiple suppressors of PTGS in the CTV genome. To address the genetic diversity of CTV, we sequenced 105 coat protein (CP) genes of Hawaiian isolates and used these data, along with the CP genes of 14 isolates from around the world present in GenBank, to create a synthetic CTV CP gene segment. This 626 bp untranslatable segment is ≥94.6% similar to the CPs of all known isolates of CTV. We used *Agrobacterium* to introduce this gene segment into Mexican lime (*Citrus aurantifolia*) in sense, antisense, and inverted repeat configurations. CTV also possesses at least 3 PTGS suppressors: p20, p23, and CP, whose expression *in planta* may prevent a resistant phenotype. To target their expression, we linked p20 and p23 gene segments and the entire 3' untranslated region to a segment of the synthetic CTV CP gene and introduced this construct into *C. aurantifolia* using *Agrobacterium*. Molecular analyses indicate these plants are indeed transgenic, and greenhouse evaluation of their resistance to CTV is currently underway.

**DP-07**

### Development of plum pox virus resistance in plum plants grown in Canada

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Plum pox virus (PPV) is the most serious viral disease of *Prunus* fruit plants, including plums, peaches, apricots, and cherries. The disease has been affecting *Prunus* fruit crops of different European countries for many years. The PPV disease has recently been found in Canada. Few natural resistance resources to PPV have been found which may be use to develop highly resistant *Prunus* varieties. Alternatively, molecular biology and biotechnology may be used to develop PPV resistance in plants. The DNA vectors containing sections of PPV genome using hairpin design were constructed and introduced into *Nicotiana benthaminana* model plants. A large number of plants transformed by the designed vectors exhibited high levels of resistance to the PPV virus. PPV specific siRNAs were detected in PPV resistant plants and there was a positive relation between siRNA presence and PPV resistance in plants. An effective genetic transformation technology was then developed for plum (*Prunus domestica*) plants grown in Canada based on studies of various aspects and factors affecting plum regeneration and transformation. Hairpin-designed vectors were introduced into plum. Plum plants expressing siRNA showed high levels of resistance to PPV but all the control plants were susceptible to the virus. The plants were subject to three cycles of cold treatment consisting of 4 $^0$C for three months. The recovered selected plants from each cycle of cold treatment consistently showed resistance to PPV. The study indicates that post transcriptional gene silencing and expression of specific siRNA is an effective approach for plum pox virus resistance in plum plants.

**DP-08**

### Towards the production of stress tolerant grapevine cultivars

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Crossbreeding to obtain frost tolerant and disease resistant grapevine varieties has been carried out in Hungary for decades. However, it has proved to be difficult to get newly processed varieties equally accepted by growers, wine industry and consumers, due to their preference for established, traditional varieties. Therefore new, cross-bred grapevine genotypes have little chance to become recognized by growers and appear on the market. This trend seems to prevail, despite time consuming and tedious procedures behind traditional breeding methods. Nevertheless, grapevine production is negatively affected by a variety of stress factors, and thus needs molecular breeding methods to improve the quality, stress- and disease resistance of cultivars. Genetic transformations achieved by expressing a stress-tolerance related gene from a plant source, thus yielding new genotypes differing in only one feature from the original cultivars may have a chance to become acceptable despite the above trend. From among stress factors, growers have practically no control over abiotic stresses brought about by recent climate changes, such as the uneven distribution of precipitation, increased ultraviolet radiation from sunlight or deviations from normal growth temperatures, adverse edaphic conditions. Effects of biotic stress factors, such as mildew or botrytis, can be moderated by chemicals, which are on the other hand, costly and often involve environmental pollutants. A common biochemical feature of all these stress conditions is oxidative cell damage, accompanied by the accumulation of reactive oxygen species (ROS). Ferritin, an iron chelating protein has been shown to regulate oxidative stress response is both mammalian (including human) and plant cells. Our experiments aimed at the production and stress endurance testing of ferritin over-expressing grapevine plants. We obtained ‘Richter 110’ transformant plants from anther-derived embryogenic culture transformed by *Agrobacterium tumefaciens* of which T-

DNA contained the different gene constructions: EHA105 (pRok2), EHA105 (pRok2Ferr) and EHA105 (pRok2FerrFLAG). The alfalfa ferritin gene construction was expressed in the chloroplasts, while an FLAG-tagged variation of the same gene was expressed in the cytoplasm (Deák *et al*., 1999). Plants were successfully regenerated from both transgenic lines and leaves contain the alfalfa ferritin protein, as shown by protein gel blot analysis. Preliminary stress tolerance results are presented, showing photosynthetic responses to excess bicarbonate treatments of roots. This experimental model was chosen to model soil salinization affecting Richter as rootstock.

**EP-01**

### Cloning and preliminary characterisation of the promoter region of the 1-aminocyclopropane-1-carboxylate synthase gene

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The 1-aminocyclopropane-1-carboxylate synthase (ACS) is a key enzyme of ethylene pathway which play an important role in apple fruit ripening. Different ACS allele sequences are available in public databases and some differences in the promoter regions were found. In particular, a 162 bp insertion was proposed to influence ethylene evolution and ripening (Harada et al., 2000). The cultivars homozygous for the 'short' ACS gene promoter (ACS1-1) showed an higher internal ethylene concentration in apple fruits compared to cultivars homozygous for the 'long' ACS gene promoter (ACS1-2). In order to better understand the functionality of the different ACS promoters, within the framework of the EU-ISAFRUIT project, the full-lenght sequences of two ACS genes were obtained from cultivar 'Florina'. This cultivar was chosen because is heterozygous for both the 'short' and 'long' alleles and because a BAC library is available at DCA-BO (Vinatzer et al., 1998). The screening of the library was done by using specific primers (Harada et al., 2000). Among the ACS-carrying BACs (seven for the 'short' and three for the 'long' allele), a clone/allele were chosen for sequencing the whole gene including the promoter region (about 3800 bp). The two ACS sequences from cv. Florina are very similar to the corresponding 'short' and 'long' allele sequences found in the public database (about 98%). The two sequences of the ACS promoters from Florina were analysed by PLACE. Some differential cis-acting elements between the two alleles were found. These preliminary information have been used to design a series of seven deletion constructs by using a pCambia 0305.1 vector. In this vector, the CaMV35S promoter, driving the expression of the GUS gene, has been eliminated to obtain a promoter-less vector to be used to insert the different sequences from the ACS promoter. The constructs are currently in the final steps of preparation and will be used for transient expression studies in apple fruits together with a 'positive' control (the original vector) and the promoter-less GUS construct. Some preliminary tests of transient expression will be also presented.

**EP-02**

### Development of transgenic citrus plants ectopically expressing ethylene-insensitive genes

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Ethylene is a hormone that affects many aspects of plant growth and development. It regulates seed germination, seedling growth, leaf and petal abscission, organ senescence, fruit maturation and ripening, and responses to abiotic stress and pathogens. Ethylene perception requires specific receptors and a signal transduction pathway to coordinate down-stream responses. The expression of genes that encode mutated ethylene receptors in plants has been used to produce ethylene insensitivity. Transgenic *Arabidopsis* plants overexpressing the mutant *etr1-1* allele displayed an ethylene-insensitive phenotype, and ethylene binding was reduced in comparison to wild-type plants. Ethylene insensitivity could also be obtained by expressing the same gene (*etr1-1*) in heterologous plants such as tomato and petunia, indicating that this strategy could be applied to inhibit ethylene responses in a wide range of plant

species. In the present study, we report the generation and characterization of transgenic citrus lines containing the *etr1-1* gene from *Arabidopsis* or the Cs-*ERS* (ethylene response sensor) from sweet orange (citrus homologue to the *Arabidopsis ERS* gene), both under the control of the constitutive and strong CaMV 35S promoter. The *etr1-1* and the *CsERS* genes were cloned in the binary vectors pBI-121 and pBIN-JIT, respectively. *A. tumefaciens* EHA 105 was transformed with the different transformation plasmids by electroporation. These strains (EHA 105/pBI-121-*etr1-1* and EHA 105/pBIN-JIT-*CsERS*) were used to genetically transform Carrizo citrange (*C. sinensis* L. Osbeck x *P. trifoliata* L. Raf.) and Mexican lime (*Citrus aurantifolia* (Christm.) Swing). Both binary plasmids contain the selectable gene cassette NOSp/*nptII*/NOSt that allowed us to select transgenic shoots in kanamycin-containing culture medium. Citrus regenerants were first characterized by PCR using primers specific for the transgenes. More than 30 putatively transgenic citrus lines were generated for each construct and the phenotypes exhibited to date were similar to those of non-transformed control plants. Different expression levels of the *etr1-1* and *CsERS* transgenes were shown by Northern blot analyses. These putative ethylene-insensitive transgenic citrus lines are allowing us studying the effects of ethylene in abscission processes and their response to certain important citrus pathogens.

## EP-03

### Papaya fruit ripening is altered by ACC oxidase cossuppresion

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Papaya is originated from Mexican tropic. Papaya is a very important crop in many tropical countries. The crop suffers from postharvest losses which are normally due to parasitic diseases, physiological disorders, mechanical damage and fruit overripening. Papaya is a climacteric fruit and ethylene plays a central role in this phenomenon. The fruit is susceptible to overripening caused by ethylene and all the strategies in use today to extend the shelf life of papaya are based in the control of ethylene action and production. In this respect, enzymes involved in ethylene synthesis represent key targets for research. In this work we report the isolation and use of a PCR fragment of the papaya ACC Oxidasa gene in the sense orientation for transformation of papaya embryogenic cells by biolistic. Sixty transgenic lines were recovered and grown under field conditions. Molecular evaluation of the lines showed the incorporation of the transgene in different copy numbers in the papaya genome. Transgenic fruits were evaluated and a reduction in ethylene and $CO_2$ production was determined in some of them. The softening pattern and the colour development of the peel were also altered. Overall the fruits showed an altered ripening process. Some of our results are similar to the obtained using 1MCP for delayed papaya fruit ripening. More studies are necessary before to use this technology at commercial level for guarantee the quality of the fruit.

## EP-04

### Flowering genetics in apple

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Apples (*Malus* × *domestica* Borkh.) are the most prevalent fruit in temperate regions and have been cultivated from ancient times in Europe and Asia. Apples rank fourth in importance after citruses, bananas and grapes. Prolonged juvenile period in apple elongates breeding cycles and makes the improvement of this crop a challenging process. To achieve the genetic manipulation of juvenility and flowering habit of apple as well as other fruit trees, it is necessary to identify the genes involved in its flowering and floral development. There are many genes already isolated from apple, most of them belonging to the MADS-box genes family with potential roles in its flowering. However, there are a lot of apple genes involved in this developmental stage yet to be identified. In the present report, flowering

genetics of apple has been elucidated through analysis of EST data available in public databanks and apple genes with potential roles in its flower development have been identified.

**EP-05**

### RNAi-silencing of *MdTFL1* induces early flowering in apple

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Efficient breeding of fruit trees such as apple is limited by the long period of juvenility lasting several years. During recent years, many factors controlling the transition period from juvenile to adult stage were identified, mainly using the model plant *Arabidopsis thaliana*. Several genes such as *LEAFY* (*LFY*), *APETALA1* (*AP1*), *TERMINAL FLOWER 1* (*TFL1*), and *FLOWERING LOCUS T* (*FT*), which control flowering time, have been isolated from Arabidopsis. The *MdTFL1* protein is homologous to TFL1 of *A. thaliana* which suppresses the floral meristem identity genes *LFY* and *AP1* and maintains the inflorescence meristem. We used an RNAi approach to induce post-transcriptional gene silencing of the *MdTFL1* gene in order to reduce the juvenile phase in apple. A binary vector was constructed which contains a constitutively expressed *nptII* gene and a hairpin RNA homologous to the coding sequence of *MdTFL1*. The vector was used to transform the apple (*Malus domestica* Borkh.) cvs. `Elstar`, `Pinova`, `Holsteiner Cox` and `Gala` via *Agrobacterium tumefaciens*- mediated gene transfer. Regenerated shoots were proven for transgenity by PCR, Southern blot and RT-PCR. Quantitative real time PCR analysis showed that the expression of *MdTFL1* was markedly reduced in transgenic lines compared to non-transformed control plants. Furthermore we studied the mRNA transcript levels of possibly affected flowering genes of apple. Some of the plants started to flower six month after the transformation under *in vitro* conditions. The plants were recently transferred to the greenhouse. Results of molecular analyses as well as flower development and morphology, pollen viability and probably fruit set will be presented.

**EP-06**

### Isolation and characterisation of almond flowering and circadian clock related genes

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Flowering is a major issue for fruit trees. *Prunoidea* is an important sub-group of Rosaceae, a family of fruit trees with high economic importance. It is known that circadian clock regulates many aspects of development, including photoperiodic induction of flowering. Almond is the fruiting tree with earlier blooming time which may compromise fruit set due to frost or to reduced pollinator availability. In fruit trees, knowledge about the molecular basis of flowering is still scarce. This is in part because of the difficulty to obtain mutants or transformed plants of Rosaceae species, which obliges to estimate gene function from comparisons with genes characterized in model plants. Following our previous research in the molecular basis of almond flowering, in which we could identify and map a number of genes putatively involved in such development (i.e. MADS1, 2 and 3) we are extending our study to cover additional genes and to perform functional analysis in a heterologous species (*Arabidopsis*). The full-length sequences of the MADS genes were individually cloned in GATEWAY® vectors under the control of a double 35S promoter and with GFP as reporter gene. Transgenic *Arabidopsis* plants were obtained by floral dipping and homozygous plants are being phenotypically analyzed. Additionally, new gene sequences were recently isolated from genomic DNA of almond *in vitro* shoots, showing homology to *cca1* and *elf4*, genes known in *Arabidopsis* as putatively involved in the regulation of the circadian clock. The full-length of these fragments is being obtained and data will be presented.

## EP-07

### Study of cell sorting for annona cherimola´s polyphenol oxidase by transient expression of GFP-based fusions

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Cherimoya (*Annona cherimola* Mill.) fruit is an attractive candidate for food processing applications as fresh cut. However, it has a marked susceptibility to browning and this condition is mainly attributed to polyphenol oxidase activity (PPO). Recent work has allowed us the cloning, sequencing and experimental analysis of the full length *Acppo* gene. The predicted protein (AcPPO) presented two candidate ATG starting codons, generating a complex N-terminal analysis about the real compartmentalization of the enzyme by simple bionformatic processing of the sequences. A detailed functional analysis of the post-translational events involved in the final AcPPO sorting and cell distribution was carried out by fusion of the two putative N-terminal signals found in AcPPO to the green fluorescent protein (GFP) gene. Constructs either with the full-length (first ATG) or the second-half (second ATG) segment of the N-terminal peptide were separately studied in transient expression assays. *Agrobacterium tumefaciens* infections with clones harboring these constructs on *Nicotiana tabacum* leaves were then evaluated by confocal microscopy. The existence of a minimal sequence in this N-terminal segment of the gene needed to direct AcPPO into chloroplasts was well established, supporting the idea that the cloned gene generates a protein that is sorted into these organelles.

## FP-01

### Development of a new strategy for apple breeding using early flowering genes

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Apple is one of the most widely grown fruit trees in the world. However, due to different apple diseases during production and postharvest, large quantities of fruits are lost every year. Thus, breeding for new disease resistant apple cultivars is of great importance. Traditional apple breeding is time-consuming and costly due to the long juvenile phase of apple. Genetic engineering offers an alternative to shorten the juvenile phase, thus speeding up the breeding process. In this study, we intend to develop a new strategy for apple breeding by introducing early flowering genes though gene technology. This strategy includes two parts. The first part is to incorporate genes which are known to reduce the time to flowering into the apple genotypes with known disease resistant traits. The early-flowering disease resistant transgenic plants will then be crossed with the already established varieties with known superior traits, but which are susceptible to the diseases. Early selection of the offspring can be obtained through its early flowering properties, thereby reducing the selection time by several years. The second part is to eliminate all transgenes from the selected seedlings using a site-specific recombination technique. The resulting trees can thus be considered as non-transgenic. So far we have cloned the apple *MdFT* or *MdSOC1* genes within the R/Rs recombination sites of the pMF1 vector. The *MdSOC1* and *MdFT* genes are under control of the TOBUbi4 or CaMV35S promoters. Four apple cultivars have also been established *in vitro*. Further cloning and transformation work are underway.

**FP-02**

## Mycorrhization of transgenic apple trees with increased resistance against fungal pathogens

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Fungal pathogens such as apple scab *Venturia inaequalis* cause severe damage in commercial apple cultivation. Using clones of the apple variety 'Pinova', transgenic apple lines with increased resistance against fungal pathogens were produced. The antifungal transgenes included the endochitinase *ech42* and the exochitinase *nag70* from the biocontrol fungus *Trichoderma harzianum*. In order to analyze the effects of transgenic chitinase genes on mutualistic fungi, one set of young apple trees was transferred to an artificial substrate and inoculated with the arbuscular mycorrhizal fungi (AMF) *Glomus intraradices* and *G. mosseae*. A second set of plants was cultivated in pots with soils from intensively and extensively managed apple orchards. Chitinase activity in leaves and roots was determined using fluorescence spectrometry and substrates 4-methylumbelliferyl N-acetyl-β-D-glucosaminide for exochitinase and 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside for endochitinase activity. Mycorrhization rates were determined microscopically. Diversity of AMF on the roots of apple seedlings cultivated in soils from intensively and extensively managed apple orchards was analyzed by molecular methods. Preliminary results indicate reduced mycorrhization rates of transgenic apple trees with increased expression of chitinases.

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