Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 3A.01

PEACH GENOMICS AND BREEDING APPLICATIONS

ARÚS P., ARANZANA M.J., HOWAD W., EDUARDO I.

IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra (Spain)

Prunus persica, rosaceae, next-generation sequencing, introgression lines

The cultivated peach (*Prunus persica*) is a major temperate fruit crop species with a diploid (2n=16) and a compact genome (approx. 220 Mbp) that has been recently sequenced. It originated in China and the variability used in European and American commercial breeding programs is low. Peach germplasm is structured and has a high level of linkage disequilibrium conservation, due mainly to its self-compatible mating system and to a recent bottleneck at the beginning of the modern breeding programs about one century ago. Genetic progress in the last 15 years has been enormous with the development of saturated linkage maps, the widespread use of markers for variability analysis, the mapping of many major genes, cloning of a few of them and the dissection of several quantitative characters of economic interest in their QTL components. The presentation will cover the current use and limitations of markers in commercial plant breeding, the development of new genomic tools, particularly those based on next-generation sequencing technology and the availability of the whole genome sequence of peach and other rosaceous crops, and the progress towards the development of an almond-peach NIL collection, as a tool for fine genetic analysis and a way towards the enrichment of the peach genome with genes coming from related species.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 3A.02

PARTITIONING AND METABOLISM OF ASSIMILATES IN DEVELOPING FRUITS

LEEGOOD R.C.

Robert Hill Institute and Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN (U.K.)

Carbohydrates, malate, PEP carboxykinase, tomato, grape

Fruits import a wide range of assimilates, such as carbohydrates and nitrogenous compounds, that are then partitioned between the fruit structures and the developing seeds. At certain stages of development many fruits accumulate organic acids, such as citric, malic, quinic, and ascorbic acids, together with their anions. These acids are a metabolically diverse group. Citric, and to a lesser extent malic acid, together with their anions, accounts for the bulk of the organic acid content of many soft fruits and there is a decrease in their content of citric and/or malic acid during ripening. In some fruits, such as Hamlin orange, this decrease can be accounted for by dilution arising from expansion of the fruit. In others, catabolism is also involved. In soft fruit, little is known about the metabolic pathways involved in this catabolism, however, this has been studied in the flesh of grapes and tomatoes, and in these a number of fates for malate/citrate have been suggested. These are oxidation by the Krebs cycle, gluconeogenesis, fermentation reactions that produce ethanol, anthocyanin synthesis, and amino acid interconversions. However, uncertainty exists as to what proportion of malate/citrate is used by each of these processes. In plant cells, PEP carboxykinase (PEPCK) is only present in the cytosol. As in other organisms, PEPCK is only present in certain tissues of plants, and in many of these only under certain conditions. Although, the occurrence of PEPCK in most fruits is uncertain, studies have been done in grapes and tomatoes. In these PEPCK appears, or increases in abundance, in the flesh at the onset of ripening, and this led to the suggestion that PEPCK might function in the catabolism of malate and/or citrate. By contrast, there are a number of forms of malic enzyme in plants, there are cytosolic and plastidic forms of NADP-malic enzyme (NADP-ME) and a mitochondrial NAD-malic enzyme. The malic enzymes have been studied in several fruits, and it has been proposed that NADP-ME may function in the catabolism of malic and citric acids in the flesh during ripening.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 3A.03

GO BACK FROM THE ADULT PHASE

CIRILLI M.*, COLAO M.C.*, RUGINI E.*, CABONI E.**, MULEO R.*

*) Department of Science and Technology for Agriculture, Forestry, Nature, and Energy, Laboratory of Woody Plant of Molecular Ecophysiology, University of Tuscia, Via C. De Lellis snc, 01100 Viterbo (Italy)
**) CRA - Fruit Tree Research Centre, Rome (Italy)

Changing phase, juvanility, Prunus persica, agamic propagation, flowering regulome

The juvenile state of woody plants manifests itself in various morphological and physiological phenomena, and the juvenile characters are often not highly correlated each other. Research in juvenility of woody plants was focused primarily on vegetative reproduction and induction of flowering, which are the most general aspects of aging in trees. Many of the morphological and physiological changes related to aging can be understood as consequences of the interaction among several genetic pathways controlling transition, which are integrated at the transcriptional level from *Flowering Time* (*FT*) and *Leafy* (*LFY*). *Twin Sister of Flowering Time* (*TSF*) acts as a flowering pathway integrator redundantly with *FT*. Searching in the genome of peach (www.peachgenome.org), one locus for *FT* and for *TSF* was found. The expression of both genes have been tested to unravel their role in plant juvenility which occurs during the growth of flowering plant *in vitro* conditions. A time scale analyses has been conducted to verify the role of *Short Vegetative Phase* (*SVP*), *Early Bolting* (*EBS*), *Gigantea* (*GI*), *Constans1* (*CO*1) and *Constans2* (*CO2*), *Tempranillo1* (*TEM1*) and *Tempranillo2* (*TEM2*). These gene are target of many endogenous and environmental factors, such as biological clock, ontogenetic development, growth regulators, temperature, photoperiod, vernalization and touch-related mechanic stimuli.

The mRNA level of *FT* was down-regulated, ranging from 50 to 100 times less, compared to the field grown donor plant. *TSF* was completely shut down in the plants *in vitro* grown. Biological clock and photoperiod doesn't seems to affect *FT* and *TSF*, and no modification in the level of gene expression of *GI*, *CO1* and *CO2* has been found. Noteworthy appears that the ontogenetic development program and the environmental factors are strongly related to the down-regulation of FT and TSF, as appear from the large up-regulation of the genes SVP EBS TEM1 and TEM2. Bioinformatic analyses of promoters of the studied genes reveal a possible cross interaction among the representative proteins of endogenous and environmental factors since the specific recognition motifs are present on the promoters of both genes *FT* and *TSF*.

Our work revealed additional complexity and temporal aspects of the regulatory network at the pathway integration level. We propose that the core genes of changing phase are the pivot that induce the plant to go back from adult phase to juvenility.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 3A.04

EXPRESSION ANALYSIS OF GENE INVOLVED IN THE IRON DEFICIENCY OF CITRUS ROOTSTOCKS

LICCIARDELLO C.*, TORRISI B.*, TONONI P.**, ALLEGRA M.*, SCIACCA F.*, DELLEDONNE M.**, INTRIGLIOLO F.*, REFORGIATO RECUPERO G.*

*) CRA-Research Centre for Citricolture and Mediterranean Crops, Corso Savoia 190, 95024 Acireale (Italy)
**) Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona (Italy)

Citrus rootstock, differential gene expression, iron chlorosis, CombiMatrix Array

At least 20 to 50% of fruit trees in the Mediterranean basin suffer from iron deficiency, due to high concentrations of calcium and bicarbonate in soils. The most common symptoms in plants consist of interveinal chlorosis in young leaves, a decrease in leaf net photosynthetic rate, leaves reduced in size, fragile and very thin. Then a growth retardation followed by death in more severe conditions, associated to the loss of yield, delayed fruit ripening and impaired fruit quality is also observed. It's possible to recover the iron deficiency adding inorganic iron salts, synthetic chelates, and natural organic compounds, even if it's difficult to correct because of the rapid transformation of iron contained in fertilizers into an unavailable form. So the easiest way to avoid iron chlorosis on calcareous soils is the use of tolerant rootstocks. In citrus even if the sour orange (*Citrus aurantium* L.) was the most diffused rootstock in the Mediterranean area, considering its tolerance to calcareous soils, the sensitivity to Citrus Tristeza Virus (CTV), one of the most serious citrus biotic stress, is producing its replacement. However the search of alternatives rootstocks remains the only strategy to react to iron deficit problem.

The aim of the work was to isolate differentially expressed genes involved in the iron deficiency using a CombiMatrix platform, made up of around 8,000 ESTs of roots extracted from NCBI database of citrus and its relatives. At first we worked on Carrizo citrange (quite tolerant) and Swingle citrumelo (sensitive) rootstocks, grown on pots with two different soils, one calcareous (9% of active lime) and another volcanic (0% of active lime). Microarray experiments were carry out with RNA samples extracted from tip roots. Foliar analysis was also performed on leaves. Expression analysis, based on the comparison between Swingle citrumelo growing on calcareous soil respect to volcanic one, showed five differential genes up-regulated, which glutathione peroxidase (GPX) is the only annotated. The GPX, such as superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase, is an antioxidant enzyme strictly involved in biotic and environmental stresses. In particular way GPXs are diverse enzymes catalyzing the reduction of hydrogen peroxide, organic and lipid hydroperoxides by reduced glutathione, helping to protect cells against oxidative damage. In a second experiment GPX was used as marker to evaluate its expression level correlated to iron deficiency on seven different rootstocks (3 sensitive and 4 tolerant), growing on soil characterized by a medium-high calcium level. The expression analysis, conducted through the Real time PCR on root and leave samples, confirmed the higher expression of GPX in citrumelo roots respect to Carrizo ones. It could be due to the reaction of sensitive rootstock against stressed and oxidant conditions. Anyway the opposite trend was evidentiated in leaves. These data are comparable to peroxidase, Fe concentration, SPAD index and chlorophyll

content performed on leaves, and to Fe chelate reductase conduced on roots. The down expression of GPX on leaves of sensitive rootstocks could be explained considering that GPX is an Fe-dependent enzyme, enable to work causing the low iron availability, and taking, as consequence, to the accumulation of free radicals. Further analysis will be addressed in array experiment on comparison of tolerant and sensitive rootstocks growing in the volcanic soil, with the aim to isolate genes involved in the protection of iron deficiency.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.05

MODELING GENETIC VARIATION FOR PHENOLOGICAL EVENTS IN *CORYLUS AVELLANA* L.

DE PACE C., CATARCIONE G., VITTORI D., RUGINI E.

Department of Science and Technologies for Agriculture, Forests, Nature, and Energy, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)

Phenology, bud sprouting, fruit development, chilling units, evergrowing

Phenological events such as bud burst, flowering, fruit development and maturity, and leaf senescence have received increased interest in the light of global climate changes. As global warming progresses and varietal turnover is not adopted, climate effects on the plant's immediate environment followed by changes of the pace of several physiological processes and time occurrence of phenological stages in hazelnut orchards, are expected, mostly in the shortest and smallest geographical scales,

The three most important factors controlling phenology in tree species are the degree of fall-winter chilling, photoperiod (day length relative to night length), and temperature. In hazelnut , the time of occurrence of winter dormancy release leading to bud sprouting may be synchronized with seasonal changes in temperature as it was observed in the phylogenetically related hornbeam plants.

An observational study based on long-term data sets from field experiments using genetically differentiated materials (a full-sib progeny composed by 135 plants), provided data for modelling the phenotypic expression of bud-sprouting under local climate changes in order to gain insight on the genetic basis of that trait. The methodology can be expanded to cover a wider range of traits and environments.

The experiment was conducted at Viterbo over seven winter seasons, starting in 2004/2005. Time of budburst was defined as the day when at least one bud on 10 terminal 1-year old stems showed the first 1-2 mm of the new leaves.

Two groups of plants, one expressing the 'evergrowing' phenotype and the other the wild-type winter-dormancy phenotype, were steadily observed. The evergrowing plants produced new shoots in the middle of October of every year and, unless frost damage occurred in February, the shoots continued to grow up to the end of June when nuts ripen (it occurred only in 2007 and 2011). The proportion between the number of plants expressing the wild-type and the evergrowing phenotype, fitted a 3:1 ratio, suggesting a simple model (1 locus, two alleles) for the inheritance of the evergrowing phenotype. Homozygosity for the evergrowing allele cause interference with the response to the signal for leaf senescence and ceasing of growth. The group of plants expressing the wild type phenotype falled in two categories: one composed by 79 FS plants with budburst centerd on March 25th, and the other composed by 40 plants with budburst on February14th. The proportion between the number of plants expressing the latest and earliest phenotype subgroups, fitted a 9:7 ratio, suggesting a simple genetic model for the inheritance of the earliest and latest phenotypes (2 loci, two alleles per locus with threshold expression). Graphical representation of the average budburst date for the 11 FS plants from each subgroup, revealed consistent phenotypic diversity over the 7 years, indicating a significant genetic divergence between the two subgroups. The year-to-year fluctuation of the average budburst date was closely associated (r=0.92) to the sum of

chilling units (CU) occurring between October 18th and Dec. 20th . Using these information on the CU necessary to budburst it is possible to devise a predictive model for forecasting the date of budburst.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.06

SIGNIFICANT LEVELS OF PHENOTYPIC AND GENETIC VARIATION FOR ERIOPHYOID MITE CONTROL IS PRESENT IN HAZELNUT (*CORYLUS AVELLANA* L.) CULTIVATED ACCESSIONS AND BREEDING POPULATIONS

CATARCIONE G., VITTORI D., RUGINI E., DE PACE C.

Department of Science and Technologies for Agriculture, Forests, Nature, and Energy, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)

Phytoptus avellanae (Nalepa), big bud mite, resistance to mites, fruit tree breeding, filbert tree

Eriophyoid mites are obligate plant parasites that feed on various plant organs of many fruit tree species causing bud swelling and/or physiological disorders. The big bud eriophyoid mite *Phytoptus avellanae* (Nalepa) (*Pa*) is considered a pest of hazelnut trees (*Corylus avellana* L.) grown in southern Europe and elsewere. The chemical control of *Pa* require the use of acaricides with long residual activities to cope with the long appearance time of the adults from the hiding sites, in the big bud-galls, to search the new bud-shelters formed during spring. Chemical control is not environmentally friendly and may cause the surge of mite strains that have developed resistance to acaricides. Biocontrol of *Pa* by predatory mites is feasible but no suitable IPM protocols for reducing *Pa* population size in Italian orchards have been developed yet.

A sustainable *Pa* control strategy may be based on hazelnut host plant resistance. Several reports have indicated resistance to *Pa* in hazelnut accessions such as 'Mortarella' and 'Barcelona'. No information are available on: (*i*) the degree of resistance to *Pa* in other Italian hazelnut accessions, (*ii*) the genetic basis of the resistance, and (*iii*) the opportunity for breeding new clonal varieties endowed of resitance to the mite pest.

Observations on the incidence of mite infestation were made before bud sprouting for three years, when the big-buds were easily seen on the terminal stems of the trees of Tonda Gentile Romana (TGR), Tonda Gentile delle Langhe (TGL), Tonda di Giffoni (TGif), S. Giovanni, Nocchione, Nociara, Longue d'Espagne, Karidati, and Sivri accessions, their half-sib (HS) progenies, and the full-sib (FS) progeny from the TGR x Nocchione hybridization. Incidence was estimated by counting total buds and big-buds on 3 two-year old stems per tree, and expressing the infestation incidence (InIn) as percentage of the big-buds over the total buds.

Alternate scions of TGR, TGif and Nocchione were planted in replicated adjacent rows, to design triangular replicated plots, each including one tree from the three accessions, in order to control sources of variation that affected the trees within the same replicate. The interval of variation for 'InIn' over 20 replicates evaluated in two years, ranged from 0 to 3% in Nocchione, 12 to 24% in TGR and 32 to 61% in TGif. This indicated clear genetic differentiation among the tested accessions. The average 'InIn' from trees of TGR, TGL, TGif, S. Giovanni, Nocchione, Nociara, Longue d'Espagne, Karidati, and Sivri evaluated for three years (2008, 2009, and 2011), consistently indicated a near 0% 'InIn' in Longue d'Espagne, Nocchione, Nociara, and Karidaty, and over 17% 'InIn' for TGif, TGL and Sivri. Out of 96 FS plants evaluated for three years, 8 plants had consistently 0% 'InIn' and 8 over 25% 'InIn'. These results suggested a significant level of genetic variation for big-bud mite control in hazelnut germplasm collection and breeding

populations. The resistance to big-bud mite can be a further and feasible criteria to be used for selecting new clones from FS progenies.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.07

INTEGRATING GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF CHESTNUT CULTIVARS: WHERE ARE WE AND WHAT FOR?

VILLANI F.*, MATTIONI C.*, MARTIN A.**

*) CNR, Institute of Agro-Environmental and Forest Biology, Viale Marconi 2, 05010 Porano (Italy)
**) Departamento de Genetica, Escuela Tecnica Superior de Ingenieros Agronomos y de Montes, Edificio Gregor Mendel, Campus de Rabanales, Universidad de Cordoba, Cordoba (Spain)

Genetic variability, varietal characterization, chestnut, Castanea sativa, environmental security

During the last few decades, one of the principal challenges in agriculture has been to combine increased productivity and competitiveness with maintenance of biodiversity. In this context, on-farm conservation has been receiving increasing attention and international initiatives have been encouraged to enhance and preserve the genetic diversity of traditional varieties (FAO, 1996; Esquinas-Alcazar, 2005). Many researches have been carried out to identify morphological and genetic standards able to univocally characterize local varieties or landraces that are encouraged to be retained either because they fill ecological, cultural and local socioeconomic niches as well as because of the increasing market requests for typical products derived from them.

Within this context, chestnut is one of the key multipurpose tree species which is considered of utmost importance in all Mediterranean countries for both timber and fruit production. Many studies have been made to identify synonymy and homonymy in the traditional classification of the local varieties.

An overview of the studies carried out using morphological as well as molecular markers is reported. Scientific results are discussed in relation to their impact on policy and marketing measures and their application in the context of genetic resources conservation, breeding, environmental security. Examples of lack of appropriate link between scientific community, governance, market, gene conservation and people are reported.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.08

IDENTIFICATION OF CANDIDATE GENES INVOLVED IN POLLEN-PISTIL INTERACTION IN *CITRUS*

CARUSO M.*, DISTEFANO G.*, MERELO P.**, LA MALFA S.*, LO PIERO A.R.*, TALON M.**, GENTILE A.*, TADEO F.R.**

*) Dipartimento di Scienze delle Produzioni Agrarie e Alimentari (DISPA), University of Catania,Via Valdisavoia 5, 95123 Catania (Italy)
**) Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Genómica, Carretera Moncada-Náquera Km. 4,5, 46113 Moncada Valencia (Spain)

Citrus, laser capture microdissection, microarray, self-incompatibility

Compared to what is known in model species, reproductive biology in citrus is still poorly understood. Although in recent years several efforts have been made to study pollen-pistil interaction and self-incompatibility, little is known about the molecular mechanisms regulating these processes. The understanding of self-incompatibility mechanism in mandarins, which is related to seedlessness, is of outstanding interest for breeding.

Here we report the identification of possible candidate genes regulating pollen-pistil interaction and self-incompatibility in clementine (*Citrus clementina* Hort. ex Tan.). These genes have been identified comparing transcriptomes of laser-microdissected stylar canal cells isolated from two clementine genotypes differing for self-incompatibility response: ‘Comune’, self-incompatible; and ‘Monreal’, a natural self-compatible mutation of ‘Comune’. These genotypes were previously characterized by histological assays, which demonstrated that the mutation leading to self-compatibility in ‘Monreal’ affected the style functions regulating pollen rejection.

Transcriptome profiling was performed using Affymetrix Citrus Genechip representing up to 33,000 citrus transcripts. Among them, only 10 genes resulted overexpressed in ‘Comune’ stylar canals and 6 genes in ‘Monreal’ ones. The results of microarray hybridizations were validated using real time PCR. Most of the differentially expressed genes are not functionally annotated in citrus or other plant species. Interestingly, 3 of the ‘Comune’ overexpressed genes clustered in a range of about 10 kb in the clementine genome. The clustered genes shows similar domains in their predicted protein sequences, and are close to a DELLA gene, previously identified in self-pollinated ‘Comune’ styles with stigmas by cDNA-AFLP transcript profiling. Moreover, a time course analysis showed different expression patterns of selected genes in virgin and self-pollinated styles with stigmas of the two genotypes during pistil maturation and pollen tube elongation (from 0 to 8 days after pollination). Since most of the candidate genes were not previously characterized, further analyses are needed to reveal their specific role in the interaction between pollen tubes and stylar canal cells.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.09

GENETIC CONTROL OF ANTHOCYANIN METHYLATION IN PETUNIA AND GRAPEVINE

PROVENZANO S.*, SPELT K.**, BRUGLIERA F.***, QUATTROCCHIO F.**, KOES R.**, FERRANDINO A.*, TANAKA Y.****, SCHUBERT A.*

*) Department Colture arboree, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (Italy)
**) Department of Molecular Cell Biology, Vrije Universiteit, University Amsterdam
***) Calgene Pacific Pty Ltd, 16 Gipps Street, Collingwood, Victoria 3066 (Australia)
****) Institute for Plant Science, Suntory Ltd, 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka 618-8503 (Japan)

Vitis vinifera, Petunia hybrida, methyltransferase, anthocyanin

Anthocyanins belong to the class of flavonoids and are the most widespread pigments in the plant kingdom. They are involved in a series of biological activities such as protecting against oxidative damage and attracting pollinators, and they can also produce benefits on the human health. The glycosylated anthocyanins can be “decorated” on the ring B through methylation and acylation. The methylation of the 3’ hydroxyl group of the anthocyanin B-ring converts cyanidin into peonidin or delphinidin into petunidin; the methylation of both the 3’ and the 5’ hydroxyl groups, convert delphinidin into malvidin. These modifications increase the stability of anthocyanins and modify their water solubility thus significantly contributing to the accumulation of coloured molecules in petals or fruits. Here we describe the cloning and characterization of two anthocyanin methyltransferases of *Petunia hybrida* and we analyse their function *in vitro* and *in vivo*. Genetic studies had previously shown that in petunia anthocyanin methylation is controlled by the two loci *MT* and *MF*. By sequence analysis of petunia mutants we show that the two genes correspond to the two loci, and we characterize the relative mutations. Expression analysis shows that the *MT* and *MF* genes are controlled by known regulators of anthocyanin biosynthesis in petunia (AN1 and AN2). Methyltransferase activity *in vitro* was demonstrated using MT recombinant protein. The complementation by transient and stable transformation of *mt* and *mf* mutants induced the production of methylated anthocyanins as analyzed by LC-MS. We also transformed the same petunia mutants with a methyltransferase from *Vitis vinifera* already studied *in vitro* (Hugueney *et al.*, 2009, Plant Physiology 150:2057-2070). The results show the presence of methylated compounds in the flower extracts and the preference of this gene for the production of di-methylated anthocyanins. Petunia is a useful model plant to study the *in vivo* function of phenylpropanoid biosynthetic genes. Moreover the possibility to use transient expression via *Agrobacterium* infiltration in petals has proven to be a fast method to test gene function in this species.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.10

DEVELOPMENT OF A METHOD FOR CONFERRING RESISTANCE TO GFL AND GLR ASSOCIATED VIRUSES THROUGH POST TRANSCRIPTIONAL GENE SILENCING

PALMA D.*, MOLESINI B.*, SABBADINI S.**, POLVERARI A.*, NAVACCHI O.***, MEZZETTI B.**, PANDOLFINI T.*

*) Dipartimento Biotecnologie, Università di Verona, Strada le Grazie 15, Verona
**) Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche, Ancona
***) Vitroplant Italia, Cesena

Grapevine, Nicotiana benthamiana, virus resistance, PTGS, genetic transformation

Grapevine (*Vitis vinifera L.)* is one of the most important crop species in the world. Main purposes of grapevine genetic improvement programs are the obtainment of plants able to stand biotic stresses and the improvement of features related to production and fruit quality. The production of resistant cultivars through classic genetic techniques such as crossing, requires very long implementation times. In contrast, the use of genetic engineering represents an effective method to increase resistance to pests and to improve fruit quality and production without altering the agronomic traits of the cultivars. The aim of this project is to develop a genetic engineering method based on post transcriptional gene silencing (PTGS) for conferring resistance against viruses responsible for grapevine fanleaf and grapevine leafroll diseases. Grapevine fanleaf disease is mainly caused by the *Nepovirus* viruses such as *Grapevine fanleaf virus* (GFLV) and *Arabis Mosaic Virus* (ArMV). The grapevine leafroll disease is ascribed to the presence of one or more infectious agents belonging to *Closterovirus* and *Ampelovirus,* "associated" to the disease (Grapevine leafroll-associated viruses, GLRaV). To curtail virus replication by gene silencing, we built a construct for the expression of hairpin transcripts homologous to selected portions of the viral genomes. The PTGS-eliciting construct (*hpViruses GFLV-GLRaV*) containes two 400 bp-long arms placed in inverted orientation, each arm consisting of two fused DNA fragments homologous to the GFLV and GLRaV-3 RNA-dependent RNA polymerase genes, respectively. The hp construct was introduced in *Nicotiana benthamiana* to test its efficacy against GFLV and ArMV infection. Several transgenic lines were identified by PCR and Southern blot analysis. The same construct has been used for *A. tumefaciens*-mediated genetic transformation of grapevine using a protocol based on organogenesis (Mezzetti *et al.*, 2002). Up to now, the material is still in the selection and regeneration phase.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.11

PURIFICATION AND PROPERTIES OF *MALUS DOMESTICA* CAROTENOID CLEAVAGE DIOXYGENASE 4

FORLANI G., GIBERTI S., BERTAZZINI M.

Department of Biology & Evolution, University of Ferrara, Via L. Borsari 46, 44100 Ferrara (Italy)

Carotenoid bleaching, functional food, Malus domestica, Prunus persica, substrate range

During recent years increasing evidence supporting the ability of the so-called functional foods to promote human well-being and reduce the risk of certain major diseases prompted a strong interest for the development of strategies to increase the levels of health-promoting bioactive compounds in fruits and vegetables. To ensure protective effects, relatively high levels of phytonutrients would be in fact required, well over those commonly taken with the dietary consumption of plant-derived foods. Among these beneficial substances are the carotenoids, powerful antioxidant tetraterpenoids able to protect against oxidative stress by quenching singlet oxygen and scavenging free radicals, therefore inhibiting lipid peroxidation. Besides the health-promoting effects, the presence of these compounds in fruits, vegetables, staple and processed foods is also attractive for the consumer. The yellow-to-orange colour rendered by high carotenoid content often increases buyer confidence and, as a consequence, product value. Typical examples are the pasta products, and the yellow-fleshed peach fruits.

Emerging data suggest a pivotal role of carotenoid-bleaching enzymes in determining the final level of these phytonutrients in foodstuffs. During pasta processing, a loss of colour as a consequence of pigment oxidation usually occurs that is mainly due to the lipoxygenase-linoleate system (Verlotta *et al.*, BMC Plant Biology 2010, 10:263). In peach, carotenoid accumulation in the mesocarp causes the difference between yellow and white genotypes. The latter are generally characterized by a peculiar and more intense aroma, because of the formation of volatiles deriving from the breakdown of the carotenoid skeleton. Dioxygenases appear to be key factors causing volatile release in fruits, and a differential expression of *carotenoid cleavage dioxygenase* (*CCD*) *4* gene was in fact found in yellow *vs.* white-fleshed isogenic peach genotypes (Brandi *et al.*, BMC Plant Biology 2011, 11:24).

Within the frame of a research project aimed at improving our knowledge on the biochemical bases of carotenoid content in peach fruits, we expressed the *CCD4* gene from *Malus domestica* in a heterologous system. The protein was purified to near electrophoretic homogeneity by sequential affinity chromatography, anion-exchange and gel-filtration FPLC. Because carotenoid breakdown by CCDs yields a coloured product, an HPLC-based assay method was set up to measure *in vitro* carotenoid cleavage reactions. However, the isolated enzyme showed an almost negligible activity toward either β-carotene or trans-β-apo-8'-carotenal, suggesting a possible cofactor requirement. Activity assay performed with extract from induced *vs.* not-induced *E. coli* transformed strains provided evidence of cleavage activity. However, contrary to previous data (Huang *et al.*, J. Exp. Bot. 2009, 20:3011), a higher affinity was found toward trans-β-apo-8'-carotenal than β-carotene.

Experiments are currently in progress in order to raise polyclonal antibodies in mice against the purified apple CCD4. In the next future these will be used as a tool in characterizing protein

levels in tissues at different stages during fruit development in contrasting genotypes of peach showing various levels of carotenoids in fruit flesh.

This work was supported by a grant from the Italian MiPAAF in the frame of the ALISAL project.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.12

MOLECULAR AND PHYSIOLOGICAL ADAPTATION OF *OLEA EUROPEAE* TO LOW TEMPERATURES

GUERRA D.*, LAMONTANARA A.*, BAGNARESI P.*, BALDONI L.**, RIZZA F.*, PAGANI D.*, CATTIVELLI L.*, MAZZUCOTELLI E.*

*) CRA - Genomics Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (Italy)
**) CNR - Perugia (Italy)

Cold stress, Olea europeae, RNA-seq, chlorophyll fluorescence

Olea europeae is a thermophilic and eliophilic plant species perfectly adapted to warm/hot temperatures of the Mediterranean basin. Growing at colder environmental conditions provides olive oil with peculiar qualitative characteristics, thus cultivation of olive trees is becoming more and more common in Northern Italy, even away from the classically devoted lakesides. This new tendency exposes olive trees of Northern Italy to winter challenges which can affect olive production till plant survival. Even if olive trees have capacity of cold acclimation, winter warm spells can break the acclimation and expose olive plants to high risk of cold damages.

Genotype specific cold tolerance has been reported for olive trees based on empiric knowledge, thus an objective characterization of genotypes based on molecular and/or physiological strategies of cold tolerance would be more suitable. However, so far little is known about the molecular changes induced by exposition of olive trees to low temperatures and no physiological tests have been developed for an *in vivo* rapid screening of cold tolerance of olive plants.

This work aims to study the short and long term molecular changes induced in leaves of olive trees exposed to progressive lowering of temperatures, till light freezing conditions. Two *O. europeae* cultivars contrasting for cold tolerance, the tolerant Dolce Agogia and the sensitive Leccino, have been compared in order to identify gene determinants of cold tolerance. RNA-seq based on Illumina GAIIx platform is being applied for whole transcriptome analysis. Contigs are being *de novo* assembled, then annotated and counted to identify genes differentially expressed in response to cold and between the two genotypes. These analyses will be early improved through sequencing and annotation of the olive genome, cv Leccino (OLEA project). The data will indicate putative candidate genes responsible of the olive adaptation to low temperatures.

In a parallel experiment we assessed the physiological status of the plants through chlorophyll fluorescence. Indeed, cold stress causes photo-inhibition of the photosynthesis, above all impacting on the efficiency of the Photosystem II. The chlorophyll fluorescence of dark adapted leaves (Fv/Fm) measures the potential photochemical activity of PSII, thus allowing the assessment of eventual photo-damages. The two cultivars showed a significant different sensitivity to cold damages on PSII, with the cold sensitive Leccino showing lower Fv/Fm values, in particular at colder temperatures. This result suggests a possible exploitation of chlorophyll fluorescence as an objective tool for *in vivo* screening of olive cultivars for tolerance to low temperature and for phenotyping of the Leccino x Dolce Agogia mapping population with the final aim to identify the genetic bases of olive cold tolerance.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.13

RNA-SEQ AND METABOLITE PROFILING OF TWO PEACH VARIETIES

PIRONA R.*, FULIGNI F.**, PEANO C.**, RIZZI E.**, LAZZARI B.*, BELEGGIA R.***, PLATANI C.***, BASSI D.****, DE BELLIS G.**, STELLA A.*, CATTIVELLI L.***, ROSSINI L.*,****

*) Parco Tecnologico Padano, Via Einstein, 26900 Lodi (Italy)
**) Institute for Biomedical Technologies , CNR-ITB, Via Fratelli Cervi 93, 20090 Segrate (Italy)
***) Cereal Research Centre, C.R.A., S.S. 16 Km 675, 71122 Foggia (Italy)
****) Dipartimento di Produzione Vegetale, Università di Milano, Via Celoria 2, 20133 Milano

Transcriptome, metabolite profiling, SNPs, Prunus persica

In order to generate a detailed description of the transcriptome, an Illumina RNA-Seq experiment was conducted on two fruit developmental stages of peach varieties Bolero and OroA, that exhibit contrasting quality traits including flesh texture, fruit weight and aroma. In addition, GC/MS analysis was conducted on 26 metabolites belonging to primary and secondary metabolism. Mapping of Illumina reads to the publicly available peach genome sequence allowed estimation of expression levels from RNA-Seq data. Considering the two maturation stages, a total of 1274 differentially expressed transcripts, within and between the two varieties, were identified. When considering currently available peach gene annotations, the data allowed the identification of 277 intronic transcripts, 5807 novel isoforms, and 992 unknown intergenic transcripts. RNA-seq data were also a source for the identification of SNPs. Comparing Bolero and OroA transcriptomes, a total of 16804 SNPs were found. These will be used for enrichment of the SSR-based genetic map previously obtained from an F1 population from the cross of the two cultivars (Eduardo et al., 2011, Tree Genetics & Genomes 7:323–335) and QTL analysis of fruit quality traits. Benefits of using an RNA-seq approach for transcriptome analysis are discussed taking into account previous microarray experiments conducted on the same cultivars.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.14

GENOMIC CHARACTERIZATION AND EXPRESSION ANALYSIS OF GENES BELONGING TO A FAMILY OF ABC TRANSPORTERS INVOLVED IN RESPONSE TO STRESS IN *VITIS VINIFERA* L.

VANNOZZI A.*, NICOLÈ S.*, DRY, I.B.**, LUCCHIN M.*

*) Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università degli Studi di Padova, Via dell'Università 16, 35020 Legnaro (Italy) - alessandro.vannozzi@unipd.it
**) CSIRO Plant Industry, Glen Osmond, South Australia 5064 (Australia)

Vitis vinifera, stress response, PDR gene family, resveratrol

This project arose from the analysis of data obtained from a whole transcriptome sequencing approach, aimed at investigating the response of grapevine (*Vitis vinifera* L.) stilbene synthase (*STS*) genes to biotic (*Plasmopara viticola* infection) and abiotic stress (wounding and UV-C exposure). By means of Next Generation Sequencing (NGS) technology (mRNA-seq, Illumina), the pattern of expression of each member belonging to this large multigenic family was analyzed. At the same time, in an attempt to identify other class of genes potentially related to the *VvSTS* expression and activity, mRNA-seq data sets were also searched for genes displaying specific expression pattern matching that observed for the majority of STS genes, using Pavlidis Template Matching (PTM) analysis, a statistic tool of the Multi Experiment Viewer (MeV) software package. Expression patterns of approximately 33000 predictions, based on the 12X V1 coverage assembly of the PN40024 grapevine genotype, were screened in the analysis, leading to the identification of a vast range of genes co-expressed with *VvSTS*. Amongst them we identified genes involved in defense, secondary metabolism (general phenylpropanoid pathway), regulation (TFs), signaling and transport. In particular, the PTM analysis revealed a significant co-expression between *VvSTS* and several members of the pleiotropic drug resistance (PDR) sub-family of ABC transporters which are an important class of membrane-bound proteins with an ATP-binding cassette thought to be involved in the transport of secondary metabolites. Analysis of the 12X V1 grapevine genome sequence indicates the presence of at least 33 *PDR* genes in grapevine. Neighbor-joining analysis of the deduced protein sequences of these 33 *VvPDR* genes together with the already annotated *Arabidopsis PDR* genes led to the identification of seven major clusters. A number of *VvPDR* genes which show similar expression patterns to *VvSTS* genes, in response to stress treatments, were found to cluster with AtPDR12 and NpPDR1, an *AtPDR12*-like gene isolated in *Nicotiana plumbaginifolia.* Previous studies have demonstrated that NpPDR1 confers resistance against *Botrytis cinerea* infection. Furthermore, the *AtPDR12*-like ABC transporter-encoding gene *BcatrB from B. cinerea*, appears to be up-regulated by treatment with resveratrol and confers resistance of this pathogen against this phytoalexin, suggesting it may be acting as a resveratrol transporter. We are currently validating the expression patterns of individual *VvSTS* and *VvPDR* genes by qPCR in leaf discs following wounding, UV-treatment and downy mildew infection and studying protein localization using GFP fusion constructs in order to determine whether any of these *VvPDR* candidates might be involved in the resveratrol transport. A resveratrol transporter on the plasma membrane of grape cells would be crucial to avoid the accumulation of this compound to toxic

concentrations within the cell while ensuring it is delivered to the extracellular space to act as a deterrent to fungal attack.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.15

DISCOVERY OF DNA POLYMORPHISMS IN OLIVE *fad7* GENE BY ECOTILLING

MONTEMURRO C., SABETTA W., RUSEK J., BLANCO A.

Department of Agro-Forestry and Environmental Biology and Chemistry, Section of Genetics and Breeding, University of Bari, Via Amendola 165/A, 70126 Bari (Italy)

Olea europea, natural variation, ω3 fatty acid desaturase, EcoTILLING

Olive (*Olea europaea* L.) is a subtropical woody species distributed throughout the Mediterranean regions whose remarkable importance is due mainly to the oil production from its drupes. The ancient origin and the ancestral hybridisation between different species of genus *Olea* and between genetically distant populations has originated numerous varieties. Unlike other crops, olive germplasm has not suffered any genetic erosion because a turnover with new genotypes has not occurred and old plants are able to survive for a long time without cultivation. Therefore a large variability has been preserved until now, but it has not been depth studied jet. Knowledge about evolution and genetic relationships within available germplasm are helpful to allow a better choice of parental lines to be used in crosses and to enlarge the genetic basis in olive breeding programs.

A new strategy for SNP detection and characterization in natural populations is represented by EcoTILLING, a variation of TILLING which has been successfully used to examine genetic variation and to genotype several species. In combination with sequencing, EcoTILLING is an high throughput and very cost-effective technology, that allows to simultaneously screen a big number of individuals and to detect natural polymorphisms in coding regions of target genes. Moreover, individuals can be grouped according to their aplotype and distinguished in homozygous and heterozygous; finally, the effect of each identified mutation on protein structure and function can be predicted.

The screening of several web-databases and the ortholog sequences of the *fad7* gene (responsible of the 18:2 to 18:3 fatty acid desaturation) led to the identification of two olive ESTs. The sequence encoding for the fad7chloroplastic isoform was chosen as candidate gene. The genomic sequence and the structure of the gene were obtained by sequencing several overlapping PCR products; moreover, bioinformatic analysis allowed to predict the most suitable region of the gene for EcoTILLING screening.

The application of the EcoTILLING strategy to olive genome was optimized for a subset of accessions, including cultivars and clones of the same cultivar. The *Cel*I-based mutation assay and the Li-COR electrophoresis were chosen as SNP detection system. The cultivar Leccino was used as reference for the constitution of the 2-fold pools. Few polymorphisms were identified between the cultivars, confirming the high level of conservation of this gene. For example, a SNP at 630bp of the analyzed fragment allowed to distinguish the Leccino, Nociara, Toscanina and Frantoio cultivars (aplotype 1) from Cima di Melfi and Ascolana Tenera cultivars (aplotype 2). In the case of Leccino, the analysis among the 5 clones has revealed no genetic variation at the screened locus, showing the uniformity of the Leccino samples.

Additionally, to detect the heterozygosity level in the target gene, single DNA from each cultivar was analysed, supposing that an heterozygous mutation in a diploid genome could be identified by the heteroduplex formation between the wildtype allele and its mutated counterpart.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.16

HIGH RESOLUTION MELTING FOR MUTATION SCANNING OF OLEATE DESATURASE ENCODING GENE

SALIMONTI A.*, VIZZARRI V.*, LOMBARDO L.**, MICALI S.*, PERRI E.*, ZELASCO S.*

*) CRA - Centro di Ricerca per l'Olivicoltura e l'Industria Olearia, Contrada Lì Rocchi, 87036 Rende (Italy)
**) Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Via Nazario Sauro 85, 85100 Potenza (Italy)

Olea europaea,high resolution melting, mutation, desaturases, fatty acid

Oleic acid is the major fatty acid in vegetable oils and its content greatly affects both their technological properties such as oxidative stability and nutritional characteristics. Olive (*Olea europaea* L.) oil shows genetic variability in oleic acid content ranging from 49% to 83%. . The biosynthetic pathway of fatty acids has been long studied in animals and critical regulation steps have been recognized, while less is known in plants. A key enzyme is oleate desaturase (FAD 2) which catalyzes the desaturation of oleic acid into linoleic acid. In order to individuate functional markers putatively associated to oleic acid content variation, we started a candidate gene approach with a microsomial oleate desaturase (FAD 2) encoding sequence *OepFAD2-2,* isolated from olive (cv. Picual) by Hernandez *et al.* (2005). Ten pairs of gene-specific primers were designed from the full-length cDNA sequence, producing short amplification fragments (150-200 bp) and a mutation scanning approach was performed by HRM-PCR assay. A set of cultivars with a very broad oleic acid content is being analyzed. A preliminary screening on a subset of cultivars with contrasting levels of oleic acid revealed the presence of sequence variations in some samples which differentiated for their melting curves shape. Work is still in progress to assay all the phenotyped cultivars and to validate by sequencing the representatives of each variant cluster obtained in order to ultimately individuate putative functional markers.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.17

AMPELOGRAPHIC AND MOLECULAR CHARACTERIZATION OF AGLIANICO ACCESSIONS (*VITIS VINIFERA* L.) COLLECTED IN SOUTHERN-ITALY

ANACLERIO A.*, ALBA V.*, GASPARRO M.**, CAPUTO A.R.**, MONTEMURRO C.*, BLANCO A.*, ANTONACCI D.**

*) Department of Agro-forestry and Environmental Biology and Chemistry, Section of Genetics and Breeding, University of Bari, Via Amendola 165/A, 70126 Bari (Italy)
**) CRA-UTV, Agricultural Research Council - Research Unit for table grapes and wine growing in Mediterranean environment, Via Casamassima 148, 70010 Turi (Italy)

AFLP, SSR, ampelography, Aglianico, biotype

To characterize 31 different Aglianico accessions randomly collected in Southern-Italy, 30 ampelographic descriptors, 13 SSRs and 10 AFLP primer combinations were analysed.

An appreciable variation of ampelographic descriptors was mainly revealed by mature leaf traits, while very few variations were recorded for shoot and berry traits. Similarly, all SSR loci revealed molecular monomorphism and AFLPs a very high genetic similarity (Dice Coefficient) among all the accessions considered.

One of the aim of this study was to clarify the genetic assessment of Aglianico Nero and Aglianico del Vulture Nero; since they are registered as two different cultivars with distinct varietal codes at the Italian Register of Grape Varieties. Registered Aglianico Nero and Aglianico del Vulture Nero were included in the analyses, compared and used as reference material.

Our plants showed that all the accessions tested, independent from the biotype, and the two registered cultivars, belong to the same genotype, suggesting that, as reported by the Vitis International Variety Catalogue, a case of synonymy occurred between Aglianico Nero and Aglianico del Vulture Nero. These cultivars could therefore be considered as a single cultivar. Moreover, the AFLP data revealed a partial match between morphological and molecular data, showing that the AFLP molecular method was able to discriminate different accessions belonging to the same cultivar.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.18

GENETIC STRUCTURE OF A GRAPE COLLECTION AND GENETIC TRACEABILITY IN WINE MAKING CHAIN

MORCIA C.*, CARLETTI G.*, CORINO L.**, REGGIANI F.*, TERZI V.*

*) CRA - Genomics Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (Italy)
**) CRA - Centro di ricerca per l'Enologia, Via Pietro Micca 35, 14100 Asti (Italy)

Vitis, genetic diversity, genetic traceability

A wide collection of grape genotypes, including both table varieties and wine making ones, has been characterized with DarT platform. Starting from several hundreds of molecular markers the genetic structure of the collection has been studied and the relationships among genotypes characterized. A set of selected markers were the starting point for the development and evaluation of a genetic traceability approach that can be applied along the wine making chain.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.19

UNDERSTANDING THE ROLES OF VvMYB5a AND VvMYB5b IN THE REGULATION OF THE FLAVONOID BIOSYNTHETIC PATHWAY IN GRAPE

CAVALLINI E.*, HARRIS N.N.**, ROBINSON S.P.**, PEZZOTTI M.*, WALKER A.R.**, TORNIELLI G.B.*

*) Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona (Italy)
*) CSIRO Plant Industry, PO Box 350, Glen Osmond, South Australia, 5064 (Australia)

Grape, transformation, flavonoids, VvMYB5a, VvMYB5b

Flavonoids belong to a class of phenylpropanoid secondary metabolites that are important for grape and wine quality. The pathway, synthesizing the three major compounds, anthocyanins, tannins and flavonols, is highly regulated in order that different flavonoids are produced in different organs and in various stages of development. Although the branches of flavonoid biosynthetic pathway have been well studied in grapes, little is known about the transcriptional mechanism that regulates it. Two MYB transcription factors, VvMYB5a and VvMYB5b, have been recently identified as regulators of the early flavonoid structural genes at different stages of berry development.

In order to gain information about their specific roles in the regulatory network, we performed functional complementation analyses of some well characterized petunia anthocyanin/pH regulatory mutants demonstrating that VvMYB5a and VvMYB5b are involved in the activation of the flavonoid pathway and in the regulation of the vacuolar acidification in the epidermal cells of petals in petunia.

However, the use of heterologous systems to study the gene function may give results that do not reflect the true role of these MYB TF in grapevine. For this reason, *V. vinifera* cv Corvina was transformed to obtain *VvMYB5a* and *VvMYB5b* over-expressing hairy roots lines. A transcriptomic analysis using a 90 K *Vitis vinifera* oligoarray revealed that they are involved in the regulation of numerous processes including phenylpropanoid biosynthesis.

Stable transformation of *Vitis vinifera* cv Shiraz has been performed to silence *VvMYB5a* and *VvMYB5b* simultaneously. The transgenic plants present stunted growth, curly leaves accumulating anthocyanins and abnormal veins. The down-regulation of genes encoding enzymes for tannin synthesis, such as LAR (leucoanthocyanidin reductase) and ANR (anthocyanidin reductase) in the silenced lines may explain the accumulation of pigmentation in leaves.

In addition, to verify if they could also exhibit different functions, each gene has been over-expressed independently. The over-expression of *VvMYB5b* leads to an accumulation of anthocyanins in leaves, which is not visible in plants over-expressing *VvMYB5a*. Taken together, these preliminary results suggest that VvMYB5b may be involved in the regulation of the anthocyanin synthesis, while VvMYB5a could be responsible for tannin production.

Transcriptomic and metabolomic analyses of the transgenic plants will provide more conclusive information about the specific roles of VvMYB5a and VvMYB5b and will help to clarify the regulatory network of the flavonoid biosynthetic pathway.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.20

GENETIC VARIABILITY OF AN ITALIAN APPLE GERMPLASM COLLECTION

DONDINI L., PARIS R., LIANG W., DE FRANCESCHI P., VENTURI S., GENNARI F., TARTARINI S.

Department of Fruit Tree and Woody Plant Sciences, University of Bologna, Viale Fanin 46, 40127 Bologna (Italy)

Malus x domestica, SSR, association study, fruit quality, breeding

Breeding apple varieties of high quality, merging grower demands and consumer satisfaction requests, is a challenging task. In this species, strict self-incompatibility, slow growth and long juvenile period of seed-derived plants hamper the efficient crossing and selection of desired genotypes. The long generation cycles make the marker-assisted selection strategic in apple breeding. By this approach molecular markers, linked to genes of desired traits are used instead of the traditional phenotypic based selection. The genetic variability among apple varieties and germplasm accessions represents the basis for association studies.

In this work, a panel of apple simple sequence repeats (SSRs) markers was chosen to study the extent and distribution of genetic variability in a collection of more than 400 apple accessions of the Italian germplasm maintained in the experimental fields of the Dept. of Fruit Tree and Woody Plant Science, University of Bologna. The cluster analysis allowed to solve practical doubts between synonymy and homonymy of genotypes with a similar phenotype and to describe the relationships among the Italian apple germplasm varieties that represent a wide source of genetic diversity in which discover allelic forms of functional genes useful for breeding purposes. This analysis made also possible the identification of various triploid genotypes. Moreover, the same set of cultivars was phenotyped for fruit quality traits.

This work was carried out within the framework of the ‘Fruit Breedomics EU project’ and represents a preliminary step in the definition of an Italian apple ‘core collection’. The wide panel of selected genotypes will be used to improve the knowledge of genetic variability distribution by exploring the phenotypic and allelic diversity available in the apple germplasm and it allows the identification of the genomic regions involved in the genetic control of major horticultural traits through genome-wide analyses.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.21

A SENSITIVE AND SPECIFIC TOOL FOR THE DETECTION OF *MAL D 1* ALLERGEN GENE TRANSCRIPTS IN APPLE

PARIS R.*, PAGLIARANI G.*,**, ARENS P.**, VAN DE WEG E.**, TARTARINI S.*

*) Department of Fruit Tree and Woody Plant Sciences, University of Bologna, Viale Fanin 46, 40127 Bologna (Italy)
**) Plant Breeding, Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen (The Netherlands)

Malus x domestica, PR-10, gene family, allergenicity, qPCR

While apples are usually recommended for an healthy diet, apple allergenicity is becoming an important issue for a growing number of European citizens. In fact, apples are one of the most allergenic foods, ranking first among the *Rosaceae* fruits. Mild oropharyngeal symptoms to fresh apples, known as Oral Allergy Syndrome, are common in north-west European population. This phenomenon is primarily due to the cross-reactivity between Bet v 1, the major allergen in birch pollen, and its homologue protein in apple, Mal d 1. *Mal d 1* is the most complex allergen gene family of apple, composed by 31 members. *Mal d 1* genes are mainly organized in two clusters on the homeologous LGs 13 and 16 but other sequences have been found also on LG6, 1 and 4. They code for different Mal d 1 isoforms that share a high sequence and structural similarity. These proteins are classified as Pathogenesis Related Proteins of class 10, for which the biological function is not well understood yet. Despite the high similarity, different Mal d 1 proteins have various functional specialisation and different binding affinities to IgE, suggesting a different importance in allergenicity. Therefore, it is necessary to understand each Mal d 1 specific characteristic and function. Up to now a fine resolution was successfully obtained at structural genomic level but functional studies are still poor and not exhaustive, often limited to few genes. The aim of this work was to develop a tool for the specific study of each single *Mal d 1* isoallergen gene from a quantitative point of view. SNPs among genes were exploited to unravel the complexity of *Mal d 1* expression and a complete set of 31 highly specific primer pairs for qPCR with the SYBR Green chemistry was developed. The expression profiling of the *Mal d 1* family in different tissues and in response to stresses will allow a wide comprehension of the feature and putative function of each one, with important implications both for basic research and for apple breeding that, through expression and association studies, can be directed to low allergenic fruits, desirable to meet nutritional needs and to diminish dietary restriction.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.22

CHARACTERIZATION OF A *VITIS VINIFERA* GH3 GENE FAMILY INVOLVED IN THE CONTROL OF HORMONE LEVELS

DI RIENZO V.*, BÖTTCHER C.**, MONTEMURRO C.***, BLANCO A.***, DAVIES C.**

*) Department of Genetics and Plant Breeding University of Bari "Aldo Moro", Via G. Amendola 165/A, 70126 Bari (Italy)
**) CSIRO Plant Industry PO Box 350 Glen Osmond SA 5064 (Australia)
***) Department of Agro-Forestry and Environmental Biology and Chemistry, Section of Genetics and Breeding, University of Bari, Via Amendola 165/A, 70126 Bari (Italy)

Vitis vinifera, GH3 enzymes, hormones

Grapevines (*Vitis vinifera* L.) produce non-climacteric fruit that exhibit a double sigmoidal pattern of growth. Ripening occurs during the second growth phase when grapes change colour, start to soften, accumulate reducing sugars, metabolise organic acids and synthesise flavour compounds. All these biochemical and physiological changes affect the quality of the fruit and therefore of the wine. Although the physiological processes underlying the ripening have been described the mechanisms that control the ripening of grape berries are not well known. Abscisic acid, ethylene and brassinosteroids are considered as promoters of ripening, as treatments of immature berries with these hormones can advance ripening. In grape, auxin levels are high early in development, then decline towards the onset of ripening (veraison). Indole-3-acetic acid (IAA) is the most abundant auxin in grape berries. Auxins can delay ripening when applied at an appropriate time prior to veraison. One important mechanism for controlling the levels of free, biologically active IAA is its enzymatic conjugation to amino acids. GH3 enzymes, encoding IAA-amido synthetases, are responsible for this conjugation. Previous phylogenetic analyses of *Arabidopsis thaliana* GH3 proteins classified them into three groups based on sequence similarity. Group II enzymes have been shown to be active on IAA and a member of group I conjugates jasmonic acid to amino acids.

In order to elucidate the involvement of GH3 genes in grape berry ripening, we studied seven GH3 genes, six of which are IAA-amido synthetases, the other is a jasmonic acid-amido synthetase. The primary objective was to determine the subcellular localization of these enzymes. GFP-protein fusion constructs for all seven enzymes were transiently expressed in capsicum by biolistic bombardment and the transformed cells were scanned by fluorescence microscopy. All of these proteins displayed a cytosolic localization, confirming the *in silico* prediction. In order to further understand the likely function of these genes their expression patterns were analysed in different tissues comparing the varieties Shiraz and Cabernet Sauvignon. All of the IAA-amido synthetase genes showed different patterns of expression suggesting that although they all conjugate IAA to amino acids there is a degree of specialisation at the organ level.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.23

CHARACTERIZATION OF THE MAIN OLIVE CULTIVARS IN MOLISE REGION BY MEANS OF SSR MOLECULAR MARKERS

D'ANDREA M.*, PILLA F.*, LIMA G.*, SCARANO M.-T.**

*) Department of Animal, Plant and Environmental Sciences, University of Molise,
Via F. De Sanctis snc, 86100 Campobasso (Italy)
**) Institute of Plant Genetics – CNR, Research Division Portici

Germplasm, barcode, Olea europeae

A very rich olive germplasm resource is present in Italy, which represents an interesting gene pool for agriculture, environment and sustenance. To this purpose, bio-molecular technology have been set up in order to clearly establish the identity of the most interesting olive cultivars in some of the Italian Regions involved in a national research project named 'OLVIVA'; characterization and exploitation studies have been carried out to the aim of eliminating cases of mislabelling and redundancies (synonymy) and to provide analytical tools for the genetic certification of the propagation plants. In particular, in the Molise region, we hav characterized eight olive cultivars from this region, cultivated at the Molise ARSIA (Regional Agency for Innovation and Development in Agriculture) catalogue field located in Larino (CB), settled for the conservation and development of local olive germplasm. Previously, the procedure of molecular characterization was optimized using 'Ring Test' method, using five DNA samples of unknown origin and 17 molecular markers (microsatellite) arranged with the specific referee. Touch down-PCR, set up for the 17 SSR loci, have been successively analyzed through capillary electrophoresis using ABI PRISM 310 automatic sequencer (Applied Biosystems). Data analysis has been carried out with Gene Mapper software (AB, version 4.0) for allele evaluation.

The same procedure has been successively transferred for the molecular characterization of the eight olive cultivar of Molise region, which were chosen in this project: Gentile di Larino, Oliva Nera di Colletorto, Aurina, Rosciola di Rotello, Cerasa di Montenero, Sperone di Gallo, Paesana Bianca e Salegna di Larino. Molecular characterization results showed that SSR locus named EMO-L was not useful for the characterization, because it produces unvaried alleles for all the analyzed samples and therefore it is not discriminating. In general, all the other loci produce different alleles (from the 3 alleles in DCA-15 locus to 11 allels for DCA18 locus) and permit thence to discriminate all the analyzed cultivars. With all the resulting sizes it was also possible to create, for each, a sort of barcode profile that quickly visualizes the different pattern of the 8 cultivars, revealing the variations. In fact, they resulted to be all different and therefore recognizable by means of the technique used in this research and therefore they do not present case of synonymy.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.24

COMPARATIVE GENOMICS FOR IDENTIFYING FLOWER ORGAN IDENTITY GENES IN PEACH AND OLIVE

GALLA G.*, BOTTON A.*, BALDONI L.**, MULEO R.***, PERROTTA G.****,
RAMINA A.*, BARCACCIA G.*

*) Dept. of Environmental Agronomy and Crop Science, College of Agriculture, University of Padova – Campus of Agripolis, Viale dell'Università 16, 35020 Legnaro (Italy)
**) CNR – Institute of Plant Genetics, Via Madonna Alta 130, 06128 Perugia (Italy)
***) Dept. of Crop Production, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)
****) ENEA Centro Ricerche Trisaia, S.S. 106 Ionica Km 419,5, 75026 Rotondella (Italy)

Olea europaea, MADS-box sequences, flower-organ identity genes

Important fruit trees as peach (*Prunus persica* L.) and olive (*Olea europaea* L.) produce fruits with closely similar features, in both cases being drupes. Despite such similarity, the flowering induction pathways of these two species are quite different. In order to shed light on phase transition and flower differentiation, specific bioinformatic analyses were carried out in peach and olive, leading to the identification of MADS-box genes (*i.e.* ABCDE model-related genes). MADS-box genes include floral homeotic genes that participate to the determination of floral organ identity. Since in peach most of the putative MADS-box genes have been already identified and the expression patterns studied, these data were used to validate our bioinformatic approach, which was carried out on the recent public release of the genome. A double experimental approach was set up, starting from available genes whose function was already characterized not only in model plants but also in crop species. HMM patterns were constructed by performing multiple sequence alignments of proteins involved in phase transition and flower differentiation, and used to query the genome for putative orthologs. Concurrently, a BLAST search was carried out using the same set of sequences. Results of both approaches were cross-checked and a list of candidate genes generated and exploited to further validate peach genes previously characterized. The same pipeline was adopted to search for olive candidate genes. Since the genome sequence of this species is not available, a 454 collection recently generated from flower buds at different developmental stages was used as a target. The nucleotide sequences were translated in all possible frames, so that the same approach adopted for peach could be carried out. In peach, the list of candidates was implemented with further members, such as four AP2- and two AP3-like genes, with a putative A and B function, respectively. In olive, a higher number of candidates was identified compared to peach, probably due both to the larger size and the polyploid origin of its genome, as well as to the presence of different alleles of the same gene (being most of the loci heterozygous in olive and homozygous in peach). It is worth mentioning that in this species a total of 21 members were identified, including four AP1, two AP3, three PI, one each of AG, STK and SHP, and eight SEP2, SEP3 and SEP4-like genes. Detailed phylogenetic analyses were performed at the amino acid level, pointing out homogeneous clusters in which candidates of the same class group together with proteins already characterized in model species (*i.e*., *Arabidopsis* and *Antirrhinum*). Expression analyses of candidates are currently in progress in order to assess the organ specificity and the timing of

expression. The role of these genes in determining discrepancies and similarities in terms of phase transition pathway and fruit type, respectively, will be presented and critically discussed.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 3A.25

CYTOLOGICAL AND MOLECULAR EVIDENCES SUPPORT A SPOROPHYTIC SELF-INCOMPATIBILITY SYSTEM IN OLIVE

COLLANI S.*, ALAGNA F.**, CACERES M.E.**, COLAO M.C.***, GALLA G.*, RAMINA A.*, BALDONI L.**, MULEO R.***, PERROTTA G.****, BARCACCIA G.*

*) Dept. of Environmental Agronomy and Crop Science, College of Agriculture, University of Padova – Campus of Agripolis, Viale dell'Università 16, 35020 Legnaro (Italy)
**) CNR – Institute of Plant Genetics, Via Madonna Alta 130, 06128 Perugia (Italy)
***) Dept. Crop Production, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo (Italy)
****) ENEA Centro Ricerche Trisaia, S.S. 106 Ionica Km 419,5, 75026 Rotondella (Italy)

Olea europaea, sporophytic self-incompatibility, OeSRK, OeSLG and OeSCR

Olea europaea L. is one of the oldest agricultural tree crop species and, in spite of the great economical and cultural impact, a few studies have been carried out on its reproductive barriers. The aim of this research was to elucidate the self-incompatibility system in olive from cyto-histological and bio-molecular standpoints. Self-incompatibility is one of the most effective systems adopted by flowering plants to prevent inbreeding, maintaining so diversity within the species. Olive is actually classified as a gametophytic self-incompatible (GSI) plant because of distinctive morphological traits, as wet-type pistil and bi-nucleated pollen. However, detailed cytological analyses of more than 34,000 pollen grains performed using pistils of self-compatible and self-incompatible cultivars under self-pollination and open-pollination conditions, were not in agreement with GSI. In fact, only 4-10% of the total pollen grains, varying with the cultivars, were germinated and none of the emerging pollen tubes was able to penetrate the stigma surface of self-incompatible cultivars (Leccino, Moraiolo and Dolce Agogia) after self-pollination. It is worth noting that GSI reaction normally occurs in the transmitting tissue of the style, being controlled by specific RNases. Furthermore, no results were achieved by molecular analyses aimed at cloning genes involved in the GSI system. Vice versa, our cytological observations were in agreement with a sporophytic self-incompatibility (SSI). The molecular attempts to isolate candidates for SSI led us to the cloning of two *OeSRK* (S-locus Receptor Kinase, the female determinant) and two *OeSLG* (S-locus Glycoprotein, an enhancer of the incompatibility response) genes. As far as the male determinant is concerned, we were not able to isolate any candidate by routine molecular approaches, such as PCR with degenerated primers and RACE experiments, because of its high intra-specific nucleotide variability. However, a screening of about 465,000 ESTs, belonging to olive flower-specific libraries, allowed us the definition of the SCR-like (S-locus Cysteine Protein, the male determinant) pattern and the isolation of 28 contigs showing SCR-like features. Then, quantitative Real-Time PCR assays enabled the identification of one *OeSCR*-like gene, showing a strong anther-specific expression at the time of pollen dispersal. Moreover, quantitative Real-Time PCR assays, replicated using different subdomain-specific primer combinations, revealed an antagonist transcriptional expression pattern in flowers of cultivars Leccino and Frantoio (the latter being self-compatible) for the genes *OeSRK* and *OeSLG*. All the genes related to a putative SSI system are currently tested by means of *in situ* hybridization in flowers of both self-incompatible and self-compatible cultivars to study their spatial gene expression patterns and domains. Yeast-

two-hybrid screenings are also in progress to test the protein-protein interaction between our male and female candidates. On the whole, a new hypothesis for the genetic system controlling the self-incompatibility reaction can be postulated for olive.