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Fruit Germplasm Characterization: Genomics Approaches for the Valorisation of Genetic Diversity

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1. Introduction

The introduction of modern varieties of staple crop in agriculture seem to lead to an overall decrease in genetic diversity, although within the released varieties themselves the data are inconsistent and no overall narrowing of the genetic base can be discerned. The situation regarding genetic erosion in landraces and crop wild relatives is equally complex. While many recent studies have confirmed that diversity in farmers' fields and protected areas has eroded, this is not universally the case.

Many reports expressed continuing concern over the extent of genetic vulnerability and the need for a greater deployment of diversity. However, better techniques and indicators are needed to monitor genetic diversity, to establish baselines and monitor trends.

In recent years, there is evidence of growing public awareness with regard to the importance of genetic diversity, both to meet increasing demands for greater dietary diversity, as well as to meet future production challenges. The increased environmental variability that is expected to result from climate change implies that in the future, farmers and plant breeders will need to be able to access an even wider range of plant genetic resources for food and agriculture than today.

The existing *ex situ* collections of fruit trees germplasm may valuably provide either a source of genes potentially useful as raw material in plant breeding, or plants directly valid for a sustainable production. With respect to the latter item, we refer to those local varieties that, having evolved for a very long period in a location, and having developed adaptative traits, well integrated with the environmental, agronomic, cultural and traditional features of the site and more or less recently have been replaced with new varieties. The requirements of modern agriculture, such as sustainability call for the cultivation of a wider range of diverse material that could better respond to the different aspects involved. Specifically, if it is necessary to obtain new varieties with a broader genetic base, capable of producing under diverse conditions and to respond to different stresses - *i.e.* pests, drought, low fertility of the soil etc. On the other hand, in some cases, the re-introduction of old local varieties and

the safeguard of traditional farming systems and landscapes can be very profitable from an economic and socio-economic point of views. In general, the lack of information about plant genetic resources conserved have the effect of limiting the use that can be made of large existing collections, restricting the value and the usefulness of a collection even within the owning institute and among other potential users. Hence, assessing the traits of the germplasm conserved in a collection is an essential prerequisite to a proper and wide utilization of the plant material conserved and it is the first step toward a further definition of the roles that the varieties can play in sustainable production, through the direct use or in breeding programmes.

In the past few years, several studies based on comparative high throughput sequencing of plant transcriptomes have, indeed, allowed the identification of new gene functions, contaminant sequences from other organisms, alterations of gene expression in response to genotype, tissue or physiological changes, as well as large scale discovery of SNPs (Single Nucleotide Polymorphisms) in a number of model and non model species, such as maize, grapevine and eucalyptus (Costa *et al.*, 2010).

Among the cultivated plants, olive (*Olea europaea* L.) is the sixth most important oil crop in the world, presently spreading from the Mediterranean region of origin to new production areas, due to the beneficial nutritional properties of olive oil and to its high economic value. The Mediterranean basin is the traditional area of olive cultivation and has 95% of the olive orchards of the world. From the Mediterranean basin, olive cultivation is presently expanding into areas of Australia, South and North America (Argentina, Chile, United States) and South Africa.

It belongs to the family of Oleaceae, order of Lamiales, which includes about 10 families for a total of about 11,000 species. Members of this order are important sources of fragrances, essential oils and phenolics claiming for numerous health benefits, or providing valuable commercial products, such as wood or ornamentals. Information on the genome sequence and transcript profiles are completely lacking. Olive is a diploid species, predominantly allogamous. In spite of its economical importance and metabolic peculiarities, very few data are available on gene sequences controlling the main metabolic pathways.

In spite of its economical importance and metabolic peculiarities, very few data are available on gene sequences controlling the main metabolic pathways in olive. With regard to oil, a range of biochemical methods to study the traceability of olive oil has to be presented. In fact, the analysis of minor and major components present in olive oil represents a valuable tool for authentication purposes.

Food authenticity has become a focal point for producers, consumers and policy markers. The DNA based technology is gaining a great attention in the field of food authenticity. This technology makes use of molecular markers such as RAPD, AFLP and SSR more efficient and constitutes promising approach for variety characterization and oil traceability in olives.

In this context, the acquisition of additional information on biochemical markers in olive represents a fundamental and indispensable step to preserve the main olive varieties and also to safeguard the minor genotypes, in order to avoid a loss of genetic diversity and offer an important genetic basis for future breeding programs.

2. Management of germplasm collection

Plant genetic resources are essential to a sustainable agriculture and food security. FAO estimates humans have used some 10,000 species for food throughout history. However, only about 120 cultivated species provide around 90% of food requirements and 4 species (Maize, Wheat, Rice and Potatoes) provide about 60% of human dietary energy for the world's population. Of the myriad of varieties of these crops developed by farmers over millennia, which form an important part of agricultural biodiversity, more than 75% have been lost in the past 100 years.

Some fear that corporate financial interests might prevent safeguarding of livelihoods, promotion of food security, biodiversity-rich farming under control of local communities.

The best way of conserving fruit germplasm collection is their utilization. However, today these resources are not only underutilized but also under conserved. The Global Plan of Action therefore supports activities improving *in situ* and *ex situ* conservation of plant collection. Regarding *ex situ* conservation, millions of accessions are already stored in hundreds of germplasm collections around the world for both conservation and utilization purposes. Find short descriptions about these germplasm databases and links to their websites by searching either by database type or by free text search.

2.1 The *in situ* management of germplasm collection

Awareness of the importance and value of crop wild relatives and of the need to conserve them *in situ* has increased. A global strategy for crop wild relatives preservation and use has been drafted, protocols for the *in situ* conservation of crop wild relatives are now available. The number and coverage of protected areas are expanding the last years and this has indirectly led to a greater protection of crop wild relatives.

Important progress has been made in the development of tools and techniques to assess and monitor plant genetic resources for food and agriculture within agricultural production systems. Countries now report a greater consideration of the amount and distribution of genetic diversity in the field, as well as the value of local seed systems in maintaining such diversity. More consideration is now being paid in several countries to increasing genetic diversity within production systems as a way to reduce risk, particularly in light of changes in climate, pests and diseases. The number of on-farm management projects is increased somewhat and new legal mechanisms have been put in place in several countries to enable farmers to market genetically diverse varieties. There is still a need for more effective policies, regulations governing the *in situ* and on-farm management of plant genetic resources for food and agriculture, both inside and outside protected areas, and closer collaboration and coordination are needed between the agriculture and environment sectors. Many aspects of *in situ* management still require further research and strengthened research capacity is required in such areas as the taxonomy of crop wild relatives and the use of molecular tools to conduct inventories and surveys.

2.2 The *ex situ* management of germplasm collection

The total number of varieties conserved *ex situ* international has reached 7.4 million. While new collecting accounted for at least 240,000 varieties, and possibly considerably more,

much of the overall increase is the result of exchange. It is estimated that less than 30% of the total number of varieties are distinct (FAO, 2010). There is still a need for greater rationalization among collections globally.

The existing *ex situ* collections of fruit tree germplasm may valuably provide either a source of genes potentially useful as raw material in plant breeding, or plants directly valid for a sustainable production. With respect to the latter item, we refer to those local varieties that, having evolved for a very long period in a location, and having developed adaptive traits well integrated with the environmental, agronomic, cultural and traditional features of the site and more or less recently have been replaced with new varieties. The needs of modern agriculture, such as sustainability call for the cultivation of a wider range of diverse material that could better respond to the different aspects involved. Specifically, if it is necessary to obtain new varieties with a broader genetic base, capable of producing under diverse conditions and to respond to different stresses – *i.e.* drought, pests, low fertility of the soil etc. –, on the other hand, in some cases, the reintroduction of old local varieties and the safeguard of traditional farming systems and landscapes, can be very profitable from an economic and socio-economic point of views. In general, the lack of information about plant genetic resources conserved have the effect of limiting the use that can be made of large existing collections, restricting the value and the usefulness of a collection even within the owning institute and among other potential users. Hence, assessing the traits of the germplasm conserved in a collection is an essential prerequisite to a proper and wide utilization of the plant material conserved and it is the first step toward a further definition of the roles that the varieties can play in sustainable production, through the direct use or in breeding programmes.

Germplasm collections established and maintained by genebanks provide for the present and future utilization of plant genetic resources. In the early stages of collection development, the focus was mainly on acquisition *per se*, and less on optimizing collection composition. Many germoplasm collections were started from working collections that had been used to support specific purposes, including breeding, crop improvement and taxonomic studies. In many cases, germplasm collections expanded their collections thereafter by including obsolete varieties, research lines or samples obtained from collecting missions to natural distribution areas of crops and their wild relatives.

There is still a need for greater rationalization among collections globally. Scientific understanding of the on-farm management of genetic diversity has increased. While this approach to the conservation and use of plant genetic resources for food and agriculture is becoming increasingly mainstreamed within national programmes, further efforts are needed in this regard. With the development of new molecular techniques, the amount of data available on genetic diversity has increased dramatically, leading to an improved understanding of issues such as domestication, genetic erosion and genetic vulnerability.

The largest total numbers of *ex situ* varieties are of wheat, rice, barley and maize accounting for 77% of the total cereal and pseudo-cereal holdings. Other large cereal holdings include sorghum (about 235,000 varieties) and pearl millet (more than 65,000 varieties; FAO, 2010). In some tropical countries, roots and tubers, including cassava, potato, yam, sweet potato and aroids, are more important as staple foods than cereals, but being more difficult to conserve, collection sizes tend to be smaller. Centro Internacional de la Papa (CIP, Spain)

holds the world's largest sweet potato collection (more than 6,400 varieties) as well as the third largest potato collection (representing about 8% of total world holdings of about 98,000 varieties) after those of the Institut National de la Recherche Agronomique (INRA, France) and N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (Russian Federation).

Other important collections of olive tree (*Olea europaea* L. subsp. *europaea* var. *europaea*) are found at several Mediterranean countries at Aegean Agricultural Research Institute of Turkey (AARI, Turkey), Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Centro di Ricerca per l'Olivicoltura e l'Industria Olearia (Agricultural Research Council - Olive growing and Oil Industry research centre, CRA-OLI, Italy), Horticulture and Subtropical Crops Research Institute (HSCRI, Azerbaijan), Junta de Andalucía, Instituto Andaluz de Investigación Agroalimentaria y Pesquera, Centro de Investigación y Formación Agroalimentaria Córdoba (CIFACOR, Spain), National Plant Gene Bank of Iran was placed in the Seed and Plant Improvement Institute (NPGBI-SPII, Iran). The largest olive collection (accounting for 17% of the total olive trees with more than 500 varieties) is held by CRA-OLI in Italy, followed by the collections of the CIFACOR in Spain.

The systematic collection of Italian olive varieties for deposit into specific catalogue fields began in Italy in the 1980s. A similar international collection was begun in 1997 by CRA-OLI of Rende, Italy. Collection entailed the following steps: a survey of the territory, individuation, basic characterization, and introduction into the gene bank field. Material identified by other international scientific institutions (International Treaty on Plant Genetic Resources for Food and Agriculture - Plant Genetic Resources RGV-FAO Projects) was also included. To date, roughly 500 varieties have been introduced into the CRA-OLI collection, and this list has been published (web site <http://apps3.fao.org/wiews/olive/oliv.jsp>).

The olive tree is one of the oldest cultivated plants, and its fruit has been used for nourishment for more than 5,000 years in the Mediterranean regions where it originated. Over the last few centuries, cultivation of the olive tree has spread to North and South America, as well as Japan, South Africa, and Australia. Due to the tree's need for a warm but not excessively hot climate, it can be cultivated in both the northern and southern hemispheres between 30 and 45 degrees latitude, with the exception of some equatorial regions where olive trees are grown at high altitude. Nowadays, olives are produced in more than 40 countries spread across all six inhabited continents, and even in exotic places like Hawaii.

A useful olive germplasm collection also requires an organizational system devoid of homonymy, synonymy and mislabelling so that a reliable classification of all varieties can be achieved without unnecessary confusion. Recent research has focused on using morphology and biochemical and molecular markers to characterize and identify olive varieties. The identification of varieties and varieties using molecular markers is a crucial aim of modern horticulture, because such a technique would greatly facilitate breeding programmes and germplasm collection management.

3. Olive germplasm characterization

The genetic patrimony of the Mediterranean Basin's olive trees are very rich and is characterised by and abundance of varieties. Based on estimates by the FAO Plant

Production and Protection Division Olive Germplasm (FAO, 2010), the world's olive germplasm contains more than 2,629 different varieties, with many local varieties and ecotypes contributing to this richness.

The olive tree is a member of the Oleaceae family, which contains the genera *Fraxinus*, *Forsythia*, *Forestiera*, *Ligustrum*, and *Syringa*, in addition to the genus *Olea*. The genus *Olea* of the sub-family *Oleideae*, includes two sub-genera, *Olea* and *Paniculatae*. According to recent revisions of the *Olea europaea* taxonomy (Green, 2002), this species is divided into the following six sub-species based on morphology and geographical distribution:

1. subsp. *europaea*, divided into the two botanical varieties: the wild olive or oleaster (var. *sylvestris*) and the cultivated olive (var. *europaea*), distributed in the Mediterranean Basin;
2. subsp. *cerasiformis*, present in Madeira Island;
3. subsp. *cuspidata*, distributed from South Africa to southern Egypt and from Arabia to northern India and south-west China;
4. subsp. *guanchica*, present in the Canary Islands;
5. subsp. *laperrinei*, localized to the Sahara region;
6. subsp. *maroccana*, present in south-western Morocco.

Commercial olives are products of *Olea europaea* subsp. *europaea* var. *europaea*, as only this species produces edible fruit. The cultivated olive tree can reach heights ranging from just a few meters to 20 meters. The trunk is irregular, and the branches bear evergreen, elliptical and/or lanceolate leaves whose upper and lower surfaces are green and silvery, respectively. The olive tree (photo 1) is a long lived evergreen and some specimens have been reported to live for nearly 2,000 years. Its wood can resist decay, and when mechanical damage or environmental extremes kill the top of the tree, new growth arises from the root system.

Olive trees were multiplied by using different explants including ovule (spheroblast) and subsequently leafy stem cutting and grafting on seedlings or clonal stocks. Vegetative reproduction potential varies, which is dependent on genotype, e.g. easy to rooting and recalcitrant to root initiation (Hartmann and Kester 1968). Micropropagation of the olive variety was successful on OM medium (Rugini, 1984) and subsequently several other researchers slightly modified the culture medium by adding different growth substances or rooting conditions (Cozza *et al.*, 1997; Mencuccini, 2003). The micropropagated materials (photo 2) can be used to screen for resistance to biotic and abiotic stress and for genetic improvement activity (Rugini *et al.*, 2000; Sasanelli *et al.*, 2000; Bartolozzi *et al.*, 2001).

When propagated by either seed or cuttings, the root system generally is shallow, spreading to only 0.9-1.2 meters even in deep soils. The above ground portion of the olive tree is recognizable by its dense assembly of limbs, short internodes, and the compact nature of the foliage. Light does not readily penetrate into the interior of an olive tree unless the tree is pruned to create light channels. If left unkempt, olive trees develop multiple branches with cascading limbs. The branches are able to bear large quantities of fruit on their terminal twigs, which are pendulous, flexible, and sway with the slightest breeze.

Olive leaves (photo 3) are thick, leathery, and oppositely arranged. The silvery green leaves are oblong in shape, measuring 4-10 centimetres long and 1-3 centimetres wide. Leaves

have stomata on their lower surfaces only. Stomata are nestled in peltate trichomes that restrict water loss and make the olive tree relatively resistant to drought. Some multicellular hairs are present on the leaf surfaces. Each leaf grows over a two year period. Olive leaves usually abscise in the spring after they are 2 or 3 years old. As with other evergreens, however, leaves older than 3 years are often present. Flower bud inflorescences (photo 4) are borne on each leaf's axil. The small white, feathery flowers, with ten cleft calyx and corolla, two stamens and bifid stigma. The bud is usually formed during one season, at which point it can remain dormant for more than a year before beginning visible growth during the subsequent season. After the buds become viable inflorescences, flowers bloom a season later than expected. Each inflorescence contains between 15 and 30 flowers, depending on the variety and on the extent of that year's development.



Photo 1. Calabrian secular olive (*Olea europaea* subsp. *europaea* var. *europaea*) trees

The olive fruit is a drupe (photo 5), botanically similar to the almond, apricot, cherry, nectarine, peach, and plum. The olive fruit consists of an exocarp, a mesocarp and an endocarp. The exocarp represents the 1.5-3.5% of the total fruit; it is free of hairs and contains stomata. The mesocarp represents the 70-80% of the total fruit; it is the tissue that is eaten, and the endocarp is woody and represents the 13-24% of the total fruit and encloses the seed (2-4% of the total fruit).

Quantitatively, the largest constituents of the drupe are water (40-70%) and oil (6-25%). The biochemical composition of olive oil consists of a major portion that includes triacylglycerols and that represents more than 98% of the total oil weight and a minor ones, that is present in very low amount (about 2% of oil weight), including more than 230 chemical compounds such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants (tocopherols and phenolic compounds).



Photo 2. *In vitro* propagation of olive (*Olea europaea* subsp. *europaea* var. *europaea*) trees through micro-grafting



Photo 3. Olive (*Olea europaea* subsp. *europaea* var. *europaea*) leaves: top side and under side



Photo 4. Olive (*Olea europaea* subsp. *europaea* var. *europaea*) inflorescence (raceme)

The phenolic compounds have shown their relevance in the production of virgin olive oil, typical food of the Mediterranean culture because of their bioactive contribution to sensory characteristics, to stability toward autoxidation, and to human health beneficial effects (Muzzalupo *et al.*, 2011, Servili *et al.*, 2004). Olive fruit pulp naturally possesses a bitter taste due to the presence of the glycoside oleuropein (photo 6), (Bianco *et al.*, 1999, 2001; De Nino *et al.*, 2005).



Photo 5. The olive fruit (drupe) at different ripening stages

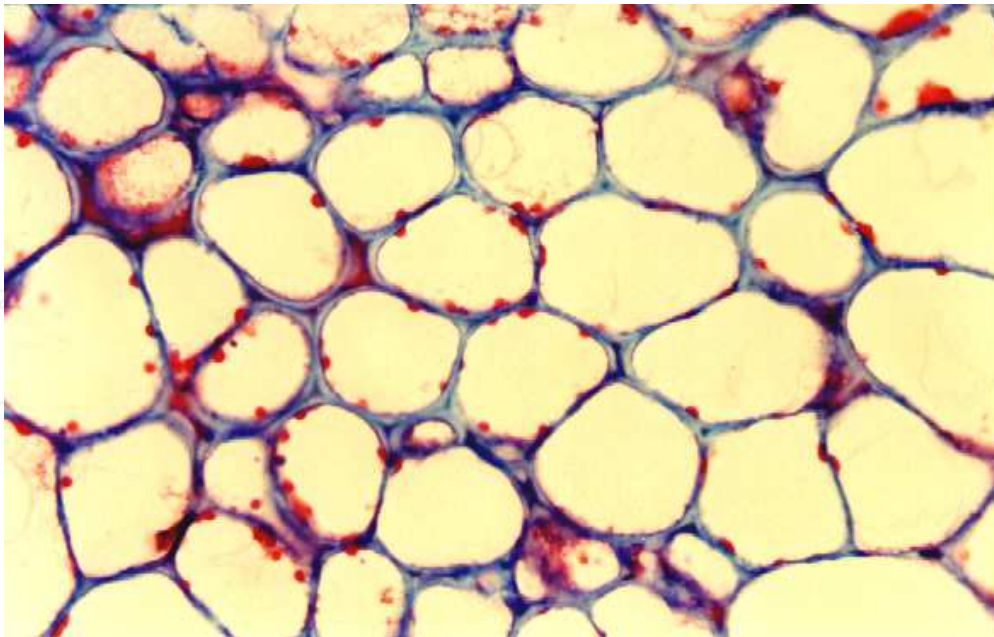


Photo 6. Cytological features of olive fruits. Fruit longitudinal sections stained with safranin O/azur II;

The olive tree and its products can be damaged from many diseases and pests. The most dangerous are the bacterium *Pseudomonas savastanoi* (photo 7), which produce tubercles forms on the branches and stems, the fungus *Cycloconium oleaginum* that damage the leaves and fruits and *Verticillium dahlie* that is destructive for the root apparatus and the growth of the plants. Among phytophagous, most harmful are the olive fruit fly (*Bactrocera olea* Gmelin), the olive moth (*Prays oleae* Bernard) and black scale (*Saissetia oleae* Olivier). Olive fruit fly is the major pest and can cause severe economic damage to olive production, which effect oil extraction and table use (photo 8).



Photo 7. *Pseudomonas savastanoi* olive or tuberculosus



Photo 8. Olive fruit fly (*Bactrocera olea* Gmelin)

Varieties are predominantly diploid ($2n = 2x = 46$) (Minelli *et al.*, 2000). The DNA content is 2.2 pg per 1C nucleus (Bitonti *et al.*, 1999), correspondent to a genome size of 2.2 Gbp (De la Rosa *et al.*, 2003).

Over the millennia, new varieties have originated by genetic mutation, by spontaneous crossing with a subsequent natural dissemination of stones. Also an important factor in the development of locally specific varietal populations was sexual reproduction, involving populations of local wild *Olea* and those selected to the criteria of local farmers (Breton *et al.*, 2006). If agreeable by humans, that new varieties were established by vegetative means. The longevity of the olive tree and the selection of a large number of varieties have contributed to the conservation of its variability and allowed to pass a large proportion of this genetic diversity (Rallo *et al.*, 2000). Another factor that has contributed to increasing the biodiversity of this species is the wide genetic variability of olive that has been created and distributed freely without any concern for loyalty to a morphologically defined archetype because the end product is not the whole fruit, such as for most other fruit trees, but the result of squeezing the fruit: the virgin olive oil. This has led, over time, to the formation of polyclonal varieties of heterogeneous phenotype (varieties-populations) rather than the formation of monoclonal varieties. Intra-varietal polymorphisms in fact, have been reported in the literature (Lopes *et al.*, 2004; Muzzalupo *et al.*, 2009b, 2010) in which the observed differences within the same variety have been suggested as somatic mutations occurring during vegetative propagation.

The problem of characterizing the olive tree germplasm is complicated not only by the richness of its genetic patrimony, but also by the absence of reference standards and a well-defined system of nomenclature that is free from homonymy and synonymy (Bartolini and Petruccelli, 2002). For olive varieties there are still no “standard reference variety” (Roselli and Scaramuzzi, 1974) and only recently, some research Italian projects (*i.e.*, “International Treaty on Plant Genetic Resources for Food and Agriculture - Plant Genetic Resources RGV-FAO”, “Improvement and qualification of nursery olive” OLVIVA and “Research and Innovation for the South Olive” RIOM projects) have been raising this issue and are trying

to achieve a “standard certificate” for each variety present in different Italian regions. The extent of this diversity has important implications for both the adaptation of varieties to their local environment and for the optimization of these varieties agronomical performance under a given set of environmental conditions. For example, every initiative promoting olive cultivation should consider the potential repercussions of such action on any local olive varieties. Every region should preserve its own plant material in order to safeguard both the adaptation and productivity of the species and the unique characteristics of the region’s olive oil. However, the study of intra-varietal polymorphisms is important since they may have traits that although not considered important in the past, might be important to meet the challenges of modern olive growing (*i.e.*, resistance to low temperatures, salinity tolerance, etc.).

The preliminary work performed in olive tree genomics is currently very far from producing results that are useful for selecting new varieties using molecular tools. This combined with the general lack of prior knowledge regarding the cultivated and wild olive germplasms, has focused attention mainly on the evaluation of the germplasm.

There is a strong need for a means of reliably identifying different olive tree varieties, partly because so many of these varieties are propagated solely via vegetative methods. This would also be of substantial benefit to nurserymen and growers, because the cost of plants represents the major investment in establishing new orchards. At the same time, it is also important to improve the *ex situ* plant germplasm collection in order to characterize adequately all varieties, and to develop future breeding programs.

Morphological and biological characteristics are widely used for descriptive purposes and are commonly used to distinguish olive varieties (Barranco *et al.*, 2000; Cantini *et al.*, 1999; Lombardo *et al.*, 2004). Agronomic characterization has also aided in the classification of different olive varieties (Barranco and Rallo 2000; Lombardo *et al.*, 2004). Morphological characterization of olive varieties is potentially unreliable, because environmental factors strongly influence the plants’ morphology. Despite this drawback, the age of trees, their training systems, and the phenological stage of the plants continues to be a key preliminary step in the description and classification of the olive tree germplasm (Lombardo *et al.*, 2004). At the same time, improving *ex-situ* olive plant germplasm collections remains an important objective, which will ultimately prove useful for characterizing all varieties and for developing future breeding programs.

Recently, a multiplicity of molecular markers as been used to characterize and distinguish between olive varieties. In light of these efforts, some combination of enzymatic markers with distinct morphological, physiological, and agronomic characteristics may ultimately provide a method for the reliable and systematic classification of olive tree varieties (Ouazzani *et al.*, 1995). Assessments of microsatellite markers, RAPD profiles, AFLPs, and RFLPs provide direct genotypic information, which has numerous, valuable applications in genetic studies. The main advantages of generating RAPD profiles are the technique’s simplicity and low cost (Bogani *et al.*, 1994; Fabbri *et al.*, 1995; Wiesman *et al.*, 1998; Belaj *et al.*, 2001; Muzzalupo *et al.*, 2007a). Nevertheless, RAPD experiments demonstrate poor reproducibility, which hampers comparison between individual studies. Experiments assessing an organism’s AFLP markers are more technically demanding than RAPD but are highly effective in detecting DNA polymorphisms (Angiolillo *et al.*, 1999; Baldoni *et al.*, 2000; Muzzalupo *et al.*, 2007a; Owen *et al.*, 2005). In contrast to a plant species’ chloroplast DNA

(cpDNA), which occasionally can be insufficiently variable for intra-species comparison (Wolfe *et al.*, 1987; Amane *et al.*, 1999; Lumaret *et al.*, 2000; Besnard *et al.*, 2002), mitochondrial DNA (mtDNA) within a given species varies enormously in terms of organization, size, structure, and gene arrangement (Brennicke *et al.*, 1996). As a result, intra-species mtDNA variation is common in plants, especially in naturally occurring populations (Besnard *et al.*, 2002). Taken together, these distinctive features make mtDNA sequencing a powerful tool for analysing a given plant population's genetic structure and phylogenetic relationships (Cavallotti *et al.*, 2003). Microsatellite markers are ubiquitous, abundant, and highly dispersed in eukaryotic genomes, but are costly to assess experimentally. Once these markers have been ascertained, the data can be readily shared among laboratories. Since not all microsatellites are identical (Baldoni *et al.*, 2009; Rallo *et al.*, 2000; Sefc *et al.*, 2000; Carriero *et al.*, 2002; Cipriani *et al.*, 2002; Muzzalupo *et al.*, 2006, 2009a), however, successful utilization of known microsatellite markers requires prior information regarding the characteristics of a particular genetic locus (Baldoni *et al.*, 2009).

Internal transcribed spacer 1 (ITS-1) sequences, RAPD profiles, and inter-SSR (ISSR) markers have been employed to evaluate the colonization history of *Olea europaea* (Hess *et al.*, 2000). A number of *Olea europaea* retroelements have also been identified (Hernandez *et al.*, 2001), and their copy number has been estimated (Stergiou *et al.*, 2002). Using previously established RAPD profiles (Hernandez *et al.*, 2001; Mekuria *et al.*, 2001) developed SCAR markers linked to leaf peacock spot tolerance. Another method to distinguish inter-variety variability and to characterize clonal variants using single nucleotide polymorphisms (SNPs) in the olive tree genome is also currently under development (Rekik *et al.*, 2011; Reale *et al.*, 2006).

All the aforementioned genetic techniques provide useful information regarding the level of olive tree polymorphism and diversity, which demonstrates their utility for the characterization of germplasm varieties (Belaj *et al.*, 2003).

3.1 Molecular approaches for olive oil quality control

The food crisis situation seen in last years and the controversy about genetically modified organisms (GMO), with a sharp increase in basic food prices, highlights the extreme susceptibility of the current agricultural and food model and the need for more strict food quality control, which should include determination of the origin of the product and the raw materials used in it. That's why a well documented traceability system has become a requirement for quality control in the food chain. The definition of traceability according to the European Council Regulation EEC 178/2002 is the ability to identify and trace a product or a batch of products at all stages of production and marketing. Traceability is important for commercial reasons and plays a considerable role in the assurance of public health.

Olive oil extraction is the process of extracting the oil present in the olive drupes for food use. The oil is produced in the mesocarp cells, and stored in a particular type of vacuole called a lipovacuole (photo 9). Olive oil extraction is the process of separating the oil from the other fruit contents. It is possible to attain this separation by physical means alone, *i.e.* oil and water do not mix, so they are relatively easy to separate.

The modern method of olive oil extraction uses an industrial decanter to separate all the phases by centrifugation. In this method the olives are crushed to a fine paste. This can be

done by a hammer crusher, disc crusher, depicting machine or knife crusher. This paste is then malaxed for 15 to 45 minutes in order to allow the small olive droplets to agglomerate. The aromas are created in these two steps through the action of fruit enzymes. Afterwards the paste is pumped in to an industrial decanter where the phases will be separated.

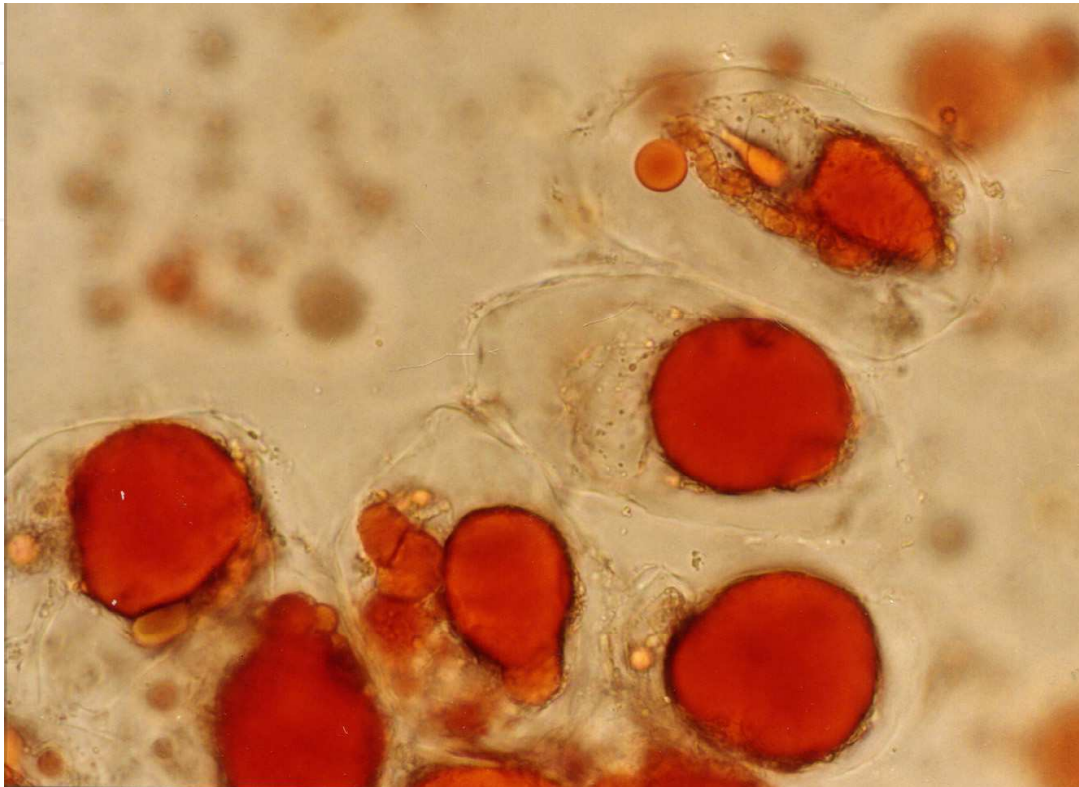


Photo 9. Lipovacuole from olive mesocarp cells stained with sudan IV

The olive oil chemical components are divided, into major and minor compounds that are briefly described below. *Major components*: glycerids correspond to more than 98% of the total weight. Abundance of oleic acid (C18:1 $n-9$), is a monounsaturated fatty acid and present in concentrations between 56 to 84% of total fatty acids, while the most essential polyunsaturated fatty acid in our diet is the linoleic acid (C18:2 $n-6$), ranges from 3 to 21% (Caravita *et al.*, 2007). *Minor components*: amounting to about 2% of the total oil weight, include compounds that are not related to lipids from a chemical viewpoint (tocopherols, polyphenols, chlorophylls, etc.) and compounds from unsaponifiable matter derived from lipids (sterols, phospholipids, waxes, ect.) (Servili *et al.*, 2004).

Almost 84% from the total olive oil production derives from the European Union, especially from Spain, Italy and Greece. The olive oil is a main constituent of the Mediterranean diet. However there has recently been an increase in olive oil consumption internationally, due to greater availability and the current consideration of its high nutritive and health benefits, including a qualified health claim from Food and Drug Administration (FDA, USA).

Some varieties of olive oil are recognized as being of higher quality because they derive from well-defined geographical areas, command better prices and generally are legally protected. Indeed, the aim of Protected Designations of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Guaranteed (TSG) is to add value to

certain specific high quality products from a particular origin. Chemical techniques have been employed for the authenticity of olive oils using a high number of variables such as glycerid composition, phenolic fraction, unsaponifiable components monitoring by statistical and mathematical analyses in order to ability the evaluation of the results. Molecular markers allow the detection of DNA polymorphisms and enable to effectively distinguish different varieties in an effective way, without any environmental influence.

When we blend olive oils of the same category, but from different provenances, most chemical analyses are of limited significance. Due to their high variability according to environmental conditions, neither morphological characteristics of different groups, nor the analyses of chemical composition of fatty acid and secondary metabolites can provide reliable results for oil traceability (Ben Ayed *et al.*, 2010; De Nino *et al.*, 2005; Papadia *et al.*, 2011). For this reason, genetic identity seems to be the most appropriate method for identifying the variety from which the olive oil under study derives. In fact, DNA in oil is not affected by the environment and is identical to the mother tree DNA since the oil containing tissues are formed by diploid somatic cells of the tree (Muzzalupo *et al.*, 2007b). However, depending on the molecular markers used correctly, extra alleles can be detected in the oil that do not correspond to the mother tree allele but to the pollinator alleles contained in the embryo, itself located inside the seed (Muzzalupo and Perri, 2002; Ben Ayed *et al.*, 2010). The use of DNA based technology in the field of food authenticity is gaining increasing attention. This technique makes use of molecular markers that mostly use polymerase chain reaction (PCR) and are thus easy to genotype. Even in a complex matrix such as olive oil, molecular marker techniques such as RAPDs (random amplified polymorphic DNA), AFLPs (amplified fragment length polymorphism) and SSRs (simple sequence repeat) are very useful in the study of the traceability of olive oil. SNP markers have been recently developed in olive and utilized to study the genetic diversity of olive trees (Reale *et al.*, 2006; Rekik *et al.*, 2010).

A recent report by Papadia *et al.*, 2011 reported a systematic effort to obtain genetic characterization by SSR amplification, soil analyses, and ¹H-NMR spectra, is carried out in order to make a direct connection between the olive tree variety (genetic information) and the NMR spectra (chemical information) of the extra virgin olive oil produced. The results reported show that a multidisciplinary approach, through the application of multivariate statistical analysis, could be used to set up a method for variety and/or geographic origin certification, based on the construction of a suitable database. Further research will be directed to the growth of an organic genetic/NMR/soil database, in order to improve the prediction ability of the LDA, and furthermore to develop a way to correlate ¹H-NMR spectra of commercial extra virgin olive oils with their geographical and genetic origin.

In the following subsections we will discuss the potential of these classes of markers in the oil traceability and in characterization of olive germplasms.

3.1.1 RAPDs (Random Amplified Polymorphic DNA)

In this technique, a PCR amplification of genomic DNA is performed using a set of arbitrary primers (Williams *et al.*, 1990). For each primer a large number of bands are generated and each DNA has the presence/absence of a band can distinguish between individuals and each individual is expected to have a specific fingerprint of bands. This molecular technique

has several advantages. It is simple, cheap, it requires small amounts of DNA (Fritsch and Rieseberg, 1996), and it can be applied without prior genetic information about the organism. Besides, it is fast, and does not require radioactivity. However, this analysis has several limitations including dominance, sensitivity to the reaction conditions, uncertain locus homology and the lack of good reproducibility. RAPDs thus combine the advantages of low technical input with almost an unlimited numbers of markers. They have proven to be very useful in the characterization of genetic diversity of plants for which few genomic data are available (Qian *et al.*, 2001; Bandelj *et al.*, 2002). RAPD markers were the first ones to be implemented to study diversity of the species *Olea europaea* (Belaj *et al.*, 2001), to discriminate olive varieties (Khadari *et al.*, 2003; Muzzalupo *et al.*, 2007a), to study inter or intra-variety genetic diversity (Wiesman *et al.*, 1998; Mekuria *et al.*, 2001, Muzzalupo and Perri, 2009; Belaj *et al.*, 2002, 2003; Gemas *et al.*, 2004), to establish genetic relationships between varieties (Belaj *et al.*, 2002, 2003; Besnard *et al.*, 2002; Khadari *et al.*, 2003; Muzzalupo *et al.*, 2007a), and to study genetic differentiation in the olive complex (Besnard *et al.*, 2001; Martins-Lopes *et al.*, 2008). As early as their use in genetic studies RAPD markers has been used for the authentication and traceability of olive oil (Pasqualone *et al.*, 2001; Muzzalupo and Perri, 2002). However, numerous authors (Pasqualone *et al.*, 2001; Sanz-Cortés *et al.*, 2001) concluded the non-reproducibility of RAPD markers in the authentication of olive oil, which resulted in inconsistent electrophoretic patterns. These unsuccessful attempts are due to the bad quality of DNA extracted from oil (Pasqualone *et al.*, 2001; Muzzalupo and Perri, 2002).

3.1.2 AFLPs (Amplified Fragment Length Polymorphism)

AFLP was described by Vos *et al.*, (1995) as a more reproducible alternative to RAPD for the genetic identification of crop plants. This technique is based on the selective PCR amplification of restriction fragments from total digests of genomic DNA. In olive, AFLP markers have been used for genetic diversity studies and variety identification. In fact, amplified fragment length polymorphism technology has been used by Angiolillo *et al.*, (1999) to obtain a large number of markers for olive. This has been used in addressing genetic relationships among wild and cultivated varieties, as well as among *Olea europaea* L. and other species from the genus (within the *Olea* complex). This technique has also been used to study the genetic diversity within and among a range of Spanish and Italian olive varieties (Sanz-Cortés *et al.*, 2003). Owen *et al.*, (2005) used AFLP markers to evaluate the structure of genetic diversity among common olive varieties cultivated in the Eastern Mediterranean. Additionally, AFLP analysis, as previously described and has been used in genetic variability studies for about 29 varieties (including oil and table olive varieties originating from Tunisia and other Mediterranean countries) of the genus *Olea* using nine AFLP primer combinations (Grati-Kamoun *et al.*, 2006). Different studies (Busconi *et al.*, 2003, Pafundo *et al.*, 2005) have reported that it is possible to use AFLP markers for genotyping olive species. As far as oil traceability is concerned, Busconi *et al.*, (2003) reported that the AFLP fingerprint of olive oil was only partially super imposable with that of the variety from which the oil was made. However, in more recent studies, Pafundo *et al.*, (2005) and Montemurro *et al.*, (2007) concluded that AFLP profiles of DNA purified from leaves and the monovarietal oil of the same variety were comparable. These latter evaluated the possibility of identifying virgin olive oil from ten different varieties by the analysis of AFLP markers using six AFLP primer combinations. For the AFLP as well as for RAPDs, the

quality of DNA isolated from olive oil seems again to be the problem (very low quantity, a high degradation and the richness in polysaccharides and phenolic compounds). Poor quality of DNA is responsible for inconsistent results and low reliability of AFLP profiles due to the inhibition of the restriction enzymes and the DNA polymerase activity.

3.1.3 SSRs (Simple Sequence Repeats)

SSRs are a class of DNA markers that consist of short tandem repeat sequences (2–6 bp), which have become one of the most successful and the most interesting markers for genotype identification due to their good properties; in addition to their high specificity, they are highly polymorphic, codominant, *locus* specific, ubiquitous, widely distributed throughout the genome and easily amenable to automated PCR-based analysis. At present, they are the most reliable DNA profiling methods in forensic investigation (Jobling and Gill, 2004). SSRs also are highly informative and reproducible tools because they use longer primer sequences (Vos, 1995).

In olive SSRs have shown high potential for resolving issues of synonymies, homonymies and misnamings. Many SSRs have been developed in olive and applied with success (Sefc *et al.*, 2000; Carriero *et al.*, 2002; Cipriani *et al.*, 2002; De la Rosa *et al.*, 2003; Sabino Gil *et al.*, 2006). All these characteristics make them ideal markers for applications in analysis of intravariety variability issues (Cipriani *et al.*, 2002; Lopes *et al.*, 2004; Muzzalupo *et al.*, 2009b, 2010), linkage mapping (Wu *et al.*, 2004) and for characterizing olive germplasm resources (Belaj *et al.*, 2004; Montemurro *et al.*, 2007; Muzzalupo and Perri, 2009). Sarri *et al.*, (2006) confirmed the power of SSR markers in the identification of 118 varieties from different Mediterranean countries to study the genetic diversities of olive varieties. A recent report by Muzzalupo *et al.*, (2009a) characterized 211 Italian olive varieties by using 11 loci microsatellite in order to study and to establish relationships of geographically-related olive-tree varieties. Microsatellites are also very useful markers for paternity analysis (Rallo *et al.*, 2000; Diaz *et al.*, 2007; Rekik *et al.*, 2008). Recently microsatellites have become available and reliable molecular markers for the traceability issues to define the olive oil origin and to detect the presence of prohibited varieties (Muzzalupo *et al.*, 2007b; Ben Ayed *et al.*, 2009). Most these publications addressed the optimization of the extraction of high quality DNA from olive oils and to identify the most interesting SSRs markers in variety discrimination. All the studies published so far, showed that the reliability and reproducibility of SSRs profiles is determined by the quality of the DNA extracted from oil (Muzzalupo *et al.*, 2007b; Breton *et al.*, 2004; Bracci *et al.*, 2011; Ben Ayed *et al.*, 2009). In fact, the amount of DNA isolated from olive oil is low and highly degraded by the nuclease present in olive oil (Muzzalupo and Perri, 2002; De la Torre *et al.*, 2004). For this reason, the extraction of DNA from olive oil is a difficult task. Several techniques of DNA preparation and immobilization for subsequent sample analysis have been developed. These methods, utilize such supports as silica, hydroxyapatite, magnetic beads, and spin columns. These supports enable the DNA to be amplified and analyzed using various quantities of oil. In particular, magnetic beads in conjunction with additional processing have proved useful. However, the defined procedure needs 2 x 40 mL of virgin olive oil, and the preparation of DNA regularly necessitates 5 h (Breton *et al.*, 2004). Besides, other authors tried various protocols of DNA extraction from olive oil such as: Wizard kit, CTAB protocol extraction, QIAamp DNA stool extraction Kit. They concluded that the most reproducible results were

obtained when the template DNA was recovered from the olive oil using QIAamp DNA stool extraction Kit (Qiagen) (Muzzalupo *et al.*, 2007b; Testolin and Lain, 2005).

3.1.4 SNP (Simple Nucleotide Polymorphism) and qRT-PCR (Quantitative Real-Time PCR)

SNP detection can be delivered in a number of ways, but the simultaneous detection of multiple SNPs from a single DNA sample is of particular interest. The “ligation detection reaction-universal array” (LDR-UA), was adopted by , to successfully genotype a panel of 49 varieties with respect to 17 SNPs. Out of the 13 amplicons containing these SNPs, 12 were successfully amplified from oil-derived template, and the resulting profiles were fully consistent with those obtained from leaf-derived DNA (Consolandi *et al.*, 2008). qRT-PCR continues to be extensively used for quantifying the amount of a specific sequence in food, with particular interest for GMOs (Marmioli *et al.*, 2009). PDO oils are typically not monovarietal, so a method for quantifying the components of the mixture is essential if conformity with certification depends on a prescribed proportion of varietal types. So far, application of Real-Time as a tool for olive oil authentication has been explored by Giménez *et al.*, (2010). The authors evidenced that Real-Time PCR is useful to quantify DNA extracted from oil, and thus to assess the yields of different methods of extraction. But the size of amplicon, is critical for the success of analysis. A possibility of utilising qRT-PCR to quantify varieties in PDO oils rests on the use of taqMan probes designed on SNPs specific of varieties entering in the oil composition (Marmioli *et al.*, 2009).

3.2 Genomics approaches for olive valorisation

The complete sequencing of the genome of *Arabidopsis* in 2000 by the Arabidopsis Genome Initiative (AGI) (Samir *et al.*, 2000) and the emerging sequence information for several other plant genomes, such as rice, *Populus*, *Medicago*, lotus, *Lycopersicon esculenum* and *Zea mays*, represent a valuable tool to determine the function of many genes (Rensink and Buell, 2005; Vij *et al.*, 2006). In the wake of these sequencing approach, plant research enters an exciting period in which genome-wide approaches are becoming an integral part of plant biology, with potentially highly rewarding but as yet unpredictable biotechnological applications. This is reflected in the growing interest of new farms that invest in the development of tools to enhance and expand this wealth of information.

Functional genomics employs multiple parallel approaches, including global transcript profiling coupled with the use of mutants and transgenics, to study genes function in a high throughput mode. The aim of these genome-wide efforts is to link the genome sequences to the phenotypic characters.

The availability of a large volume of genomic data has provided information about the genes content of plants. Partial or complete sequences of cDNAs often provide a firm basis of the dimension of the transcriptome. The all plant expression sequence tags (ESTs) available are organized together with well characterized genes, into non-redundant gene clusters in three main databases (National Center for Biotechnology Information, NCBI; Unigenes, <http://www.ncbi.nlm.nih.gov/>; The Institute for Genomic Research, TIGR; Gene Indices, www.tigr.org; and Sputnik, <http://mips.gsf.de/proj/sputnik>) accessible via the Internet. It is worth noting that several companies possess large private EST databases for

various crop plants such as *Zea mays* and soybean; in this case the access can be negotiated on a case by-case basis.

The ESTs are single-pass sequences of 300 to 500 bp determined from one or both ends of randomly chosen cDNA expressed genes. The sequences are sufficiently accurate to unambiguously identify the corresponding gene in most cases. Thousands of sequences can thus be determined with a limited investment. EST information present in public databases is available for a variety of species, including a number of plants (Cooke *et al.*, 1996; Yamamoto and Sasaki, 1997).

ESTs are important for the accurate genome annotation and provide information about gene structure, alternative splicing, expression patterns and transcript abundance (Umezawa *et al.*, 2004). Recent progress in DNA sequencing technology, the rapid growth of EST and cDNA sequence resources and the large amount of genetic variation at the nucleotide level can be exploited to generate various types of molecular markers for variation analysis, marker-assisted selection (MAS) and quantitative trait locus analysis (QTL) for desirable traits and to identify genetic loci involved in phenotypic changes of model and non-model plant species (Lee *et al.*, 2007).

In the absence of the complete genome sequence, EST databases are a good resource for finding genes and for interspecies sequence comparison, and have provided markers for genetic and physical mapping and clones for expression analyses. The relative abundance of ESTs in libraries prepared from different organs and plants in different physiological conditions also provides preliminary information on expression patterns for the more abundant transcripts.

Limitations at the EST approach are represented by the rare transcripts that are induced only under specific condition and consequently they are not present in EST database. In this case the only sure way to gain access to the entire set of genes is to determine the complete genomic sequence. The genomic sequence also provides information on the global structure of the genome, including the relative order of genes on the chromosomes, which is extremely valuable for positional cloning strategies. The major problem with genomic sequences is how to distinguish coding regions from noncoding intergenic sequences and introns. In this case, the comparisons between genomic sequences, ESTs and cDNA sequences can help to assign intron positions for many genes. However, for the genes that do not match sequences in the databases, the coding sequences need to be predicted from the genomic sequence. Therefore, sequencing technology applied to crop species represent the first step to identify the genes involved in the control of important agronomic traits. Rice was the first crop genome to be sequenced (Yu *et al.*, 2002; Matsumoto *et al.*, 2005), after the sequencing of the first model plant genome, *Arabidopsis thaliana* (Arabidopsis Genome, 2000). Current crop genome sequencing projects are rapidly changing pace with the new technology and researchers are quickly adopting second generation sequencing to gain insight into their favourite genome. Roche 454 technology is being used to sequence the 430 Mbp genome of *Theobroma cacao* (Scheffler *et al.*, 2009), while a combination of Sanger (old school sequencing) and Roche 454 one of the "2nd generation" technologies of sequencing is being used for the apple genome (Velasco *et al.*, 2009). A similar approach is being applied to develop a draft consensus sequence for the 504 Mbp of grape genome (Velasco *et al.*, 2007) A combined Illumina Solexa and Roche 454 sequencing approach has been used to characterize the genome of cotton (Wilkins *et al.*, 2009). Roche 454 sequencing has been used

to survey the genome of *Miscanthus* (Swaminathan *et al.*, 2009), while Sanger, Illumina Solexa and Roche 454 sequencing are being used to characterize the genome of banana (Hribova *et al.*, 2009).

3.2.2 Gene identification in crop species

The sequencing and assembly of large and complex crop genomes remains a valuable goal, but at the moment, a significant amount of knowledge can be gained from low coverage shotgun sequencing of these genomes. In this contest, the second generation technologies of sequencing are particularly suitable to know genes and gene promoters in crop plants that are homologous to related species. Therefore, designing polymerase chain reaction (PCR) primers to the read pairs enables the amplification and sequencing of the gene and corresponding genomic region in the target species. This approach to gene discovery offers the potential to identify genes, gene promoters and polymorphisms in a wide range of agronomically important crop species (Bracci *et al.*, 2011).

Microarray represents functional genomic approaches that have revolutionized global gene expression profiling. In fact they allow studying the entire gene complement of the genome in a single experiment (Duggan *et al.*, 1999; Li *et al.*, 2005). At the moment, cDNA and oligonucleotide microarrays have been widely used in plants, such as *Arabidopsis*, rice, maize, strawberry, petunia, ice plants and lima bean, to study and compare global gene expression levels in specific organs and/or tissues under controlled physiological conditions.

In olive, the genomics information present on the international database NCBI, concerning the identification and characterization of functional genes are prevalently based on EST identification and they are predominantly related to pollen allergens and characteristics of olive fruit.

Olea europaea trees are widely distributed throughout the Mediterranean basin and therefore their pollen is one of the most prevalent causes of respiratory allergy such as allergic rhinitis and allergic asthma in the Mediterranean region and some other countries between late April and early June (Kalyoncu *et al.*, 1995). Olive pollen is also responsible of allergic inflammation of the upper and/or lower airways that may persist after the pollination season is over (Quiralte *et al.*, 2005).

Allergenic proteins name	Molecular mass (kDalton)	Family
Ole e 1	~ 19	Unknown
Ole e 2	~ 15	Profiling
Ole e 3	~ 9	Polcalcin
Ole e 4	~ 32	Unknown
Ole e 5	~ 16	Cu/Zn superoxide dismutase
Ole e 6	~ 6	Unknown
Ole e 7	~ 10	Lipid transfer protein
Ole e 8	~ 19	Ca++ binding protein
Ole e 9	~ 46	1,3 β glucanase
Ole e 10	~ 10	Carbohydrate binding protein

Table 1. The olive pollen allergens (from Villalba *et al.*, 2007)

At the moment 10 olive pollen allergens have been purified and characterized from *Olea europaea* pollen extract (Table 1). Several of these allergenic proteins, eg, Ole e 6, fail to show any homology to known protein sequences and, therefore, the biochemical function of these gene products remains unknown. Many other allergens belong to well-known families of proteins, such as profilin (Ole e 2), superoxide dismutase (Ole e 5), calciumbinding proteins (Ole e 3 and Ole e 8), lipid transfer proteins (Ole e 7) and 1,3- β -glucanases (Ole e 9) (Villalba *et al.*, 2007).

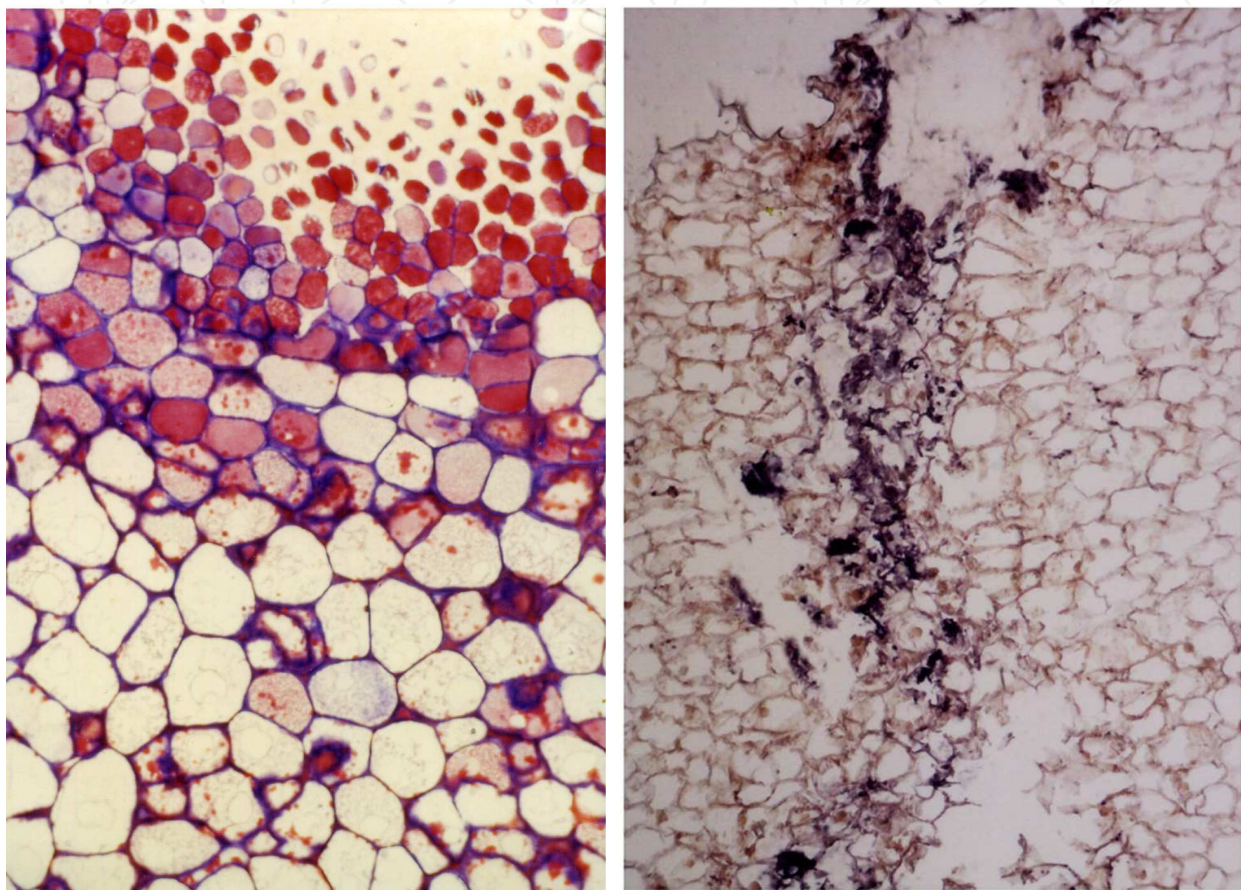


Photo 10. Cross sections of mesocarp olive fruit at level of insect injury (*Bactrocera oleae*, right: sections stained with safranin O/ azur II; left: localization of *OeCHLP* transcripts by *in situ* hybridization with dig-labelled *OeCHLP* antisense probe).

The biochemical composition of olive fruit is variable because it depends on olive variety, soil, climate, and cultivation. The virgin olive oil is overwhelmingly composed of triglycerides (>98%), along with traces of other compounds. The dominant triglyceride fatty acid species are the oleic acids (57-78%) such as palmitic, stearic, linoleic and linolenic acids (Caravita *et al.*, 2007). The other minor constituents such as alcohols, polyphenols, chlorophyll, carotenoids, sterols, tocopherols and flavonoids, contribute to the olive's organoleptic qualities, taste, flavour, and nutritional value (Perri *et al.*, 2002; Servili *et al.*, 2004). These constituents may also serve to distinguish olive oils originating from different regions. Olive oil, especially extra-virgin oil also contains small amounts of hydroxytyrosol, secoiridoids, lignans (Bianco *et al.*, 1999, 2001; De Nino *et al.*, 2005) and other compounds thought to possess anticancer properties (*i.e.*, squalene and terpenoids) (Fabiani *et al.*, 2002;

Owen *et al.*, 2004). In spite of its economical importance and metabolic peculiarities, very few data are available on gene sequences controlling the main metabolic pathways. Particular attention has been paid to the genes encoding the key enzymes involved in fatty acid biosynthesis, fatty acid modification, triacylglycerol synthesis, and fat storage (Hatzopoulos *et al.*, 2002; De la Rosa *et al.*, 2003; Banilas *et al.*, 2005).

In recent years, much attention has turned to the olive fruit. In this contest, the parallel sequencing of different fruit cDNA collections has provided large scale information about the structure and putative function of gene transcripts accumulated during fruit development (Alagna *et al.*, 2009).

A nuclear gene, named *OeCHLP* (*Olea europaea* GERANYLGERANYL REDUCTASE) was isolated and characterized by Bruno *et al.*, (2009). This gene encodes a chloroplastic enzyme involved in the formation of phytolic side chain of tocopherols chlorophyll, and plastoquinones. In olive fruits *OeCHLP* gene expression was enhanced in dark fruit very likely in relation to the increase in mature fruits of the level of total tocopherols suggesting a role in the synthesis of the antioxidant. It is noteworthy that the variations in gene transcript levels that occurred during the ripening of olive fruits depend on the genotype analyzed (Muzzalupo *et al.*, 2011). In this contest, in olive fruits tocopherols confer not only nutritional value (Valk and Hornstra, 2000), but also contribute to product stability and post harvesting shelf life (Goffman and Bohme, 2001) by protecting storage oil from oxidative damage (Sattler *et al.*, 2004). *OeCHLP* was also detected in fruits attacked by *Bactrocera oleae* pathogen as well as in fruits wounded by needle suggesting a role in protection mechanisms related to cell damage and oxidative burst induced by pathogen (photo 8 and 10) (Ebel, 1998; Klessig *et al.*, 2000; Bruno *et al.*, 2009).

4. Conclusion

Although many efforts have been made in the last years, genome studies in *Olea europaea* L. are currently behind those of other crops. Several groups have started to work on the olive genome sequencing and, thanks to the rapid development of the new sequencing technologies; hopefully soon the complete sequence of olive genome will be available. Identification of all genes within a species permits an understanding of how important agronomic traits are controlled, knowledge of which can be directly translated into crop improvement.

The availability of reliable genotype data of olive varieties and oils deriving from them, in publicly accessible curate and regularly update databases will be the challenge for the next few years. Recent advances in DNA sequencing technology are radically changing biological and biomedical research and will have a major impact on crop improvement. The new information on genome sequence will be very useful to identify genes involved in agronomical traits that could be used to improve the nutritional characteristics and the productivity of this crop. A possible application could be, for example, the studies of molecular mechanisms of drought and salinity tolerance of olive, in order to improve the cultivation of this important fruit crop also in the most arid and semiarid areas of the world. The knowledge of genome nucleotide sequences also could be useful to identify new sequence polymorphisms, which will be very useful in the development of many new variety-specific molecular markers and in the implementation of more efficient protocols for tracking and protect olive oil origin.

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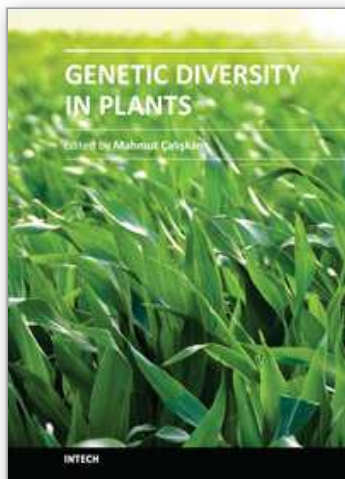
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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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