Chapter 11
Olive

Aurora Díaz

Abstract The scarce knowledge about the genetics of the olive tree is not comparable to the great impact of its cultivation on the economy and culture of Mediterranean countries. Actually, the polyploid nature of some *Olea europaea* subspecies has been recently confirmed by the use of new techniques and methodologies, like microsatellite markers and flow cytometry analyses.

The most extended idea among the researchers is that the origin of olive cultivation goes back to the Prehistory in the Eastern Mediterranean. The use of cytoplasmic DNA markers to trace olive migration routes has allowed identifying, at least, two possible centres of origin for the olive tree, located to the east and the west of the Mediterranean Sea, Near East and Maghreb. Nowadays, the olive tree cultivation is concentrated in Mediterranean-type climate regions with benign winters and dry and hot summers.

Modern olive oil industry requires more competitive cultivars better adapted to the new trends in olive growing. Breeding programmes undertaken have focused in obtaining new cultivars with a combination of superior characteristics, like high productivity, low vigour and compact plant architecture, earliness of flowering and fructification, resistance to pathogens and pests (i.e., leaf spot, Verticilium wilt and olive knot), among agronomic traits; and high oil content and quality, as oil traits.

The detection of a large number of mislabellings, homonyms and synonyms has raised the need of easy and accurate cultivar identification methods to manage properly the rich olive biodiversity. Up to date, morphological traits are the only markers accepted and used by the International Plant Genetic Resources Institute (IPGRI, Rome) and the International Olive Oil Council (IOOC), though their usefulness is being constantly strengthened by molecular markers to unambiguously discriminate among individuals. The use of molecular markers can speed the breeding programmes up, not only being used in identification and compatibility studies, but in

A. Díaz (✉)
Instituto de Biología Molecular y Celular de Plantas-CSIC/Universidad Politécnica de Valencia, Laboratory 0.08 Ciudad Politécnica de la Innovación, Ingeniero Fausto Elio, s/n-Escalera 8G, 46022, Valencia, Spain
e-mail: audiaber@ibmcp.upv.es

S.K. Gupta (ed.), *Technological Innovations in Major World Oil Crops, Volume 1: Breeding*, DOI 10.1007/978-1-4614-0356-2_11,

the selection of individuals with desirable agronomic characteristics in an early stage (marker-assisted selection, MAS). Isozymes became the biochemical markers most widely used in plant breeding, though they have been superseded by genetic markers. Most of them have been used with identification purposes, some cases of homonyms and synonyms being solved, and to estimate the genetic distances among very diverse sources of material (wild, feral and cultivated forms). In this sense, microsatellite markers have revealed the exotic germplasm as a source of new variability, wild genotypes being grouped together in a different gene pool than the cultivated forms. Clusterings of olive cultivars according to economically important traits have been described, what could be very useful when it comes to design breeding crosses. And the genetic relationships among olive cultivars and genotypes selected from a breeding programme that ultimately has rendered a new variety have been elucidated. Furthermore, microsatellites have become tremendously useful for checking the paternity of olive progenies from controlled crossings and exploring the compatibility relationships among olive cultivars, which is vital to design effective crosses in breeding programmes. Linkage maps in olive are needed, so markers linked to the traits of interest can be identified. Up to date, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and microsatellite markers have been used to construct linkage maps.

Genetic transformation can significantly contribute to plant breeding by generating additional genetic diversity and introducing alleles that encode desirable traits into superior cultivars. The progress in the genetic transformation methodologies in olive must be accompanied by the design of efficient regeneration protocols, via organogenesis and somatic embryogenesis.

Real-time quantitative PCR (qPCR) and real-time quantitative reverse-transcription PCR (qRT-PCR) have contributed to monitor the sanitary status of olive plants that is essential to undertake successful breeding programmes. These techniques have also been used to infer the resistance or susceptibility level of particular cultivars to olive leaf spot, this application being very valuable as a breeding tool.

From MAS to expression studies, without forgetting genetic transformation, the olive research community has used these technological innovations to acquire a deeper knowledge of the species and to transfer it to breeding programmes, what is providing the first promising results.

Keywords Olive • Morphological markers • Biochemical markers: isozymes • Genetic markers • RFLPs • RAPDs • AFLPs • SCARs • Real-time quantitative PCR • Genetic transformation

1 Introduction

The olive tree (*Olea europaea* L.) is a subtropical, evergreen oil-producing tree belonging to the family *Oleaceae*, the subfamily Oleoideae, the tribe Oleeae, the genus *Olea* and the subgenera *Olea* (Heywood 1978). The genus *Olea* comprises

more than 40 species including the cultivated, wild and feral forms under the name *O. europaea* L. (section *Olea*). However, *O. europaea* is the only species producing an edible fruit. Enormous confusion prevails around the taxonomical classification in this family. There is lack of consensus over the nomenclature adopted to distinguish the cultivated forms from the wild forms. At least three different ways of naming the olive tree, cultivated in the Mediterranean region, can be found in the literature (*O. europaea* subspecies *sativa*, *O. europaea* subspecies *europaea* var. *sativa* and *O. europaea* subspecies *europaea* var. *europaea*). A similar situation can be found in case of the wild olive trees, popularly known as acebuches (*O. europaea* subespecies *sylvestris*, *O. europaea* subespecies *europaea* var. *sylvestris* and *O. europaea* subespecies *europaea* var. *oleaster*).

Despite the huge impact of the olive tree cultivation on the economy and culture of Mediterranean countries, the knowledge about the genetics of this species is very limited. High number of chromosomes ($2n=46$) is an indicator of its polyploid (tetraploid) origin (Taylor 1945; Brousse 1987). Stergiou et al. (2002) speculated on the possible role of tropical and subtropical species, like *Olea chrysophylla* Lam. and *Olea excelsa* Ait., in its evolution. The hypothesis that domesticated olive comes from an ancient polyploid is supported by molecular data, as some microsatellite markers have been reported to show multi-locus amplification in modern olive cultivars (Cipriani et al. 2002; Diaz et al. 2006a). An alternative explanation is argued by Minelli et al. (2000), who point out the existence of chromosome fusion and rearrangements in a primitive genome, consisting of 48 chromosomes, as the probable origin of the current chromosome set. The range of possibilities (contradictory, in many cases) about the olive genome structure and origin exemplifies the scarcity of knowledge about the species and the need for undertaking a deeper investigation on its genetic behaviour. Actually, microsatellite patterns and flow cytometry analyses have confirmed the hexaploid and tetraploid nature of *O. europaea* subspp. *maroccana* and *cerasiformis*, respectively (Besnard et al. 2008; Brito et al. 2008), and the triploid status of some individuals belonging to *O. europaea* subsp. *laperrinei* (Batt. & Trab.) Ciferri (Besnard and Baali-Cherif 2009).

2 Origin and History

Some authors date the beginning of olive cultivation around the Copper Age, 4000–3000 BC (Loukas and Krimbas 1983). This long lapse of time was more delimited by Zohary and Hopf (1994), suggesting that olive domestication took place between 5,500 and 5,700 years ago. In any case, its origin is very ancient and its cultivation goes back to the Prehistory, even if it has not been possible yet to determine certainly the course of its progressive or intermittent propagation through the time (Civantos 1999).

Different hypothesis have been proposed regarding the place in which the olive tree was used as a crop for the first time (Chevalier 1948; Moazzo 1994). One of them postulates that it comes from the coasts of Syria, Lebanon and Israel while another one considers the olive tree native to Asia Minor. The historian and man of

letters De Candolle is among those supporting Syria as the centre of origin of *O. europaea* L. (De Candolle 1884). Some other authors locate the beginnings of the olive cultivation in a region around Palestine, Crete and Egypt. There are still others who maintain that the olive tree originated in Ethiopia. However, the most extended idea among the researchers is that the olive tree originated in Eastern Mediterranean, more precisely, to the north of the Dead Sea (Zohary and Spiegel-Roy 1975; Loukas and Krimbas 1983). The first documentary evidences of the olive tree cultivation go back to 4,000 years before the present era when it appeared in the oriental regions of the current Syria and Iran. More recently (2500 BC), the Minoic small clay boards named "Ebla boards" show the olive trees and bear witness to the use of the olive oil at the court of king Minos.

In the last decade, the use of cytoplasmic DNA markers to elucidate migration routes of some plant species has become very common (Tomaru et al. 1998; Sinclair et al. 1999; Gugerli et al. 2001). The study of the mitochondrial DNA polymorphism using amplified fragments length polymorphism (AFLP) markers has helped to conclude that there are, at least, two possible origins for the Mediterranean olive tree (Besnard and Berville 2000; Besnard et al. 2001a). These are, on one hand, the extensive area known as Near East and, on the other hand, Maghreb. So, two different centres of origin are proposed, located to the east and the west of the Mediterranean Sea. This conclusion is based on the discovery of a unique mitotype (ME1) to the populations of wild olive trees from Near East, whereas other two out of the four possible mitotypes (MOM and MCK) could only be found among the wild forms from the west area.

3 Olive Tree Cultivation Spread

At first, it is assumed that olive tree spread from its centre of origin, on one hand, to Cyprus and Anatolia and, on the other hand, to Crete and Egypt. There are some discrepancies in this theory, since some authors support that the olive tree went from Crete to Egypt, Asia Minor, Palestine and Greece, spreading from there to the whole Mediterranean from the second century BC. Some of the researchers consider Palestine being a primary centre of origin of the olive tree. However, Besnard et al. (2001a), who proposed two clearly differentiated areas for its origin, postulated that dispersion of olive tree took place from the oriental region of the Mediterranean toward the western shore, in clear parallel with the migratory movements of men during colonization of the European continent. This would explain why the mitotypes MOM and MCK are only present in the western area.

During the fifteenth, sixteenth and seventeenth centuries, olive cultivation spread all over the Iberian Peninsula. At the beginning of the nineteenth century it was taken to Australia by Italian emigrants. It is grown in many other countries of Latin America and has been spread even up to California. At present the countries around the Mediterranean Basin represent more than 99% of the total hectares dedicated to cultivating olive trees all around the world, Spain being the world's largest olive oil-producing country (FAOSTAT 2008). World olive oil production is around

2.9 million tonnes, the Mediterranean countries being the major contributors (International Olive Council 2009, http://www.internationaloliveoil.org/downloads/production1_ang.PDF). Up to date, there are 103 germplasm banks disperse around five continents and 25 countries which aim to preserve these resources (Olea databases 2008, http://www.oleadb.it).

4 Breeding Objectives and Procedures

The olive tree is the sixth most important oil crop in the world and in spite of its great economic importance, the most widely planted cultivars are ancient and come from the empirical selection made by growers throughout the centuries (Rallo et al. 2005). Its long juvenile period delays development of new cultivars through breeding methods involving selection among progenies generated from crossing of parents (Rallo 1995). However, the modern olive oil industry requires new and more competitive cultivars that have high oil content, better oil quality, low alternate bearing, increased productivity, suitability for mechanical harvesting and resistance to pests. In the case of table olives, other features to be considered are the shape and size of fruit; uniformity in ripening and a high pulp:stone ratio (Lavee 1994). Some cultivars, such as 'Barnea' (Lavee 1986) in Israel and 'FS 17' (Fontanazza et al. 1998) and 'Briscola' (Roselli and Donini 1982) in Italy, were obtained as a result of breeding programmes. Nevertheless, they are still not widely grown. A new cultivar has been obtained from the crossbreeding programme started in Cordoba (Spain) in 1990 (Rallo 1995). The traits considered for selection were earliness of bearing, high productivity, oil production efficiency, increased oleic acid content, a certain degree of resistance to olive leaf spot and suitability to mechanical harvesting. For this, 'Arbequina,' 'Frantoio' and 'Picual' were chosen as progenitors. More recently, another cultivar called 'Chiquitita,' which combines the outstanding characteristics of both parentals ('Arbequina' and 'Picual') has been developed (Luis Rallo et al. 2008a; Pilar Rallo et al. 2008b). It shows early bearing, high oil content and yield efficiency on one hand and, at the same time, it has low vigour and a compact architecture.

Breeding programmes undertaken have focused on obtaining new cultivars with a combination of superior characteristics. The traits of interest in breeding and improvement of olive are shown below.

4.1 Agronomic Traits

4.1.1 Productivity

Many factors can affect the productivity of an olive tree, ranging from environmental and cultural management to genetic factors. The alternate bearing conditions greatly affect the productivity. Some cultural practices contribute to diminish this

phenomenon; and there is a genetic component also as in some cultivars the alternate bearing effects are less pronounced. Another genetic characteristic (though influenced, to some extent, by the environmental conditions) is the rate of bud differentiation to reproductive inflorescences or vegetative shoots. The fruit set is also determined by the self- and cross-compatibility of the cultivars (and the nature of the pollen available), apart from other factors having an impact on the tree reproductive ability, like those causing ovary abortion.

4.1.2 Vigour and Plant Architecture

Dwarf olive trees or shrubs are desirable in order to be cultivated in a hedgerow design that would facilitate the mechanical pruning and harvesting. Several studies have evaluated the suitability of some olive cultivars to the modern high-density orchards (De la Rosa et al. 2007; Leon et al. 2007b). As a result, low-vigour cultivars, like 'Arbosana' and 'Arbequina' showed the highest productivity and oleic acid content (De la Rosa et al. 2007), the latter rending no differences in fruit oil content and moisture when grown at different densities (Leon et al. 2007b).

4.1.3 Earliness of Flowering and Fructification

The reduction of the juvenile period or the lapse of time in which the plant is not able to produce flowers, is one of the most promising olive breeding objectives. As in many other trees, the juvenile period in olive is very long, which is a handicap for growers and breeders. In an attempt to predict the juvenile/adult shift, Moreno-Alias et al. (2009) recorded a number of leaf parameters, finding an organized layer of subepidermal cells in the abaxial mesophyll exclusive to adult trees. So, the authors proposed to use this feature as a criterion of phase change in the olive tree. From a breeding perspective, there is need of an early and easy to measure trait as an indicator of the juvenile period length. In this sense, a correlation between seedling vigour parameters (i.e., height and stem diameter) and the length of the juvenile period has been observed in different studies (De la Rosa et al. 2006; Luis Rallo et al. 2008a; Pilar Rallo et al. 2008b) and is being suggested as precocity selection criterion at early developmental stages. However, Pritsa et al. (2003) observed no such correlation between vigour parameters and earliness of first flowering. Interestingly, in a comparative field assay including 15 selections, a high number of early-bearing genotypes has been reported (Leon et al. 2007a).

4.1.4 Resistance to Pathogens and Pests

Among the most important diseases affecting the olive tree, in terms of economical losses, the olive leaf spot, the *Verticilium* wilt and the olive knot are the most remarkable ones.

Olive leaf spot (also named peacock spot, peacock eye and bird's eye spot) is caused by the fungus *Spilocaea oleaginea* and it dramatically decreases the olive productivity. An extensive classification of more than 300 olive cultivars according to their resistance or susceptibility to this disease has been carried out (Trapero and Lopez-Doncel 2005). More precisely, 28 and 20 cultivars were described as resistant and highly resistant, respectively. In Israel, a cultivar resistant to *S. oleagin*a was obtained (Lavee et al. 1999) and a molecular marker linked to the locus conferring the resistance to the disease identified (Mekuria et al. 2002) which could assist in the early selection of resistant individuals in subsequent breeding programmes.

The disease known as Verticilium wilt is caused by the fungal pathogen *Verticillium dahliae*. An evaluation of 23 olive cultivars carried out by Lopez-Escudero et al. (2004) revealed three cultivars ('Frantoio,' 'Oblonga' and 'Empeltre') being moderately susceptible and resistant to the defoliating (highly virulent) and non-defoliating (mildly virulent) pathotypes, respectively. As expected, 'Empeltre' showed a remarkable recovering ability to both fungal isolates, as it is considered a valuable cultivar for inclusion in breeding programmes aimed to develop new genotypes resistant to *Verticillium* wilt. In this context, new technologies, like the real-time PCR (qPCR), have been optimized to be used as a way to identify the resistant or susceptible nature of individuals in early developmental stages (Mercado-Blanco et al. 2003) and this will undoubtedly facilitate the selection in breeding programmes.

The bacterium *Pseudomonas savastonoi* is the causal agent of olive knot disease, which infects through wounds. In an attempt to classify olive cultivars into resistant or susceptible types to knot disease Peñalver et al. (2006) inoculated 29 cultivars with two pathogen strains at two different doses and found that none of them was immune to the disease. They categorized olive cultivars as high, medium, or low susceptible to knot disease based on their reaction upon inoculation. According to these authors, some cultivars, widely included in breeding programmes for having outstanding characteristics, like 'Frantoio' and 'Picual', were among those showing low susceptibility. Nonetheless, these results should be taken cautiously, as 'Frantoio' has been reported to be the most susceptible cultivar among those tested by Hassani et al. (2003). The use of potent diagnostic tools, as the qPCR, already employed to detect *P. savastonoi* in olive samples (Bertolini et al. 2003a, b), will hopefully be useful in such studies.

4.2 Oil Traits

4.2.1 Oil Content

In all selection schemes, oil quantity and quality are the criteria invariably taken into consideration. In this context, the work carried out in the Olive Germplasm Banks of Cordoba and Catalonia (both in Spain), to measure several olive fruit characteristics is very valuable (Del Rio et al. 2005; Tous et al. 2005). The oil content (among other fruit characteristics) was measured in 112 (Del Rio et al. 2005) and 30

(Tous and Romero 2005) olive cultivars. In both cases, the cultivars were classified into five different categories according to the oil content, weight and pulp:stone ratio of their fruits, ranging from "very high" to "very low."

4.2.2 Oil Quality and Composition

Similarly to the work carried out in the Olive Germplasm Banks of Cordoba and Catalonia (both in Spain) on oil content, the composition of virgin oils coming from 74 (Uceda et al. 2005) and 28 (Tous et al. 2005) olive cultivars has been analysed. Though there were differences among the cultivars with respect to oil quality and composition obtained in both locations (as expected, due to the influence of environment and cultural practices), some important cultivars, like 'Picual', show excellent values (i.e., monounsaturated:polyunsaturated fatty acids ratio) in both cases.

Furthermore, in order to identify the relevant genes regulating the fruit metabolism and phenolic content (quality trait) during ripening, Alagna et al. (2009) undertook a transcriptomic study of 'Coratina' (cultivar with a very high phenolic content) and 'Tendellone' (an oleuropein-lacking cultivar), in both cases using material at the beginning and at the end of fruit development. Some of the genes expressed differentially at both stages coded for enzymes involved in the metabolic pathway for terpenoid biosynthesis.

5 Genetic Engineering and Molecular Biology

5.1 Types of Markers and Their Use in Olive

The genetic variability existing in the cultivated olive is enormous. To date, 2,600 different olive cultivars have been described (Rugini and Lavee 1992) and large numbers of mislabelling, homonyms and synonyms have been reported (Barranco and Rallo 2000). The preservation of this valuable genetic patrimony is of paramount importance to avoid its erosion, which would lead to an irreversible narrowing of the genetic background, as it is occurring in many other crops. During the last few decades, considerable exploration, harvesting, characterization and evaluation works of the most outstanding olive cultivars have been performed. Therefore, easy and accurate cultivar identification is an urgent necessity to manage properly the rich olive biodiversity. All the markers listed below have played an important role in the identification and evaluation of the variability present in the species, as well as in the breeding programmes and incompatibility studies.

Besides, even if the juvenile period in olive tree has been considerably shortened by forced growth techniques (Santos Antunes et al. 1999), it is still too long to make a breeding programme viable. The use of molecular markers can speed up this process. They are being used not only in identification and compatibility tasks, but also in the selection of individuals with desirable agronomic characteristics in an

early stage (marker assisted selection, MAS). For this, obtaining linkage maps in olive is needed, so that markers linked to the traits of interest can be identified.

5.1.1 Morphological Markers

Before the availability and routine use of molecular markers, a great number of morphological traits were studied in an attempt to identify and characterize the enormous variety of forms existing in most of the species of agricultural importance. In olive, the organs most commonly used for this purpose are leaves, inflorescences, whole fruits and seeds (Barranco and Rallo 1984), though pollen grains are also being used as the exine pattern seems to be a valuable discriminating character (Lanza et al. 1996). Important morphological traits used for different studies include the size, the area, the perimeter, or the longitudinal diameter (in leaves, fruits, seeds and pollen), weight (in fruits and seeds), and other organ-specific measurements, like the number of flowers or their density, in the case of the inflorescences. From these data, new parameters can be developed, like the pulp:seed weight ratio or the weight ratio in the whole fruit (pulp and stone) and the seed. Categories based on qualitative characters can also be established, like the leaf or fruit colour and shape. At present, the International Olive Oil Council (IOOC, Madrid) employs the cultivar classification system described by Barranco and Rallo (1984).

The difficulty in evaluation and the influence of general (cultural management and environmental conditions) and plant-specific (age, phenological state, etc.) factors are among the most important handicaps in the use of phenotypic characters as markers. Massei and Hartley (2000) reported a clear example of these effects. Furthermore, these authors observed that the selection aimed to increase the yield and the growth rate in the species *O. europaea* has led to a decrease in the defence mechanisms deployed by the plant. Another additional problem, considering the high cost of the orchard maintenance, is that the olive tree shows a prolonged unproductive period and most of the phenotypic characters are evaluated when the plant has reached the adult state.

At first, morphological markers were employed to discriminate olive cultivars (Barranco and Rallo 1984, 1985; Leitão 1988; Cimato et al. 1993; Prevost et al. 1993; Tous and Romero 1993; Cantini et al. 1999; Barranco et al. 2000a). Up to date, they are the only markers accepted by the International Plant Genetic Resources Institute (IPGRI, Rome) and the IOOC, though its usefulness is being constantly strengthened by molecular markers (Claros et al. 2000; Sanz-Cotes et al. 2001; Roselli et al. 2002; Rotondi et al. 2003; Corrado et al. 2009; Rao et al. 2009), as sometimes they reveal themselves insufficient to discriminate among olive forms, cultivated and oleaster trees (Hannachi et al. 2008).

5.1.2 Biochemical Markers: Isozymes

Undoubtedly, the biochemical markers most widely used in plant breeding have been the isozymes. They represent the different biochemical forms of an enzyme,

easily distinguishable by electrophoresis and coded by different alleles of the same gene (Soltis and Soltis 1989).

The methodology needed to develop and use these codominant markers in the olive tree is relatively easy, quick and affordable (Trujillo et al. 1995). They have been extensively used in olive cultivar identification due to their high level of polymorphism (Pontikis et al. 1980; Loukas and Krimbas 1983; Trujillo et al. 1990; Ouazzani et al. 1993, 1995, 1996; Hilali and El Antari 1994; Trujillo et al. 1995). However, in recent times, breeders are making use of DNA-based markers to acquire genetic knowledge about the olive tree because isozymes have many disadvantages. They are gene products, so their expression is affected by the environment, sometimes is tissue-specific and can be subjected to selective processes. Besides, less than one percent of the genetic variations involve changes in the electrophoretic mobility of the proteins, the number of isozyme systems is limited and many species are monomorphic for these markers, particularly those with a high degree of endogamy or with a narrow genetic base.

5.1.3 Genetic Markers

In last two decades, plant breeding has experimented major advances in the field of genetics with the development of new molecular markers. Genetic markers analyse directly the genotype (DNA), quite the opposite than the previous ones, which were based on the phenotypes and the gene products. For this reason, they are the most extensively used presently. Among them, PCR-based markers, amenable to automation, seem to be the most suitable ones for breeding purposes.

Restriction Fragment Length Polymorphisms

This type of markers detects the polymorphism present in the DNA strand when the target site of a restriction enzyme is altered by a mutation or by changes in a segment of the molecule, like deletions and insertions (Botstein et al. 1980). They are codominant and robust markers but their development is time consuming and expensive, and show little polymorphism in species with a small gene pool.

In the last decade, DNA coming from cytoplasmic organelles has been used to unravel origin of the olive tree and the history of its domestication. Mitochondrial (Besnard and Berville 2000; Khadari et al. 2001b; Besnard et al. 2002a; Bronzini de Caraffa et al. 2002) and chloroplastic (Amane et al. 1999; Lumaret et al. 2000; Besnard and Berville 2002; Besnard et al. 2002a, b) RFLPs have been the markers most widely used for this purpose. The discoveries reported by Amane et al. (1999) are especially interesting as they have found a correlation between a concrete chloroplastic genotype (clorotype V) and the olive male sterility. Similar results were reported by Besnard et al. (2000), who associated the male sterility with the CCK chlorotype and the MCK mitotype. The value of these markers linked to male sterility in olive breeding is enormous as they allow establishing the fertility status in

early stages of the trees. There are also works in which the polymorphism revealed by nuclear RFLPs is used to elucidate the moment of the genetic divergence of the different taxons included in the species *O. europaea* (Besnard et al. 2001a). Finally, they have been employed to construct the first olive linkage map, along with other molecular markers (De la Rosa et al. 2003).

Random Amplified Polymorphic DNAs

Random amplified polymorphic DNAs (RAPDs) were the first PCR-based DNA markers developed (Welsh and McClelland 1990; Williams et al. 1990). Arbitrary, short primers (around ten nucleotides) are used to amplify genomic DNA, what renders a band profile considered as a dominant marker.

In olive, a massive use of RAPDs has been made to achieve cultivar identification (Bogani et al. 1994; Vergari et al. 1996; Claros et al. 2000; Bandelj et al. 2001; Belaj et al. 2001; Besnard et al. 2001b; Khadari et al. 2001a, b; Sanz-Cotes et al. 2001; and a lot more besides), some cases of homonym and synonym being solved (Wiesman et al. 1998; Mekuria et al. 1999; Barranco et al. 2000a, b). It has been even possible to detect intra-cultivar polymorphism in some of the most important Portuguese cultivars intended for oil production (Gemas et al. 2000, 2004). RAPDs have been also used to estimate the genetic distance among the wild, feral and cultivated forms and within those groups (Besnard and Berville 2000; Belaj et al. 2001, 2002; Besnard et al. 2001a, b, c; Bronzini de Caraffa et al. 2002; Sesli and Yegenoglu 2009), as well as to study the olive propagation in the Macaronesian region (Hess et al. 2000). In this last study, the authors employed ISSR (inter-simple sequence repeat) markers too. The results obtained by Mekuria et al. (2002), in which a clear segregation of the RAPD band patterns in the progeny from crosses among resistant, semi-resistant and sensitive cultivars to olive leaf spot is observed. Thus, markers linked to these disease-resistance gene(s) have been identified are becoming of great interest to the olive breeding programmes. As mentioned above, the data coming from the use of different sorts of molecular markers, RAPDs among them, have been used to construct the first linkage maps of the *O. europaea* L. genome (De la Rosa et al. 2003; Wu et al. 2004).

Amplified Fragment Length Polymorphisms

The methodology necessary to develop amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995) is more complicated than the one used in the case of the RAPD markers. Firstly, the total genomic DNA of the species must be digested and the resulting fragments ligated to adaptors used as priming sites in the following rounds of PCR.

In the olive tree, AFLP markers have been used to study the intra-cultivar (Belaj et al. 2004) and inter-cultivar variation (Sanz-Cortes et al. 2003) showed by different Spanish varieties and to explore the genetic diversity and relationships of Slovene

(Bandelj et al. 2004) and southern Italian cultivars (Rao et al. 2009). Owen et al. (2005) extended the study including cultivars from Eastern, Central and Western Mediterranean Basin and found a grouping fashion that supports the East–West divergence of olive. Interestingly, Grati-Kamoun et al. (2006) reported the clustering of olive cultivars grown in Tunisia but coming from different areas of the Mediterranean Basin according to their fruit size but not to their geographical origin when they studied their genetic relationships using AFLPs. Montemurro et al. (2005) observed cultivars from different Mediterranean countries grouped according to their end-use (oil, table or dual purpose) when they analysed data generated from AFLP and microsatellite makers. The associations based on traits of such economic importance could be very useful when it comes to decide crosses in breeding programmes, as sometimes the genitors are chosen not only because of their outstanding characteristics but also because they are not genetically close, minimizing in this way inter-incompatibility issues. Together with RAPDs, AFLPs have been employed to investigate the relationship between the feral and the cultivated olives (Angiolillo et al. 1999; Baldoni et al. 2000). Gallitelli et al. (2001) carried out the same kind of study about the genetic distance among cultivated varieties, including an evaluation of the usefulness of AFLPs compared to RAPDs. Finally, AFLPs are among the markers used for obtaining the first linkage map in olive (De la Rosa et al. 2003).

Sequence Characterized Amplified Regions

Any of the DNA markers mentioned above can be transformed into an easier to use and more specific and robust sort of marker named sequence characterized amplified region (SCAR) (Paran and Michelmore 1993). For this purpose, it is necessary to clone and sequence one of the fragments obtained previously and design specific primers to amplify the region by PCR. In the olive tree, the development of several SCAR markers from RAPD (Hernandez et al. 2001a, b; Bautista et al. 2003), AFLP (Busconi et al. 2006; Pafundo et al. 2007) and selective amplification of microsatellite polymorphic loci (SAMPL, Busconi et al. 2006) bands have been reported. Though these markers are less polymorphic than others due to their dominant nature, they can be successfully used for identification purposes. In this way, the ten markers obtained by Bautista et al. (2003) were sufficient to unambiguously discriminate the 22 geographically related cultivars in study.

Microsatellites

Microsatellites or “simple sequence repeats” (SSRs) are short (1–6 bp) tandemly repeated DNA motifs (Hamada et al. 1982). They are multi-allelic, hypervariable, codominant and amenable to automation by PCR markers.

Up to date, 120 microsatellites are available in the olive tree (Rallo et al. 2000; Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; De la Rosa et al. 2002; Wu et al. 2004; Diaz et al. 2006a; Sabino-Gil et al. 2006). Even if a great effort has

been made to develop good, reliable molecular tools such as microsatellite markers that could assist breeding programmes, compared to other fruit trees, like the apple tree (*Malus domestica* B.) or the peach tree (different species of *Prunus*), it is inevitable to reach to the conclusion that they are still insufficient. All of them have been used for cultivar identification, detecting even intra-cultivar variation in some cases (Cipriani et al. 2002). Their high discrimination power have made possible to solve many cases of homonyms, synonyms and misnamings (La Mantia et al. 2005; Cantini et al. 2008). Additionally, microsatellites have been widely employed for elucidating genetic relationships among olive cultivars (Carriero et al. 2002; Belaj et al. 2003; Bandelj et al. 2004; Diaz et al. 2006a) and among the first 17 selections of an olive breeding programme (Diaz et al. 2007a) that ultimately has rendered a new variety (Luis Rallo et al. 2008a; Pilar Rallo et al. 2008b). Microsatellites have revealed a certain tendency of the cultivars to group together according to their geographical origin and routes of propagation (Rallo et al. 2003; Bandelj et al. 2004; Diaz et al. 2006a; Sarri et al. 2006), though some clustering based on their end-use have been reported too (Montemurro et al. 2005; Rekik et al. 2008). Furthermore, it has been verified that microsatellites can be transferred to related species belonging to the genus *Olea* (Rallo et al. 2003) or even to other genera in the family *Oleaceae* (De la Rosa et al. 2002), since microsatellites flanking sequences are highly conserved. And, more interestingly, the microsatellites developed in other species belonging to the same family have been employed, together with other markers, for elaborating a linkage map of the olive genome (De la Rosa et al. 2003). Different types of DNA markers, including microsatellites, have been used to construct a new olive linkage map (Wu et al. 2004).

Microsatellites have revealed themselves to be very useful for checking parentage of olive progenies from controlled crossings (De la Rosa et al. 2004; Diaz et al. 2006b, 2007a, b) since their great polymorphism makes it possible to obtain high parentage exclusion probabilities and, in some cases, to assign the paternity to concrete genotypes. De la Rosa et al. (2004) proved the enormous contamination present among the offspring coming from selfings and out-crosses within an olive breeding programme (64.4% of the seedlings had a different pollen donor from the nominal one) using this methodology. Similarly, Diaz et al. (2006b) found that none of the seeds coming from the self-pollination of 'Picual' and 'Arbequina' olives were really products of self-fecundations. Interestingly, when the offspring from controlled crosses was analysed, the pollen contamination rate was either almost total or almost null depending on the cultivars chosen as genitors (Diaz et al. 2007b). All this supports the idea of an incompatibility system acting in some olive cultivars. Microsatellites have also been used to assign the paternity to olive seeds coming from free-pollination in Australia (Mookerjee et al. 2005). However, these results themselves are not sufficient to affirm that the cultivars chosen as mother trees are self-incompatible, since their flowers were not subjected to self-pollinations; they only corroborate that foreign pollen competes favourably with its own pollen, as it has been extensively reported in the literature (Fernandez-Escobar and Rallo 1981). The same can be argued for the intercompatibility relationships established. The knowledge of the cross-compatibility

relationships in olive (Diaz et al. 2006c, 2007b) is vital to design effective crosses in breeding programmes and the microsatellites seem to be the suitable tools to verify the paternity of the seedlings. Genotyping the individuals at an early developmental stage means time and effort savings since it makes possible to discard the unwanted ones (i.e. those coming from pollen contamination) before reaching the adult phase, when it is feasible to carry out a morphological characterization.

The wild olive germplasm represents a valuable source of variability with a huge potential in breeding programmes. The transfer of both qualitative and quantitative traits from wild into domesticated forms could become an attractive objective in olive breeding programmes. In this sense, an attempt to elucidate the genetic relationships within and between wild and cultivated olives using microsatellites has been made (Erre et al. 2010). This study shows the wild genotypes clustering together in a different gene pool than the cultivated forms, revealing the exotic germplasm as a source of new variability. Regarding the structure of wild populations from north-western Mediterranean, Belaj et al. (2007) observed high and low levels of diversity within and among populations, respectively, using microsatellite markers. They hypothesized that the hybridization with cultivars and the exchange of cultivated genetic resources among different Mediterranean regions could be behind the limited genetic differentiation among populations and the lack of grouping according to their geographical origin.

Actually, microsatellites have shown a higher level of polymorphism when they were compared to other markers, like AFLPs (Belaj et al. 2003; Bandelj et al. 2004; Montemurro et al. 2005) and RAPDs (Belaj et al. 2003). In this context, enterprises like the molecular database, included in the olea databases (http://www.oleadb.it), where the allelic profile of a wide set of olive cultivars for 12 microsatellite markers can be consulted, facilitate access to information continuously being expanded. At the same time, a standardization of some of the microsatellites available is starting to be carried out (Doveri et al. 2008; Baldoni et al. 2009), with the same cultivars being genotyped with a set of markers in different laboratories. This kind of work is aimed to compare the results obtained using diverse methodologies in different laboratories and to rank the markers according to their usefulness in cultivar discrimination.

Single Nucleotide Polymorphisms

In recent years, a new generation of molecular markers has entered into the molecular biology field, particularly in the human diseases diagnostic area. These are termed as single nucleotide polymorphisms (SNPs) and consist of single DNA base differences (single base pair changes or deletions) between homologous genomes in which the minor allele is present in 1% of the cases or more (Cooper et al. 1985). The considerable increase in sequences available in databases has revealed the high frequency of these DNA variations in genomes. This abundance turns SNPs into good genome coverage supplier markers, their frequency in several crop species being an order of magnitude higher than that of microsatellites (Kwok et al. 1996). Other desirable characteristic also present in SNPs is their codominant inheritance.

The first SNP-based markers were recently developed in olive (Reale et al. 2006). They were used for cultivar identification purposes, allowing the authors to verify the authenticity of samples coming from the same cultivar but collected in different geographical locations. They made possible to unambiguously discriminate 77% of the cultivars studied. Additionally, an assumed case of synonym between 'Ottobratica' and 'Mirtolia' was clarified, as both rendered different genotypes for the 11 markers tested (nine SNPs among them). This confirms the usefulness of molecular markers in clearing confusion in olive nomenclature. Methodologies to process a high number of samples with a large number of markers (due to their low polymorphism) are complicated. In this context, Consolandi et al. (2007) have used a microarray-based approach to identify 49 olive cultivars using 17 new SNPs. Similarly, Muleo et al. (2009) successfully used a high-resolution melting (HRM) analysis for identification purposes. Nonetheless, the most important drawback in scoring SNPs is the high cost and the necessity of sophisticated equipments for employing the majority of the methodologies developed. For this, the transformation of SNPs into codominant PCR and gel-based markers, like cleaved amplified polymorphic sequences (CAPS), as proposed by Reale et al. (2006), seems to be a good way of getting an easy to use and low-cost method.

Although the development and use of SNPs in olive are still in an early phase, these are markers with an enormous potential in a broad range of applications such as genetic diversity studies, evolutionary and population genetics, mapping, quantifying linkage disequilibrium and marker-assisted plant breeding. In this sense, SNPs located in coding or functional regions of the genomes are especially useful in MAS, since associations between the markers and particular traits allow a more efficient and cost-effective phenotype selection. In short, olive SNPs can be appropriate for the study of genetic diversity and cultivar identification at first, and in future for studies of associations with economically valuable traits.

5.2 Genetic Transformation

Genetic transformation can significantly contribute to plant breeding by generating additional genetic diversity, which can be subsequently subjected to selection through classical and molecular approaches, but also introducing alleles that encode desirable traits into superior cultivars. Successful genetic transformation has been undertaken in some economically important crop species, such as maize, rice, cotton and soybean. Concisely, two methodologies have been developed to transfer an engineered gene into a plant chromosome, the *Agrobacterium*-mediated transformation and the microprojectile bombardment. The first strategy employed in the case of the olive tree was the agro-transformation (Rugini 1986). This technique has been used with the aim of reducing the olive tree size and enhancing its rooting ability (Rugini and Fedeli 1990). The authors employed *Agrobaterium rhizogenes* to transform immature zygotic embryos of 'Morailo' cultivar. Though the transgenic nature of the calli selected was confirmed molecularly, the regeneration

was a limitation. Mencuccini et al. (1999) succeeded in obtaining transgenic calli using adult material (leaf petioles) from 'Dolce Agogia' cultivar as starting material. However, they failed to regenerate the whole plant. Regarding the biolistic technique, Lambardi et al. (1999) detected GUS transient expression in somatic embryos derived from 'Canino' cultivar. Though encouraging results were obtained with cotyledon explants, nowadays, the protocols for olive genetic transformation through biolistic methods are still under optimization (Perez-Barranco et al. 2009).

What becomes clear from above is that the progress in the genetic transformation methodologies in olive must be accompanied by the design of efficient regeneration protocols, via organogenesis and somatic embryogenesis.

5.3 Real-Time Quantitative PCR and Real-Time Quantitative Reverse-Transcription PCR

It is well known that plant diseases impact negatively on yield and fruit quality. For this reason, to develop efficient methods aimed to monitor the sanitary status of the plants is essential to undertake successful breeding programmes. The development of real-time quantitative PCR (qPCR) and real-time quantitative reverse-transcription PCR (qRT-PCR) has allowed the routine and reliable quantification of PCR products with a great specificity and sensitivity, becoming a valuable diagnosis tool (Schaad and Frederick 2002). The qRT-PCR has become a powerful diagnosis tool as in many cases, the viruses can be latent or the symptoms are cultivar-specific, the visual inspections being unreliable. Faggioli et al. (2002) employed one-step qRT-PCR protocol to correlate the infection of olive trees by the strawberry latent ring spot virus (SLRSV) with leaf symptoms. Furthermore, multiplex qRT-PCR has been optimized in olive, allowing to detect a number of different viruses infecting the tree in a single step (Bertolini et al. 2001; Luigi et al. 2009; Varanda et al. 2010). A variant of this technique, the nested qRT-PCR has been successfully used to detect four RNA viruses and the bacterium *Pseudomonas savastanoi* simultaneously in more than 240 olive samples belonging to 15 different cultivars (Bertolini et al. 2003a; Bautista et al. 2003). The high sensitivity of qRT-PCR made possible to diagnosis infection even when the amount of the viral RNA was below the minimum threshold required by other techniques (Grieco et al. 2002; Alabdullah et al. 2009; Varanda et al. 2010). Interestingly, the quantification of DNA coming from highly virulent (defoliating) and mildly virulent (nondefoliating) *Verticillium dahliae* by qPCR has allowed the establishment of a correlation between those values and the susceptibility of olive cultivars to Verticillium wilt (Mercado-Blanco et al. 2003). This methodology has rendered satisfactory results when combined with others, such as doubled-stranded RNA (dsRNA) analysis and dot blot hybridization, complementing and/or improving the data shed by the latter (Montemurro et al. 2008; Alabdullah et al. 2009).

Due to its sensitivity, among many other applications, qRT-PCR can be used to compare the gene expression of samples subject to different treatments. Benitez et al. (2005), coupling this technique to differential display, identified olive genes involved in signalling, transcriptional control and stress response, whose transcript levels were significantly raised after the infection with the fungus *Schinia oleagina*. Interestingly, the induction of those genes was higher and earlier in the resistant cultivar 'Lechin de Sevilla' compared to the susceptible cultivar 'Picual'. Additionally, the basal expression of some of those genes was increased in the resistant cultivar compared to the susceptible one even when uninfected, suggesting that a constitutive activation of the response pathways could be under its invulnerability. The authors propose the measure of the basal expression of those genes as a way of inferring the resistance or susceptibility level of a particular cultivar, as they observed a correlation between both parameters when different cultivars were analysed. The potential use of this type of assays as a breeding tool to identify and select resistant individuals is obvious.

6 Conclusions

Genetic engineering techniques cannot be considered as substitutes for classical methods in plant breeding. Quite the opposite, the new advances in Genetics and Molecular Biology should be used in combination with conventional breeding, which will facilitate the work of breeders, as it is actually happening in the case of the olive tree. From MAS to expression studies, without forgetting genetic transformation, groups working in olive have known how to incorporate them to their fields of study to generate basic knowledge and to apply it to the breeding of the species.

References

Alabdullah A, Elbeaino T, Minafra A, Digiaro A, Martelli GP (2009) Detection and variability of olive latent virus 3 in the Mediterranean region. J Plant Pathol 91:521–525

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 10:339

Amane M, Lumaret R, Hany V, Ouazzani N, Debain C, Vivier G, Deguilloux MF (1999) Chloroplast-DNA variation in cultivated and wild olive (*Olea europaea* L.). Theor Appl Genet 99:133–139

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

Baldoni L, Pellegrini M, Mencuccini M, Angiolillo A (2000) Genetic relationships among cultivated and wild olives revealed by AFLP markers. Acta Hort 521:275–283

Baldoni L, Cultrera NG, Mariotti R, Ricciolini 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 microsatellite markers for olive genotyping. Mol Breed 24:213–231

Bandelj D, Jakse J, Javornik B (2001) Identification of olive (*Olea europaea* L.) cultivars by molecular markers. Zbornik Biotehniske Facultete Univerze v Ljubljani. Kmetijstvo 77:11–17
Bandelj D, Jakse J, Javornik B (2004) Assessment of genetic variability of olive varieties by microsatellite and AFLP markers. Euphytica 136:93–102
Barranco D, Rallo L (1984) Las variedades de olivo cultivadas en Andalucía. Ministerio de Agricultura, Junta de Andalucía, Madrid
Barranco D, Rallo L (1985) Las variedades de olivo cultivadas en España. Olivae 9:16–22
Barranco D, Rallo L (2000) Olive cultivars in Spain. Hort Technol 10:107–110
Barranco D, Cimato A, Fiorino P, Rallo L, Touzani A, Castañeda C, Serafin F, Trujillo I (2000a) World Catalogue of Olive Varieties. International Olive Oil Council, Madrid
Barranco D, Trujillo I, Rallo P (2000b) Are Oblonga and Frantoio olives the same cultivar? Hort Sci 35:6
Bautista R, Crespillo R, Cánovas FM, Claros MG (2003) Identification of olive-tree cultivars with SCAR markers. Euphytica 129:33–41
Belaj A, Trujillo I, De la Rosa R, Rallo L (2001) Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an olive germplasm bank. J Am Soc Hort Sci 126:64–71
Belaj A, Satovic Z, Rallo L, Trujillo I (2002) Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theor Appl Genet 105:638–644
Belaj A, Satovic Z, Cipriani G, Baldoni L, Testolin R, Rallo L, Trujillo I (2003) Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing relationships in olive. Theor Appl Genet 107:736–744
Belaj A, Rallo L, Trujillo I, Baldoni L (2004) Using RAPD and AFLP markers to distinguish individuals obtained by clonal selection of 'Arbequina' and 'Manzamila de Sevilla' olive. Hort Sci 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
Benitez Y, Botella MA, Trapero A, Alsalimiya M, Caballero JL, Dorado G, Muñoz-Blanco J (2005) Molecular analysis of the interaction between *Olea europaea* and the biotrophic fungus *Spilocaea oleagina*. Mol Plant Pathol 6:425–438
Bertolini E, Olmos A, Martinez MC, Gorris MT, Cambra M (2001) Single-step multiplex RT-PCR for simultaneous and colourimetric detection of six RNA viruses in olive trees. J Virol Methods 96:33–41
Bertolini E, Olmos A, Lopez MM, Cambra M (2003a) Multiplex nested reverse transcription-polymerase chain reaction in a single tube for sensitive and simultaneous detection of four RNA viruses and Pseudomonas savastanoi pv. savastanoi in olive trees. Phytopathology 93:286–292
Bertolini E, Peñalver R, Garcia A, Olmos A, Quesada JM, Cambra M, Lopez MM (2003b) Highly sensitive detection of *Pseudomonas savastanoi* pv. *savastanoi* in asymptomatic olive plants by nested-PCR in a single closed tube. J Microbiol Methods 52:261–266
Besnard G, Baali-Cherif D (2009) Coexistence of diploids and triploids in a Saharan relict olive: evidence from nuclear microsatellite and flow cytometry analices. Comptes Rendus Biologies 332:1115–1120
Besnard G, Berville A (2000) Multiple origins for Mediterranean olive (*Olea europaea* L. ssp. *europaea*) based upon mitochondrial DNA polymorphisms. Life Sci 323:173–181
Besnard G, Berville 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, Berville A (2000) Cytoplasmic male sterility in the olive (*Olea europaea* L.). Theor Appl Genet 100:1018–1024
Besnard G, Baradat P, Berville A (2001a) Genetic relationships in the olive (*Olea europaea* L.) reflect multilocal selection of cultivars. Theor Appl Genet 102:251–258

Besnard G, Baradat P, Chevalier D, Tagmount A, Berville A (2001b) 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, Breton C, Baradat P, Khadari B, Berville A (2001c) Cultivar identification in olive based on RAPD markers. J Am Soc Hort Sci 126:668–675

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

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

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

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

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

Brito G, Loureiro J, Lopes T, Rodriguez E, Santos C (2008) Genetic characterisation of olive trees from Madeira Archipelago using flow cytometry and microsatellite markers. Genet Resour Crop Evol 55:657–664

Bronzini de Caraffa V, Maury J, Gambotti C, Breton C, Berville A, Giannettini J (2002) Mitochondrial DNA variation and RAPD mark oleasters, olive and feral olive from Western and Eastern Mediterranean. Theor Appl Genet 104:1209–1216

Brousse G (1987) Olive. In: Robbelen G, Downey RK, Ashri A (eds) Oil crops of the world, their breeding and utilization. McGraw Hill Publishing Company, New York, pp 462–474

Busconi M, Sebastiani L, Fogher C (2006) Development of SCAR markers for germplasm characterisation in olive tree (*Olea europea* 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

Cantini C, Cimato A, Autino A, Redi A, Cresti M (2008) Assessment of the Tuscan olive germplasm by microsatellite markers reveals genetic identities and different discrimination capacity among and within cultivars. J Am Soc Hort Sci 133:598–604

Carriero F, Fontanazza G, Cellini F, Giorio G (2002) Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theor Appl Genet 104:301–307

Chevalier A (1948) L'origine de l'olivier cultivéet ses variations. Rev Int Appl Agric Trop 28:1–25

Cimato A, Cantini C, Sani G, Marranci M (1993) II Germoplasma dell'Olivo in Toscana. Regione Toscana, Florence

Cipriani G, Marrazzo MT, 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

Civantos D (1999) La olivicultura en el mundo y en España. In: Barranco D, Fernandez-Escobar R, Rallo L (eds) El cultivo del olivo. Mundiprensa, Madrid, pp 19–33

Claros GM, Crespillo R, Aguilar ML, Canovas FM (2000) DNA fingerprinting and classification of geographically related genotypes of olive-tree (*Olea europaea* L.). Euphytica 116:131–142

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 reactionin a universal array format. J Biotechnol 129:565–574

Cooper DN, Smith BA, Cooke H, Niemann S, Schmidtke J (1985) An estimate of unique sequence heterozygosity in the human genome. Hum Genet 69:201–205

Corrado G, La Mura M, Ambrosino O, Pugliano G, Varricchio P, Rao R (2009) Relationships of Campanian olive cultivars: comparative analysis of molecular and phenotypic data. Genome 52:692–700

De Candolle A (1884) Origin of cultivated plants. Kegal Paul Trench & Co., London
De la Rosa R, James CM, Tobutt KR (2002) Isolation and characterization of polymorphic microsatellites in olive (*Olea europaea* L.) and their transferability to other genera in the Oleaceae. Mol Ecol Notes 2:265–267
De la Rosa R, Angiolillo A, Guerrero C, Pellegrini M, Rallo L, Besnard G, Berville 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 KR (2004) Using microsatellite markers to check parentage of some olive progenies. Hort Sci 39:351–354
De la Rosa R, Kiran AI, Barranco D, Leon L (2006) Seedling vigour as a preselection criterion for short juvenile period in olive breeding. Aust J Agric Res 57:477–481
De la Rosa R, Leon L, Guerrero N, Rallo L, Barranco D (2007) Preliminary results of an olive cultivar trial at high density. Aust J Agric Res 58:392–395
Del Rio C, Caballero JM, Garcia-Fernandez MD (2005) Rendimiento graso de la aceituna (Banco de Germoplasma de Córdoba). In: Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Trujillo I (eds) Variedades de olivo en España (Book II: Variabilidad y selección). Junta de Andalucía, MAPA and Ediciones Mundi-Prensa, Madrid, pp 347–356
Diaz A, De la Rosa R, Martin A, Rallo P (2006a) Development, characterization and inheritance of new microsatellites in olive (*Olea europaea* L.) and evaluation of their usefulness in cultivar identification and genetic relationships studies. Tree Genet Genomes 2:165–175
Diaz A, Martin A, Rallo P, Barranco D, De la Rosa R (2006b) Self-incompatibility of 'Arbequina' and 'Picual' olive assessed by SSR markers. J Am Soc Hort Sci 131:250–255
Diaz A, Rallo P, De la Rosa R (2006c) Self- and cross-incompatibility mechanisms: a strategy to ensure a great variability in olive (*Olea europaea* L.) populations. Olea 25:29–33
Diaz A, De la Rosa R, Rallo P, Muñoz-Diez C, Trujillo I, Barranco D, Martín 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 the main factor for successful olive (*Olea europaea* L.) breeding crosses. J Am Soc Hort Sci 132:1–6
Doveri S, Sabino-Gil F, Diaz A, Reale S, Busconi M, da Câmara Machado A, Martin A, Fogher C, Lee D (2008) Standardization of a set of microsatellite markers for use in cultivar identification studies in olive (*Olea europaea* L.). Sci Hort 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
Faggioli F, Ferretti L, Pasquini G, Barba M (2002) Detection of Strawberry latent ring spot virus in leaves of olive trees in Italy using a one-step RT PCR. J Phytopathol 150:636–639
FAOSTAT (2008) http://faostat.fao.org/.
Fernandez-Escobar R, Rallo L (1981) Influencia de la polinización cruzada en el cuajado de frutos de cultivares de olivo (*Olea europaea* L.). ITEA 45:51–58
Fontanazza G, Bartolozzi F, Vergara G (1998) Fs-17. Riv Frutticol 5:61
Gallitelli M, Cifarelli RA, Giorio G, Cellini F (2001) Analysis of olive (*Olea europaea* L.) cultivars using AFLP markers and RAPD markers. Plant and animal genome IX conference. San Diego, California, p 325
Gemas VJV, Rijo-Johansen MJ, Tenreiro R, Fevreiro P (2000) Inter- and intra-varietal analysis of three *Olea europaea* L. cultivars using the RAPD technique. J Hort Sci Biotechnol 75:319–321
Gemas VJV, 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
Grati-Kamoun N, Mahmoud FL, Rebai A, Gargouri A, Panaud O, Saar A (2006) Genetic diversity of Tunisian olive tree (*Olea europaea* L.) cultivars assessed by AFLP markers. Genet Resour Crop Evol 53:265–275

Grieco F, Alkowni R, Saponari M, Pantaleo V, Savino V, Martelli GP (2002) Molecular detection of olive-infecting viruses. Proc Fourth Intl Symp Olive Growing, Acta Hort (ISHS) 586:737–740

Gugerli F, Sperisen C, Buchler U, Magni F, Geburek T, Jeandroz S, Senn J (2001) Haplotype variation in a mitochondrial tandem repeat of Norway spruce (*Picea abies*) population suggests a serious founder effect during postglacial re-colonization of the western Alps. Mol Ecol 10:1255–1263

Hamada H, Petrini MG, Kakunaga T (1982) A novel repeated element with Z-DNA-forming potential is widely found in evolutionary diverse eukaryotic genomes. Proc Natl Acad Sci U S A 79:6465–6469

Hannachi H, Breton C, Msallem M, Ben El Hadj S, El GM, Berville A (2008) Differences between native and introduced olive cultivars as revealed by morphology of drupes, oil composition and SSR polymorphisms: a case study in Tunisia. Sci Hort 116:280–290

Hassani D, Buonaurio R, Tombesi A (2003) Response of some olive cultivars, hybrid and open pollinated seedlings to *Pseudomonas savastanoi* pv. *savastanoi*. In: Iacobellis NS, Collmer A, Hutcheson SW, Mansfield JW, Morris, CE, Murillo J, Schaad NW, Stead DE, Surico G (eds) 6th International conference on *Pseudomonas syringae* and related pathogens: biology and genetics, Maratea

Hernandez P, De la Rosa R, Dorado G, Martin A (2001a) 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

Hernandez P, De la Rosa R, Rallo L, Martin A, Dorado G (2001b) First evidence of a retrotransposon-like element in olive (*Olea europaea*): implications in plant variety identification by SCAR-marker development. Theor Appl Genet 102:1082–1087

Hess J, Kadereit JW, Vargas P (2000) The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequences repeat (ISSR). Mol Ecol 9:857–868

Heywood HU (1978) Flowering plants in the world. Oxford University Press, London

Hilali S, El Antari A (1994) Varietal polymorphism in fruit-bearing olive cultivars in Marrakesh: a study. Olivae 50:45–47

International Olive Council (2009) http://www.internationaloliveoil.org/downloads/production1_ang.PDF

Khadari B, Berville A, Dore C, Dosba F, Baril C (2001a) Genetic diversity of Moroccan cultivated olive using RAPD markers. Acta Hort 546:439–442

Khadari B, Breton C, Besnard G, Roger JP, Berville A, Dore C, Dosba F, Baril C (2001b) Molecular characterization and genetic structure of olive germplasm collection in Conservatoire Botanique National Mediterranean de Porquerolles using nuclear RAPD markers and RFLP of mitochondrial DNA. Acta Hort 546:433–437

Kwok PY, Deng Q, Zakeri H, Taylor SL, Nickerson DA (1996) Increasing the information content of STS-based genome maps: identifying polymorphisms in mapped STSs. Genetics 31:123–126

La Mantia M, Lain O, Caruso T, Testolin R (2005) SSR-based DNA fingerprints reveal the genetic diversity of Sicilian olive (*Olea europaea* L.) germplasm. J Hort Sci Biotechnol 80:628–632

Lambardi M, Amorosi S, Caricato G, Benelli C, Branca C, Rugini E (1999) Microprojectile-DNAdelivery in somatic embryos of olive (*Olea europaea* L.). Acta Hort 474:505–509

Lanza B, Marsilio V, Martinelli N (1996) Olive pollen ultrastructure: characterization of exine pattern through image analysis scanning electron microscopy (IA-SEM). Sci Hort 65:283–294

Lavee S (1986) Olive. In: Monselise SP (ed) Handbook of fruit set and development. CRC, Boca Raton, FL, pp 261–276

Lavee S (1994) Por qué la necesidad de nuevas variedades de olivos? Fruticultura Profesional 62:29–37

Lavee S, Harshemesh H, Haskal A, Avidan B, Ogrodovich A, Avidan N, Trapero A (1999) 'Maalot' a new cultivar for oil extraction resistant to *Spilocaea oleagina* (Cast.). Acta Hort 474:125–128

Leitão F (1988) Contributo para o conhecimiento de cultivares de (*Olea europaea* L.) que sobre o aspecto de caracterizaçao, quer da productividade, determinante do seu valor económico. Estaçao Agronómica Nacional, INIA, Oeiras
Leon L, De la Rosa R, Barranco D, Rallo L (2007a) Breeding for early bearing in olive. Hort Sci 42:499–502
Leon L, De la Rosa R, Rallo L, Guerrero N, Barranco D (2007b) Influence of spacing on the initial production of hedgerow 'Arbequina' olive orchards. Span J Agric Res 5:554–556
Lopez-Escudero FJ, Del Rio C, Caballero JM, Blanco-Lopez MA (2004) Evaluation of olive cultivars for resistance to *Verticillium dahliae*. Eur J Plant Pathol 110:79–85
Loukas M, Krimbas CB (1983) History of olive cultivars based on their genetic distances. J Hort Sci 58:121–127
Luigi M, Manglli A, Thomaj F, Buonaurio R, Barba M, Faggioli F (2009) Phytosanitary evaluation of olive germplasm in Albania. Phytopathol Mediterr 48:280–284
Lumaret R, Amane M, Ouazzani N, Baldoni L, Debain C (2000) Chloroplast DNA variation in the cultivated and wild olive taxa of the genus *Olea* L. Theor Appl Genet 101:547–553
Massei G, Hartley SE (2000) Disarmed by domestication? Induced responses to browsing in wild and cultivated olive. Oecologia 122:225–231
Mekuria GT, Collins GG, Sedgley M (1999) Genetic variability between different accessions of some common commercial olive cultivars. J Hort 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 Hort Sci 127:673–676
Mencuccini M, Micheli M, Angiolillo A, Baldoni L (1999) Genetic transformation of olive (*Olea europaea* L.) using *Agrobacterium tumefaciens*. Acta Hort 474:515–519
Mercado-Blanco J, Collado-Romero M, Parrilla-Araujo S, Jimenez-Diaz RM (2003) Quantitative monitoring of colonization of olive genotypes by *Verticillium dahliae* pathotypes with real-time polymerase chain reaction. Physiol Mol Plant Pathol 63:91–105
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* hybridisation of highly repeated DNA sequences. Chromosome Res 8:615–619
Moazzo GP (1994) Les plantes d'Homère et de quelques autres poètes de l'Antiquité. V. L'olivier (Elaie). Annales du Musei Goulandris 9:185–223
Montemurro C, Simeone R, Pasqualone A, Ferrara E, Blanco A (2005) Genetic relationships and cultivar identification among 112 olive accessions using AFLP and SSR markers. J Hort Sci Biotechnol 80:105–110
Montemurro C, Simeone R, Blanco A, Saponari M, Bottalico G, Savino V, Martelli GP, Pasqualone A (2008) Sanitary selection and molecular characterization of olive cultivars grown in Apulia. Proc Fifth Intl Symp Olive Growing 791:603–609
Mookerjee S, Guerin J, Collins G, Ford C, Sedgley M (2005) Paternity analysis using microsatellite markers to identify pollen donors. Theor Appl Genet 111:1174–1182
Moreno-Alias I, Leon L, De la Rosa R, Rapoport HF (2009) Morphological and anatomical evaluation of adult and juvenile leaves of olive plants. Trees 23:181–187
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
Olea databases (2008) http://www.oleadb.it/.
Ouazzani N, Lumaret R, Villemur P, Di Guito F (1993) Leaf alloenzyme variation in cultivated and wild olive trees. J Hered 84:34–42
Ouazzani N, Lumaret R, Villemur P (1995) Apport du polymorphisme alloenzymatique à l'identification variétale de l'Olivier (*Olea europaea* L.). Agronomie 15:1–7
Ouazzani N, Lumaret R, Villemur P (1996) Genetic variation in the olive tree (*Olea europaea* L.) cultivated in Morocco. Euphytica 91:9–20
Owen CA, Bita EC, Banilas G, Hajjar SE, Sellianakis V, Aksoy U, Hepaksoy S, Chamoun R, Talhook SN, Metzidakis I, Hatzopoulos P, Kalaitzis P (2005) AFLP reveals structural details of

genetic diversity within cultivated olive germplasm from the Eastern Mediterranean. Theor Appl Genet 110:1169–1176
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
Paran I, Michelmore RM (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:958–993
Peñalver R, Garcia A, Ferrer A, Bertolini E, Quesada JM, Salcedo CI, Piquer J, Perez-Panades J, Carbonell EA, Del Rio C, Caballero JM, Lopez MM (2006) Factors affecting *Pseudomonas savastanoi* pv. *savastanoi* plant inoculations and their use for evaluation of olive cultivar susceptibility. Phytopathology 96:313–319
Perez-Barranco G, Torreblanca R, Padilla IMG, Sanchez-Romero C, Pliego-Alfaro F, Mercado JA (2009) Studies on genetic transformation of olive (*Olea europaea* L.) somatic embryos: I. Evaluation of different aminoglycoside antibiotics for nptII selection; II. Transient transformation via particle bombardment. Plant Cell Tiss Organ Cult 97:243–251
Pontikis CA, Loukas M, Kousounis G (1980) The use of biochemical markers to distinguish olive cultivars. J Hort Sci 55:333–343
Prevost G, Bartolini G, Messeri C (1993) Italian olive cultivars and their synonyms. Menegazzo edition, Lucca
Pritsa TS, Voyiatzis DG, Voyiatzi CJ, Sotiriou MS (2003) Evaluation of vegetative growth traits and their relation to time to first flowering of olive seedlings. Aust J Agric Res 54:371–376
Rallo L (1995) Selección y mejora genética del olivo en España. Olivae 59:46–53
Rallo P, Dorado G, Martin A (2000) Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). Theor Appl Genet 101:984–989
Rallo P, Tenzer I, Gessler C, Baldoni L, Dorado G, Martin A (2003) Transferability of olive microsatellite loci across the genus *Olea*. Theor Appl Genet 107:940–946
Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Trujillo I (2005) Las variedades de olivo cultivadas en España. Consejería de Agricultura y Pesca, Ministerio de Agricultura, Pesca y Alimentación. Ediciones Mundi-Prensa, Madrid
Rallo L, Barranco D, De la Rosa R, Leon L (2008a) 'Chiquitita' olive. Hort Sci 43:529–531
Rallo P, Jimenez R, Ordovas J, Suarez MP (2008b) Possible early selection of short juvenile period olive plants based on seedling traits. Aust J Agric Res 59:933–940
Rao R, La Mura M, Corrado G, Ambrosino O, Foroni I, Perri E, Pugliano G (2009) Molecular diversity and genetic relationships of southern Italian olive cultivars as depicted by AFLP and morphological traits. J Hort Sci Biotechnol 84:261–266
Reale S, Doveri S, Diaz A, Lucentini L, Angiolillo A, Pilla F, Martin A, Donini P, Lee D (2006) SNP-based assessment of genetic relationships among *Olea europaea* L. cultivars. Genome 49:1193–1205
Rekik I, Salimonti A, Kamoun NG, Muzzalupo I, Lepais O, Gerber S, Perri E, Rebai A (2008) Characterization and identification of Tunisian olive tree varieties by microsatellite markers. Hort Sci 43:1371–1376
Roselli G, Donini B (1982) Briscola, nuova cultivar di olivo a sviluppo comatto. Riv Ortoflorofrutt It 66:103–104
Roselli G, Petruccelli R, Polsinelli L, Cavalieri D (2002) Variability in five Tuscan olive cultivars. J Genet Breed 56:51–60
Rotondi A, Magli M, Ricciolini M, Baldoni L (2003) Morphological and molecular analyses of the characterization of a group of Italian olive cultivars. Euphytica 132:129–137
Rugini E (1986) Olive. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 10. Springer, New York, pp 253–267
Rugini E, Fedeli E (1990) Olive (*Olea europaea* L.) as an oilseed crop. In: Bajaj YPS (ed) Legumes and oilseed crops I. Biotechnology in agriculture and forestry, vol 10. Springer, New York, pp 593–641
Rugini E, Lavee S (1992) Olive. In: Hammerschlag FA, Litz RE (eds) Biotechnology of perennial fruit crops. CAB, Wallingford, pp 371–382

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

Santos Antunes AF, Mohedano A, Trujillo I, Rallo L, Metzidakis IT, Voyiatzis DG (1999) Influence of the genitors on the flowering of olive seedlings under forced growth. Acta Hort 474:103–105

Sanz-Cortes F, Parfitt DE, Romero C, Struss D, Llacer G, Badenes ML (2003) Intraspecific olive diversity assessed with AFLP. Plant Breed 122:173–177

Sanz-Cotes F, Badenes ML, Paz S, Iñiguez A, Llacer G (2001) Molecular characterization of olive cultivars using RAPD markers. J Am Soc Hort 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

Schaad NW, Frederick RD (2002) Real-time PCR and its application for rapid plant disease diagnosis. Can J Plant Pathol 24:250–258

Sefc KM, Lopes MS, Mendonça D, Rodrigues Dos Santos M, da Câmara L, Machado M, da Câmara Machado A (2000) Identification of SSR loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. Mol Ecol 9:1171–1173

Sesli M, Yegenoglu ED (2009) Standardization of RAPD assay for genetic analysis of olive. Afr J Biotechnol 8:6772–6776

Sinclair WT, Morman JD, Ennos RA (1999) The postglacial history of Scots pine (*Pinus sylvestris* L.) in Western Europe: evidence from mitochondrial DNA variation. Mol Ecol 8:83–88

Soltis DE, Soltis PS (1989) Isozymes in Plant Biology. Dioscorides Press, Portland

Stergiou G, Katsiotis A, Hagidimitriou M, Loukas M (2002) Genomic and chromosomal organization of Ty1-*copia*-like sequences in *Olea europaea* and evolutionary relationships of *Olea* retroelements. Theor Appl Genet 104:926–933

Taylor H (1945) Cyto-taxonomy and phylogeny of the Oleaceae. Brittonia 5:337–367

Tomaru N, Takahashi M, Tsumura Y, Takahashi M, Ohba K (1998) Intraspecific variation and phylogeographic patterns of *Fagus crenata* (Fagaceae) mitochondrial DNA. Am J Bot 85:629–636

Tous J, Romero A (1993) Variedades del olivo. Fundación "La Caixa", Barcelona

Tous J, Romero A (2005) Rendimiento graso de la aceituna (Banco de Germoplasma de Cataluña). In: Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Trujillo I (eds) Variedades de olivo en España (Book II: Variabilidad y selección). Junta de Andalucía, MAPA and Ediciones Mundi-Prensa, Madrid, pp 347–356

Tous J, Romero A, Diaz I (2005) Composición del aceite (Banco de Germoplasma de Cataluña). In: Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Trujillo I (eds) Variedades de olivo en España (Book II: Variabilidad y selección). Junta de Andalucía, MAPA and Ediciones Mundi-Prensa, Madrid, pp 357–372

Trapero A, Lopez-Doncel LM (2005) Resistencia y susceptibilidad al repilo. In: Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Variedades I (eds) Trujillo de olivo en España (Book II: Variabilidad y selección). Junta de Andalucía, MAPA and Ediciones Mundi-Prensa, Madrid, pp 321–328

Trujillo I, Rallo L, Carbonell EA, Asins MJ (1990) Isoenzymatic variability of olive cultivars according to their origin. Acta Hort 286:137–140

Trujillo I, Rallo L, Arus P (1995) Identifying olive cultivars by isozyme analysis. J Am Soc Hort Sci 120:318–324

Uceda M, Beltran G, Jimenez A (2005) Composición del aceite (Banco de Germoplasma de Córdoba). In: Rallo L, Barranco D, Caballero JM, Del Rio C, Martin A, Tous J, Trujillo I (eds) Variedades de olivo en España (Book II: Variabilidad y selección). Junta de Andalucía, MAPA and Ediciones Mundi-Prensa, Madrid, pp 357–372

Varanda C, Cardoso JMS, Felix MD, Oliveira S, Clara MI (2010) Multiplex RT-PCR for detection and identification of three necroviruses that infect olive trees. Eur J Plant Pathol 127:161–164

Vergari G, Patumi M, Fontanazza G (1996) Use of RAPDs markers in the characterisation of olive germplasm. Olivae 60:19–22

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

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

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 Hort Sci 123:837–841

Williams JGK, 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-B, 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

Zohary D, Hopf M (1994) Domestication of Plants in the Old World. Clarendon, Oxford

Zohary D, Spiegel-Roy P (1975) Beginnings of fruit growing in the old world. Science 187:319–327