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Poster Abstract – G.01

EXPRESSION OF TWO MAL D 3 (NON-SPECIFIC LIPID TRANSFER PROTEINS) GENES IN FRUIT OF 'GALA' AND 'FLORINA'

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apple, allergen, nsLTP, Mal d 3, cultivar

A non specific lipid transfer protein (nsLTP), called Mal d 3, is one of the major allergene in apple, which causes a class I food allergy. Two distinct genes, named *Mal d 3.01* and *Mal d 3.02*, were previously identified and mapped in homeologous segments of linkage groups 12 and 4.

Genomic sequences and cDNAs of both *Mal d 3* genes were cloned from two apple cultivars, 'Gala' and 'Florina'. Specific primers were disegned for the two genes, in order to study their expression in apple fruit by PCR analyses, in three different ways: end point, semi-quantitative and quantitative (Real Time). Levels of *Mal d 3.01* and *Mal 3.02* were determined in peel and flesh of apple as a function of apple growth and cultivar.

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Poster Abstract – G.02

THE INVOLVEMENT OF THE AEC MULTIGENE FAMILY DURING APPLE FRUITLET ABSCISSION

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abscission, auxin, ethylene, Malus x domestica, PIN

Throughout development, plants shed entire organ systems through the process referred to as abscission witch provides a mechanism for the removal of senescing or otherwise damaged organs and for the release of fruit as it ripens . Many fruit tree species bear an abundance of flowers which may produce a surplus of fruit that trees are unable to support. Malus X domestica L. Borkh, like many others fruit tree species, has developed a self regulatory-mechanism whereby it sheds part of the fruit load at an early fruit development period. For many years it has recognized that the balance between the plant hormones ethylene and auxin has an important role in the regulation of the abscission process.

Several auxin efflux carriers (PINs) and auxin responding transcription regulators (Aux/IAA) partial clones were isolated from immature apple fruit along abscission induction. The clones encode proteins with a high level of similarity, indicating the presence of several members belonging to big gene families. In the case of PINs, two of the clones, likely to be hortologues to AtPIN1, were named MdPIN1 and MdPIN10, and were characterized by 98% aminoacid identity. The isolation of the genomic clones and the analysis of the introns indicated the presence of two MdPIN10 alleles, these findings were further supported by the corresponding cDNA clones. The expression analysis performed on different tissues indicated a differential pattern of transcript accumulation suggesting that MdPIN1 and MdPIN10 are likely to be paralogous. The other PINs clones, according to the highest level of similarity to AtPINs were named MdPIN4 and MdPIN7, the latter one displaying two allelic forms. Concerning Aux/IAA clones, blastX analysis indicated high level of similarity with AtIAA3, AtIAA7, AtIAA8, AtIAA16 and AtIAA27. The transcript amount of these clones was exploited as an indicator to assess the level of auxin during abscission in seed, cortex, peduncle and abscission zones of apple fruitlet. The data indicated a differential expression of both PIN and Aux/IAA clones depending on both tissue and abscission magnitude of the fruitlet population.

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Poster Abstract – G.03

CHARACTERIZING THE TRANSCRIPTOME OF HcrVf12-TRANSFORMED RESISTANT APPLE IN RESPONSE TO *VENTURIA INAEQUALIS* INOCULATION

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Malus x domestica, Venturia inaequalis, Vf, resistance, SSH

Background: To understand the gene networks that underlie plant defense responses, it is necessary to identify and characterize the genes that respond to the pathogen infection. My research investigated the incompatible interaction between apple and the fungal pathogen *Venturia inaequalis*, causal agent of apple scab. A transcriptomic approach was used.
As plant material, scab resistent transgenic apple lines of cv Gala for the R gene *HcrVf 2* were used, in comparison to Gala *wild type* (scab susceptible). *HcVf2* codify for a putative LRR receptor-like protein, involved in the pathogen recognition, and confers resistance to apple cv. Gala.
A PCR-based suppression subtractive hybridization was used to collect ESTs (Expression Sequence Tags) that are differentially expressed in apple resistant genetically modified genotypes after infection with *V. inaequalis*.

Results: A subtractive library of 524 ESTs was constructed from infected scab-resistant apple leaves. This collection is enriched in sequences involved in the incompatible interaction between Gala GM lines and *V. inaequalis*.
A putative function was assigned to 331 ESTs (representing the 63.2% of the total collection) by BlastN and BlastX search against public database. The other clones had no homologies with known sequences or were similar to not annotated expressed or putative proteins. Genes of known functions were sorted into 12 primary functional categories. The largest set of genes was assigned to Primary metabolism (14%) and the smaller one to Cell Growth (less than 1%). Other important categories were Disease Defence, Signal Transduction, Transcription ande Cell Wall in which many putative resistance genes were classifed (40%).
Although functional assignment based only on sequence homology needs experimental verification, it nonetheless provides a measure of the diversity of the genes in the stress cDNA collection. In fact, genes from all the major functional categories are represented in the collection.

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Poster Abstract – G.04

MONITORING MESSAGE LOCALISATION OF *KNOPE3L*, A CLASS II *KNOTTED*-LIKE GENE, DURING STEM, LEAF AND DRUPE DEVELOPMENT OF PEACH (*PRUNUS PERSICA* L. BATSCH)

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class II knotted-*like gene, organ development, transcript localisation.*

The *knotted-like* homeobox (*KNOX*) genes encode transcription factors which play a role in the regulation of plant development. They have been divided into two classes: the class I *KNOX* genes control meristem identity; whereas the class II messages (*KNOX II*) are more ubiquitous, and their function is poorly investigated in crop species for an exiguous number has been characterised so far. However, *KNOX II* are thought to be involved in shoot vigour, fruit architecture and fibre content in conifers: these traits are also important in tree fruit production and their control is crucial in breeding programmes.

Two full length cDNAs, *KNOPE3L* and *KNOPE3S*, were cloned from peach fruit and the deduced products were 98% and 83% identical to arabidopsis KNAT3 and KNAT4, respectively.

KNOPE3L harboured 5 introns which maintained conserved positions as compared to other plant *KNOX II* members. Southern analyses suggested that each gene is represented by a single copy in the peach genome. *KNOPE3L* was located on the linkage group 1 of *Prunus* map by following the segregation of a PCR-RFLP marker in a F2 population of almond (cv. Texas) X peach (cv. Earlygold).

Message localisation was monitored in herbaceous stems performing sections located under the shoot apical meristem and proceeding for 5 internodes downstream. *KNOPE3L* transcript was spread thoroughly, with peak signal in vascular bundles, and was absent in the epidermis. In sections of stem secondary structure, *KNOPE3L* message was just phloem-associated. The latter pattern was also observed in leaf petioles. In leaves, the message was absent in the epidermis, but signalled in all the other cell layers. However, in leaves at very early developmental stages the transcript was less abundant than in those at later stages. Finally, an intense signal constantly occurred in bundles of drupes at various stages but not detected in cells of pericarp, mesocarp and endocarp.

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Poster Abstract – G.05

PEACH FRUIT SOFTENING IS STRICTLY ASSOCIATED WITH THE LOSS OF CELL ADHESION AND TURGOR AND WITH THE ENDOPOLYGALACTURONASE LOCALIZATION IN THE CELL WALL

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Prunus persica, fruit softening, endopolygalacturonase, immunolocalization, cell adhesion

Peach fruit quality consists of many characteristics among which flesh firmness is of great concern. Firmness and texture of ripe fruit are considered to result, in large part, from the disassembly of the primary cell wall (Rose and Bennett, 1999, Brummel and Harpster, 2001). This process involves several biochemical and physiological factors, among which proteins able to plasticize (expansins) or degrade (endopolygalacturonases [endoPGs], pectin methyl esterases, cellulases) the cell wall components are of great importance. The involvement of endoPG proteins in peach softening, was investigated by our group which demonstrated that both protein transcription and protein modification, in particular glycosylation, are mechanisms used by plant to regulate endo-PG activity (Ardolino et al., 2005).

In this study, which is part of a project aimed to understand the molecular mechanisms regulating fruit softening, morphological analyses of peach mesocarp and esocarp have been carried out by producing semi-thin sections (0.5 µm). The following different cultivars have been analysed at different ripening stages: *(i)* "melting flesh" peaches (MF) showing a pronounced decrease in fruit firmness during the final stages of ripening; *(ii)* "non melting flesh" peaches (NMF) which remain relatively firm since the lack of the final melting phase of softening; *(iii)* "Stony Hard" peaches (SH) which maintain flesh firmness for several days after harvest even at room temperature; *(iv)* "Slow ripening" peach mutant characterized by the fruit development arrest in the pre-climateric phase with the absence of the typical maturation processes. Sections used for morphological analyses have been also probed with a rabbit polyclonal antibody specifically produced and tested for peach endopolygalacturonase (endoPG).

Image analysis results showed a progressive loss of cell adhesion and turgor in parallel with softening and with endoPG localization in cell wall. The analysis of the different peach cultivar showed that the loss of cell adhesion and turgor are characteristics strictly associated with the loss of flesh firmness and with the endo-PG accumulation in the cell wall.

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Brummel DA and Harpster MH (2001) *Plant Molecular Biology* **47**, 311-340.
Ardolino S et al. (2005) 49° Annual Siga Congress.

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Poster Abstract – G.06

CLASS I *KNOTTED1*-LIKE GENES IN PEACH (*P. PERSICA* L. BATSCH): GENOMIC FEATURES OF THREE NEW MEMBERS AND EXPRESSION PATTERNS DURING STEM DEVELOPMENT

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Prunus persica, stem development, class I knotted1-like genes

Members of the class 1 *Knotted1*-like homeobox (*KNOX*) gene family regulate the processes of organ morphology and function, and subsequently of plant architecture. They are differentially required for meristem development and function to inhibit cell expansion and differentiation associated with organogenesis. The architecture of aerial organs and vegetative habit of fruit trees are important traits for productivity and quality. In the model species *A. thaliana*, *KNAT1* (or *BP*) was demonstrated to play a role in internode patterning and lignin deposition, however *KNOX* function in trees has been poorly investigated so far, though there is evidence that they play a role in wood formation and bud dormancy. The determinism and variability of these traits is alleged to be under the control of *KNOX* genes and their mapping and use as markers may have a potential for assisted breeding.

We have cloned three new cDNAs from herbaceous stems using RT-PCR based on conserved functional domains and they were named *KNOPE2*, *KNOPE4* and *KNOPE5*. The deduced product of the first was 75% identical to *A. thaliana* KNAT6, whereas the latter two were 80% and 87% identical to STM, respectively. The genomic features of class I *KNOPE* genes indicated that intron position was conserved throughout plant species. Patterns of transcript abundance and localisation (by in situ analysis) were also monitored during stem development and putative roles are discussed.

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Poster Abstract – G.07

ANTHER AND ISOLATED MICROSPORE CULTURE IN THE FRUIT TREE SPECIES *PRUNUS AVIUM* L.

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in vitro morphogenesis, pollen embryogenesis, cherry

Woody plants are generally characterized by a high degree of heterozygosity, an extremely long reproductive cycle and sometimes strong self-incompatibility. The generation of inbred lines via back-crossing series does therefore not constitute a practical means to facilitate plant breeding. As a consequence, the establishment of haploid technology would be of particular advantage in such species.

In vitro anther or isolated microspore culture are usually the most effective and widely used approaches to produce haploids and doubled haploids. Anther culture is also an efficient tool to produce highly embryogenic somatic callus (Germanà, 2003).

Studies regarding anthers and isolated microspore cultures of two genotypes of *Prunus avium* L. (cvs. Van and Celeste) are reported.

In anther culture experiments, flower buds from each genotype were collected from the field and pretreated at 4ºC. Then they were surface sterilized, rinsed three times with sterile distilled water and anthers were isolated and placed on solid media (about 30 to 40 anthers per Petri dish). Experiments with different genotypes and different media have been carried out in several years.

In isolated microspore cultures, different starvation and temperature treatments were tested to induce androgenetic development. FDA and DAPI staining were applied to characterise the cultures regarding viability and nuclear or cell division, respectively. Under appropriate conditions, initiation of androgenesis, indicated by the formation of multinucleated pollen grains in the isolated microspore cultures and by the production of haploid calli in anther cultures, was obtained.

Acknowledgements:

This work was supported by PRIN 2004 Project: “In vitro morphogenesis and synthetic seed as innovative tool to propagate and to conserve the Mediterranean woody plant germplasm” and *by Vigoni project:* “Gametic embryogenesis for Citrus and other fruit trees breeding”.

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Poster Abstract – G.08

STEPS TOWARDS THE PRODUCTION OF A FUNCTION MAP IN PEACH (*PRUNUS PERSICA*)

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Prunus persica, Expressed Sequence Tag (EST), Single Nucleotide Polymorphism (SNP), ESTree DB, Candidate gene (CG)

Peach is currently considered a model species for genomics studies in Rosaceae. An international effort is aimed to the improvement of the available EST collections, to the sequencing of gene rich regions and to the production of high-density maps, for integration of QTLs, monogenic traits and functional maps.

We report the development and mapping of genetic markers based upon expressed sequence tags (ESTs) polymorphisms and the positioning of ESTs in a physical framework map for peach genome. Based on ESTree DB (a collection of 20924 cDNA sequences), contigs and EST were selected as candidate genes (CGs) based on sequence similarity with genes relevant for fruit quality, already characterized in other related species like apple, apricot and strawberry.

To rapidly identify SNPs, the ESTs generated from six different peach genotypes (Suncrest, Bolero, Oro, Loring, Fantasia, Redhaven) and from almond were aligned by AutoSNP, a program that allows *in silico* SNP (isSNP) detection. A total of 1863 isSNP was identified and further analysis concentrated on a subset of 67 isSNPs, derived from ESTs representing genes putatively involved in important aspects of the secondary metabolism. Confirmed SNPs were genotyped in selected individuals of the segregating population *Texas* [almond] x *Earlygold* [peach]. In a parallel approach a strategy based on the identification of contigs representing putative genes potentially affecting fruit quality was adopted using online resources offered by the ESTree web site. SNPs were thus scanned by sequencing of amplified products from parental lines of mapping populations. SNPs scoring was conducted on segregating populations by minisequencig.

Approximately 200 ESTs were selected for mapping on a physical framework map and 17 out of 46 ESTs which hybridized to the filters containing the BACs clones were localized on physically mapped contigs.

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Poster Abstract – G.09

STUDY OF GENETIC DIVERSITY IN APRICOT (*PRUNUS ARMENICA*) USING SIMPLE SEQUENCE REPEATS

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microsatellites, SSRs, genetic diversity

Apricot (*Prunus armenica)* is an edible fruit cultivated all over the world, but the 60% of its production is concentrated into the Mediterranean basin, where it finds the best climate for its growth: hot-dry weather, fresh soil, and bright position. Italy with an extension of 17.000 hectares is one of the most productive European countries, whose national production supremacy is held by Campania (above all Vesuvian aerea) and Emilia Romagna Regions.

The aim of the present study is to characterize an apricot germplasm collection organized by the Regional Office for Agricultural Development and grown in the farm Improsta (SA) by highlighting the presence of simple sequence repeat polymorphism in genomic DNA.

Twelve primers (kindly supplied by Raffaele Testolin) related to 6 SSR loci were used to amplify 42 genomic DNA of apricot samples. Amplification products from these reactions were separated on 2% agarose gel and by capillary elettrophoresis on ABI PRISM 3100 automated sequencer (Applera).

Some SSR exhibited high level of genetic polymorphism. The size of fragments ranging from 76 to 218 base pair and the total number of alleles per locus varied from four to eleven, with a mean of 8.0. The most polymorphic locus was UDAp-407 with eleven alleles. The CONVERT, MICROSAT and PHYLIP programs were used to analyze the data and to construct a neighbor-joining dendrogram based on allele frequency data calculated by Nei's genetic distance. The genetic relationship exhisting among the analysed samples are discussed as a mean to identify apricot varieties.

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Poster Abstract – G.10

PIDICEUVE: A PROJECT FOR COMPARA TIVE LARGE-SCALE GENE EXPRESSION ANALYSIS OF GRAPE CULTIVARS GROWN IN THE OLTREPO AREA IN NORTHERN ITALY

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Vitis vinifera, microarray, fruit ripening, bioinformatics, cultivar

The genetic determinants of grape quality are largely unknown in *Vitis*, as the factors that interact at the cellular and molecular level to cause differences in fruit quality are not understood.

We describe a project (acronym: PIDICEUVE) aimed at studying fruit ripening in grapevine using large-scale gene expression analysis. This project is part of a larger one which integrates transcriptomics, proteomics and metabolomics. Five different cultivars widely cultivated in Northern Italy, including four red-skinned cultivars (Croatina, Barbera, Pinot noir, Pinot gris), and one white-skinned cultivar (Riesling italico) have been compared during the ongoing of ripening starting from veraison and until full maturation.

Gene expression analysis has been performed using the Affymetrix GeneChip™ arrays. Preliminary results of intra- and intervarietal comparison of gene changes are reported and discussed.

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Poster Abstract – G.11

APPLYING LASER MICRODISSECTION TECHNOLOGY TO STUDY THE MOLECULAR MECHANISMS INVOLVED IN PLANT - PATHOGEN INTERACTIONS IN GRAPE LEAVES

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Oidium, grape, laser capture microscopy

Here we present our preliminary results on the application of Laser Capture Microdissection (LCM) technology for the isolation of mRNA from single cells derived from leaves of grape (*Vitis vinifera* L.), to be used for addressing crucial aspects of the complex molecular interactions occurring between plant cell and fungal pathogens. As a model we are using *Uncinula necator*, the ascomycetes casual agent of powdery mildew in grape. Powdery mildew of grape, like other powdery mildew fungi, grows on the surface of leaves. In grape it also infects the fruit and other young tissue including flowers, shoots and petioles. During infection, the fungus grows its hyphae on the surface of the leaf and the hyphal tip (haustorium) penetrates into epidermal cells from which the fungus drags nutrients. However, infection of epidermal cells is not massive, instead haustoria are produced only in a limited number of susceptible upper epidermal cells. Expression analysis of infected cells in comparison to non infected cells could provide useful information on the molecular messages both from the fungus and the plant cell. Whole leaf expression profiling in this case might not be appropriate, since it is complicated by the multiple cell types present in an infected leaf: non epidermal, non infected cells, infected and non infected epidermal cells, uninfected cells adjacent to a cell in which a haustorium is present. For this reason we are interested in implementing cell specific RNA profiling, taking advantage of the LCM technology. Laser capture microdissection (LCM) is a rapid way of isolating substantially pure cellular preparations directly from heterogeneous tissues, based on conventional histological identification. By this technique, individual cells can be harvested from tissue sections while they are viewed under the microscope.

The fixation method, paraffin embedding, was utilized to isolate epidermal cells from adult grave leaves. RNA was extract and amplified.

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Poster Abstract – G.12

ANALYSIS OF 5S rDNA SEQUENCES IN *VITIS VINIFERA* L.

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Vitis vinifera, 5S ribosomal genes, FISH

The 5S ribosomal genes and their associated non-transcribed spacer (NTS) regions were investigated in five genotypes of grapevine. The 5S rDNA sequences were isolated by PCR using two primer designed in such a manner to isolate the complete NTS sequence and the adjacent transcribing regions. Genomic DNA amplification revealed two fragments of different length in all plants analyzed with the exception of one cultivar in which an additional fragment, notably shorter, was found. The analysis of the clones revealed the presence of three types of 5s rDNA repeats which differ for the length of the non-transcribed spacer. These variants were denominated Long-repeat, Short-repeat and DEL-short-repeat. The Long and Short type differ each other not only for the length but also for nucleotide composition which showed a remarkable heterogeneity. Instead DEL-short-repeat appeared a variant of the short repeat type from which differs for a deletion of 60 bp. In order to verify the organization of the 5S repeat variants the primers SC and SL were designed on the sequence of the short and long spacer, respectively, and used for PCR amplification. The sequence analysis of the obtained fragments demonstrated that the three variants coexist into the same array, moreover fluorescent *in situ* hybridization showed that they are clustered into a single locus.

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Poster Abstract – G.13

TOWARDS AN ALTERNATIVE HIGH-RESOLUTION NON-MEIOTIC LINKAGE MAP OF GRAPE (*VITIS VINIFERA* L.)

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linkage map, Vitis vinifera, HAPPY mapping

Classic linkage genetic maps are based on meiotic recombination and segregation. Resolution of such maps is limited by the dimension of the segregating population. Unfortunately, biological and reproductive characteristics of grape do not allow easily producing and analyzing large populations required for high-resolution linkage maps. For this reason, it would be useful to develop mapping approaches not strictly dependent on meiotic recombination events to build high-resolution maps to be used in assisting the assembly of physical maps. In this line of action, we propose the application of Haploid Polymerase (HAPPY) mapping technique to the construction of a high-resolution linkage map in grape. This method has been tested with success in human and fungi, allowing map resolutions equivalent to that obtainable with *radiation hybrids* (in the order of hundreds of Kb). HAPPY mapping approach is based on analyzing the segregation of markers amplified from high molecular weight genomic DNA artificially "segregated" by limiting dilution into sub-haploid samples. After random breakage of genomic DNA and size selection of fragments, DNA aliquots are opportunely arranged to generate a mapping panel where every sample contains a casual sub-aliquot of the genome (about 0.7 equivalent genomes). Sub-fractions are tested by PCR for the presence of specific genes or markers. Co-segregation frequencies, reflecting the physical proximity between any pair of markers, allow a map to be computed.

In particular, here we discuss the set up of a grape (Pinot Noir, clone ENTAV115) HAPPY map at a resolution of 0.8-1.6 Mb. Specific primers are being designed on available genomic and BAC-end sequences, corresponding to a minimum of 1,200 markers that will be scored on the mapping panel. The resulting HAPPY map will be used to assist the assembly of the physical map currently being produced by fingerprinting of the same BAC library.

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Poster Abstract – G.14

QTLS AND CANDIDATE GENES FOR BERRY QUALITY AND RIPENING TRAITS IN GRAPE

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QTLs, candidate genes, berry quality, ripening, grapevine

The identification of genes underlying agriculturally important traits is a major goal of plant functional genomics, which has been significantly aided by the explosive growth of large-scale expressed sequence tag (EST) sequencing projects. Co-localization of candidate genes and QTLs represents a valuable strategy to find associations between genes involved in relevant pathways and trait variation. Here we report on mapping of QTLs and candidate genes for berry quality and ripening traits in the 163 F_1 individuals derived from the cross between the *Vitis vinifera* cultivars Italia and Big Perlon. A number of genes, mainly related to aromatic compound metabolism, were selected according to predicted functions and gene ontologies. About 40 EST-based markers were developed by revealing molecular polymorphisms through SSCP analysis and minisequencing and placed on a linkage map based on approximately 350 markers, mainly AFLPs and SSRs. Segregating characteristics were scored as quantitative traits in three growing seasons by recording flowering, veraison and ripening dates, by measuring number and weight of seeds, berry size, and finally by quantifying through HRGC-MS the free monoterpenes responsible for Muscat aroma. QTL analysis, based on single marker and interval mapping methods, revealed the existence of QTLs for all the investigated traits, a number of which were repeatedly obtained in the same genetic regions by independently analysing field data from the different years. Interestingly map co-localization of some CG markers and QTLs was observed, which provides clues regarding the possible role of these genes in the regulation of the investigated traits. The existence of linkage disequilibrium between these sequence polymorphisms and quantitative traits, as well as the predictive value of some SSR markers in MAS processes, is now under evaluation by testing allelic variation in a germplasm collection representing most of the phenotypic variance.

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Poster Abstract – G.15

DIFFERENTIAL EXPRESSION OF DEHYDRIN GENE (*OeDHN*) IN OLIVE FRUITS AND LEAVES

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Olea europaea, dehydrin, fruit quality, real-time

Dehydrins (DHN) belong to a subgroup of late-embryogenesis-abundant (LEA) proteins and accumulate in various plant tissues during cellular dehydratation (1). DHN proteins exhibit a wide range of molecular masses (9-200 kDa) but are all characterized by glycine and lysine rich domains and lack of cysteine and tryptophan (2-3-4). It has been demonstrated that DHN genes (*DHN*) are modulated during the late stages of embryogenesis and are induced by environmental stress such as water scarcity, salinity and freezing (1-5). *DHNs* triggering during cell dehydratation suggests their involvement in protection and defence mechanisms even though their specific roles is not yet fully understood.

In the present work a fragment of gene encoding for DHN belonging to a multiple member family was isolated in *Olea europaea c.v. carolea* L. (*OeDHN*). The deduced partial polypeptides sequence showed the highest identity with DHN of *Salvia miltiorrhiza*. Gene specific quantitative PCR was performed to monitor *OeDHN* expression and its transcript was more abundant in fruit than in leaves and in both organs it appeared to be developmentally modulated. In leaf, message abundance was associated to leaf age, peaking in old leaves. In drupe, the transcript level was low at early stage of development, rose to the maximum at green mature stage and decreased after ripening. Moreover, *OeDHN* expression was two fold higher in epicarp than in endocarp. These preliminary data suggest that *OeDHN* may play a role during fruit maturation and ripening affecting fruit production and quality. Hence, *DHN* genes may have an impact as markers in assisted breeding as well their biotechnological control may be a tool to affect such traits.

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Poster Abstract – G.16

DEVELOPMENT OF AN OLIVE MAPPING POPULATION: *IN VITRO* CULTURE AND SELECTION OF CROSS-DERIVED EMBRYOS BY SSR MARKERS

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embryos in vitro culture, SSR markers, F_1 paternity test

Olive (*Olea europaea* L.) is one of the most important species in the Mediterranean basin. Despite its importance, olive has a lack of genetic knowledge and breeding programmes. Nevertheless, up to present time, there is a serious need for developing competitive production systems.

The aim of this experimental work was to develop in short time (six months) an olive population suitable for the construction of genetic linkage map. Traditionally olive germination and seedlings growing take a long time (1-2 years) with an high lost of material (20-30% germination capacity). For these reasons we presented results about an *in vitro* germination protocol and preliminary F_1 paternity test performed directly on F_1 endosperm to select cross-derived embryos. We realized a controlled cross between Coratina and Oliastro and collected 328 F_1 seeds 100 days after blooming time. Seeds were stored in cool chamber at 4°C for 5 months to get over the physiological dormancy of olive. For embryos *in vitro* culture we collected 260 (75%) complete embryos placed individually in sterile test tubes containing 20 ml of hormone free medium in a growth chamber at 19°C with a 16h photoperiod. Germination started after 10 days with an high percentage (85%). During the germination period the contamination of bacteria and fungi was very low (2-3%). After 2 months we started to transfer seedlings in soil in growing chamber at 23°C. The survive percentage was 95%. After 3 months plantlets (12-14 leaves) were transferred in a conditioned green-house.

Subsequently to the evaluation of Coratina self-compatibility (by field analyses), we verified the percentage of cross derived seeds in the F_1 population in order to exclude self-pollinated derived seedlings. To select cross derived embryos, we analyzed the DNA endosperm, recovered from embryos dissection, by SSR markers. We selected the SSR UDO99-039 (Cipriani *et al.*, 2002) that presents a simple profile with any overlapping of parents bands We recognized 80% of embryos originated by cross-pollination and 20% derived by self- pollination of Coratina.

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Poster Abstract – G.17

IDENTIFICATION AND CHARACTERIZATION OF A SEQUENCE FROM *CITRUS SINENSIS* RELATED TO GENES OF THE MYC TRANSCRIPTION FACTOR FAMILY

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anthocyanins, regulatory genes, blood oranges

Anthocyanins are a class of pigments widespread in the various organs of most plants. The anthocyanin's regulatory genes are responsible for regulating transcription of genes in the anthocyanin biosynthetic pathway. Moreover they play an important role in plant evolution. We have identified a sequence, *csMyc2*, from Moro [*Citrus sinensis* (L.) Osbeck], a blood orange, showing high homology with genes of the Myc transcription factor family. We have analysed the cDNAs, from both the blood and blond orange flesh, by SemiQ RT-PCR. Preliminary results seem to indicate a major expression of *csMyc2*, in the flesh of blood orange.

We are also tempting to construct a binary vector containing the Myb-like gene *Rosea* and the Myc-like gene *Delila* (both from *Anthirrinum majus*, kindly provided from K. Martin), in order to transform either fruits and young plantlets of lemon and orange.

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Poster Abstract – G.18

VALIDATION OF DIFFERENTIALLY EXPRESSED GENES IN THE FLESH OF BLOOD AND BLOND CULTIVARS OF SWEET ORANGE [*C. SINENSIS* (L.) OSBECK]

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SSH-PCR select, anthocyanins, PCR Real time

A subtractive cDNA library of sweet orange was constructed through Suppression Subtractive Hybridization - PCR Select on Moro (blood cultivars, tester) and Cadenera (blond cultivars, driver). After reverse Northern screening, a total of 230 clones were found to be up-regulated in blood orange, while 30 were up-regulated in common one. Some genes are involved in the anthocyanins pathway, biosynthetic (44%) and regulatory (1%) mechanism; some others are related to flavour's biosynthesis, signal transduction mechanisms, defense and primary metabolism. Almost the 9% of clones encoded proteins with insufficient similarity to proteins of known function, and we classified them as being of unknown and unnamed function (most of them are incomplete and not full length). Almost the 36% of ESTs produced from the subtracted library were redundant and this situation could be an indication of the high level of gene expression. EST identities were determined by sequence comparison to the nonredundant GenBank database using BLASTN and BLASTX and sequence homology informations were used to assign putative functions. We validated the result of the different expression of some clones (GST, cytochrome b5, PAL, alcohol acyl transferase, 10-hydroxigeraniol oxidoreductase, valencene synthase, bHLH, MADs box, putative Ser receptor kinase and pectinesterase) firstly through semiquantitative RT-PCR. Then we used Real-time PCR to confirm the differential expression pattern of selected candidate genes (GST, putative Ser receptor kinase and pectinesterase). We chose four samples harvested in different periods to investigate the behaviour of gene expression level during maturity period. Real-time PCR showed that GST transcript levels in 'Moro' orange increased constantly during the entire period of maturation, while in common orange no detectable levels of transcripts could be detected at the first time point of sampling, according also semiquantitative RT-PCR results. We isolated many clones of different kinases (10%) and expecially for the clone of a putative Ser receptor kinase (isolated 25 times), we can suppose that maybe it could be involved in regulation mechanisms. Semiquantitative RT-PCR and Real time results revealed that this clone is slightly up-regulated in blood oranges during the late phases of sampling (even if a lower level of transcripts are detected also in common oranges samples). According Real time data of pectinesterase, it is clear that in all ripening sampling of 'Cadenera' and in the first two sampling of 'Moro' there's no traces of gene expression of pectinesterase, but in 'Moro' become evident in the third sampling and increase in ripening sampling. We also concentrated our attention on lenghthen and verifying the sequence of alcohol acyl transferase, 10-hydroxigeraniol oxidoreductase and a clone of an unknown sequence,

respectively, using 5'-3' RACE PCR technique, cloning and sequencing. Our object is to obtain the complete ORF sequence and to verify the difference with the corresponding sequences depositated in GenBank. According the 'unknown' clones, the possibility to lengthen and verfying them could allow to obtain many informations about their sequences and their functions. Recently it was constructed a microarray, in which there were spotted all sequences isolated with SSH and cDNA-AFLP procedures and other regulatory genes (MYC and MYB): our aim is using differential sampling period of Moro and Cadenera oranges to will allow a better understanding of their role during ripening. First results will be presented.

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Poster Abstract – G.19

PRODUCTION OF NEW SEEDLESS CULTIVARS FOR MANDARIN IMPROVEMENT

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Citrus, triploid, flow cytometric analysis, PCR-ISSR

Seedlessness is one of the most important characteristics for marketing *Citrus* fruit. In the past 10 years, the main goal of the *Citrus* improvement programs developed by CNR IGV-Palermo has been to select new seedless and high quality mandarin cultivars. The production of triploids (2n=3x=27) has proved to be a useful and efficient strategy for obtaining seedless cultivars. In order to obtain triploid varieties of mandarin, a wide interploid cross program has been conducted choosing an autotetraploid (2n=4x=36) clone of Dancy tangerine (*Citrus reticulata* B.) as pollen parent and three different diploid (2n=2x=18) varieties of mandarin and tangerine (Fortune mandarin, Wilking mandarin and Monreal clementine) characterized by sweet, juicy, easy pealing but, also, seedy fruit.

Crosses were conducted following emasculation of the female parents; the derived fruits were harvested 105 days after pollination for embryo rescue and culture. Flow cytometric analysis (FCM), using a FACStarPLUS flow cytometer and sorter, was used to analyse the relative nuclear DNA content of the cells of the regenerated plants. A molecular marker analysis, inter-simple sequence repeat polymerase chain reaction (ISSR-PCR), provided a definitive characterization of the hybrids obtained.

DNA ploidy evaluation by FCM revealed the presence of triploid plants in all three different progenies obtained. When compared with the diploid and tetraploid controls, the cell lines demonstrated the fluorescence intensity intermediate between the controls, which indicated that they were triploids. Eight triploids were detected in the progeny obtained from the cross between Monreal clementine and Dancy tangerine, ten in the cross Fortune mandarin x Dancy and five in the cross Wilking mandarin x Dancy.

In order to determine the hybrid origin of these progenies, 6 ISSR primers were selected to give polymorphic patterns between the parents of each cross and they were used to analyse the relative offspring. In any case, the ISSR analysis showed specific markers from both parents to segregate in all components of the progeny. In our study, ISSR analysis demonstrated to be a useful and simple method to recognize the hybrid nature of the offspring.

All triploid plants grew very vigorously and have been grafted onto several rootstoks for selection.

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Poster Abstract – G.20

MOLECULAR CLONING AND EXPRESSION ANALYSIS OF GLUTATHIONE S-TRANSFERASE IN BLOOD AND BLOND ORANGE FRUITS

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anthocyanin, blond and blood oranges, cold stress, glutathione S- transferase, RT-real time PCR

Glutathione S-transferases (GSTs) are ubiquitary enzymes which have a defined role in xenobiotic detoxification, but a deeper knowledge of their function in endogenous metabolism is still lacking. In this work, we isolated the cDNAs, the genomic clones and the promoter regions of pigmented and non-pigmented orange *gsts*. Having considered gene organization and homology data, we believe that the isolated GST gene is probably involved in the vacuolar import of anthocyanins. In order to confirm this hypothesis we have shown that a strong reduction in GST expression occurs in the non-pigmented orange cultivar [*Citrus sinensis* L. (Osbeck)] (Navel and Ovale) compared to that of the pigmented orange (Tarocco). In accordance with this data, in the crude extracts of both pigmented and non pigmented orange fruit, GST activity was reproducibly detected by providing cyanidin-3-O-glucoside as substrate. However, crude extract of non-pigmented orange showed only 8.5% of GST activity compared to that of pigmented orange suggesting that the GST enzyme involved in cyanidin-3-O-glucoside conjugation to GSH is not largely represented. Moreover, we have shown that cyanidin-3-O-glucoside acted as a powerful competitive inhibitor of 1-chloro-2,4 dinitrobenzene conjugation to GSH in the pigmented orange, whereas it behaves as an in-competitive inhibitor of the enzyme in non-pigmented orange. In addition, we have reported here the successful *in vitro* expression of orange GST cDNAs leading to a GST enzyme which is active against cyanidin3-O-glucoside thus confirming the involvement of the isolated genes in the tagging of anthocyanins for vacuolar import. Finally, after putting together our findings, we have concluded that the low expression level of non pigmented orange *gst* is the result of a likely mutation in a regulatory gene controlling the expression of *gst*.

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Poster Abstract – G.21

GM POPLAR CULTIVATION: EVALUATION OF AGRONOMIC PERFORMANCE, MOLECULAR AND BIOCHEMICAL INVESTIGATIONS, TRANSGENE- SOIL INTERACTIONS.

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bar, StSy, resveratrol, herbicide tolerance,horizontal gene transfer

In vitro grown GM poplar plants (*Populus alba* L.) expressing the *bar* and *StSy* transgenes and the *nptII* marker gene (Confalonieri et al., 2000; Giorcelli et al., 2004) were transferred to the greenhouse and cultivated in pots containing soil collected from agricultural land. We studied the stability of *StSy* transgene expression over different seasons by evaluating the susceptibility of the *StSy* GM poplars to different leaf diseases and the amount of resveratrol-like compounds produced by different plant tissues. We also investigated the tolerance of *bar* GM poplars to a non selective herbicide (BASTA) during three years. Plants from each transgenic and control line were monitored to evaluate the steady-state level of the *bar* and *StSy* transcripts in apical and basal leaves under conditions of full vegetative growth and dormancy. The evaluation of the i*n planta* expression pattern over a two-year period showed significant fluctuations in the steady-state level of the *bar* and *StSy* transcripts. Investigations are currently in progress to check the methylation state of the transgenic sequences, in order to find out the molecular processes responsible for the transient silencing of both transgenes. From the agronomic point of view, neither *bar*-transformed line showed any damage due to the application of the herbicide, either with the field standard concentration and with the double concentration. The analysis of leaf extracts showed the presence of resveratrol-like glucosides in all the tested tissues (leaves, stems and roots) in the *StSy* GM poplars. The concentrations of the *cis* and *trans* isomers in the leaves are alike, whereas the total concentration varies, from 150 up to 1300 mg/kg, in different plantlets of the same transgenic line. The same results were found in all *StSy* lines.

Different soil samples from *bar* and *StSy* pots were collected to monitor the persistence of recombinant DNA sequences in soil and to assess the possible occurrence of horizontal gene transfer from GM poplars to soil microrganisms. Molecular analysis allowed the detection of recombinant DNA sequences derived from GM poplar tissues. On the same soil samples the total culturable bacterial population and the fraction of kanamycin-resistant bacteria were also analysed. No significant variation was detected in the microbial flora of the soil cultivated with GM poplars in comparison with the soil before GM poplar cultivation.

The reported data will contribute to a better understanding of the agronomical response and environmental impact of different classes of GM white poplars cultivated on a large scale, as a result of detailed analyses carried out at biochemical, molecular and cellular level.

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Poster Abstract – G.22

MOLECULAR CHARACTERIZATION OF *PISTACIA* GENUS USING RAPD MARKERS

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Pistacia, RAPDs, germplasm, sex determination, Monoecious plants

The genus *Pistacia* of the Anacardiaceae family consists of at least eleven species with dioecious plants. Some cases of monoecism have been reported for *P. vera*, *P. atlantica* and *P. terebinthus. P. vera* is an important crop tree that produces commercially valuable nuts. Dioecism represents an inconvenience to pistachio breeding since the long juvenile period (5-8 years) hampers sex determination until flowering. In this outline a molecular marker linked to sex could facilitate breeding allowing early seedling selection with the saving of time and economic resources. Very little work has been done at molecular level on the *Pistacia* genus and no linkage map and only very few codominant markers (SSRs) are so far available. As to this in the last years most of the researches have been based on RAPDs (Random Amplified Polymorphic Dna) for the identification of a marker able to early determine plant gender. The RAPD fragment $OPO08_{945}$ was found to distinguish male from female plants within *P. vera*.

In the present study 34 *Pistacia* genotypes have been characterised with RAPD markers: 2 M and 6 monoecious from *P. terebinthus*, 1 M from *P. integerrima*, 3 M unknown, 15 F and 6 M from *P. vera* and 1 M *P. vera* x *P. terebinthus* hybrid. All the accessions analysed belong to the germplasm collection of the CRA- ISF of Rome and among the *P. terebinthus* genotypes four are monoecious and were found in the Rodopi Mountains in Bulgaria in 2003. The monoecious trees bear male and female flowers in different inflorescences and in one case both are present in the same cluster. Nine RAPD primers, namely OPA01, OPA03, OPA12, OPAD16, OPK09, OPK19, OPL11, OPO08 and OPP03 have been employed for the analysis. A total of 195 polymorphic fragments were obtained considering for the scoring only the bands between 500 and 1500 bp. A high level of polymorphism was found within the genus. As expected from literature the marker $OPO08_{945}$ was present in all the females and absent in all *P. vera* males, but it was also present in one *P. terebinthus*, one *P. integerrima* and in the three unknown males. This marker was absent in all but one the monoecious genotypes. The primer OPK19 showed a characteristic amplification pattern of a single 1100 bp band in the monoecious *P. terebinthus*. The same fragment was also present as a major amplification product in dioecious *P. terebinthus* plants, in *P. integerrima*, in two unknown wild type and in the *P. vera* x *P. terebinthus* hybrid.

In the attempt to find a strict association to sex further RAPD primers will be tested and new molecular markers will be developed from reproductive tissues.

Abbreviations: M, male; F, female

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Poster Abstract – G.23

ISOLATION AND CHARACTERIZATION OF RNA FRACTIONS INVOLVED IN THE MATURATION PROCESS IN *OPUNTIA FICUS-INDICA* (L.) MILL.

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prickly pear, nutraceuticals, biophenol

Recently, an increasing interest towards the genetic mechanism regulating fruit ripening process has been registered (Bartley et al., 2002). Genes involved in different pathways, characterising this process, hold a key role to produce fruits of high nutritional and commercial quality.

This preliminary study has been carried out in order to study, to analyse and to characterize differentially expressed gene fragments during the prickly pear ripening process,. Particularly, we focused on biophenol biosynthesis pathways because of the growing interests towards these metabolites, very important as nutraceuticals, due to their antioxidant activity.

Opuntia ficus-indica (L.) Mill. is an important crop, native of Mexico and widespread in Mediterranean basin. It is characterised by a high adaptability to the semi-arid environment and by multiple uses of the plants (fruit, vegetable, forage, fence, etc.).

Fruits of three prickly pear genotypes ("Bianca", "Rossa" and "Gialla") at different ripening stages (green, ripening and full ripe) have been collected.

Total RNA extraction from the pulp and peel of cactus fruits at the different ripening stages and stored at -80°C has been performed. Afterwards, cDNA has been synthesised and processed to isolate differentially expressed fragments.

Differentially amplified products have been obtained either among genotypes and the different sampling stages of the same genotype. Differentially expressed fragments have been cloned and sequenced. The sequence were compared to database to evaluate similarity with genes all ready isolated.

The next step will be directed to better outline the trascriptomic activity for some fragments isolated involved in secondary metabolites biosynthesis. In particular, further studies will be directed to carry out a quantitative analyses of the transcript fragments.