Plant Cell Rep (2011) 30:449–462
DOI 10.1007/s00299-010-0991-9

REVIEW

Molecular studies in olive (*Olea europaea* L.): overview on DNA markers applications and recent advances in genome analysis

T. Bracci · M. Busconi · C. Fogher · L. Sebastiani

Received: 13 December 2010 / Accepted: 21 December 2010 / Published online: 7 January 2011

Abstract Olive (*Olea europaea* L.) is one of the oldest agricultural tree crops worldwide and is an important source of oil with beneficial properties for human health. This emblematic tree crop of the Mediterranean Basin, which has conserved a very wide germplasm estimated in more than 1,200 cultivars, is a diploid species ($2n = 2x = 46$) that is present in two forms, namely wild (*Olea europaea* subsp. *europaea* var. *sylvestris*) and cultivated (*Olea europaea* subsp. *europaea* var. *europaea*). In spite of its economic and nutritional importance, there are few data about the genetic of olive if compared with other fruit crops. Available molecular data are especially related to the application of molecular markers to the analysis of genetic variability in *Olea europaea* complex and to develop efficient molecular tools for the olive oil origin traceability. With regard to genomic research, in the last years efforts are made for the identification of expressed sequence tag, with particular interest in those sequences expressed during fruit development and in pollen allergens. Very recently the sequencing of chloroplast genome provided new information on the olive nucleotide sequence, opening the olive genomic era. In this article, we provide an overview of the most relevant results in olive molecular studies. A particular attention was given to DNA markers and their application that constitute the most part of published researches. The first important results in genome analysis were reported.

Keywords Olive tree · Molecular markers · Genetic variability · Functional genomics

Communicated by R. Reski.

T. Bracci and M. Busconi have contributed equally to this paper.

T. Bracci · L. Sebastiani (✉)
Biolabs, Scuola Superiore Sant'Anna,
Piazza Martiri della Libertà 33, 56127 Pisa, Italy
e-mail: l.sebastiani@sssup.it

M. Busconi · C. Fogher
Istituto di Agronomia, Genetica e Coltivazioni Erbacee,
Università Cattolica del Sacro Cuore, Via Emila Parmense 84,
29122 Piacenza, Italy

Why the olive?

Olive (*Olea europaea* L.) is a typical and widespread tree of the Mediterranean region, where its cultivation started in the third millennium B.C. (Loukas and Krimbas 1983). Olive is the second most important oil fruit crop cultivated worldwide after oil palm. Its cultivation covers over eight million hectares of land, predominantly concentrated in the Mediterranean basin, where 70% of the olive oil produced is consumed (Baldoni and Belaj 2009). The olive tree is a glycophytic species that shows a high tolerance to drought and salt stresses, if compared with other fruit trees that are generally salt sensitive (Gucci and Tattini 1997).

Several studies have highlighted the beneficial effects of olive oil on human health (Keys 1995; Pérez-Jiménez et al. 2007). As consequence of the increased interest for this crop, the consumption of olive oil has expanded also in non-traditional producer countries such as the United States, Australia and Japan (Pinelli et al. 2003).

Some minor constituents, that differentiate olive oil from all the other vegetable oils used in the human diet, seem to be responsible for the major effects on health. These components, named secoiridoids, represent the most important class of the olive phenolics and they are exclusively present in the *Oleaceae* family that includes *Olea europaea* L. The protective activity of olive oil against

chronic degenerative diseases and tumours is credited to this group of secondary metabolites, which are also responsible for the agreeable sensory properties of virgin olive oil (Servili et al. 2004).

The olive tree belongs to the *Oleaceae* family that comprises 30 genera and 600 species (Cronquist 1981). Within the genus *Olea*, which includes 30 species and has spread to Europe, Asia, Oceania and Africa, only *Olea europaea* is cultivated. The wild olive or oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris*) and the cultivated olive (*Olea europaea* subsp. *europaea* var. *europaea*) are two co-existing forms of the subspecies *europaea* (Green 2002). Other five subspecies constitute the *Olea europaea* complex: (a) *laperrinei*, present in Saharan massifs; (b) *cuspidata*, present from South Africa to southern Egypt and from Arabia to northern India and south-west China; (c) *guanchica* present in the Canary Islands; (d) *maroccana* present in south-western Morocco; (e) *cerasiformis* present in Madeira (Green 2002).

The cultivated olive is an evergreen, out-crossing, vegetatively propagated tree with a very wide genetic patrimony that is the result of both plant longevity and the scarcity of genotype turnover through centuries of cultivation. The large number of cultivars, added to the many cases of synonymous and homonymous name, makes particularly difficult the description and classification of olive varieties (Fabbri et al. 2009). As a result the size of this germplasm is controversial: about 1,250 varieties, cultivated in 54 countries and conserved in over 100 collections, were included in the FAO olive germplasm database (Bartolini 2008), also if this number is certainly higher because the lack of information on many local cultivars and ecotypes (Cantini et al. 1999). The most part of these cultivars comes from southern European countries such as Italy (538 varieties), Spain (183), France (88) and Greece (52) (Baldoni and Belaj 2009). Due to this richness of the germplasm, olive is an unusual case among horticultural crops and its biodiversity can represent a rich source of variability for the genetic improvement of this plant (Baldoni and Belaj 2009).

Cytogenetic studies

Olive is a diploid species having 46 chromosomes ($2n = 2x = 46$) (Breviglieri and Battaglia 1954). However, karyological studies in this plant are very complex due to the small size, similar morphology and large number of chromosomes, which make difficult a satisfactory characterisation of the chromosome complement. In order to solve this problem differential staining of the chromatin and in situ hybridization of highly repeated DNA sequences and ribosomal cistrons were used in combination and were able to distinguish the most part of the olive chromosomes pairs (Minelli et al. 2000). The determination of nuclear DNA content was employed for characterising the olive genetic resources. Nuclear DNA content was determined by cytometric methods in several Italian cultivars. Rugini et al. (1996) found 2.26 and 2.20 pg of DNA per haploid nucleus, respectively, in Frantoio and Leccino, while Bitonti et al. (1999) found 3.90 pg/2C in Dolce Agogia and 4.66 pg/2C in Pendolino. The genome size of six Portuguese cultivars and a wild olive was estimated by Loureiro et al. (2007) using flow cytometry methods. The obtained data were slightly different from those of the Italian cultivars, with a nuclear DNA content ranging between 2.90 ± 0.020 and 3.07 ± 0.018 pg/2C for the Portuguese cultivars and 3.19 ± 0.047 pg/2C DNA for the wild olive. These studies proved an intraspecific variation of genome size in *Olea europaea* and confirmed the nuclear DNA content analysis as an useful tool for characterising genotypes within species.

The analysis of ploidy level was also made in order to study the *Olea europaea* complex. A different level of polyploidy was highlighted by Besnard et al. (2008) in the six subspecies, using flow cytometry and six highly variable nuclear microsatellites. Four subspecies appeared to be diploids, while subspp. *cerasiformis* was tetraploid and *maroccana* hexaploid. Concerning the subspp. *europaea* and *cuspidata*, Rallo et al. (2003) assessed a partial polyploidy by microsatellites analysis, although this hypothesis was not confirmed by Besnard et al. (2008). With regard to the olive tree relatives, recently Besnard and Baali-Cherif (2009) reported the first evidence of the coexistence of two ploidy types (diploid and triploid genotypes) in a relict *laperrinei*'s olive population from Algeria.

DNA-based molecular markers in olive studies

Molecular markers revealing polymorphisms at the DNA level are very useful tools in genetics studies and in the improvement of crop plants. Indeed they can be applied to a variety of purposes including DNA fingerprinting, genetic screening and chromosome mapping.

In olive DNA based markers were widely used both in theoretical and applied research fields, such as the revision of species *Olea europaea*, the characterisation of the huge olive germplasm, the breeding programs and the cultivars traceability of olive oil. A description of the main genetic markers used in olive studies and their use in this crop is presented below and summarised in Table 1.

RAPDs (Random Amplified Polymorphic DNA)

This procedure, developed in 1990 by Williams et al., detects nucleotide sequence polymorphisms by using

Table 1 Applications of DNA-based molecular markers in *Olea europaea* studies

Molecular marker	Developers	Application in *Olea europea* L.	References
RAPD	Williams et al. (1990)	DNA fingerprinting of cultivars	Bogani et al. (1994), Fabbri et al. (1995), Wiesman et al. (1998), Belaj et al. (2001), Guerin et al. (2002)
		Genetic correspondence of plant material from nursery	Rubio and Arus (1997), Belaj et al. (1999)
		Detection of intra-cultivar variability	Gemas et al. (2000), Belaj et al. (2004)
		Construction of linkage map	De la Rosa et al. (2004)
		Cultivar traceability in olive oil	Muzzalupo and Perri (2002), Martins-Lopes et al. (2008)
		Phylogenetic studies	Hess et al. (2000), Bronzini de Caraffa et al. (2002)
AFLP	Vos et al. (1995)	DNA fingerprinting of cultivars	Angiolillo et al. (1999), Owen et al. (2005)
		Detection of intra-cultivar variability	Belaj et al. (2004)
		Phylogenetic studies	Baldoni et al. (2006), Rubio de Casas et al. (2006)
		Cultivar traceability in olive oil	Busconi et al. (2003), Montemurro et al. (2007)
		Construction of linkage map	De la Rosa et al. (2003)
SCAR	Paran and Michelmore (1993)	DNA fingerprinting of cultivars	Busconi et al. (2006)
		Cultivar traceability in olive oil	De la Torre et al. (2004), Pafundo et al. (2007)
SSR	Morgante and Olivieri (1993)	DNA fingerprinting of cultivars	Sefc et al. (2000), Cipriani et al. (2002), Sabino Gil et al. (2006), Sarri et al. 2006), Baldoni et al. 2009)
		Construction of linkage map	De la Rosa et al. (2003), Wu et al. (2004)
		Paternity analysis	De la Rosa et al. (2004), Diaz et al. (2006), (2007a, b), Mookerjee et al. (2005)
		Cultivar traceability in olive oil	Martins-Lopes et al. (2008), Alba et al. (2009)
		Phylogenetic studies	Belaj et al. (2007), Erre et al. (2010)
ISSR	Zietkiewicz et al. (1994)	Phylogenetic studies	Hess et al. (2000), Vargas and Kadereit (2001)
		Detection of intra-cultivar variability	Gemas et al. (2004)
		Cultivar traceability in olive oil	Pasqualone et al. (2001), Martins-Lopes et al. (2008)
SNP	Wang et al. (1998)	DNA fingerprinting of cultivars	Reale et al. 2006, Muleo et al. (2009), Santos Macedo et al. (2009), Hakim et al. (2010)
Ribosomal DNA polymorphism			
Direct sequencing		Phylogenetic studies	Hess et al. (2000), Besnard et al. (2007b), Baldoni et al. (2009)
RFLP	Botstein et al. (1980)	Phylogenetic studies	Besnard et al. (2001, 2007a)
Chloroplast and mithocondrial polymorfphism			
Direct sequencing		Effect of prolonged vegetative propagation on cytoplasmic genome segregation	García-Díaz et al. (2003)
		Cultivar traceability in olive oil	Intrieri et al. (2007)
RFLP	Botstein et al. (1980)	Phylogenetic studies	Besnard and Bervillé (2002), Besnard et al. (2002a, b), Baldoni et al. (2009)
		Male sterility analysis	Besnard et al. (2000)

primers (usually 8–10 bp long) of arbitrary sequence. A single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template and, if these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through PCR (Polymerase Chain Reaction) amplification.

Due to their simplicity of application and low cost, these markers were the first used to evaluate the genetic variability in olive (Bogani et al. 1994) Subsequently RAPDs has been widely used for characterisation of varieties from the collections of several countries such as Italy (Fabbri et al. 1995; Cresti et al. 1997), Spain (Belaj et al. 2001; Sanz-Cortés et al. 2001; Belaj et al. 2004), Israel (Wiesman et al. 1998) and Australia (Mekuria et al. 1999; Guerin et al. 2002). This technique was also very useful in the identification of plant material from the nursery (Rubio and Arus 1997; Belaj et al. 1999). The high discriminating power of these markers allowed their use in the detection of intra-

cultivar variability (Belaj et al. 2004; Gemas et al. 2000). Applications of these markers to phylogenetic studies in *Olea europaea* species was also reported (Hess et al. 2000). Moreover, their applicability to the traceability of cultivars in the olive oil was evaluated by Muzzalupo and Perri (2002). Finally, this kind of markers was used, together with other DNA-based markers, in the construction of the first linkage maps (De la Rosa et al. 2004; Wu et al. 2004).

AFLPs (Amplified Fragment Length Polymorphism)

This technique developed by Vos et al. (1995) is based on the detection of the variation among genomic restriction fragments by PCR amplification. The procedure consists of a double digestion of genomic DNA with two restriction enzymes. The fragments generated are ligated with adaptors (short double stranded oligonucleotide with a known sequence) and then reduced in number by a selective amplification with arbitrary primers containing a core sequence that is a part of the adapter. This technique, which does not require any prior knowledge of the sequence, is very useful in the detection of polymorphisms between closely related genotypes (Belaj et al. 2004). In olive, AFLPs have been widely used for DNA fingerprinting of cultivars (Angiolillo et al. 1999; Owen et al. 2005), to analyse the relationships between wild and cultivated olive (Baldoni et al. 2006), for the construction of linkage maps (de la Rosa et al. 2003) and for cultivar traceability of olive oil (Busconi et al. 2003; Pafundo et al. 2005).

SCARs (Sequence Characterised Amplified Region)

This technique, that was introduced by Paran and Michelmore (1993), involves the conversion of single RAPD or AFLP products in sequence-characterised amplified regions by the development of specific primers, drawn on the nucleotide sequence of the RAPD or AFLP fragment. SCARs are PCR-based markers representing genetically defined loci that have been widely and successfully used in crop plants for marker-assisted selection (MAS) (Zhang and Stommel 2001).

In olive, SCARs have been used for cultivar identification (Busconi et al. 2006) and in olive oil traceability (De la Torre et al. 2004; Pafundo et al. 2007). Putative associations of several SCAR markers with fruit characteristics (Mekuria et al. 2002) and resistance to pathogenic fungi (Hernández et al. 2001) were found, suggesting the applicability of this kind of marker for marker-assisted breeding programs.

SSRs (Simple Sequence Repeats)

Microsatellites or SSR markers are regions of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units, which are arranged throughout the genomes of most eukaryotic species (Powell et al. 1996). The number of repetitions of these nucleotide units generates a polymorphism among genotypes. As for SCARs, the development of these molecular markers requires prior knowledge of the DNA sequences of the SSRs flanking regions. Nowadays, microsatellites are likely the markers of choice for genetic studies in olive because of their high polymorphism and reproducibility. Many authors have reported on SSR development in olive and several of them are currently available for DNA analysis (Sefc et al. 2000; Cipriani et al. 2002; De la Rosa et al. 2002; Sabino Gil et al. 2006).

In *Olea europaea*, these markers have been used for different applications such as cultivar discrimination (Sarri et al. 2006; Fendri et al. 2010), study of relationships between wild and cultivated olive tree (Belaj et al. 2007), construction of association maps (De la Rosa et al. 2003), paternity analysis (Mookerjee et al. 2005) and identification of olive oil varietal composition (Alba et al. 2009; Ayed et al. 2009). Recently, some attempts to improve the application of SSRs have been made in order to compare results among different laboratories. A list of recommended SSR markers and protocols for olive genotyping has been provided with the aim to develop a robust method to track the origin of olive cultivars (Doveri et al. 2008; Baldoni et al. 2009).

ISSRs (Inter Simple Sequence Repeats)

ISSRs are DNA fragments of about 100–3,000 bp located between adjacent, oppositely oriented microsatellite regions. This technique, reported by Zietkiewicz et al. (1994), based on the amplification of inter-SSR DNA sequences by using microsatellite core sequences as primers for PCR reaction. About 10–60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size.

ISSRs were applied both in phylogenetic analysis within the *Olea europaea* species and in olive cultivar identification. In a study about the structure of *Olea europaea* complex in the oceanic islands of Macaronesia, molecular evidence, provided by ISSRs and RAPDs markers, clearly indicates that Madeiran and Canarian populations of ssp. *Cerasiformis* do not form a monophyletic group, supporting for the hypothesis of two independent dispersal events of *Olea* in Madeira and Canary Islands (Hess et al. 2000). In an other study, Vargas and Kadereit (2001) confirmed the wild status of some olive trees populations in the Iberian Peninsula by means ISSRs analysis. These markers were also used with success to distinguish 10 Italian varieties, by analysing genomic DNA extracted from the olive

fruit (Pasqualone et al. 2001), and for the study of intra-cultivar variability of 201 accessions belonging to 11 Portuguese cultivars (Gemas et al. 2004).

SNPs (Single Nucleotide Polymorphism)

A Single Nucleotide Polymorphism is a small genetic variation that can occur within a DNA sequence. SNPs are the most abundant and ubiquitous type of polymorphisms in living organisms, since they occur in virtually unlimited numbers as differences in single nucleotides between individuals (Ganal et al. 2009). Due to their abundance along the genome, coupled with the development of next-generation high-throughput genomic sequencing technologies, they could be the marker system of choice in the future. The development of this kind of marker requires a high level of genome sequence information: it is therefore not surprising if only a few SNPs have been reported in olive, where only a small amount of sequence data was available before the year 2009.

To overcome this lack in sequence knowledge, in 2006 Reale et al. (2006) used both a sequence-based and an arbitrary approach to identify eight SNPs in olive. In the second approach, products from a generic fingerprinting technique were sequenced, amplified by specific primers in several olive cultivars and then compared to found polymorphisms. Muleo et al. (2009) found several SNPs in the Phytochrome A gene by means of high-resolution melting (HRM) analysis of DNA. Using this technique, the authors were able to easily detect the presence of mutations for substitution, either homozygous or heterozygous status of the gene. All the SNPs were confirmed by subsequent analysis. Finally, they concluded that HRM analysis has a very high reproducibility and sensitivity for detecting SNPs, allowing olive cultivar genotyping and resulting in an informative, easy, and low-cost method able to greatly reduce the operating time. Five SNPs were also identified by Santos Macedo et al. (2009) in the partial sequence of the gene for alternative oxidase *Oe*AOX2. Recently, Hakim et al. (2010) discovered nine new SNPs by direct sequencing of the lupeol synthase (OEW) and cycloartenol synthase (OEX) genes in 16 Tunisian olive cultivars.

Sequence variation of ribosomal and cytoplasmic DNA

The polymorphism of ribosomal and cytoplasmic non-coding DNA, such as internal transcribed spacer (ITS) and intergenic spacer (IGS), is widely used for phylogenetic studies. The lack of strict mechanisms of conservation in function for these sequences followed indeed in a high nucleotide variability, promoting the usefulness of these markers for evolutionary purposes. Variations in these regions can be detected in several ways: by direct sequencing and by digestion of amplified sequences with restriction enzymes (RFLP—Restriction Fragment Length Polymorphism—Botstein et al. 1980; Neale and Williams 1991).

Ribosomal DNA polymorphism

The sequence variation in the internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal genes 18S, 5.8S and 26S has been analysed for the description of *Olea europaea* complex. Hess et al. (2000) used different nuclear markers, including sequence variations inside the ITS1 region, to reconstruct the colonisation history of *Olea europea* L. in the Macaronesian islands. Besnard et al. (2001) studied the genetic differentiation of cultivated olive from its wild relatives recovered from different geographic areas using RAPD and RFLP of rRNA genes. Additionally, the structure of invasive populations of olive tree (belonging to subspp. *europaea* and *cuspidata*) from Australia and Hawaii was studied by Besnard et al. (2007a), using different markers, including ribosomal DNA polymorphism. The authors determined that East Australian and Hawaiian populations (subsp. *cuspidata*) have originated from southern Africa while South Australian populations (subsp. *europaea*) have mostly derived from western or central Mediterranean cultivars. Besnard et al. (2007b) used ribosomal DNA sequences from genes and pseudogenes (18S and 5.8S) and ITS1 in order to evaluate the relationships between taxa and populations inside the genus *Olea europaea* and to allow the reconstruction of evolutionary patterns involved in the differentiation of the olive complex. More recently, Besnard et al. (2009) revised the relationships within the *Oleaceae* family by using both ribosomal and cytoplasmic sequences.

Cytoplasmic DNA polymorphism

Polymorphisms in chloroplast (cpDNA) and mitochondrial DNA (mtDNA) have been used in olive for different purposes.

In 2000 Besnard et al. associated the male sterility displayed several olive cultivars with particular chloroplast and mitochondrial RFLP polymorphisms. Besnard et al. (2002a) analysed the genetic structure of Mediterranean olive trees by comparing the chlorotypes of oleasters and cultivated forms. A chlorotype-specific marker from the Eastern basin was found in several cultivated forms throughout the Mediterranean basin, suggesting a strong presence of human influence on the phylogeography of olive trees. García-Díaz et al. (2003) tested the effect of prolonged vegetative multiplication in the maintenance of mitochondrial homoplasmy and the generation of heteroplasmy. By using an intergenic spacer of the mitochondrial genome, the authors

found that several sequence changes were detected in 88.5% of the investigated genomes after several rounds of vegetative reproduction. Analysis of the same sequence in clones from olive trees obtained by sexual reproduction showed only a few changes, confirming the role of sexual reproduction in the maintenance of mitochondrial homoplasmy. Finally, Intrieri et al. (2007) used chloroplastic markers for cultivar identification and oil traceability. The authors analysed 13 cultivars and 1 feral accession of *Olea europaea* for polymorphisms in the intergenic spacer of chloroplast DNA. Four out of the 13 cultivars analysed were discriminated, and an identification protocol for these cultivars, based on the amplification and subsequent sequencing of the chloroplast trnT-trnD intergenic spacer, was suggested.

Applications of DNA-based molecular markers

In this section, we reported the main results achieved in olive studies by using DNA molecular markers. An overview on the most relevant fields of application is given, with the aim to highlight the relevant contribute that these molecular techniques gave to improve the knowledge about some theoretical and practical aspects of olive tree.

Studies on genetic variability of *Olea europaea* complex

Genetic variability study is a key step in acquiring knowledge on the resources available for the genetic improvement of a crop. This is particularly important for olive where a high number of different genotypes are currently cultivated.

In order to supplement and refine the traditional morphological description of cultivars, which show some limitations because of environmental influences, molecular markers have been applied for characterisation of the olive germplasm (Dorado et al. 2005; Trujillo et al. 2005; Ganino et al. 2006). Information on the classification of *Olea europaea* L. has resulted in very interesting advances in our understanding of the share of *Olea* taxa intercompatibility with the Mediterranean olive tree. The phylogenetic reconstruction by AFLPs of this complex confirmed *O. europaea* L. as a monophyletic group having six subspecies recognised in Eurasia and Africa (Rubio de Casas et al. 2006).

RFLP analysis of ribosomal sequences (IGS-RFLP) confirmed that the Mediterranean basin is the area of olive domestication and that the taxa more related to the olive tree are the subspecies *laperrinei*, *maroccana* and *cerasiformis* (Besnard et al. 2001). Further analysis of the subspecies of *Olea europaea* L. by RFLP analysis of chloroplast sequences highlighted a strong differentiation between chlorotypes from the Eastern and Western parts of the Mediterranean area and confirmed the close relatedness of olive to the subspecies *laperrinei* (Besnard et al. 2002b).

Several analyses regarding the patrimony of Mediterranean wild olive have been performed with the aim to characterise this as a source of genetic traits potentially useful for olive improvement programs (Hannachi et al. 2009). Mitochondrial RFLP analysis confirmed a clear genetic distinction between wild olives from the Eastern and Western parts of the Mediterranean area (Bronzini de Caraffa et al. 2002; Besnard and Bervillé 2002), while the pattern of genetic variability and the genetic relationships among different populations of wild olive from the northwestern Mediterranean basin area were clarified by SSR markers (Belaj et al. 2007). With regard to the relationships between wild and cultivated olive trees, RAPD (Bronzini de Caraffa et al. 2002), AFLP (Angiolillo et al. 1999; Baldoni et al. 2006) and SSR analysis (Erre et al. 2010) showed that cultivars had originated only in few cases by selection of local wild olive and that they have been prevalently introduced in their area of cultivation from the outside.

In olive the richness of the germplasm, coupled with the absence of references and mistakes made on cultivar denominations, remarkably complicates the classification of varieties. With the aim of overcoming these problems, numerous studies on cultivar identification have been made in the last 15 years by means of different molecular markers. RAPDs were the first molecular technique used with this purpose (Fabbri et al. 1995; Belaj et al. 2001). This approach was also used to evaluate intra- and intercultivar variability (Gemas et al. 2000). RAPD studies revealed the wide genetic variability existing at the regional (Sanz-Cortés et al. 2001), national (Belaj et al. 2003) and Mediterranean levels (Belaj et al. 2001). AFLP analyses have been used to study relationships among cultivars both from wide (Owen et al. 2005) and restricted areas of cultivation (Angiolillo et al. 2006). Among the available molecular markers, SSRs are becoming the preferred choice in olive cultivar identification because of their high discriminatory power and usually straightforward interpretation. They have been used for genotyping cultivars from different areas of the Mediterranean basin (Sarri et al. 2006), but also to characterise the local germplasm from small areas of cultivation (Poljuha et al. 2008; Bracci et al. 2009).

Olive oil traceability

Food traceability is important in order to prevent deliberate or accidental mislabelling during food production processes (Marmiroli et al. 2009). As for other products, the

introduction of certifications of origin and quality for virgin olive oil as PDO (protected designation of origin) makes necessary the implementation of traceability procedures. At the moment, DNA analysis seems to be a promising approach to this problem, since it is less influenced by environmental and processing conditions in respect to other methods (i.e., metabolites). DNA recovery methods from olive oil have been developed by several authors (Busconi et al. 2003; Doveri et al. 2006; Pasqualone et al. 2007; Consolandi et al. 2008). In addition several commercial kits, providing adapted protocols, were used in different works (Martins-Lopes et al. 2008; Spaniolas et al. 2008; Ayed et al. 2009; Pafundo et al. 2010). All of these studies confirmed that the DNA of the cultivars is recoverable from extra virgin olive oils, but it is low in quantity and quality. The first works, carried out using genomic DNA extracted from drupes, showed the possibility to make amplification using RAPDs markers (Cresti et al. 1997). By means of SCAR and AFLP markers, Busconi et al. (2003) were able to show that DNA recovered from olive oil had both organellar and nuclear origin. Pafundo et al. (2005) traced the cultivar composition of monovarietal olive oils by AFLPs, suggesting that DNA extraction is the most critical step affecting the procedure. Pafundo et al. (2007), starting from AFLPs amplified in olive oil, developed some SCARs that amplified successfully on DNA extracted from olive oil. Using SSR analysis, Pasqualone et al. (2007) demonstrated that microsatellites are useful in checking the presence of a specific cultivar in a PDO oil, thus verifying the identity of the product. However, they obtained only the marker profile of the main cultivar in the oil: no signal was detected for the secondary varieties. Montemurro et al. (2007) analysed by AFLP markers 10 virgin monovarietal olive oils prepared in the laboratory. They were able to distinguish all the olive oils examined, even if only a partial correspondence with the AFLP profile obtained from the leaves was obtained. Martins-Lopes et al. (2008) evaluated the efficiency of RAPD, ISSR and SSR molecular markers for olive oil varietal identification and their possible use in certification purposes.

Consolandi et al. (2007, 2008) reported the development of a semi-automated SNP genotyping assay to verify the origin and the authenticity of extra-virgin olive oils. The authors developed a Ligation Detection Reaction (LDR)/Universal Array (UA) platform by using several olive SNPs. They found that 13 accurately chosen SNPs were sufficient to unequivocally discriminate a panel of 49 different cultivars.

Finally, in a recent study, Pafundo et al. (2010) investigated the effect of the storage time on the degradation of the DNA purified from the oil, a negative correlation between storage time and quality–quantity of recovered DNA has been observed. The authors showed that 1 month after the production of the oil the degradation increases making harder traceability efforts.

Considering all these investigations, it is possible to note that DNA-based olive oil traceability is a topic of great importance, but in addition to studies reporting good results using DNA analysis, Doveri et al. (2006) published a cautionary note on the use of DNA markers for provenance testing. Their observations were based on non-concordance between the genetic profiles of olive oil and fruit. The authors suggested that this could be due to the contribution of pollen donors in DNA extracted from the paste obtained by crushing whole fruits. They concluded that care needs to be taken in the interpretation of DNA profiles obtained from DNA extracted from oil for resolving provenance and authenticity issues.

Paternity analysis and molecular linkage maps

Similar to other woody species, olive is characterised by a long juvenile phase that ranges between 10 and 15 years. This represents a great obstacle to breeding programs and makes the genetic improvement of olive very difficult and expensive. Although seedling-forcing growth protocols have been developed to reduce the length of this phase, the evaluation of the agronomic performance of mature olive plants still requires at least 5 years of experimentation (Santos-Antunes et al. 2005). For this reason, the application of molecular markers both to confirm the parental origins of the progeny and to select early agronomical characteristic-associated markers (Martín et al. 2005) can be very useful to reduce the time and cost of the development of new genotypes.

With regard the paternity analysis, SSRs are the most suitable to trace the genetic contribution of alleles from the parents to the offspring, being codominant and highly polymorphic markers (Mookerjee et al. 2005). The effectiveness of SSRs in the identification of paternity contribution to progeny obtained from olive breeding programs has been demonstrated by several authors (de la Rosa et al. 2004; Diaz et al. 2007a, b). The results demonstrated that SSR analysis is a convenient technique to routinely assess the crosses made in breeding programs and to for check self-incompatibility in olive cultivars (Diaz et al. 2006). These studies have highlighted that no contamination by self-pollen was found, indicating that placing pollination bags well before anthesis is important and that emasculation to avoid selfing is unnecessary (de la Rosa et al. 2004). The analysis also revealed that the main factor affecting the success of crosses seems to be the inter-compatibility among the parental cultivars, since this significantly influences the rate of contamination from external pollen donors. These results indicate that knowledge of cross-compatibility among cultivars is

necessary to plan efficient olive breeding crosses (Diaz et al. 2007a, b).

The possibility of associating genetic characteristics and DNA-based molecular markers is very important to select the progeny showing interesting agronomical traits at the first stages of development. However, this technique, called marker-assisted selection (MAS), requires some knowledge on the co-segregation of molecular markers and genetic characteristics in the progeny.

Several efforts to build an association map in olive have been ongoing in the last several years. The first attempt to construct a linkage olive map by means of RAPDs, AFLPs, RFLPs and SSRs was conducted using 95 seedlings from the cross Leccino × Dolce Agogia (de la Rosa et al. 2004). Two maps, one for each parent, were drawn and a partial coverage of the olive genome was obtained with the molecular markers used. The stearoyl-ACP desaturase gene, an important enzyme in the production of oleic acid from stearic acid (Baldoni et al. 1996), was linked to Leccino group four. Wu et al. (2004) constructed an integrated map, using linkage data from the two parents of the cross (Frantoio × Kalamata), based on RAPDs, SCARs and SSRs sequences in the progeny of 104 individuals. In this map, the gene for resistance to peacock disease, discovered by Mekuria et al. (2002), linked with the integrated linkage group 2 and with the linkage group 1 of the Frantoio cultivar.

Genomics studies

In olive, the knowledge of genome is back if compared with those of other crops (Table 2). As for other plant species, studies about olive nucleotide sequences identification started in nineties. The first DNA sequence of *Olea europaea* L. was released in NCBI database in 1994 but, in the following years, the olive genomic research has been slower than in other plants. Probably due to the renewed interest of the market for this crop and its products, recently many efforts have been made to fill this gap and from 2009 many several thousand of EST (expressed sequence tag) have been identified (Table 3). In this section, we described the most relevant results obtained in the last years in olive genome analysis.

ESTs identification

Understanding the function of genes and other parts of the genome is known as functional genomics. In olive, efforts to improve the identification and annotation of genes are prevalently based on EST identification (Table 3), which are predominantly related to pollen allergens and characteristics of olive fruit.

Respiratory allergy caused by olive pollen is an important health problem in several geographic areas worldwide (the Mediterranean basin, North America, South America and Australia) affecting more than 30% of the Mediterranean population (Bousquet et al. 1984). The relevance of this problem on human health induced to identify olive pollen allergens: it is not surprising that the first nucleotide sequences isolated in 1994 in olive (Table 3) coded for allergenic proteins (Villalba et al. 1994). To date, 10 different allergens (named Ole e 1 to Ole e 10) have been found in olive pollen (Rodríguez et al. 2002; Hamman-Khalifa et al. 2008), and for almost all these genes, with the exception of Ole e 7 and Ole e 8, the nucleotide sequences are also available. Recently, Hamman-Khalifa et al. (2008) showed the relationship between cultivar origin and high heterogeneities present in the nucleotide sequence of Ole e 1 gene among different genotypes. The

Table 2 Comparison of development of genomic studies between *Olea europaea* and several major crop plants

Species	First sequences released on NCBI (years and No. of sequences)	Accessions on NCBI in 1994	Accessions on NCBI in 2010	Genome sequence available	No. chromosomes (*n*)
Olea europaea	1994 (3)	3	7,157	No	23
Vitis vinifera	1994 (10)	10	690,548	Yes in 2007	19
Malus × *domestica* Borkh	1994 (9)	9	327,243	Yes in 2010	17
Prunus persica	1993 (3)	8	128,466	No	8
Populus trichocarpa	1996 (4)	–	180,216	Yes in 2006	19
Arabidopsis thaliana	1992 (717)	5,394	2,329,137	Yes in 2000	5
Glycine max	1993 (333)	417	1,890,141	Yes in 2010	20
Zea mays	1993 (869)	1,938	4,495,510	Yes in 2007	10
Mendicago truncatula	1993 (9)	17	467,161	Yes in 2007	8

Data of *Populus trichocarpa* and *Arabidopsis thaliana* were also introduced for a comparison, being the genetic model species, respectively, for tree and herbaceous plants. Year of first submission and number of sequences released initially, number of released nucleotide accessions in 1994 (years of the first publication of olive DNA sequences) and 2010 (to October) on NCBI database were shown

Table 3 Olive genomics information present on NCBI database (http://www.ncbi.nlm.nih.gov) from 1994 (years of the first released sequence on database of *Olea europaea*) to October 2010

Year	*Olea europaea* accessions on NCBI database				Notes
	Total nucleotide sequences	Nucleotide	*EST*	*GSS*	
1994	3	3			Nucleotide sequences referred to pollen allergen (OLE1, OLE3, OLE5)
1995	1	1			*Olea europaea* main olive allergen (Ole e I)
1996	1	1			*Olea europaea* fruit stearoyl-ACP desaturase, complete cds
1997	13	13			Pollen allergens, cloroplast NADH dehydrogenase, cytochrome b5, tandem repeat sequences, ribulose 1,5 bisphosphate carboxylase (large subunit)
1998	1	1			*Olea europaea* calcium-binding pollen allergen (OLE3), complete cds
1999	11	11			Lupeol synthase, cycloartenol syntase, RAPD sequences
2000	39	39			Cu/Zn superoxide dismutase, calcium-binding pollen allergen, internal transcribed spacer (ITS1), microsatellite sequences, tandem repeats
2001	57	57			Chloroplast intergenic spacers, polyubiquitins, hexose transporter, anthocyanidin synthase, chalcone syntase, microsatellite sequences, pollen allergen (Ole e 9), Ty1-copia-like retrotransposons, 18S ribosomal gene
2002	57	57			Microsatellite sequences, photosystem II protein D1, oleosin, β-actin, fatty acid desaturase, β-gluconidase, monosaccharide transporter, Fe superoxide dismutase
2003	88	64	24		Cox3 mitochondrial intergenic spacer, ESTs involved in response to *S. Oleagina* infection
2004	23	23			Phenylalanine ammonia-lyase (PAL), chloroplast fatty acid desaturase, microsatellite sequences, β- 1,3-glucanase, acylCoA: diacylglycerol acyltransferase
2005	213	213			Pollen allergens, chloroplast intergenic spacers, red/far-red receptor (phyA), oleate desaturase (FAD2-2), internal transcribed spacer (ITS2), 5.8S ribosomal gene, 26S ribosomal gene, aquaporins (pip1, pip2, tip), SNPs, sucrose transport-like, zeaxanthin epoxidase
2006	44	44			Geranylgeranyl reductase, B-type cyclin, microsatellites equences, ω-3 fatty acid desaturase, mannitol transporter, glycosyl transferase
2007	334	332		2	GSS are AFLP fragments from cv Hojiblanca leaves, pollen allergens, nitrate reductase, mixed amyrin synthase, plastid inter genic spacers, internal transcribed spacers, chloroplast t-RNA genes, maturase K, MADS-box, mitogen-activated protein kinase, ATPase subunits, pollen allergens
2008	186	186			Flavonoid 3-*O*-glucosyltransferase (UFGT), ribulose 1,5 bisphosphate carboxylase (small subunit), farnesyl pyrophosphate synthase-like (FPPS), microsatellite sequences, lipoxygenase, IAA transcription factor, internal transcribed spacer (ITS1), isoflavone reductase-like, photosystem I reaction center subunit XI, AcylCoA synthase, sorbitol dehydrogenase-like protein, zinc finger protein, genes involved in juvenile-adult transition, α-tubulin, Na/H antiporter, lipoxigenase, ATP binding chaperonin, cytochrome P450, catalase, c-myc binding protein, defensin, polygalacturonase
2009	4,891	55	4,836		ESTs involved in fruit development and from cDNA library of olive leaves and fruits
2010	1,195	36	1,159		3 out of the 36 nucleotide sequences are the complete sequence of olive chloroplast (cv Bianchera and Frantoio)
					ESTs involved in flower development
Total sequences	7,157	1,136	6,019	2	

Typologyof sequence records recovered from database screening: Nucleotide, EST (expressed sequence tags), GSS (genome survey sequences)

authors found that the origin of an olive cultivar is a major factor determining the diversity of Ole e 1 variants among different olive pollens. Sequence polymorphisms can influence the folding of the corresponding protein which leads to variability in the allergenicity of Ole e 1. This agrees with previous in vivo and in vitro observations that different olive cultivars differ in their capacity to bind IgE antibodies. Notably, sequence polymorphisms within the Ole e 1 gene are so high that closely related cultivars can be recognised as different, as has been reported in the case of the cultivars Picholine marocaine and Menara (considered a clonal selection of the former).

With regard the characteristics of fruits and olive oil, particular attention has been focussed on genes involved in fatty acid biosynthesis, including enoyl-ACP reductase, stearoyl-ACP desaturase, omega 6 plastidial desaturase, omega 3 plastidial desaturase, cytochrome b5, omega 6 cytoplasmatic desaturase, omega 3 cytoplasmic desaturase, acyl-CoA diacylglycerol acyltransferase and oleosin enzymes (Hatzopoulos et al. 2002). Several studies have dealt with the cloning, characterisation and spatial/temporal activation of genes involved in these pathways (Banilas et al. 2005; Poghosyan et al. 2005; Hernández et al. 2005; Giannoulia et al. 2007). In particular, very recently Banilas et al. (2010) deeply investigated the triacylglycerols (TAGs) biosynthesis. They showed that DGAT1 and DGAT2 (two families of diacylglycerol acyltransferase, the last and key enzyme of the triacylglycerols pathway) contribute differentially to the TAGs storage in the olive tissues, highlighting DGAT2 to be more involved than DGAT1 in oil accumulation in the mesocarp.

Other than fatty acid composition, the presence of minor components with antioxidant activity also has great value for human health, in terms of protecting DNA, proteins and lipids from oxidative damage. Among the minor components olive, phenolic compounds have been the most studied. As reviewed by Hatzopoulos et al. (2002), to achieve high quality olive oils with resistance to oxidation, it is crucial to enhance the quantity and efficiency of antioxidants in olive. Some studies concerning the clarification of the biosynthetic pathways for antioxidant biosynthesis have been carried out (Shibuya et al. 1999; Hatzopoulos et al. 2002; Saimaru et al. 2007).

A monosaccharide transporter (*Oe*MST2), whose expression increases during fruit maturation has been also cloned (Conde et al. 2007). In 2009, significant progress in our understanding of the olive transcriptome were achieved by the identification of genes differentially expressed during fruit development, with particular attention to those involved in lipid and phenolic metabolism. By using the 454 sequencing platform, Alagna et al. (2009) sequenced four different cDNA libraries obtained at the beginning and at the end of fruit development from two cultivars, Coratina and Tendellone, characterised, respectively, by high and low phenolic content. A total of 261,485 reads were obtained, for an output of about 58 Mb. The EST sequences generated from this study are available at the Olea EST database web site (http://140.164.45.140/oleaestdb/).

Galla et al. (2009) also identified large sets of differentially expressed genes at three different stages (i.e., initial fruit set, completed pit hardening and veraison) of fruit development in the Leccino cultivar. Four subtractive hybridisation libraries were constructed and all sequenced clones (1,132 in total) were analysed by bioinformatic tools; 60% of these showed similarities to known proteins.

Other investigations concerning the fitness of olive in responding to different environmental conditions have been carried out. Secchi et al. (2007) investigated the effect of water stress on olive by analysing the change in the expression level of genes related to the aquaporin family in plants subjected to drought treatment. The authors found a strong downregulation in these genes following drought stress, probably resulting in reduced membrane water permeability and preventing the loss of water in periods of water stress. Additionally, Bruno et al. (2009) isolated the gene encoding a geranylgeranyl reductase (*Oe*CHLP) and hypothesised its role in organ development and responses to abiotic and biotic stresses in relation to tocopherol action.

Chloroplast genome sequencing

A very important results, recently published, in *Olea europaea* L. genomic studies have been the DNA sequencing of the entire plastome of the Italian cultivar 'Frantoio' (Mariotti et al. 2010). This sequence has a length of 155,889 bp and showed an organisation and gene order that is conserved among numerous Angiosperms. The olive chloroplast contains 130 genes and 644 repetitive sequences (among which 633 mono-nucleotide SSRs, 6 di-, 3 tetra- 2 penta-nucleotide SSRs were identified).

Forty polymorphic plastid markers were identified by using eight cultivars for a comparative study. Among these, 10 markers were previously reported while 30 new cpDNA markers were identified. All these information about the chloroplast sequence will be used to better understand the evolutionary and ecological processes involved in olive domestication, the function of plastid genes on plant metabolism and they will be applied in olive cultivar identification with particular relevance to the application of DNA-based tracking of olive oil, in which one of the problems is the possibility to amplify in DNA extracted from oil and also the genomic DNA of pollinators' varieties.

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 (i.e., OLEAGEN genomics project, Fundacion Genoma, Spain, www.chirimoyo.ac.uma.es/oleagen/) and, thanks to the rapid development of the new sequencing technologies, soon the complete sequence of olive genome will be available.

The new informations on genome sequence will be very useful to identify genes involved in agronomical traits that could be used to improve the productivity and the nutritional characteristics 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 cultivar-specific molecular markers (e.g., SNPs) and in the implementation of more efficient protocols for tracking and protect olive oil origin.

References

Alagna F, D'agostino N, Torchia L, Servili M, Rao R, Pietrella M, Giuliano G, Chiusano ML, Baldoni L, Perrotta G (2009) Comparative 454 pyrosequencing of transcripts from two olive genotypes during fruit development. BMC Genomics 10:349–353

Alba V, Sabetta W, Blanco A, Pasqualone A, Montemurro C (2009) Microsatellite markers to identify specific alleles in DNA extracted from monovarietal virgin olive oils. Eur Food Res Technol 229:375–382

Angiolillo A, Mencuccini M, Baldoni L (1999) Olive genetic diversity assessed using amplified fragment length polymorphisms. Theor Appl Genet 98:411–421

Angiolillo A, Reale S, Pilla F, Baldoni L (2006) Molecular analysis of olive cultivars in the Molise region of Italy. Genet Resour Crop Evol 53:289–295

Ayed RB, Grati-Kamoun N, Moreau F, Rebaï A (2009) Comparative study of microsatellite profiles of DNA from oil and leaves of two Tunisian olive cultivars. Eur Food Res Technol 229:757–762

Baldoni L, Belaj A (2009) Olive. In: Vollmann J, Rajean I (eds) Oil crops. Handbook of plant breeding, vol 4. Springer Science + Business Media, New York, pp 397–421. doi 10.1007/978-0-387-77594-4_13

Baldoni L, Georgi LL, Abbott AG (1996) Nucleotide sequence of a cDNA clone from *Olea europaea* encoding a stearoyl acyl carrier protein desaturase. Plant Physiol 111:1353

Baldoni L, Tosti N, Ricciolini C, Belaj A, Arcioni S, Pannelli G, Germana MA, Mulas M, Porceddu A (2006) Genetic structure of wild and cultivated olives in the central Mediterranean basin. Ann Bot 98:935–942

Baldoni L, Cultrera NG, Mariotti R, Riccioloni C, Arcioni S, Vendramin GG, Buonamici A, Porceddu A, Sarri V, Ojeda MA, Trujillo I, Rallo L, Belaj A, Perri E, Salimonti A, Muzzalupo I, Casagrande A, Lain O, Messina R, Testolin R (2009) A consensus list of microsatellites markers for olive genotyping. Mol Breed 24:213–231

Banilas G, Moressis A, Nikoloudakis N, Hatzopoulos P (2005) Spatial and temporal expressions of two distinct oleate desaturases from olive (*Olea europaea* L.). Plant Sci 168:547–555

Banilas G, Karampelias M, Makariti I, Kourti A, Hatzopoulos P (2010) The olive DGAT2 gene is developmentally regulated and shares overlapping but distinct expression patterns with DGAT1. J Exp Bot. doi:10.1093/jxb/erq286

Bartolini G (2008) *Olea* databases. Available at: http://www.oleadb.it

Belaj A, Trujillo I, De la Rosa R, Rallo L (1999) Marcadores de ADN para identificacion de variedades de olivo. Agricultura 799:166–167

Belaj A, Trujillo I, de la Rosa R, Rallo L, Gimenez MJ (2001) Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an olive germplasm bank. J Am Soc Hortic Sci 126:64–71

Belaj A, Caballero JM, Barranco D, Rallo L, Trujillo I (2003) Genetic characterization and identification of new accessions from Syria in an olive germoplasm bank by means of RAPD markers. Euphytica 134:261–268

Belaj A, Rallo L, Trujillo I, Baldoni L (2004) Using RAPD and AFLP markers to distinguish individuals obtained by clonal selection of 'Arbequina' and 'Manzanilla de Sevilla' olive. HortScience 39:1566–1570

Belaj A, Muñoz-Diez C, Baldoni L, Porceddu A, Barranco D, Satovic Z (2007) Genetic diversity and population structure of wild olives from the North-western Mediterranean assessed by SSR markers. Ann Bot 100:449–458

Besnard G, Baali-Cherif D (2009) Coexistence of diploids and triploids in a Saharan relict olive: evidence from nuclear microsatellite and flow cytometry analyses. CR Biol 332:1115–1120

Besnard G, Bervillé A (2002) On chloroplast DNA variations in the olive (*Olea europaea* L.) complex: comparison of RFLP and PCR polymorphisms. Theor Appl Genet 104:1157–1163

Besnard G, Khadari B, Villemur P, Bervillé A (2000) Cytoplasmic male sterility in the olive (*Olea europaea* L.). Theor Appl Genet 100:1018–1024

Besnard G, Baradat PH, Chevalier D, Tagmount A, Bervillé A (2001) Genetic differentiation in the olive complex (*Olea europaea*) revealed by RAPDs and RFLPs in the rRNA genes. Genet Resour Crop Evol 48:165–182

Besnard G, Khadari B, Baradat P, Bervillé A (2002a) Combination of chloroplast and mitochondrial DNA polymorphism to study cytoplasm genetic differentiation in the olive complex (*Olea europaea* L.). Theor Appl Genet 105:139–144

Besnard G, Khadari B, Baradat P, Bervillé A (2002b) *Olea europaea* (*Oleaceae*) phylogeography based on chloroplast DNA polymorphism. Theor Appl Genet 104:1353–1361

Besnard G, Henry P, Wille L, Cooke D, Chapuis E (2007a) On the origin of the invasive olives (*Olea europaea* L., *Oleaceae*). Heredity 99:608–619

Besnard G, Rubio de Casas R, Vargas P (2007b) Plastid and nuclear DNA polymorphism reveals historical processes of isolation and reticulation in the olive tree complex (*Olea europaea*). J Biogeogr 34:736–752

Besnard G, Garcia-Verdugo C, Rubio De Casas R, Treier UA, Galland N, Vargas P (2008) Polyploidy in the olive complex (*Olea europaea*): evidence from flow cytometry and nuclear microsatellite analyses. Ann Bot 101:25–30

Besnard G, Rubio de Casas R, Christin P, Vargas P (2009) Phylogenetics of Olea (Oleaceae) based on plastid and nuclear

ribosomal DNA sequences: tertiary climatic shifts and lineage differentiation times. Ann Bot 104:143–160

Bitonti MB, Cozza R, Chiappetta A, Contento A, Minelli S, Ceccarelli M, Gelati MT, Maggini F, Baldoni L, Cionini PG (1999) Amount and organization of the heterochromatin in *Olea europaea* and related species. Heredity 83:188–195

Bogani P, Cavalieri D, Petruccelli R, Roselli G (1994) Identification of olive tree cultivars by using random amplified polymorphic DNA. Acta Hortic 356:98–101

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Bousquet J, Cour P, Guerin B, Michel FB (1984) Allergy in the Mediterranean area I. Pollen counts and pollinosis of Montpellier. Clin Allergy 514:249–258

Bracci T, Sebastiani L, Busconi M, Fogher C, Belaj A, Trujillo I (2009) SSR markers reveal the uniqueness of olive cultivars from the Italian region of Liguria. Sci Hortic 122:209–215

Breviglieri N, Battaglia E (1954) Ricerche cariologiche in *Olea europaea* L. Caryologia 6:271–283

Bronzini de Caraffa V, Giannettini J, Gambotti C, Maury J (2002) Genetic relationships between cultivated and wild olives of Corsica and Sardinia using RAPD markers. Euphytica 123:263–271

Bruno L, Chiappetta A, Muzzalupo I, Gagliardi C, Iaria D, Bruno A, Greco M, Giannino D, Perri E, Bitonti MB (2009) Role of geranylgeranyl reductase gene in organ development and stress response in olive (*Olea europaea*) plants. Funct Plant Biol 36:370–381

Busconi M, Foroni C, Corradi M, Bongiorni C, Cattapan F, Fogher C (2003) DNA extraction from olive oil and its use in the identification of the production cultivar. Food Chem 83:127–134

Busconi M, Sebastiani L, Fogher C (2006) Development of SCAR markers for germplasm characterisation in olive tree (*Olea europaea* L.). Mol Breed 17:59–68

Cantini C, Cimato A, Sani G (1999) Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109:173–181

Cipriani G, Marazzo M, Marconi R, Cimato A, Testolin R (2002) Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. Theor Appl Genet 104:223–228

Conde C, Agasse A, Silva P, Lemoine R, Delrot S, Tavares R, Geros H (2007) *Oe*MST2 encodes a monosaccharide transporter expressed throughout olive fruit maturation. Plant Cell Physiol 48:1299–1308

Consolandi C, Palmieri L, Doveri S, Maestri E, Marmiroli N, Reale S, Lee D, Baldoni L, Tosti N, Severgnini M, De Bellis G, Castiglioni B (2007) Olive variety identification by ligation detection reaction in a universal array format. J Biotechnol 129:565–574

Consolandi C, Palmieri L, Severgnini M, Maestri E, Marmiroli N, Agrimonti C, Baldoni L, Donini P, Bellis G, Castiglioni B (2008) A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR-universal array analysis. Eur Food Res Technol 227:1429–1438

Cresti M, Linskens HF, Mulchay DL, Bush S, Di Stilio V, My X, Vignani R, Cimato A (1997) Preliminary communication about the identification of DNA in leaves and in olive oil of *Olea europeae*. Olivae 69:36–37

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, NY

De la Rosa R, James CM, Tobutt KR (2002) Isolation and characterisation of polymorphic microsatellites in olive (*Olea europaea* L.) and their transferability to other genera in the *Oleaceae*. Mol Ecol 2:265–267

De la Rosa R, Angiolillo A, Guerrero M, Pellegrini M, Rallo L, Besnard G, Bervillé A, Martin A, Baldoni L (2003) A first linkage map of olive (*Olea europaea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. Theor Appl Genet 106:1273–1282

De la Rosa R, James CM, Tobutt KH (2004) Using microsatellites for paternity testing in olive progenies. HortScience 39:351–354

De la Torre F, Bautista R, Canovas FM, Claros MG (2004) Isolation of DNA from olive oil and oil sediments: application in oil fingerprinting. J Food Agric Environ 2:84–86

Diaz A, Martin A, Rallo P, Barranco D, de la Rosa R (2006) Self-incompatibility of 'Arbequina' and 'Picual' olive assessed by SSR markers. J Am Soc Hortic Sci 131:250–255

Diaz A, de la Rosa R, Rallo P, Muñoz-Diez C, Trujillo I, Barranco D, Martin A, Belaj A (2007a) Selections of an olive breeding program identified by microsatellite markers. Crop Sci 47:2317–2322

Diaz A, Martin A, Rallo P, de la Rosa R (2007b) Cross-compatibility of the parents as main factor for successful olive breeding crosses. J Am Soc Hortic Sci 132:830–835

Dorado G, de la Rosa R, Rallo P, Martin A (2005) Marcadores moleculares. In: Rallo L, Barranco D, Caballero JM, del Rio C, Martín A, Tous J, Trujillo I (eds) Variedades del olivo en España, Junta de Andalucía. MAPA and Ediciones Mundi-Prensa, Madrid

Doveri S, O'Sullivan DM, Lee D (2006) Non-concordance between genetic profiles of olive oil and fruit: a cautionary note to the use of DNA markers for provenance testing. J Agric Food Chem 54:9221–9226

Doveri S, Sabino Gil F, Díaz A, Reale S, Busconi M, da Câmara Machado A, Martín A, Fogher C, Donini P, Lee D (2008) Standardization of a set of microsatellite markers for use in cultivar identification studies in olive (*Olea europaea* L.). Sci Hortic 116:367–373

Erre P, Chessa I, Muñoz-Diez C, Belaj A, Rallo L, Trujillo I (2010) Genetic diversity and relationships between wild and cultivated olives (*Olea europaea* L.) in Sardinia as assessed by SSR markers. Genet Resour Crop Evol 57:41–54

Fabbri A, Hormaza JI, Polito VS (1995) Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. J Am Soc Hortic Sci 120:538–542

Fabbri A, Lambardi M, Ozden-Tokatli Y (2009) Olive breeding. In: Mohan Jain S, Priyadarshan PM (eds) Breeding plantation tree crops: tropical species. Springer Science + Business Media LLC, New York, pp 423–68

Fendri M, Trujillo I, Trigui A, Rodriguez-Garcia IM, De Dios Alche Ramirez J (2010) Simple sequence repeat identification and endocarp characterization of olive tree accessions in a Tusinian germplasm collection. Hortscience 45:1429–1436

Galla G, Barcaccia G, Ramina A, Collani S, Alagna F, Baldoni L, Cultrera NG, Martinelli F, Sebastiani L, Tonutti P (2009) Computational annotation of genes differentially expressed along fruit development. BMC Plant Biol 9:128–144

Ganal MW, Altmann T, Röder MS (2009) SNP identification in crop plants. Curr Opin Plant Biol 12:211–217

Ganino T, Bartolini A, Fabbri A (2006) The classification of olive germplasm—a review. J Hortic Sci Biotechnol 81:319–334

García-Díaz A, Oya R, Sánchez A, Luque F (2003) Effect of prolonged vegetative reproduction of olive tree cultivars (*Olea europaea* L.) in mitochondrial homoplasmy and heteroplasmy. Genome 46:377–381

Gemas VJ, Rijo-Johansen MJ, Tenreiro R, Fevereiro P (2000) Inter and intra-varietal analysis of three *Olea europaea* L. cultivars using the RAPD techniques. J Hortic Sci Biotechnol 75:312–319

Gemas VJ, Almadanim MC, Tenreiro R, Martins A, Fevereiro P (2004) Genetic diversity in the Olive tree (*Olea europaea* L.

subsp. europaea) cultivated in Portugal revealed by RAPD and ISSR markers. Genet Resour Crop Evol 51:501–511
Giannoulia K, Banilas G, Hatzopoulos P (2007) Oleosin gene expression in olive. J Plant Physiol 164:104–107
Green PS (2002) A revision of *Olea* L. (Oleaceae). Kew Bull 57:91–140
Gucci R, Tattini M (1997) Salinity tolerance in olive. Hortic Rev 21:177–213
Guerin JR, Sweeney SM, Collins GG, Sedgley M (2002) The development of a genetic database to identify olive cultivars. J Am Soc Hortic Sci 127:977–983
Hakim RI, Grati-Kammoun N, Makhloufi E, Rebaï A (2010) Discovery and potential of SNP markers in characterization of Tunisian olive germplasm. Diversity 2:17–27
Hamman-Khalifa AM, Castro AJ, Jímenez-López JC, Rodríguez-García MI, de Dios Alché J (2008) Olive cultivar origin is a major cause of polymorphism for Ole e 1 pollen allergen. BMC Plant Biol 8:10–18
Hannachi H, Sommerlatte H, Breton C, Msallem M, El Gazzah M, El Hadj SB, Berville A (2009) Oleaster (var. sylvestris) and subsp. cuspidata are suitable genetic resources for improvement of the olive (*Olea europaea* subsp. europaea var. europaea). Genet Resour Crop Evol 56:393–403
Hatzopoulos P, Banilas G, Giannoulia K, Gazis F, Nikoloudakis N, Milioni D, Haralampidis K (2002) Breeding, molecular markers and molecular biology of the olive tree. Eur J Lipid Sci Technol 104:574–586
Hernández P, de la Rosa R, Rallo L, Dorado G, Martin A (2001) Development of SCAR markers in olive (*Olea europaea*) by direct sequencing of RAPD products: applications in olive germplasm evaluation and mapping. Theor Appl Genet 103:788–791
Hernández ML, Mancha M, Martínez-Rivas JM (2005) Molecular cloning and characterization of genes encoding two microsomal oleate desaturases (FAD2) from olive. Phytochemistry 66:1417–1426
Hess J, Kadereit W, Vargas P (2000) The colonization history of *Olea europea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD) and intersimple sequence repeats (ISSR). Mol Ecol 9:857–868
Intrieri MC, Muleo R, Buiatti M (2007) Chloroplast DNA polymorphisms as molecular markers to identify cultivars of *Olea europaea* L. J Horticult Sci Biotechnol 82:109–113
Keys A (1995) Mediterranean diet and public health: personal reflections. Am J Clin Nutr 61:1321S–1323S
Loukas M, Krimbas CB (1983) History of olive cultivars based on their genetic distances. J Hortic Sci 58:121–127
Loureiro J, Rodriguez E, Costa A, Santos C (2007) Nuclear DNA content estimations in wild olive (*Olea europaea* L. ssp. europaea var. sylvestris Brot.) and Portuguese cultivars of *O. europaea* using flow cytometry. Genet Resour Crop Evol 54:21–25
Mariotti R, Cultrera NGM, Munoz Diez C, Baldoni L, Rubini A (2010) Identification of new polymorphic regions and differentiation of cultivated olives (*Olea europaea* L.) through platome sequence comparison. BMC Plant Biol 10:211
Marmiroli N, Maestri E, Pafundo S, Vietina M (2009) Molecular traceability of olive oil: from plant genomics to Food Genomics. In: Berti L, Maury J (eds) Advances in olive resources. Research Signpost, Kerala (India), pp 1–16
Martín A, Rallo P, Dorado G, Valpuesta V, Botella MA, de la Rosa R (2005) Utilización de marcadores en la mejora genetic del olivo. In: Rallo L, Barranco D, Caballero JM, del Rio C, Martín A, Tous J, Trujillo I (eds) Variedades del olivo en España, Junta de Andalucía. MAPA and Ediciones Mundi-Prensa, Madrid
Martins-Lopes P, Gomes S, Santos E, Guedes-Pinto H (2008) DNA markers for Portuguese olive oil fingerprinting. J Agric Food Chem 56:11786–11791
Mekuria GT, Collins GG, Sedgley M (1999) Genetic variability between different accessions of some common commercial olive cultivars. J Hortic Sci Biotechnol 74:309–314
Mekuria GT, Sedgley M, Collins G, Lavee S (2002) Development of a sequence-tagged site for the RAPD marker linked to leaf spot resistance in olive. J Am Soc Hortic Sci 127:673–676
Minelli S, Maggini F, Gelati MT, Angiolillo A, Cionini PG (2000) The chromosome complement of *Olea europaea* L.: characterization by differential staining of the chromatin and in situ hybridization of highly repeated DNA sequences. Chromosom Res 8:615–619
Montemurro C, Pasqualone A, Simeone R, Sabetta W, Blanco A (2007) AFLP molecular markers to identify virgin olive oils from single Italian cultivars. Eur Food Res Technol 226:1439–1444
Mookerjee S, Guerin J, Collins G, Ford C, Sedgley M (2005) Paternity analysis using microsatellite markers to identify pollen donors in an olive grove. Theor Appl Genet 111:1174–1182
Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182
Muleo R, Colao MC, Miano D, Cirilli M, Intrieri MC, Baldoni L, Rugini E (2009) Mutation scanning and genotyping by high-resolution DNA melting analysis in olive germplasm. Genome 52:252–260
Muzzalupo I, Perri E (2002) Recovery and characterisation of DNA from virgin olive oil. Eur Food Res Technol 214:528–531
Neale DB, Williams CG (1991) Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Can J For Res 21:545–554
Owen CA, Bita EC, Banilas G, Hajjar SE, Sellinakis V, Aksoy U, Hepaksoy S, Chamoun R, Talhook SN, Metzidakis I, Hatzopoulos P, Kalaitzis P (2005) AFLP reveals structural details of genetic deiversity within cultivated olive germoplasm from the Eastern Mediterranean. Theor Appl Genet 110:1169–1176
Pafundo S, Agrimonti C, Marmiroli N (2005) Traceability of plant contribution in olive oil by amplified fragment length polymorphisms. J Agric Food Chem 53:6995–7002
Pafundo S, Agrimonti C, Maestri E, Marmiroli N (2007) Applicability of SCAR markers to food genomics: olive oil traceability. J Agric Food Chem 55:6052–6059
Pafundo S, Busconi M, Agrimonti C, Fogher C, Marmiroli M (2010) Storage-time effects on olive oil DNA assessed by amplified fragments length polymorphisms. Food Chem 123:787–793
Paran I, Michelmore R (1993) Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993
Pasqualone A, Caponio F, Blanco A (2001) Inter-simple sequence repeat DNA markers for identification of drupes from different *Olea europaea* L. cultivars. Eur Food Res Technol 213:240–243
Pasqualone A, Montemurro C, Summo C, Sabetta W, Caponio F, Blanco A (2007) Effectiveness of microsatellite DNA markers in checking the identity of protected designation of origin extra virgin olive oil. J Agric Food Chem 55:3857–3862
Pérez-Jiménez F, Ruano J, Pérez-Martinez P, Lopez-Segura F, Lopez-Miranda J (2007) The influence of olive oil on human health: not a question of fat alone. Mol Nutr Food Res 51:1199–1208
Pinelli P, Galardi C, Mulinacci N, Vincieri FF, Cimato A, Romani A (2003) Minor polar compound and fatty acid analyses in monocultivar virgin olive oils from Tuscany. Food Chem 80:331–336
Poghosyan ZP, Giannoulia K, Katinakis P, Murphy DJ, Hatzopoulos P (2005) Temporal and transient expression of olive enoyl-ACP reductase gene during flower and fruit development. Plant Physiol Biochem 43:37–44

Poljuha D, Sladonja B, Šetić E, Milotić A, Bandelj D, Jakše J, Javornik B (2008) DNA fingerprinting of olive varieties in Istria (Croatia) bymicrosatellite markers. Sci Hortic 115:223–230

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222

Rallo P, Tenzer I, Gessler C, Baldoni L, Dorado G, Martini A (2003) Transferability of olive microsatellite loci across the genus *Olea*. Theor Appl Genet 107:940–946

Reale S, Doveri S, Díaz A, Angiolillo A, Lucentini L, Pilla F, Martín A, Donini P, Lee D (2006) SNP-based markers for discriminating olive (*Olea europaea* L.) cultivars. Genome 49:1193–1205

Rodríguez R, Villalba M, Batanero E, Gonzáles EM, Monsalve RI, Huecas S, Tejera ML, Ledesma A (2002) Allergenic diversity of the olive pollen. Allergy S71:6–16

Rubio MJ, Arus P (1997) Un vivero "Agromelliora Catalana" aplica en su producion de planta de olivo un control basado en las tecnologias de RAPDs y ELISA-DAS. Fruticultura 88:14–18

Rubio de Casas R, Besnard G, Schönswetter P, Balanguer L, Vargas P (2006) Extensive gene flow blurs phylogeographic but not phylogenetic signal in *Olea europaea* L. Theor Appl Genet 113:575–583

Rugini E, Pannelli G, Ceccarelli M, Muganu M (1996) Isolation of triploid and tetraploid olive (*Olea europaea* L.) plants from mixoploid cv. 'Frantoio' and 'Leccino' mutants by in vivo and in vitro selection. Plant Breed 115:23–27

Sabino Gil F, Busconi M, da Câmara Machado A, Fogher C (2006) Development and characterization of microsatellite loci from *Olea europaea*. Mol Ecol Notes 6:1275–1277

Saimaru H, Orihara Y, Tansakul P, Kang YH, Shibuya M, Ebizuka Y (2007) Production of triterpene acids by cell suspension cultures of *Olea europaea*. Chem Pharm Bull (Tokyo) 55:784–788

Santos Macedo E, Cardoso HG, Hernández A, Peixe AA, Polidoros A, Ferreira A, Cordeiro A, Arnholdt-Schmitt B (2009) Physiologic responses and gene diversity indicate olive alternative oxidase as a potential source for markers involved in efficient adventitious root induction. Physiol Plant 137:532–552

Santos-Antunes F, León L, de la Rosa R, Alvarado J, Mohedo A, Trujillo I, Rallo L (2005) The length of the juvenile period in olive as influenced by vigor of the seedlings and the precocity of the parents. Hortscience 40:1213–1215

Sanz-Cortés F, Badenes ML, Paz S, Iniguez A, Llacer G (2001) Molecular characterization of olive cultivars using RAPD markers. J Am Soc Hortic Sci 126:7–12

Sarri V, Baldoni L, Porceddu A, Cultrera NGM, Contento A, Frediani M, Belaj A, Trujillo I, Cionini PG (2006) Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. Genome 49:1606–1615

Secchi F, Lovisolo C, Uehlein N, Kaldenhoff R, Schubert A (2007) Isolation and functional characterization of three aquaporin from olive (*Olea europaea* L.). Planta 225:381–392

Sefc KM, Lopes MS, Mendonça D, Rodrigues dos Santos M, Laimer da Câmara Machado M, da Câmara Machado A (2000) Identification of microsatellite loci in olive (*Olea europaea* L.) and their characterization in Italian and Iberian olive trees. Mol Ecol 9:1171–1193

Servili M, Selvaggini R, Esposto S, Taticchi A, Montedoro G, Morozzi G (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. J Chromatogr A 1054:113–127

Shibuya M, Zhang H, Endo A, Shishikura K, Kushiro T, Ebizuka Y (1999) Two branches of the lupeol synthase gene in the molecular evolution of plant oxidosqualene cyclases. Eur J Biochem 266:302–307

Spaniolas S, Bazakos C, Awad M, Kalaitzis (2008) Exploitation of the chloroplast trnL (UAA) intron polymorphisms for the 554 authentication of plant oils by means of a Lab-on-a-chip capillary electrophoresis system. J Agric Food Chem 16: 6886–6891

Trujillo I, Morales A, Valpuesta V, Botella MA, Belaj A, Rallo P, Martín A, Dorado G (2005) Identificación de variedades de olivo por marcadores moleculares. In: Rallo L, Barranco D, Caballero JM, del Rio C, Martín A, Tous J, Trujillo I (eds) Variedades del olivo en España, Junta de Andalucía. MAPA and Ediciones Mundi-Prensa, Madrid

Vargas P, Kadereit JW (2001) Molecular fingerprinting evidence (ISSR, Inter-Simple Sequence Repeats) for a wild status of *Olea europaea* L. (Oleaceae) in the Eurosiberian North of the Iberian Peninsula. Flora 196:142–152

Villalba M, Batanero E, Monsalve RI, Gonzalez de la Pena MA, Lahoz C, Rodriguez R (1994) Cloning and expression of Ole e I, the major allergen from olive tree pollen. Polymorphism analysis and tissue specificity. J Biol Chem 269:15217–15222

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeu M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang DG, Fan JB, Siao C-J, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, TopalogloT HubbellE, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES (1998) Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280:1077–1082

Wiesman Z, Avidan N, Lavee S, Quebedeaux B (1998) Molecular characterization of common olive varieties in Israel and the West bank using randomly amplified polymorphic DNA (RAPD) markers. J Am Soc Hortic Sci 123:837–841

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wu S, Collins G, Sedgley M (2004) A molecular linkage map of olive (*Olea europaea* L.) based on RAPD, microsatellite, and SCAR markers. Genome 47:26–35

Zhang Y, Stommel JR (2001) Development of SCAR and CAPS markers linked to the beta gene in tomato. Crop Sci 41:1602–1608

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183