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**Poster Abstract – G.01**

# JUVENILITY AND GENETIC FIDELITY IN CITRUS REGENERATED THROUGH STIGMA/STYLE SOMATIC EMBRYOGENESIS

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*juvenility, somatic embryogenesis, Citrus, somaclonal variations, flow cytometric analysis*

The biotechnology applications in *Citrus* are often limited because of the morphological reversion to the juvenile state of plants. Investigations on the morphological traits of the fruits take long periods of time and become rather costly when applied on new mutant somaclones, on regenerants and on micropropagated plants.

In this work, the degree of juvenility that occurs in plants regenerated from *in vitro* stigma/style culture of lemon, mandarin, sour orange and sweet orange was investigated. Comparisons were made between somatic embryo-derived scion and mature-phase scion, both grafted onto sour orange. Growth conditions in screenhouse and in field were also compared.

The plants were examined during the first 3 years of growth after grafting, for differences in stem and leaf growth and the presence or absence of reproductive structures and thorns.

Plants regenerated from stigma/style culture were initially morphologically different from mature-scion propagated plants, and showed many features being characteristic of seedlings. However, under screenhouse conditions, juvenile characters were lost during the second year after culture initiation mainly in the terminal portion of some shoots, even after their grafting onto rootstocks. Regenerated plants began fruiting on some branches after three years with different grade according to the species: 50, 20 and 10 % with mandarin, sour orange and lemon, respectively. Flowering usually occurred 1-2 years later in plants growing in the field.

Flow cytometric analysis and two different DNA-based techniques (ISSR and RAPD) were used to detect the genetic fidelity in regenerated plants. In these experimental conditions, somaclonal variations in regenerants were never observed.

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**Poster Abstract – G.02**

# GENETIC DIVERSITY OF SICILIAN LEMON GERMPLASM IDENTIFIED BY MOLECULAR MARKERS

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*Citrus limon, genetic diversity, ISSR, RAPD*

The lemon cultivation (*Citrus limon* L.) is spread in the Southern Italy, specially in Sicily, the first Italian region for lemon production. Whereas in the other Citrus growing countries, one cultivar prevails on the others ('Eureka' in U.S.A., 'Genoa' in Argentina, 'Galego' in Brasil and 'Berna' in Spain), on the contrary, in Italy, each lemon cultivated area (Palermo, Catania, Syracuse, Messina, Reggio Calabria, Salerno and Naples) has a wide range of genotypes mostly derived from bud mutation from 'Femminello' cultivar.

Over the past century, also the Italian lemon has suffered a dramatic reduction in the gene pool, because of 'malsecco' disease, that imposed the tolerance as necessary feature. So, some genotypes getting interesting breeding characters risk to disappear because of this disease. Currently, in Sicily, there are yet several lemon varieties with interesting agronomical traits.

In the last years, the collection and the characterization of fruit crop germplasm is becoming a common concern among geneticists and breeders, to identify and preserve the genetic diversity of a specie for setting up new breeding programmes for genetic improvement.

In the present study, 35 phenotypically diverse genotypes, including 'Femminello', 'Monachello' and 'Lunario' varieties, were collected from different parts of Sicily and used in the investigation. Polymerase Chain Reaction (PCR)-based molecular markers techniques have been used to characterize the phenotypic-based selection of Sicilian lemon and to analyse their genetic variability. Twenty RAPD primers and twenty ISSR primers were used to detect genetic polymorphisms. Twenty four genotypes were discriminated and the relative Nei's genetic distance (GD) estimates were used to carry out a cluster analysis by unweighted pair-group method using an arithmetic averaging (UPGMA) algorithm.

The accessions analysed could be clustered in two principal groups, including indistinctly plants coming from different collecting areas. Several phenotypically-different but genetically-similar plants belonged to the same subgroup, representing the Sicilian base 'Femminello' genotype.

The Nei's analysis of genetic diversity showed a low grade of variability ($H_T = 0.02$). Higher genetic variability was identified in genotypes of Palermo zone, having a tighter relationship with the genotypes of Messina zone. Data obtained from this study have been also used to provide several other information about genetic relationships among the cultivars examined. This information, with morphological and phenological descriptors, could be useful for assessing the basis of breeding programmes aimed at the genetic improvement of lemon.

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**Poster Abstract – G.03**

# STRUCTURAL AND REGULATORY GENES INVOLVED IN ANTHOCYANIN BIOSYNTHESIS IN CITRUS ORGANS AND TISSUES

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*flavonoids, Citrus, Real-Time PCR, in-situ hybridization*

The peculiar characteristic of blood oranges' and their hybrids' fruits is to present anthocyanin pigmentation in flesh and rind at maturity. In other species (lemons, *Papeda*, etc.) only flower buds and young shoot growth are anthocyanin purplish-red tinted.

Therefore assuming that in each tissue or organ anthocyanin biosynthesis may be regulated in a different way, the aim of this work was to test the differential expression level of some of the structural genes of the pathway, previously characterized [chalcone synthase (CHS), anthocyanidin synthase (ANS) and UDP-glucose-flavonoid 3-O-glucosyltransferase (UFGT)] and of a myc-like regulator (*csmyc2*), recently isolated and characterized.

The experimental work was conducted by mean of quantitative Real time RT PCR on young shoot growth of Zagara Bianca and Femminello lemons, Avana mandarin and Moro and Biondo oranges, on rind and flesh of Moro and Biondo oranges and of Avana mandarin and of *in-situ* hybridization on Moro and Biondo oranges.

An important distinction can be made on the basis of our results between the expression of target genes in the flesh of blood oranges and in the other tissues and organs. In particular in the flesh the different genes are strongly correlated among each other and with anthocyanin content, while only ANS transcript levels are related to pigment presence in the other tissues tested. Presumably the presence of CHS, UFGT and *csmyc2* even in non pigmented tissues suggests their involvement in an alternative pathway.

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**Poster Abstract – G.04**

# ON THE GLUTATHIONE S-TRANSFERASE GENE FAMILY IN *CITRUS SINENSIS*

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*Citrus sinensis (L) Osbeck, glutathione S-transferase, differential tissue gene expression*

This study aimed to achieve a comprehensive characterization of the glutathione S-transferase (GST) gene family in *Citrus sinensis (L.) Osbeck*.

In plants, GST activity protects cells from a wide range of biotic and abiotic stresses, including pathogen attack, xenobiotic and heavy-metal, toxicity and oxidative stress. In addition, plant GSTs have been shown to be important in binding secondary metabolites like anthocyanins and cinnamic acid and hormones.

A collection of 94.127 orange Expressed Sequence Tags (ESTs) was screened in order to identify members of the gluthatione S-transferase gene family.

A total of 370 ESTs, putatively encoding GST proteins, were identified by similarity search against the UniProtKB/Swiss-Prot database. This set of sequences is submitted to a clustering/assembling procedure resulting in 62 distinct transcripts: 28 tentative consensus sequences (TCs) and 34 singletons (sESTs). Then, for each transcript the GST membership class is determined, according to the classification schema developed by *Dixon et al.* (2002). Finally, the identification of the longest Open Reading Frame (ORF), permitted to describe some transcripts as full-length mRNAs. Tissue specific expression patterns of the GST transcripts identified in this study were inferred by quering the dbEST database with respect to different tissues/developmental stages. SemiQuantitative Reverse Transcription Polymerase Chain Reaction (SemiQ RT-PCR) analyses were performed to assess the expression levels of the *in silico* assembled mRNAs in different tissues such as the albedo, flavedo, flesh, young and adult leaf and ovary tissues. Tissue samples were collected from the Moro nucellare 58-8D-1 (blood orange) and the Cadenera (common orange) cultivars.

The experimentally defined expression patterns confirmed the existence of the *in silico* predicted mRNAs, and that the GST family is composed of genes that revealed a tissue specific expression as well as of genes that are differentially expressed in the two cultivars.

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**Poster Abstract – G.05**

# THE USE OF MICROSATELLITE MARKERS FOR THE CHARACTERIZATION OF ITALIAN OLIVE GERMPLASM

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*DNA, molecular markers, Olea europaea L., SSR, genetic diversity*

Olive (*Olea europaea* L.) is a species of great economic importance in the Mediterranean basin, where 95% of world production is concentrated. Among Mediterranean countries, Italy occupies a very important place in the olive industry. Italy is the main exported of olive oil in the world. The genetic patrimony of this country is very rich and is characterised by the abundance of varieties, most of them landraces vegetatively propagated at the farm level since ancient times. The existence of many varieties maintained by vegetative propagation reinforces the need of a reliable identification of varieties, for nurserymen and growers benefit as plant cost represents the major investment in the new orchards. At the same time, it is important to improve the *ex-situ* plant germplasm collection and adequately to characterise all accessions and to develop future breeding programs.

The Italian olive germplasm is estimated to include over 650 varieties and over 1300 synonyms, most of which are landraces vegetatively propagated at the farm level since ancient times. For years, the Consiglio per la Ricerca e Sperimentazione in Agricoltura - Istituto Sperimentale per l'Olivicoltura (C.R.A.-I.S.Ol.) of Rende in Cosenza, Italy, has made significant efforts in the individuation and collection of olive germplasms, generally within Italy. For each species, cuttings have been collected with the aid of local experts for successive propagation. During the second year following grafting, the plants were numbered using a unique code and placed in the varietal collection area localizated at Mirto-Crosia (CS). To date, over 450 Italian accessions have been collected.

In this work 125 olive tree of CRA-Experimental Institute for Olive Growing germplasm, were analysed by molecular markers, corresponding to the widespread olive germplasm of Italy. The olive trees were genotyped using nine nuclear SSR loci: GAPU59, GAPU71A, GAPU71B, GAPU103A, UDO01, UDO03, UDO12, UDO28 and UDO39. The nine SSR primers produced polymorphic amplification products in the cultivars studied. Dice's coefficient was used and the accessions were grouped by cluster analysis using the UPGMA method. A few cases of homonymy and presumable synonyms were identified.

This study allowed us to construct a molecular data-base for the reference collection and to analyse genetic diversity for further prospecting and for olive germplasm collection management.

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**Poster Abstract – G.06**

# MOLECULAR FINGERPRINTING OF OLIVE CULTIVARS OF SOUTHERN ITALY

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*SSR, Olea europea, genetic variability, variety identification*

Olive is one of the oldest cultivated plants and it is one of the most important oil-producing crop in Italy. Such plant is characterised by a high degree of genetic diversity because of its ancient domestication, the simplicity of the vegetative propagation, the long life span and the high self-incompatibility. A very large number of varieties are described in the Mediterranean countries, although it is likely that the variability of the morphological traits in different areas of cultivation, has contributed to the description of several synonymous. This large olive collection is valuable for germplasm resources as well as for research, and the evaluation and characterisation of these genetic resources is necessary to maximise the efficiency of germplasm management, preservation and pre-breeding programs. The development of various molecular marker techniques and their application in genetic diversity studies have resulted in improved discrimination among or within several olive cultivars. Here we are reporting on the molecular characterisation of 22 cultivars (for a total of 39 samples) locally cultivated in Southern Italy, and many of these cultivars are listed in the regulations for the production of DOP olive oil. The identification was carried out on fluorescent-based capillary electrophoresis and automated size estimation of six polymorphic DNA microsatellites (SSR), four of them belonging to the microsatellites set selected to carry out a ring-test on olive cultivar identification coordinated at national level by the IGV, Perugia. The total number of detected alleles was 48, with a minimum of 7 in three loci. The allele size range of all amplified fragments was from 100 to 250 bp. The average observed heterozigosity was 0,793. The results indicated that there is sufficient genetic diversity to distinguish all but two cultivars, as only Sant'Agostino and Uova di Pavone have an identical allelic pattern in all the loci analysed. However, principal co-ordinate analysis on the SSR data could not clearly cluster the cultivars according to the regions of diffusions or the destination of use. In conclusion, our data indicated the presence of genetic variability among the cultivars analysed and, furthermore, this work can provide data to enforce legislation for the control of olive oil genetic origin.

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**Poster Abstract – G.07**

# SNPs DETECTED ON CANDIDATE GENES OF OLIVE

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*SNPs, linkage disequilibrium, Olea europaea, candidate genes*

Single Nucleotide Polymorphisms (SNPs) were detected on three candidate genes of olive. One of them, Acyl Carrier Protein (*acp*), is involved in lipid synthesis, Lupeol synthase (*lup*) in terpene synthesis, and Sucrose transporter (*sut*) is part of carbohydrate metabolism.

The polymorphism analysis and SNP frequency were evaluated at all loci on 90 cultivars collected along the Mediterranean area. About 800 bp fragments were analyzed for each gene.

In order to identify different haplotypes and evaluate the presence of polymorphic multilocus genes, PCR products, amplified on a subset of six varieties, were cloned and re-sequenced. Where necessary, primers have been re-designed on polymorphic regions for the amplification of single-locus genes.

SNP frequency, haplotype structure and genetic differentiation among populations have been calculated on the entire set of varieties.

Linkage Disequilibrium (LD) decay was estimated along each locus showing a rapid decline within few hundreds of base pairs distance.

Data are currently being analysed for their possible application on studies of association mapping.

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**Poster Abstract – G.08**

# OLIVE CHLOROPLAST GENOME SEQUENCING AND IDENTIFICATION OF INTERVARIETAL POLYMORPHISMS

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*chloroplast genome, Olea europaea, pbsk, trnT-trnD, trnK-rps16*

The cultivar Frantoio has been used for the sequencing of the first complete *Olea europaea* L. chloroplast genome. Conserved primers designed by Grivet et al. (2001) have been used to sequence the large single copy (LSC) region. For the small single copy (SSC) and the inverted repeat (IR) regions, primers were constructed on conserved sequences homologous to *Jasminum*, the closest genus to *Olea*, and other fully sequenced plant chloroplast genomes.

Availability of the cpDNA sequence has allowed to detect areas of major polymorphism among cultivars useful for cultivar characterization, DNA barcoding and ancient DNA studies. In particular, cultivar-specific SNPs or indels were found on the *psbk* gene (A/G), on the intergenic *trn*K-*rps*16 area (poly-T 11 or 12) and on the *trn*T-*trn*D intergenic spacer (A/G). These polymorphisms allow to distinguish cultivars of different geographical origin as well as cultivated from wild plants.

Valuable information on the organization, gene arrangement and nucleotide substitution within the Oleaceae family will derive from the cpDNA molecule sequence, while phylogenetic relationships among lineages within the *Olea europaea* species are currently under assessment.

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**Poster Abstract – G.09**

# EFFECT ON ORGANOGENESIS OF THE *VITREOSCILLA* HAEMOGLOBIN GENE IN GRAPEVINE

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*Vitis vinifera, Vitreoscilla stercoraria, VHb haemoglobin gene, genetic transformation, morphogenesis*

In a framework of a study aiming to enhance the morphogenesis potential in grapes, we are investigating the effect of the VHb haemoglobin gene of *Vitreoscilla stercoraria.* Such gene is expressed in this bacterium in response to oxygen-limited conditions, being VHb haemoglobin capable of increasing the oxygen supply (Ramandeep *et al*., J. Biol. Chem., 2001, 276: 24781-24789). In various plant species expressing *vhb*, interesting physiological effects (such as accelerated cell proliferation, enhanced metabolite production and a general improvement of hypoxic stress tolerance) has been reported (Zhang *et al*., Biotechnol. Adv., 2007, 25: 123-136). No data are however available for grapes.

Embryogenic calli of *Vitis vinifera* cv. Brachetto were co-cultured with *Agrobacterium tumefaciens* carrying the pPLT7000 construct (kindly provided by Prof. C. Fogher, Catholic University, Piacenza), containing the *vhb* and the *nptII* genes under the control of CaMV35S promoters. Selection on kanamycin (100 $mgl^{-1}$) has been applied to the cultures and putatively transgenic plantlets have been regenerated. During embryo conversion into plantlets, single embryos of putatively transgenic cultures produced meristematic highly proliferative tissues rather then germinate. Shoots were dissected and transferred to solid medium for root development and plant elongation, and several lines were micropropagated. Moreover, compared to control plantlets, while nodes exhibited canonical morphology and development, anomalies were observed in the radical system, such as thickness, apical swellings and enhanced rates of growth and of secondary root production. Qualitative PCR screenings and Southern Blot analysis are in progress. Preliminary results proved the insertion of *nptII* and *vhb* genes into the genome of all the analysed plantlets. Our preliminary results suggest morphogenic modifications associated with the insertion of the *vhb* gene in grapevine. Further assays will enable us to better associate *vhb* expression with the observed phenotype, and to understand the role of VHb in the cell metabolism.

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**Poster Abstract – G.10**

# HIGH-RESOLUTION HAPPY MAPPING IN GRAPE: PROGRESS

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*genetic and physical map, grape*

Haploid Polymerase (HAPPY) mapping technique is an *in vitro* approach for defining the order and spacing of DNA markers directly on native genomic DNA. It is a cloning-free technique which allows analyzing the segregation of markers amplified from high molecular weight (HMW) genomic DNA randomly broken and segregated by limiting dilution into sub-haploid samples. Co-segregation frequencies, reflecting the physical proximity between any pair of markers, allow a map to be computed.

We set out to apply HAPPY mapping technique to the construction of high-resolution linkage maps of grape, for which production of extended segregating populations for classic linkage genetics maps is not trivial. In particular, our aim is the production of two HAPPY mapping panels with different resolutions to anchor the upcoming physical map to available genetic maps and assist the ordering of draft genomic sequences.

We report a progress on the testing procedures aimed at the successful application of HAPPY mapping to the grape genome (Pinot Noir, clone ENTAV 115). HMW-DNA extraction protocol and PFGE run were optimized for tissues with high polyphenolic content. Appropriate grape HAPPY panels were built to verify the applicability of the technique on the grape genome. Five markers designed on a grape BAC sequence and one non-linked marker (NL) were used. In each experiment, we first tested the proportionality of positive samples to the presumed genome content per aliquot. Co-segregation analysis was then performed on dilutions whose estimated genome content was close to 0.7X (i.e. 50% of positive aliquots), which is the optimal condition to estimate marker linkage.

A first testing panel was built using diluted DNA in solution at 5 different concentrations. The number of positive bands was below 50% for all dilutions and, as expected, increasing with DNA concentration. Analysis of raw data confirmed linkage between the 5 markers designed on the BAC sequence, whereas, as expected, NL marker resulted unlinked. The same results were obtained in experiments carried out using HMW-DNA recovered from PFGE. In this case five capillaries with increasing diameters were first used to set different DNA dilution into each aliquot. We were then able to determine the right combination of nuclei concentration and capillary diameter necessary to obtain 50% positives through the analysis of a third HAPPY panel (three different nuclei dilutions with two different picking volumes).

We are currently in the process of designing additional markers at different distances on a longer grape sequence contig (500-700 kb), which will allow performing a final test for linkage on a 500 kb HMW-DNA panel. Specific primers will be then designed on available genomic and BAC-end sequences, corresponding to a minimum of 1,200 markers to be scored on a 100-800 kb resolution mapping panel.

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**Poster Abstract – G.11**

# MOLECULAR CHARACTERIZATION OF *VITIS VINIFERA* ACCESSIONS FROM UMBRIA REGION

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*Vitis vinifera, molecular markers, SSR, AFLP, biodiversity*

We will report the genotyping of some *Vitis vinifera* L. accessions from Umbria region (Central Italy). In particular we are interested in assigning the correct genotype to a so called "Greco nero" accession traced in the surroundings of the town Todi which shows interesting traits in micro-wine making trials. The mentioned Greco nero accession is compared to other Greco nero accessions found in Umbria region and maintained in the grapevines collection of the Dept. of Agricultural and Environmental Sciences, University of Perugia, and to the standard registered grapevine cultivar "Greco nero" from Calabria region. At the same time we are attempting the characterization of the "Cornetta" grapevine cultivar which is traditionally used for the production of the Vernaccia di Cannara wine. From ampelographic measurements, the cv. Cornetta was assigned to the cv. Fortana (Cartechini and Moretti, 1989); we intend to verify if these two cultivars are synonymous. Genotyping is being carried out by molecular markers such as AFLPs and SSRs as reported by This *et al.*, 2004.

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**Poster Abstract – G.12**

# BERRY AND PHENOLOGY RELATED TRAITS IN GRAPEVINE (*VITIS VINIFERA* L.): FROM QTLS TO UNDERLYING GENES

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*grapevine, ripening time, berry size, seedlessness, QTL analysis*

The timing of grape ripening initiation, length of maturation period, berry size and seed content are major concerns to viticulturalists and wine makers. Understanding the genetic control of grapevine phenology is desirable for staggering harvest along growing season, expanding the production towards periods when the fruit gets a higher value in the market and finally ensuring an optimal adaptation to climatic and geographic conditions. Fruit size determines grape productivity and has a role in wine quality. Seedlessness is especially demanded in the table grape market and is negatively correlated to berry size. All these traits result from complex developmental processes modified by genetic, physiological and environmental factors. In order to elucidate their genetic determinism we carried out a quantitative analysis in a $F_1$ segregating progeny obtained by crossing two table grape cultivars. Molecular linkage maps covering most of the genome were generated for each parent and integrated into a consensus map. Segregating traits were evaluated in three growing seasons by recording flowering, veraison and ripening dates and by measuring berry size, seed number and weight. QTL (Quantitative Trait Loci) analysis was carried out based on single marker and interval mapping methods. QTLs could be identified for all the investigated traits, a number of which were stable over more than one year. In order to characterize the most significant QTLs at the gene level, the underlying SSR markers were used as anchors to identify the corresponding Pinot noir genomic sequence, which was analysed by means of bioinformatic tools. Gene prediction and protein similarity search suggested the involvement in the studied phenotypes of some interesting proteins, whose role in flower and fruit development is reported also in literature. These results will be validated by analysing the gene expression profile in contrasting phenotypes at different phenological stages and by testing allelic variation at the gene trait-associations in a grapevine germplasm collection.

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**Poster Abstract – G.13**

# IRAP, REMAP AND S-SAP MOLECULAR MARKERS IN GRAPEVINE GENOTYPE IDENTIFICATION AND PHYLOGENETIC STUDIES

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*grapevine varieties, IRAP, REMAP, retrotransposon, Vitis vinifera*

Long-terminal-repeat (LTR) retrotransposons represent a large proportion of repetitive DNA in plant genome. The ubiquitous nature of retrotransposons and their activity in creating genomic diversity by stably integration of large DNA segments into dispersed chromosomal loci make these elements ideal for development as molecular markers. In grapevine genome the complete sequence of one *gypsy*-like retrotransposon (*Gret1*) and the partial sequences of two *copia*-like retrotransposons (*Vine-1* and *Tvv1*) have been isolated, and some molecular markers based on the insertion polymorphisms of the above mentioned retrotransposons have been recently tested for identification of grapevine species and varieties.

In this study we analysed the capacity of Inter-Retrotransposon Amplified Polymorphism (IRAP), Retrotransposon-Microsatellite Amplified Polymorphism (REMAP) and The Sequence-Specific Amplified Polymorphism (S-SAP) molecular markers in the discrimination of 18 *Vitis* genotypes: 6 *Vitis* species (*Vitis arizonica*, *Vitis cinerea*, *Vitis labrusca*, *Vitis rupestris*, *Vitis rotundifolia* and *Vitis vinifera* subsp. *sylvestris*) and 12 *Vitis vinifera* subsp. *sativa* varieties ("Cabernet Franc", "Cabernet Sauvignon", "Chasselas", "Fiano", "Malvasia Bianca Cenaia 2", "Moscato bianco di Canelli", "Pinot Nero", "Rkatzitely", "Sauvignon Blanc", "Sangiovese", "Sultanina", "Vermentino"). The insertion polymorphisms of *Gret1*, *Vine-1* and *Tvv1* retrotransposons and the allocation of the *Vitis* genotypes in IRAP, REMAP and S-SAP phylogenetic trees are discussed.

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**Poster Abstract – G.14**

# ANALYSIS OF MOLECULAR DIVERSITY AT CANDIDATE GENES FOR AROMA DETERMINATION IN GRAPE GERMPLASM

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*association analysis, candidate genes, germplasm collections, Muscat flavour, QTL analysis*

Many traits of agricultural interest are defined by a multiplicity of genes with a different and partial contribution on phenotypic variation. Testing the role of candidate genes (CGs) in the expression of a trait could be carried out either by a conventional co-segregation analysis in structured segregating populations or by looking for allelic variation at the gene-trait associations in germplasm collections. In this work CGs were selected a priori based on their hypothetical biological function. The public EST database TIGR was then searched for berry gene sequences with Gene Ontology (GO) annotation related to berry quality and ripening. SNP markers were developed on these sequences and segregation analysis was carried out in a F1 mapping population of *V. vinifera* which had previously been genotyped with AFLP and SSR markers. QTL analysis in three years showed significant associations between three SNP markers (corresponding to 3 different genes) and ln-transformed contents of nerol, geraniol and linalool which are the main determinants of Muscat flavour. In order to confirm these associations in different genetic backgrounds we started to exploit the natural genetic variation of a grape collection with an approach of association genetics. Individual grapevines, for which phenotypic characterization was available, were sampled from Vassal collection (INRA, Montpellier). A first candidate gene, DOXP-synthase, was preliminary sequenced on 25 varieties representing Muscat aromatic and non aromatic cultivars. SNPs and INDELs were detected and used to reconstruct haplotypes. Preliminary association tests, which do not consider population structure, showed statistically significant correlations between some SNPs and Muscat flavour. A few number of haplotypes also emerged to be present only in Muscat aromatic varieties. Sequence analysis is being extended to a larger collection of 150 samples including Muscat varieties, highly diverse accessions, and non-aromatic grapevines genetically related to Muscat varieties. On the basis of SSR marker data, population structure and kinship will be then investigated in order to avoid false positive associations. Thus, structured association tests that include population structure and kinship estimation will enable to search for statistically significant association between CG haplotypes and Muscat flavour. This approach will be applied to each selected candidate gene and may lead to the identification of the alleles responsible for the variation of the trait under investigation.

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**Poster Abstract – G.15**

# DEVELOPMENT AND MAPPING OF FUNCTIONAL MOLECULAR MARKERS FOR FRUIT QUALITY TRAITS IN *MALUS X DOMESTICA*

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*Malus x domestica, marker development, EST, functional map, linkage*

The understanding of the genetic mechanism underlying fruit quality traits in apple is one of the main steps to improve marker-assisted selection and breeding of apple cultivars. Therefore, molecular markers derived from fruit modulated EST and from other candidate genes controlling fruit traits are a useful tool to map genes and QTL involved in fruit development and quality. In order to develop functional markers for fruit quality traits we followed two main approaches. On the one hand, the public sequence database was screened for transcription factors and 425 *Malus* EST, putatively involved in DNA binding or transcript regulation, were identified. These genes are supposed to play a key role in fruit quality related metabolic pathways: 28 microsatellite containing sequences were tested and 13 were placed on 'Fiesta' x 'Discovery' reference map. On the other hand, in a previous microarray analysis performed in our lab, 300 genes, differentially expressed during different fruit developmental stages, were identified. The corresponding EST sequences provided the second source of functional markers and were assembled to identify unique transcripts: 153 sequences resulted to be unique while 147 redundant sequences were assembled in 36 contigs. In total 189 sequences were screened for the presence of microsatellites (SSR), the sequences not containing repeated elements were BLAST searched against PlantGDB-assembled Unique Transcript database (PUT) and the resulting PUTs were analyzed to identify repeated motif. Furthermore, insertion/deletion (INDEL) and single nucleotide polymorphism (SNP) were searched in the assembled sequences. Finally, more then 50 PCR primer pairs were designed flanking SSR, INDEL or SNP containing regions or including most of the differentially expressed sequences and, in case of such differences were not identified, sequence reactions on parental genotypes were performed. SNP detection was performed digesting PCR products with different restriction enzymes while SSR and INDEL were scored as length polymorphism. Twenty-seven polymorphic markers were placed on 'Fiesta' x 'Discovery' or 'Fiesta x Prima' reference maps, increasing the total number of genes mapped in *Malus x domestica* to 40.

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

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**Poster Abstract – G.16**

# LRPKM MULTIGENE FAMILY EXPRESSION PROFILE IN *HcrVf2*-TRANSGENIC AND WILD-TYPE APPLE PLANTS TREATED WITH *VENTURIA INAEQUALIS*

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*resistance gene, plant disease, Malus x domestica, real time RT-PCR, Venturia inaequalis*

The interaction of *Malus* genotypes and *Venturia inaequalis*, causal agent of apple scab, is nowadays the most studied plant-pathogen interaction involving a woody non-model plant. The cloning of an apple scab resistance gene, named *HcrVf2*, and the identification of apple genes differentially expressed after pathogen recognition represent the basis for further investigation of the resistance mechanisms. The *Malus x domestica* gene *LRPKm1*, isolated from the scab resistant cv. Florina, belongs to a multigene family and encodes for a putative leucine-rich repeat (LRR) receptor-like protein kinase which transcripts accumulate in response to *V. inaequalis* infection or salicylic acid treatment (Komjanc *et al.*, 1999).

*LRPKm* genes could be divided in two groups, according to their expression profiles in *HcrVf2*-transgenic (Belfanti *et al.*, 2004) and wild-type apple plants treated with *V. inaequalis.* One group (*LRPKm1* and *3*), giving a response related to the presence of *HcrVf2*, is probably involved in the recognition of a pathogen-derived signal, while the other group (*LRPKm2* e *4*), with an expression profile unrelated to the *HcrVf2* gene, is putatively involved in the plant innate immune system.

The possible involvement of these receptor-like protein kinases in apple scab resistance and in the plant innate immune system makes those genes attractive for a better comprehension of the molecular mechanisms of the signal transduction pathways activated after pathogen recognition.

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**Poster Abstract – G.17**

# *KNOPE1*, A CLASS 1 *KNOTTED-LIKE* GENE OF PEACH, PLAYS A ROLE IN STEM DEVELOPMENT

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*Prunus persica, stem development, class 1 knotted-like genes.*

The reservoirs of meristematic cells of the shoot apical meristem and the cambium have to be guaranteed to support indeterminate growth and require the expression of class 1 *KNOX* genes. The arabidopsis class1 *BREVIPEDICELLUS* (*BP*) regulates internode development and wood formation by direct interaction with promoters from genes acting in the lignin synthesis. In the past, we characterised peach *KNOPE1*, a *BP*-like gene, which was located onto linkage group 1 of the *Prunus* reference genetic map, in association with a quantitative trait locus controlling the internode length. Hence, we addressed *KNOPE1* role in caulis development. In stem portions sited 0.2 mm below the SAM, the *KNOPE1* message featured in the cortex and marked the borders of petiole bundles, recalling *BP* behaviour. In internodes at 4 mm below the apex, it mainly localised to the phloem and intra fascicular cambium. In 5 month old shoots, the *KNOPE1*transcript abundance decreased from top to basal stem portions, while key lignin biosynthesis genes increased the expression, suggesting that *KNOPE1* activity and lignin deposition were inversely correlated. Arabidopsis lines overexpressing *KNOPE1* exhibited decreased lignin content in the stem, together with typical modifications of leaf margins and vascular system, similarly to those overexpressing *BP*. These results strongly suggest that *KNOPE1* has a role in regulating internode length and stem maturation in peach. In addition, KNOPE1 protein was demonstrated to bind to the TGACAGG/CT sequence recognised by the major class1 KNOX. The arabidopsis *COMT1* and *CCoAOMT* genes encode crucial lignin methyl-transferases, whose promoters contain such motif. Consequently, we set up binding experiments between KNOPE1 and these promoters, and results will be presented.

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**Poster Abstract – G.18**

# *EX-SITU* CONSERVATION OF WILD ALMOND *AMYGDALUS WEBBII* SPACH FROM PUGLIA REGION

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*genetic resources, wild almond, fruit weight, introgression*

The wild almond *Amygdalus webbii* (Spach) Vierh. (sin. *Prunus webbii* Spach, 2n= 2x= 16) is a close relative of cultivated almond (*A. communis* L.): both belong to the section *Eumygdalus*, of subgenus *Amygdalus* (genus *Prunus, Rosaceae*). *A. webbii* is the only wild relative of almond growing in Italy, and can be found only in Sicily and Apuly, as scattered trees or small populations at the edge of denser maquis formations. Interest in this species rises from both an environmental conservation perspective and its putative role as a pool of genetic diversity for cultivated almond. *A. webbii* can spontaneously hybridize with cultivated almond and it has been speculated that the Apulian almonds derived their distinctive self-compatibility trait by introgression from this wild species. *A. webbii* has also received some attention for its possible use as rootstock. Within the framework of EcoMeMaq project (INTERREG IIIB ARCHIMED), a trans-national initiative geared toward sustainable development of Mediterranean areas, specific objectives for this species are the detection and characterization of natural stands in Puglia region, and the establishment of an *ex-situ* collection.

Up to now, team explorations have focused on the Murgian hills: a few stands of different sizes were found, and their GPS localization is in progress. Nuts were collected from these stands from individual plants. Nuts from the same plant appeared quite uniform in size and shape; the weight distribution of nuts from different plants appeared asymmetrical. Although sample size was actually too small for formal testing, the distribution was compared against the normal distribution by standardization and $\chi^2$ test, and it was rejected at $P$= 1%. Progressive removal of a few samples with larger nut weight showed a clear trend toward goodness of fit. This preliminary data suggests indeed the prospect of a larger sampling, to test if lack of fit from normal distribution is indeed due to the presence of specimens with exceedingly large nuts. Almonds produce much larger nuts than *A. webbii*, and while findings of wild plants with varying levels of similarity to the cultivated almond has been repeatedly reported, other accounts point also to the occurrence of feral forms which are escapes from cultivation.

Meanwhile, other analyses are being planned on the collected material. To establish sampling parameters of populations, the required sample size for nut weight has been estimated. Over twelve hundred *A. webbii* seedlings are currently being grown in nursery for further characterization, and the amplification of *webbii* SSR loci by means of specific Almond primers is under evaluation. With regard to the *ex-situ* collection, a parcel of the Martucci experimental farm in Valenzano (BA) has been set aside, and is being arranged to suit general public environmental education purposes and germplasm conservation.

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**Poster Abstract – G.19**

# A NOVEL ROOTSTOCK TOLERANT TO FLOODING STRESS USABLE FOR *PRUNUS* SPP.

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*carbohydrates, hypoxia, regulated genes, root vitality, waterlogging tolerance*

A novel somaclonal variant tolerant of prolonged soil waterlogging, was regenerated from callus derived from leaf explants of *in vitro* grown plantlets of rootstock Mr.S.2/5 originated from free pollination *Prunus cerasifera*. In vitro pressure selection conditions were induced by adding N-methyl-D-glucamine to the medium. Among regeneration events, only 4 cases of conversion into shoots were recorded. Under *in vivo* conditions, plants of line S.4 exposed to waterlogging survived for up to 15 days (*plus variant*) of hypoxic stress, plants of the line S.1 (*minus variant*) showed a lower capacity to survival at waterlogging (4 days), while Mr.S2/5 wild type and Barrier1 rootstock plants died after 7 days of continuous exposure to stress.

Physiological and morphological modifications occurred in line S.4 and line S.1 as change in stem and leaf water retention, chlorophyll stability, formation of adventitious roots and modifications in leaf tissue free hexoses. The data collected on free carbohydrates indicated that line S4 is able to maintain its metabolic activity and sugar transport during waterlogging, whereas the other lines are affected by treatment. Root and leaf mitochondrial activity, as relieved by TTC test, was retained for longer period, in line S.4 than Mr.S2/5 wild type. From histological studies was observed that after 6 days, under hypoxia stress, the roots of line S.4 plants maintained the tissue and cell integrity of their structure, while it was loosed in wild type and as early event in line S.1. The histological observations suggest that genes codifying for enzyme responsible for the cell wall architecture and for protein related to cell-cell adesion are regulated differently in the tolerant line S.4 as compared to the Mr.S2/5 wild type and minus variant line S.1. The natural ability of Mr.S2/5 wild type to generate adventitious roots was strongly increased in the line S.4, under stress conditions, forming large roots even in the deep layer of root system. This new property could indicate an acquired new strategy of adaptation to the stress by the line S.4.

Differential gene expression studies suggested that ethylene biosynthesis and signalling pathway components, ATP1 and NAD mitochondria gene expression, ADH1, and glycosil transferase like gene (ltg4) are differently expressed, by the waterlonging stress, in the plants of line S.4 respect to the plants of line S.1 and MrS2/5 wild type. The sorbitol transporter, SOT1, was differently affected in the wild type and line S.4 by the waterloging The expression pattern of lgt4 is particularly interesting, because of, after six days of waterlogging stress, was down-regulated in the tolerant plants of line S.4 while did not change in plant of wild type.

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**Poster Abstract – G.20**

# GENETIC DIVERSITY AND EVOLUTIONARY STUDIES OF *FICUS CARICA* L. FROM SYRIA

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*Ficus carica, SSR, ISSR*

*Ficus carica* L. is one of the about 1400 species in 53 genera of the *Moraceae* Family. The fig is believed to be indigenous to western Asia and to have been distributed throughout the Mediterranean.

De Candole mentioned that Syria and Anatolia are the natural habitats of the fig tree; from there it would have been transferred to other Mediterranean countries, Mexico, Chile, Peru and California.

The fruit usually is consumed locally, fresh or in dried, canned, or used for preparing cakes and other bakery products, jams, jellies, etc.

There are many other uses for fig (Fruits, leaves, wood). Alcohol is obtained from fermented Figs. Fig leaves as a source of perfume material called "fig-leaf absolute" and seed oil is both edible and used as lubricant.

An ecogeographical survey of wild species and cultivated varieties was carried out in Syria. It produced a collection of 82 accessions, presently maintained at Edleb-250KM North of Damascus.

A survey of the variation in collected material was undertaken using morphological descriptors and molecular markers (eight Simple Sequence Repeat DNA (SSR) and four Inter Simple Sequence Repeat DNA (ISSR)).

The analysis showed a significant variation among varieties and the possibility of grouping the accessions according their geographical origin.

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**Poster Abstract – G.21**

# RESISTANCE BEHAVIOR TO ANTHRACNOSE DISEASE BY *GNOMONIA LEPTOSTYLA* (FR.) CES. IN *JUGLANS* SPP.

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*Juglans* spp., *resistance, breeding, Gnomonia leptostyla, NBS-profiling*

Among fast growing *Juglans* species, *J. regia* L. is an indigenous species of Eurasia, characterised by high quality wood, *J. nigra* L. is native to North America, with valuable wood, and the *J. major* (Torr.) Heller is close to *J. nigra* species. Anthracnose by *Gnomonia leptostyla* (Fr.) Ces. is one of the most important diseases of *Juglans regia* L. reducing both nut and wood production. In order to select resistant genotypes toward the anthracnose disease, the performance of the two interspecific hybrids, NG23 (*J. nigraN23 x J. regia*) and MJ209 (*J. major x J. regia*), was compared with the pure species, *Juglans nigra* (Eastern black walnut) and *Juglans regia* (Persian walnut). The correlation between this resistance and the growth of the genotypes was also taken into account. During the 2002 summer seventy five 15-years-old plants for each species and hybrids, were sampled in a experimental field of CRA-Forest Research Unit, Poplar Research Institute, in Rome. In each tree, fifteen leaves from upper and lower part of crow were collected, in order to score the number of necrosis spots and detect the percentage of leaf necrosis area. The tree growth was quantified measuring the height and the diameter (DBH). In addiction all necessary steps for a preliminary NBS (Nucleotide binding site)-profiling application in *J. regia*, *J.nigra* and hybrids were carried out in order to provide molecular markers tightly linked to R-gene and RGAs involved in anthracnose resistance. NBS-profiling approach is based on PCR amplification using simultaneously, an adapter primer matching a restriction enzyme site and a degenerate primer targeting the conserved domains present in the NBS.

During the vegetative season of 2002, the rainfall was abundant and therefore anthracnose incidence was very significant. Both the average number of necrosis spots and the percentage of leaf necrosis area were useful tools to evaluate the disease incidence. Our resulted proved that *J. regia* was highly susceptible species, although a wide variability was observed among genotypes; in addiction they proved that *J. nigra* is always resistant. The interspecific hybrids showed an intermediate behaviour toward *Gnomonia leptostyla* infection. Particularly NG23 hybrid behaviour was similar to *J. nigra*, while MJ209 to *J. regia*. A significant correlation between the disease incidence and growth ability was found only in *J. regia* and NG23. It proved that in case of severe attacks, anthracnose disease can limit the growth of tree more attacked in *J. regia* and hybrid NG23 and consequently affect their timber production. The practical application of these results are discussed.

A total of 4 primer-enzyme combinations (RsaI-NBS1, RsaI-NBS5A6, MseI-NBS1 and MseI-NBS5A6) amplified in *Juglans* spp. These primers were designed from a part of the conserved P-loop motif for NBS1 and of kinase-2 for NBS5A6. Out of the total (341 bands), 89 fragments were

common to *J. nigra* and *J. regia* and were labelled as “common”. In addition, 254 fragments amplifying in *J. nigra* and 128 in *J. regia* only, were classified as species “private NBS-bands”. No private NBS-bands were observed in the interspecific hybrids. This is the starting point to find an correlation between the NBS markers amplified in walnut germplasm and the resistance for anthracnose diseases.

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**Poster Abstract – G.22**

# GENERATION AND ANALYSIS OF EXPRESSED SEQUENCE TAGS FROM CAROB (*CERATONIA SILIQUA* L.) FLOWERS

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*EST random sequencing, gene identification, markers development, microsatellites*

Carob is a trioecious cesalpinoid legume tree which is mainly used for production of gelling agents (from endosperm) in food industry, for agroforestry and soil preservation in marginal dry areas, and as ornamental. This species is highly plastic in sexuality and is considered an anomalous cesalpinoid, having unusual flower feautures (missing floral organs, absence of petals, variability in organ number per whorl).

With the main aims of identifying flower-expressed genes and of developing specific markers, 1056 clones from a cDNA library of carob flowers at different developmental stages were bidirectionally sequenced. A total of 1376 high quality ESTs clustered into 1095 unigenes, consisting of 213 contigs and 882 singletons. Carob ESTs were subjected to BLAST search against the GenBank nr database, and GO annotation tool from the TAIR website (http://www.arabidopsis.org/tools/bulk/go/index.jsp) was used for the functional classification of the unigenes. Several homologs of genes involved in flower development and sexual reproduction (MADS-box genes, AP2, YABBY, etc.) were identified. Furthermore, using Msatfinder (http://www.genomics.ceh.ac.uk/msatfinder/) we identified thirty-eight di, tri and tetranucleotide EST-SSR, which could be potentially useful markers. A preliminary test of some carob genic microsatellites on Italian and Spanish carob accessions suggests their usefulness for genotyping and genetic diversity analysis.

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**Poster Abstract – G.23**

# SSR AND EST-SSR MARKERS TO ASSESS GENETIC DIVERSITY IN EUROPEAN CHESTNUT POPULATIONS

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*microsatellites, adaptive genetic variation, climate change, Castanea sativa*

The genetic diversity is the basis for the adaptability of organisms against environmental changes through natural selection. Populations with low genetic variation are more vulnerable to pollution, changes in climate and habitat alteration due to the human activities. In recent years microsatellites have become the most used markers for population genetics analysis. These markers measure neutral DNA variation, but they are not useful for measuring the adaptive genetic diversity. Recently the increased availability of the DNA sequences has given the possibility to develop EST-derived SSR markers, a new type of functional genomic markers. EST-derived SSR were found to be more than three times as transferable across species as compared with anonymous SSRs.

*Castanea sativa* is one of the most widespread tree species in Europe. It belongs to the *Fagaceae* family together with *Quercus* and *Fagus* sp. Previous studies, aimed to evaluate the genetic and adaptive variation, have been carried out in a large number of populations spanning the whole distribution area of the species. Genetic variation was estimated by ISSR and isozyme markers (Mattioni et al.2007). Adaptive variation was estimated at traits related to climate change (i.e. drought tolerance, bud burst, bud set ). The results showed a high degree of variation within and among chestnut populations both at genetic as well as phenotypic level (Lauteri et al.2004).

The aim of the present work is to compare the genetic differentiation, based on neutral SSR markers and EST-SSR markers, of chestnut European populations collected through a climate gradient. EST-SSR were developed from oak EST data base and the transferability was tested in chestnut. Six SSR markers were used to assess genetic diversity in natural chestnut populations from Italy, Greece and Turkey. Some of these EST-SSR have been used to estimate functional diversity in the same populations analysed by neutral SSR. The results obtained are compared and discussed.

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**Poster Abstract – G.24**

# EVALUATION OF AGRONOMIC PERFORMANCE, MOLECULAR AND BIOCHEMICAL INVESTIGATIONS AND TRANSGENE- SOIL INTERACTIONS OF GM POPLARS

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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 investigated the tolerance of *bar* GM poplars to a non selective herbicide (BASTA) over three years: neither *bar*-transformed line showed any damage due to the application of the herbicide,

We studied also 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..The trials were performed in 2004 and 2005. The results did not show significant differences between GM and control lines. *In vitro* investigations on the effects of *trans*-piceid, expressed by the tested white poplar lines, against some main fungal parasites of cultivated poplars were performed, to assess how it may affect the growth of fungi without plant mediation. *Venturia populina*, *Discosporium populeum* and *Rosellinia necatrix* were tested on potato-dextrose agar medium containing different amounts of *trans*-piceid. The *trans*-piceid was inhibitory on *V. populina* colonies, especially at concentrations over 100 mg/l of medium, but, on the contrary, its presence was associated with an increased growth of colonies of one *D. populeum* isolate and of two *R. necatrix* isolates. *V. populina*, however, is a quite specific pathogen, so its response to the presence of *trans*-piceid may be triggered by the activation of certain inducible genes. The metabolic pathways of such compound in fungal parasites need to be clarified HPLC-DAD analysis mainly showed the presence of the *trans*-piceid (*trans*-resveratrol 3-glucoside) in all the tested tissues (leaves, stems and roots) in the *StSy* GM poplars.. Seasonal variations of the trans-piceid content were found, opposite during time in leaves and roots. Its highest amount (364 mg/kg fresh weight) was detected in leaves during the full vegetative growth whereas in roots just after dormancy (330 mg/kg)

The 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 three-year period showed significant fluctuations in the steady-state level of the *bar* and *StSy* transcripts. Further

studies were carried out in order to clarify the molecular mechanisms underlying the seasonal fluctuactions of the *bar* and *StSy* transcripts.. One possible explanation might involve microRNAs (miRNA). To this purpose, a bioinformatic approach was used to check for the presence of miRNA target sites within the *bar* and *StSy* nucleotide sequence

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 analyses carried out on both the *bar* and *StSy* trials confirmed the presence of recombinant DNA sequences derived from GM poplar tissues in soil over three year 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 contribute to a better understanding of the agronomical response and environmental impact for a potential cultivation of GM poplars on a large scale.

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**Poster Abstract – G.25**

# A SURVEY ON GENE EXPRESSION AFTER LOW TEMPERATURE STRESS IN WHITE POPLAR (*POPULUS ALBA*)

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*cold stress tolerance, gene expression, Populus alba, suppression-subtractive hybridisation*

Temperature is one of the abiotic factors limiting growth and productivity of plants. Exposure of plants to low temperature induces reduction of membrane fluidity and affects kinetic parameters and protein folding. Low temperature stress is one of the most serious problems for forest crops, since it affects yield, quality, and survival. Forest crops are especially susceptible to low temperatures in spring, after dormancy release, when the newly flushed shoots are vulnerable, and cold stress causes serious loss of biomass production.

During last years, an increased incidence of warm episodes has determined early onset of spring phenophases, so that it has been calculated that an anticipation of 6 days has occurred from the sixties to today. The predicted global warming should further induce earlier bud flushing. Due to earlier dormancy release, forest crops are more exposed to frost injury in spring. Hence, breeding for cold tolerance is based on the development of late flushing genotypes, for which the possibility to cope with spring frost is reduced. The aim of our study was to improve the knowledge of the genetical bases of low temperature tolerance in forest trees: such a knowledge can be useful to improve the selection process.

White poplar plants in the stage of leaf burst were maintained for two weeks at 25°C, then transferred to 4°C for 6, 12, 24, 48 hours, and mRNAs were isolated from leaves. Two differential SSH cDNA libraries were constructed, after short- (6 hours) or long- (48 hours) cold treatments. We have isolated 162 genes, that can be grouped into six categories: i) encoding stress and defence proteins (containing genes already associated to cold stress); ii) involved in signal transduction; iii) related to regulation of gene expression; iv) encoding proteins involved in cell cycle and DNA processing; v) encoding proteins involved in metabolism and energetic processes; vi) involved in the protein fate. Genes encoding not yet characterized proteins were also found.

Genes encoding proteins involved in signal transduction, in cell cycle and DNA processing and in protein fate were especially induced in the early stages of stress. On the contrary, stress and defence proteins encoding genes and genes related to regulation of gene expression were more represented after the long-term treatment. The percentage of genes encoding proteins involved in metabolism and energy processes remained constant in both treatments.

The expression of isolated genes was analysed at 6, 12, 24, 48 h of cold treatment and after 24 h of recovery by reverse northern hybridisation. Sixty percent of genes were transiently expressed (i.e. their expression ceased with recovery), indicating that their function should be crucial for stress response. Almost all genes involved in signal transduction and the majority of genes encoding cell cycle and DNA processing related proteins and involved in protein fate belong to this category. Stress and defence proteins encoding genes were early activated and expressed also after recovery. In conclusion, we have established a reference transcriptome that is being used for

comparisons of *P. alba* genotypes from different altitudes and presumably differently tolerant to cold stress. This approach will allow to identify candidate genes to be used for the selection of cold tolerant poplar genotypes.

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**Poster Abstract – G.26**

# GENETIC DISSECTION OF PHENOLOGICAL TRAITS DURING BUDSET IN POPLAR

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*photoperiod, budset, heritability, Populus spp.*

The work has the objective to contribute to the knowledge of the genetic control of budset and to identify genomic region associated to this trait in *Populus* spp. For this purpose two full-sib families of black and white poplar obtained from parents divergent for phenology have been measured. The study has been realized on the basis of a protocol designed to monitor the dynamic of different phenological phases during budset in the black poplar. Only the phase of transition (1.5) from the structure of the shoot to the bud has been measured in white poplar. Data analysis have allowed to decompose the contribution of the different phases to the dynamic of bud set in black poplar and to select 5 phases characterizing the process (phase 2.5, phase 1.5, subprocess 1 and 2, 50% of individuals in phase 1.5). The results have shown the significant genotypic differences in the two full-sib families and the budset traits were characterized by high coefficients of genetic variation and broad sense heritability. The obtained results will be discussed in relationship to the answer of the two full-sib families to the photoperiod and temperature introducing, in prospect, the utility of this work for genetic improvement and the mapping of QTLs associated to the bud set in poplar.

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**Poster Abstract – G.27**

# BEECHWOOD SPATIAL GENETIC STRUCTURE IN SOUTHERN ITALY

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*Fagus sylvatica, landscape genetics, microsatellite marker, refuge area*

Beechwood forest of the Mediterranean mountain is the main provider of ecosystem services associated to water quantity and quality resources. The beechwood persistence and resilience, at a low latitudinal level, in the coming years will be a dependent variable of local and global environmental changes. Therefore a detailed knowledge of the spatial genetic structure of this species is required to generate updated guidelines of beechwood management.

Buds and leaves have been harvested *in situ* from parents and progeny plants (where present) - based on a systematic sampling design (every 200 m) stratified over accessible homogenous landscapes (woods within mountains and mountains within sub-regions) – over the heterogeneous geographical range comprising Basilicata Apennine, Cilento (Campania), Foresta Umbra (Apulia). Basilicata Apennine deserved a special attention as it has been considered a refuge area. Ecological data (pedology, vegetation, management, exposition and altitude) were recorded during this survey. A control sub-population from Stavanger (Norway) was used as the northernmost site. The DNA from the whole population (861 individuals) was analysed with 5 nuclear microsatellite loci and 2 chloroplast microsatellite loci using capillary electrophoresis.

Overall, 6 identified chloroplast clusters showed a non casual/obvious spatial distribution. They revealed specific patterns of seed migration as well as the effect of ecological fragmentation. Within twenty one beech sub-populations, when comparing the overlapping old generation *versus* the most recent (represented by plants of 3 to 10 years old from seeds), 9 showed an average reduction of the observed heterozygosity (*Ho*) of 8.5% and 12 an average increase of 7.6%. Either the minimum (Stavanger, $Ho$ = 0.35 and Foresta Umbra, $Ho$ = 0.45) or the maximum heterozygosity (Mount Vulture, $Ho$ = 0.73 and Mount Vetrice, $Ho$ = 0.75) were specific to ecologically very fragmented sites. The integration of genetic and landscape data will provide new beech woods management tools.