Chapter 13
Olive

Luciana Baldoni and Angjelina Belaj

13.1 Introduction

13.1.1 Overall Importance of the Crop and Production Areas

The olive (*Olea europaea* L.), grown on over 8 million hectares, is the second most important oil fruit tree crop worldwide after oil palm and its cultivation is traditionally concentrated in the Mediterranean area. The total olive oil production for the 2006–2007 season was 2,859,500 tons (International Olive Oil Council (IOOC) data). Southern European countries account for about 74.9% of the world production, with Spain being the main producer (38.7%), followed by Italy (21%) and Greece (12.9%). Other important olive oil producers are Turkey, Tunisia and Syria (17.1%) as well as Jordan, Morocco and Algeria.

Seventy percent of the olive oil produced globally is consumed in the Mediterranean area, but its demand is rapidly increasing in other countries. For example, the consumption of olive oil in the USA grew from 88,000 tons in 1990 to 251,000 tons in 2007, in Japan from 4,000 to 31,000, and in Australia from 13,500 to 40,000 tons (IOOC data).

13.1.2 Major Problems of Olive Cultivation

The income from olive production is quite low due to the low mean production per unit area of about 2 t/ha of olives, the low content of oil rarely exceeding 24% of fresh weight (depending on variety, agro-climatic conditions and extraction method) and the high costs of cultivation. Fruit production starts 3–5 years after planting, and olive orchards can survive almost indefinitely due to the longevity of the species. However, alternate bearing and the costs of cultivating old trees generally force the reduction of the lifetime of olive orchards to no more than 50–60 years. In order to minimize the costs of production,

L. Baldoni (✉)
CNR, Istituto di Genetica Vegetale, Perugia, Italy
e-mail: luciana.baldoni@igv.cnr.it

J. Vollmann, I. Rajcan (eds.), *Oil Crops*, Handbook of Plant Breeding 4,
DOI 10.1007/978-0-387-77594-4_13,

new models of cultivation have been developed based on the complete mechanization of harvesting and pruning, the most time-consuming steps in oil production.

The olive is a long-living evergreen tree that grows up to 15 m at maturity. Its life span is typically longer than 500 years, and trees older than 2,000 years are still under cultivation in numerous regions (Fig. 13.1). Flowers are generally hermaphroditic and wind pollinated, and most cultivars are self-incompatible.

The olive fruit is a drupe (Fig. 13.2) and, in contrast to other oil crops, the oil accumulates in the mesocarp, and only 3–4% of the oil is derived

Fig. 13.1 Ancient olive still under cultivation in Apulia (Italy). Olive trees may survive for thousands of years and many of them can be found along the entire Mediterranean area of cultivation. Plants like this represent the most ancient cultivated living plants of the world

Fig. 13.2 Green olive drupes; at ripening the colour will turn to reddish or black

from the seed. The best olive oils are extracted from olives harvested at the green-turning-to-black stage of ripeness; they have to be immediately milled to minimize oxidation and enzymatic reactions.

13.1.3 Types of Olive Oil and Characteristics

Virgin olive oil is mechanically extracted by pressing or centrifuging crushed fruits without any chemical treatment. Main component of olive oil triglycerides is the mono-unsaturated oleic acid (18:1), which represents 57–80% of total fatty acids, followed by linoleic (18:2) (7–19%), linolenic (18:3) (0.6–0.8%), palmitic (16:0) and stearic (18:0) acids (Salas et al. 2000).

Due to the mechanical processing, virgin olive oil represents a fresh-squeezed fruit juice containing, other than triglycerides, a vast range of microconstituents (more than 230 compounds) including phenolics, tocopherols, aliphatic and triterpenic alcohols, sterols (and their precursor squalene), hydrocarbons and volatile compounds, representing up to 20% of the fresh olives and about 2% of virgin olive oil (Obied et al. 2008). The phenolic compounds of olive and virgin olive oil show a peculiar composition that can not be found in any other vegetable oil. They include different types of hydrophilic phenols, such as secoiridoids, lignans, flavonoids, phenolic alcohols, and phenolic acids. Secoiridoids are the most important class of phenolics and they arise from simple structures, like tyrosol and hydroxytyrosol, to quantitatively more important,

conjugated forms, like oleuropein and ligstroside (Servili et al. 2004). The protective activity of olive oil on the prevention of a variety of tumors and important chronic and degenerative diseases may be credited to these minor constituents unique to virgin olive oil. There is extensive background demonstrating the effectiveness of olive oil and some of its compounds on human health.

Oleuropein, exclusively present in olive and in a few other related taxa, is a non-toxic secoiridoid known to exhibit several biological properties, many of which may result from their antioxidant and free radical scavenger activity. A number of observations elevate oleuropein from a non-toxic antioxidant into a potent anti-tumor agent with direct effects against tumor cells (Hamdi and Castellon 2005; Giamarellos-Bourboulis et al. 2006). Beauchamp et al. (2005) have recently identified *oleocanthal* (deacetoxy ligstroside aglycon), a compound present in newly pressed extra-virgin olive oils that was shown to be a 3–4 times more potent inhibitor of Cox-1 and Cox-2 than ibuprofen, a potent modulator of inflammation and analgesia (Galli 2006). *Squalene* is a triterpene containing six isoprene units and representing a key intermediate in the biosynthetic pathway to steroids in plants and animals. It has been demonstrated to protect against skin cancer (Newmark 1999). *Minor compounds* are also responsible for the organoleptic qualities, taste and flavor of extra virgin olive oils, which may distinguish oils originating from different regions.

As defined by the European Union Council Reg. (EC) 1513/2001, there are additional categories of olive oils: *Refined oil* is obtained from low quality virgin olive oil after chemical and physical refining treatments, which lead to removal of contaminants or degraded compounds. *Olive oil* consists of a blend of refined and virgin olive oil. Finally, *olive-pomace oil* is obtained by treating olive pomace with solvents.

Within the category of virgin olive oil the extra virgin is far more valuable than most other vegetable oils, due to the low percentage of free fatty acidity ($<0.8\%$) and the superior taste, but its production is costly and time consuming. For this reason adulteration is very common, mainly represented by blending premium extra virgin olive oil with seed oils or cheap olive oil. In order to reduce frauds, valorize excellence and defend the origin of best products, a number of regulations and standards have been defined by the International Olive Oil Council and by the Codex Alimentarius Commission. Moreover, the European Union has established the option of a protected designation of origin (EU Commission Reg. (EC) 1898/2006) for olive oils of important regional and traditional origins.

The quality of extra virgin olive oils strongly depends on the cultivar of origin, which affects the content of specific polyphenols and aromatic compounds controlling taste and flavour of the oil. Agro-climatic factors, harvesting time and oil extraction technologies may have a negative effect on the olive oil quality, altering the fatty acid and phenolic composition.

DNA fingerprinting is a powerful aid for the identification of olive oil provenance, and fingerprinting methods have been applied to trace the varietal origin of batches of olive oil (Muzzalupo et al. 2007; Busconi et al. 2003; Breton et al. 2004; Pasqualone et al. 2004; Testolin and Lain 2005).

13.1.4 Ancient and Recent History of Olive Cultivation

Olives have been extensively cultivated along the Mediterranean basin since 3000 BC and have had an enormous impact on the economy, history, culture, and environment of the area. Ancient Greeks and Romans considered olive oil a sacred substance and used it as food, medicine, soap, fuel for lamps and as base for perfumes. There are a great number of archaeological remains showing the cultivation, extraction, commerce, and consumption of olive oil from the main civilizations along the Mediterranean region.

The first evidence for olive cultivation is seen during the Minoan age, when olives were cultivated on the island of Crete (3000–1500 BC). They were then cultivated by the Egyptians (2000 BC) and grown in an almost specialized form. In 1000 BC olive started to be cultivated in Palestine and, between the ninth and eighth centuries BC, olive growing was seen in Greece and in North African coasts surrounding the Mediterranean Sea, where it was introduced by the Phoenicians. Thanks to the Phoenician and Greek shipping routes, olive trees reached the coasts of Sicily and Spain, where they were widely diffused in the fifth century BC. Between the sixth and fourth century BC their cultivation was established in many regions of Italy and Spain by the Romans. By the first century AD, olives were a cash crop for the Romans, who imported oil from the most remote colonies of the empire, mainly Spain and North Africa. Olive cultivation declined during the Medieval age but increased again after the 15th century, up to the present extension of cultivated area in the Mediterranean (Mastrangelo 1982).

After several unsuccessful attempts to introduce olive cultivation in Central and North America during the 18th century, olive production has recently started in new countries such as Argentina, Chile, Mexico, USA (California), New Zealand, Australia, and South Africa, that now represent 1.4% of world olive production.

13.2 Origin and Domestication

The poor historical documentation on cultivar pedigrees and the fragmented information available on olive paleobotany have failed to provide definitive conclusions on the origin of the cultivated olive, but numerous hypotheses are currently under evaluation. Palynological, anthropological, and archeological evidence (Watts et al. 1996; Carrion and Dupré 1996) have demonstrated the presence of sporadic forms of olive during the last glaciation (18000 BC) in the western and eastern Mediterranean regions.

Oleasters have been directly exploited for oil extraction since 4500–4300 BC as evidenced by archaeological and paleobotanical findings from Spain (Terral et al. 2004; Rodríguez-Ariza and Montes Moya 2005). Some studies on olive domestication have asserted that cultivars moved westward with human migrations

(Besnard et al. 2001a; Belaj et al. 2002). Other recent evidence has pointed out that the domestication process started simultaneously at both ends of the Mediterranean (Lumaret et al. 2004; Breton et al. 2006). Analyses of archaeological charcoal and olive stones have effectively dated domestication to the end of the Bronze Age in the north-western Mediterranean area (Terral 2000; Terral et al. 2004; Rodríguez-Ariza and Montes Moya 2005).

The first cultivars were probably selected from trees bearing large fruits and/or high oil content and were vegetatively propagated, either via cutting or grafting onto indigenous oleasters. However, considering the long life of olive plants, there has been relatively little selection and probably only a few generations separate the presently cultivated forms from their progenitors (Liphschitz et al. 1991).

Taking into account recent results obtained by molecular analysis of sets of wild olives and cultivars from different Mediterranean regions, it has been assumed that olive trees have undergone different selection/domestication processes in different regions. The contribution of local wild plants to the development of varieties has been demonstrated only in restricted areas where wild olives, very ancient cultivated trees, and local varieties shared a large portion of variability. In other areas of the western Mediterranean, in contrast, the clear distinction between oleasters and cultivars has confirmed that local cultivars did not develop from local oleasters but were introduced from abroad and propagated by grafting onto local oleasters (Baldoni et al. 2006).

13.3 Varietal Groups

13.3.1 Cultivated Olive Germplasm

Olive germplasm has not suffered significant genetic erosion, maintaining almost intact its entire variability, given that turnover with new genotypes has not occurred, and that the species has great longevity and a good capacity to survive without cultivation. Thus, more then 1,200 varieties are still under cultivation, 79 national and international collections hold about 4,200 genotypes and more than 5,300 cultivar names are recognized (Bartolini et al. 1998). Over two thirds of cultivars are present in the southern European countries (538 in Italy, 183 in Spain, 88 in France, 52 in Greece and 45 in Turkey) (Bartolini et al. 1998), and many other local varieties and ecotypes contribute to the richness of the olive germplasm. Olive represents, therefore, an unusual case among horticultural crops and its germplasm could constitute a particularly rich source of variability to be used directly or for future breeding.

Olive cultivars can be considered as varieties of unknown origin, most of them originating from empirical selections made by growers from naturally cross-bred genotypes over many centuries and propagated by cutting or grafting. Evidence for multilocal selection of most cultivars has been repeatedly demonstrated (Besnard et al. 2001b; Rotondi et al. 2003).

The genetic diversity of cultivated populations shows a complex patchy pattern (Baldoni et al. 2006; Owen et al. 2005). Few cultivars are dispersed over widespread areas, whereas the majority of varieties are highly localized. Cultivars may either have a non-autochthonous origin or have been derived by selection from local oleasters (Lumaret et al. 2004; Breton et al. 2006).

Up to now, identification of each variety has been delayed by confusion about the names given to each genotype, the low intervarietal genetic distances, the intra-cultivar variability and the putative presence of asymptomatic viruses that may affect the plant phenotype.

13.3.2 Cultivar Classification

The geographical distribution of olive cultivars and their economical importance are considered the main criteria for their classification. According to this system, Barranco and Rallo (2000) divided olive cultivars into four categories: main, secondary, dispersed and local. Main cultivars are those which account for either a large portion of the acreage or predominate in one or more olive districts. Secondary cultivars are not predominant in any district but form the basic cultivars of some orchards. Dispersed and local cultivars are isolated trees in various or single districts, respectively. The agronomical performance of main cultivars has been widely evaluated, while for most of the others information is unavailable.

13.3.3 Identification of Olive Cultivars

Until recently, the variability of *O. europaea* germplasm has been reported with respect to morphological descriptors. Understanding the amount and distribution of genetic variability among cultivars by means of molecular markers has been the main goal for most of research on olive trees.

Various types of molecular markers have been widely applied over the last decade, particularly in investigations aiming at studying the variability of olive cultivars and at developing tools to determine their origin and detecting frauds on olive oil varietal composition (Hess et al. 2000; Rallo et al. 2000; Sefc et al. 2000; Guerin et al. 2002; Owen et al. 2005; Montemurro et al. 2005; Vargas and Kadereit 2001; Carriero et al. 2002; Cipriani et al. 2002; Bandelj et al. 2002; Sarri et al. 2006).

Isozymes were the first molecular markers used (Trujillo et al. 1995). Since then, cultivar identification has been based on DNA markers such as RAPDs using different protocols (Mekuria et al. 1999; Belaj et al. 2002; Gemas et al. 2000; Sanz-Cortés et al. 2001; Gonzalo-Claros et al. 2000). AFLP data are available on a wide number of cultivars (Angiolillo et al. 1999; Baldoni et al. 2000; Sanz-Cortés et al. 2003; Sensi et al. 2003; Owen et al. 2005), ISSRs (Pasqualone et al. 2001; Vargas and Kadereit 2001) have also been used and SCAR markers have been developed from RAPDs (Hernandez et al. 2001).

To date, very few SNP markers have been identified in olive. Based on the sequence of candidate genes and their frequency along coding sequences, it has been estimated that there is one SNP in every 190 base pairs (Reale et al. 2006; Consolandi et al. 2007).

For cultivar characterization simple sequence repeats (SSRs) have become the most popular kind of marker (Sefc et al. 2000; Rallo et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; De la Rosa et al. 2002; Díaz et al. 2006a; Sabino Gil et al. 2006), and it has been demonstrated that only three SSR markers are able to distinguish more than a hundred olive genotypes (Sarri et al. 2006). In a recent paper a set of the most effective SSR markers has been selected and proposed for varietal characterization (Baldoni et al. 2009).

DNA markers used to identify olive cultivars have also been applied for DNA tracking of olive oils to test their varietal composition (Breton et al. 2004; Pasqualone et al. 2004; Pafundo et al. 2005; Testolin and Lain 2005; Doveri et al. 2006; Muzzalupo et al. 2007; Consolandi et al. 2008).

13.4 Genetic Resources

13.4.1 Taxonomy and Distribution of Olea europaea

The genus *Olea* belongs to the Oleaceae family, sub-family Oleideae. The genus includes two sub-genera: *Olea* and *Paniculatae*, the former is divided into sections *Olea* and *Ligustroides*. According to recent revisions of *Olea europaea* taxonomy, the species includes six sub-species based on morphology and geographical distribution (Green and Wickens 1989; Green 2002):

- subsp. *europaea*, represented by two botanical varieties: cultivated olive (var. *europaea*) and wild olive (var. *sylvestris*), both present throughout the whole Mediterranean basin;
- subsp. *cuspidata*, distributed along south Asia, on the Arabian peninsula, and throughout east and south Africa;
- subsp. *laperrinei*, restricted to the Sahara region;
- subsp. *maroccana*, present in Morocco;
- subsp. *cerasiformis*, typical of Madeira island;
- subsp. *guanchica*, restricted to the Canary Islands.

Wild and cultivated olives are diploid ($2n = 2x = 46$), predominantly allogamous and their genome size is about 1,800 Mb (Loureiro et al. 2007; Besnard et al. 2008).

13.4.2 Natural Diversity of Olive

The geographic distribution of variability within the *Olea* genus and the genetic relationships among the wild (oleasters) and cultivated olives have been studied using various molecular methods (Angiolillo et al. 1999;

Besnard et al. 2002a,b; Lumaret et al. 2004; Baldoni et al. 2006; Breton et al. 2006; Belaj et al. 2007; Rubio de Casas et al. 2006).

The wild relatives of cultivated species exhibit traits such as biotic stress resistances, adaptation to extreme environmental conditions, plant vigour and architecture, which could be utilized in olive breeding. Model plant genetic systems and the molecular genetic resources that are currently available are greatly enhancing our ability to identify adaptive or stress-responsive genetic determinants. But natural diversity is still an underexploited sustainable resource for olive that could enrich the genetic basis of cultivated plants with novel alleles to improve both productivity and adaptation.

The potential value of wild olive trees and related species as a source of agronomically interesting traits has never been evaluated, thus severely restricting the ability of breeders to develop new genotypes by introgression of superior alleles into cultivated varieties. Oleasters growing under the drought-salt-heat complex conditions and ultra-millennially aged olive trees surviving in adverse environments have only occasionally been submitted to phenotypic evaluation, and there is a lack of serious prospecting surveys to characterize wild olive populations (Mulas et al. 2004; Belaj et al. 2007).

Up to now, most work has concentrated on evaluating the distribution of variability between cultivated and wild olives and on establishing the genetic relationships among the different *O. europaea* subspecies that are distributed beyond the Mediterranean area.

13.4.3 Wild Olives

Wild olive (*Olea europaea* subsp. *europaea* var. *sylvestris*), also known as oleaster, has colonized diverse environments along the Mediterranean basin, characterised by semi-arid climatic conditions with different altitudes, vegetative communities and soils, including those with extreme levels of drought, low temperatures and salinity (Baldoni et al. 2006). Wild plants occur in the same areas as domesticated olive, in the *maquis* and in uncultivated sites, and show some morphological differences from cultivars, such as a bushy plant shape and small fruit size (Terral and Arnold-Simard 1996).

The contribution of oleasters to the evolution of cultivated olive is still questionable, and a widely debated problem relates to the distinction between real oleasters and feral plants derived from the natural dissemination of cultivars. In fact, both forms occur in the same ecological sites and their appearance is very similar. Different criteria have been proposed to clearly distinguish the two forms based on geo-ecological parameters (Lumaret et al. 2004) or on molecular markers (Baldoni et al. 2006; Breton et al. 2006). It is believed that oleasters have originated in the eastern Mediterranean, and that wild olives present in the western Mediterranean basin could be feral (Besnard et al. 2002b).

In previous studies performed through the analysis of chloroplast, mitochondrial, and nuclear DNA polymorphisms, it has been shown that eastern and western Mediterranean oleaster populations are strongly differentiated from one another (Besnard et al. 2001b, 2002b; Lumaret et al. 2004).

It has been hypothesized that humans could have brought eastern-specific chlorotypes to the west, probably bringing plant material (or olive fruits) from the eastern to western Mediterranean (Besnard et al. 2002b). The linkage disequilibrium between widely-spread chlorotypes and nuclear markers characteristic to eastern oleasters can be explained by the common origin of these polymorphisms in a wild population from the east. These results support the hypothesis that western oleasters could be feral forms as a result of an eastern introduction and a gene flow from olive groves towards wild populations.

Studies carried out by the use of allozyme markers (Lumaret et al. 2004) have pointed out the genetic evidence that genuine oleasters still survive locally in the west Mediterranean, as shown by west-specific alleles found in wild olives collected in forests potentially containing genuinely wild forms according to environmental, historical, and demographic criteria. Western populations are more closely related to the wild populations of the Canary Islands. Populations of wild olive seem to be restricted to a few isolated areas of the native Mediterranean forests, where pollen and stones may be wind/bird-distributed.

Other studies, performed on oleaster populations of restricted areas on both sides of the Mediterranean pointed out significant differences between east and west areas (Rubio de Casas et al. 2006). The highest genetic diversity was found at the extreme western side of Mediterranean and in the Balearic islands. Additionally, long-lasting isolation of the northern populations of the Iberian Peninsula appears to be responsible for a significant divergence. Gene flow estimates demonstrated that genetic material seems to be exchanged frequently among populations in accordance with the predominant outcrossing between wild and cultivated individuals. Birds eating olives (Rey and Alcántara 2000) may enable long distance dispersal and make exchange of migrants common even between distant regions. Pollen circulation can also occur over long distances, enhancing lineage admixture.

The cline of genetic diversity revealed by chloroplast and SSR markers was explained by oleaster re-colonization of the Mediterranean basin from refugees after the last glacial event, located in both eastern and western regions (Breton et al. 2006). Based on different population analysis methods, it has been shown that oleasters are equally present in the eastern and the western Mediterranean, and that they are native and not derived from cultivars. It is also likely that gene flow has occurred in oleasters mediated by cultivars spread by human migration or through trade and animals.

The evaluation of olive differentiation at a microscale regional level has shown more complex results (Baldoni et al. 2006). Levels of interpopulation genetic variance between wild olive populations present in distant islands and highly differentiated from mainland oleasters, which only partially represent

real wild olives, were very low, even if the populations were clearly distinguishable from those of other areas. Other wild olives represent feral plants, spread in the same uncultivated areas where real oleasters still survive. The low level of differentiation among wild plants of distant isolated regions is difficult to explain for natural populations. Factors that may be considered include a common ancestral genetic pool, a lack of differential selective pressure and a reduced number of divergent generations between the different populations, likely representing refugial relics of the same population.

13.4.4 Related Subspecies

Analyses performed on rDNA, cpDNA, and mtDNA polymorphisms have characterized three distinct main clades: *O. europaea – O. laperrinei – O. maroccana – O. cerasiformis (= europaea* phylum) of the Mediterranean region, Sahara and northwest Africa, *O. cuspidata – O. chrysophylla (= cuspidata* phylum) of Asia, and *O. africana (= africana* phylum) for east and south Africa (Besnard and Bervillé 2002; Besnard et al. 2002a,b).

AFLP analyses showed that subsp. *laperrinei* and *maroccana*, from northwest Africa, showed a high similarity with the Mediterranean cultivated and wild olive and a clear distinction of the Australian taxa from those of east Africa and Asia, which clustered together (Angiolillo et al. 1999).

The phylogenetic relationships between the *Olea europaea* subspecies and other related taxa were assessed by nucleotide variation in non-coding cpDNA regions and by cpDNA RFLPs, distinguishing four groups: the taxa of northwest Africa and Mediterranean region (including the cultivated olive), the *O. europaea* forms from southeast Africa, those from Asia, and finally *Olea capensis* and *O. lancea,* both belonging to a distinct subgenus *Ligustroides* (Baldoni et al. 2002; Lumaret et al. 2000).

Two tandemly repeated sequences isolated by Katsiotis et al. (1998) localized on the chromosomes by in situ hybridization are present in oleasters and in subsp. *chrysophylla* and *africana*, but are absent in other genera of the Oleaceae family.

13.5 Major Breeding Achievements

Despite the pressure to improve productivity and agronomic performance of olive cultivars and in spite of the economic importance of the crop, there have been few efforts to produce new olive cultivars.

Exploration of phenotypic variability in agronomic characters has led to the identification of valuable clones within numerous olive cultivars of various Mediterranean countries (Suárez et al. 1990; Lavee et al. 1995; Bartolini et al. 2002; Grati-Kammoun et al. 2002). However, in spite of the significant efforts made towards clonal selection, very few clones have shown outstanding performance (Loussert and Berrichi 1995; Tous et al. 1998).

Very few studies have addressed the selection of clonal rootstocks. Preliminary work addressed the influence of rootstocks on scion performance. Clonal rootstocks have been selected with high rooting ability and the capacity to control scion vigour (Baldoni and Fontanazza 1990). Other selected rootstocks can control scion vigour and resistance to frost injury (Pannelli et al. 2002). The use of cvs. Souri, Muhasan and Barnea as rootstocks under dry conditions did not show any significant effect on tree vigour, shape and fruit production after ten years from planting (Lavee and Schachtel 1999).

Similarly to clonal selection, the use of induced mutagenesis has not been encouraging, and so far has succeeded in producing of only a compact mutant of the cv. Ascolana Tenera (Roselli and Donini 1982).

The evaluation of minor local cultivars has recently been exploited to identify individuals highly adapted to extreme environmental conditions (Pannelli et al. 2003; Rotondi et al. 2003).

The long generation time of olives has severely hindered both classical breeding and genetic studies (León et al. 2004a; Santos-Antunes et al. 2005). But now the development of new protocols to force seedling growth has made it possible to greatly reduce the length of the juvenile phase, even if the evaluation of the agronomic performance of mature plants still requires at least five years of experimentation (Santos-Antunes et al. 2005). Furthermore, the genetic control of major traits under selection is still unknown (De la Rosa et al. 2003). Tree vigour, leaf size, and fruit shape seem to be controlled by major genes showing dominance (Bellini et al. 2002a), while the inheritance of other characters such as fruit size, flowering intensity, fruit set, ripening time, and yield remains uncertain (Bellini et al. 2002a; Parlati et al. 1994).

In spite of these drawbacks, various classical breeding programs, performed by intervarietal crossing have been reported from different countries such as Turkey (Arsel and Cirik 1994), Morocco, Spain (Rallo 1995; León et al. 2004a, 2007), Tunisia (Trigui 1996), Israel (Lavee et al. 1999, 2003), Greece (Pritsa et al. 2003), Italy (Fontanazza et al. 1998; Bellini et al. 2002b) and Iran. In Australia, the University of Adelaide has recently established a breeding program to select for quality oil production in feral olive populations derived from natural dissemination of cultivars previously introduced in Australia and well adapted to that environment (Sedgley 2004). In any case, at present, the procedures of selection are still in progress and very few new genotypes have arisen from these breeding programmes.

Maalot, a new cultivar resistant to *Spilocaea oleagina*, has been selected from a selfed F_1 progeny of a semi resistant seedling probably of cv. Chemlali (Lavee et al. 1999). From seedling populations obtained by unknown parents, two other cultivars were selected, 'Barnea' with vigorous and upright growth and 'Kadesh' as a table olive (Lavee 1978; Lavee et al. 1986). The new cultivar 'Askal', a hybrid from the cross 'Barnea' × 'Manzanillo', was selected for its adaptation and good performance in high-density olive orchards (Lavee et al. 2003).

Three new olive cultivars ('Arno', 'Tevere' and 'Basento') were released from the progeny of the cross 'Picholine × Manzanillo' (Bellini et al. 2002b) and their

performance is still under evaluation. The new cultivar 'Fs 17' was selected among seedlings obtained from free pollination of the Italian olive cultivar 'Frantoio' (Fontanazza et al. 1998).

Very recently, the new cultivar 'Chiquitita', derived from a cross between 'Picual' and 'Arbequina', was obtained in a Spanish cross-breeding program. This new cultivar is characterized by early bearing, high oil content and high yield efficiency, while its low vigour, compact canopy and pendulous branches make it very suitable for high density orchards (Rallo et al. 2008).

In the same olive breeding program, preliminary results obtained from a comparative trial of the first 15 selections (León et al. 2007) indicated that some early bearing genotypes could be released as new cultivars. Additionally, some selections have shown a low vigour and could be kept for high-density hedgerow orchards.

13.6 Current Goals of Breeding

The primary goals in olive breeding are directed towards overcoming current limiting factors for production. These include: shortening the unproductive period, increasing fruit number and size, increasing oil content and quality (fatty acid composition, phenol content, etc.), reduction of alternate bearing, dwarfing or modifying tree architecture to facilitate mechanical pruning and harvesting, improving resistance to pests, in particular olive fruit fly, *Bactrocera oleae,* and diseases such as leaf peacock spot, caused by *Spilocaea oleagina*, Verticillium wilt, *Verticillium dahliae* and olive knot, *Pseudomonas savastanoi*. Other important objectives are to improve cold tolerance to allow cultivation in colder areas and to promote self-fertility in order to reduce reliance on pollinators.

Tree architecture and vigour are particularly important because the height of the trees prevents mechanical harvesting and pruning, thereby increasing the costs of cultivation.

Although olive is considered a species adapted to semi-arid climates, its productivity is strongly reduced under dry conditions, and thus there is great interest in developing new drought-tolerant cultivars as well as those that can thrive on saline and heavy soils.

Rootstock selection is focused on the ability to control scion vigour, to improve the resistance to pathogens, mainly *Verticillium*, and to abiotic stresses, namely water stress.

13.7 Breeding Methods and Techniques

13.7.1 Classical Breeding

Main steps for olive breeding are: (i) establishing the inventory of the existent varieties and determining their agronomic value, and (ii) breeding by hybridisation and selection. The first point has received particular attention and is

under way in various Mediterranean countries. The second step has been initiated in Spain and in a few other countries.

13.7.2 Clonal Selection

Clonal selection aims at uncovering valuable genotypes within a variety. Most selection programs in olive have so far relied on clonal selection and are based on the assumption that natural mutations generating any positive alteration in traits of agronomic interest may occur in long-living plants, in which they may be maintained by vegetative propagation (Rallo 1995; Belaj et al. 2004). Prospecting surveys to identify outstanding trees within a variety, either for agronomical or technological characters, are the first steps for clonal selection. Vegetatively propagated progenies of these individuals are then tested in comparative trials. Individuals performing better than standard samples of the corresponding cultivar for specific characteristics are selected and their vegetatively propagated clones represent the new clonal selections. The selected individuals most commonly retain the original cultivar name and acquire an identifying clone code. Most of the reported works on clonal selection in olive have followed this methodology, but most of them have stopped at the first step. This method of selection has demonstrated a low efficiency, and only a few selected clones have gained commercial relevance and have been propagated by the nursery industry (Loussert and Berrichi 1995; Tous et al. 1998).

13.7.3 Sanitary Selection

Systemic pathogens, such as viruses and phytoplasms, are sources of variation in vegetatively propagated plants. The application of molecular diagnostic techniques (such as RT-PCR) has allowed to detect the presence of different viruses (Faggioli et al. 2005) which may be symptomless but affecting plant morphology. Little information on the incidence of these diseases on agronomic characteristics is available and has given contradictory results (Clara et al. 1997; Martelli 1998; Bartolini et al. 1998). For that reason in the last years the sanitary selection, i.e. original plant material free from systemic pathogens, has become a technique of olive selection (Bottalico et al. 2004).

13.7.4 Breeding by Intervarietal Crossing

The adequate choice of parents is of great importance for the achievement of the objectives of a breeding program. Thus, a detailed knowledge of cultivars' identity and of their agronomic performance as well as the amount and distribution of their genetic variability is crucial to broaden the genetic base of new cultivars (Belaj et al. 2004; León et al. 2004a).

In olive breeding, the length of the juvenile period has traditionally been one of the main drawbacks. Under normal conditions olive seedlings begin to set fruits 15–20 years after germination (Santos-Antunes et al. 2005). This may

explain the various attempts by olive breeders to study the juvenile period and to develop protocols to shorten it (Lavee et al. 1996; Santos-Antunes et al. 2005; De la Rosa et al. 2006).

The protocols under use to perform intervarietal crosses in the olive consist of adding pollen of the paternal variety to bagged branches of the maternal parent, chosen among self-sterile cultivars (Lavee 1990; Fontanazza and Baldoni 1990; De la Rosa et al. 2004). Fruits are collected at ripening and, after removing the endocarp, seeds are germinated under controlled temperature and humidity. Plantlets are grown in a greenhouse under continuous light until they reach a minimum height, then they are moved to the field where they undergo the procedures of agronomic evaluation and selection of outstanding genotypes. After the pre-selection phase, plants are vegetatively propagated and compared in experimental trials for the final selection (Lavee et al. 1999; León et al. 2004b; Santos-Antunes et al. 2005; León et al. 2007).

An alternative way to overcome the problems of the long juvenile period is the selection of early bearing genotypes (Pritsa et al. 2003; De la Rosa et al. 2006). The relationships between seedling phenotypes and their agronomic behaviour at the mature phase are also very important. The initial results of a comparative trial of some genotypes under selection indicate that seedlings with a short juvenile period also show a short unproductive period when vegetatively propagated (León et al. 2007). It has also been demonstrated that there is a strong correlation between the resistance to *Spilocaea oleagina* of seedlings and that of adult plants (De la Rosa et al. 2006; Lavee et al. 1999; León et al. 2007).

The first evaluations of olive progenies have shown wide ranges of variation for all evaluated characteristics (Lavee et al. 1999; León et al. 2004a,b). Significant correlations have been observed among many traits. The most relevant correlations were found between oil content and oleic acid concentration, which was negatively correlated with palmitic, palmitoleic and linoleic acid percentages (León et al. 2004b).

Up to now, crossing programs have been performed only between cultivars, but the use of wild olives in future crosses should introduce useful variability, as wild genotypes may contain characteristics rare or absent in cultivated olive germplasm. So far, only one case of an interspecific cross has been reported using *Olea chrysophylla* Lam (Lavee 1990).

At the moment, olive breeding programs using intervarietal or interspecific crosses are mainly carried out in Spain, Israel and Australia.

13.7.5 Marker Assisted Breeding

The very preliminary works performed on olive genomics are far from producing effective results toward the selection of new cultivars by the use of molecular tools. For that reason and considering the lack of knowledge on the real useful variability already present in the cultivated and wild olive germplasm, attention has been focused in the last ten years on the evaluation of such germplasm.

As far as the use of molecular markers in olive breeding programs, a SCAR marker was proved to be linked to leaf peacock spot tolerance, as reported by Mekuria et al. (2001). Recently SSR markers have proved to be useful for paternity testing in olive progenies (De la Rosa et al. 2004; Díaz et al. 2006a; Mookerjee et al. 2005) as well as for the study of parental cross compatibility (Díaz et al. 2006b). These studies have evidenced the high frequency of contamination with undesirable pollen in seedlings (Santos-Antunes et al. 2005; León et al. 2004b). SSR markers have also proved to be useful for unequivocally identifying selections from a breeding program (Díaz et al. 2007).

One of the major contributions of molecular markers in breeding is the construction of genetic maps and the detection of QTLs. The first mapping population in olive consisted of a progeny derived from the cross between two highly heterozygous cultivars, Leccino and Dolce Agogia. Dolce Agogia is resistant to the most important olive pathogens such as *Spilocaea oleagina* and *Verticillium dahliae* (Bartolini et al. 1998; Gonzales-Lamothe et al. 2002), whereas Leccino is susceptible or medium tolerant to these biotic stresses. The linkage map was based on dominant (RAPDs and AFLPs) along with a small number of codominant markers (RFLPs and SSRs, De la Rosa et al. 2003). The Leccino map covered 2,765 cM and included 249 markers, falling into 22 major and 17 minor linkage groups (the latter each involving less than four markers). The Dolce Agogia map was of similar length (2,445 cM) and included 236 markers arranged in 27 major and three minor linkage groups. Besides, a candidate gene for stearoyl-ACP desaturase, which is a key enzyme for the conversion of 18:0 stearic acid to 18:1 oleic acid, the main component of olive oil was mapped on a linkage group of cv. Leccino (De la Rosa et al. 2003). At present, with the aim to construct a reference linkage map, this first map is being completed by a wider use of codominant markers such as SSRs and SNPs.

A second linkage map was constructed by Wu et al. (2004) based on RAPDs, SCARs and SSRs, exploiting the progeny of a cross between the cultivars Frantoio and Kalamata. The greater use of codominant markers allowed the integration of the two parental maps to generate 15 linkage groups covering 101 loci and 879 cM with a mean inter-marker distance of 10.2 cM.

At present, no further olive genetic mapping data is available, no QTLs have been detected, and neither is there any detailed analysis known on genome organization.

13.8 Integration of New Biotechnologies in Breeding Programmes

The very long generation time of olive has delayed the recovery of superior olive cultivars through conventional breeding. Genetic transformation represents a powerful alternative technique for accelerating the development of superior cultivars. For the introduction of genes controlling specific traits via transgenetics, many studies have been performed in the 1980s through 2000 in order to develop the biotechnological tools necessary to transform and regenerate olive

plants. But the restrictions from the European legislation and the public concern about the use of genetically modified plants, especially for traditional products such as olive oil, have dramatically decreased investigation on such topic during the recent years.

Here, the goals and main achievements obtained on the different aspects of olive genetic transformation will be summarized. Most importantly, olive transformation research has to address the identification and evaluation of genes and specific promoters for useful traits and the development of efficient protocols for regeneration from cell and tissue cultures of elite cultivars. Many potentially useful genes have been isolated from different species which could be introduced into olive separately or in combination. However, genetic transformation also requires the development of genotype-independent procedures based on the transformation of meristematic cells with high regeneration potential and/or the use of regeneration-promoting genes.

13.8.1 Organogenesis and Regeneration

Organogenesis represents a first step to somatic embryogenesis, as adventitious buds developing from explants of micropropagated plantlets may generate embryogenic cultures.

Shoot organogenesis and complete plants from mature phase explants of important cultivars have been achieved by Mencuccini and Rugini (1993), and thereafter applied routinely with minor modifications on the media and regeneration conditions. New rooting has been induced by inoculating the basal part of in vitro microcuttings with *Agrobacterium rhizogenes*. The application of putrescine increased rooting rate and basal callus formation (Rugini 1992).

Significant progress has also been achieved on the improvement of regeneration systems, in particular by somatic embryogenesis. Somatic embryos have been induced from immature zygotic embryos of various cultivars (Leva et al. 1995), from cotyledons of mature zygotic embryos (Pritsa and Voyiatzis 1999) and from seeds using radicle segments as explants. Somatic embryogenesis from tissues of elite olive cultivars has been difficult to achieve and has been reported only for two cultivars, 'Canino' and 'Moraiolo' (Rugini and Caricato 1995). Secondary somatic embryos originate from the epidermis or from the first sub-epidermal layer of embryos, mainly from their basal part. A limited number of cells of the primary explant seems to be involved in the formation of embryo's primordia (Benelli et al. 2001).

13.8.2 Genetic Manipulation

Olive explants have been transformed by the use of *Agrobacterium rhizogenes* through microprojectile-mediated DNA delivery on sporophytic explants or zygotic embryos (Mencuccini and Rugini 1993). Even if transgenic callus has been produced from leaf petioles of in vitro growing shoots, somatic embryos represent the most suitable tissue for transformation, because they may continuously develop secondary embryos (Rugini et al. 2000).

Genes used for transformation of olive were mainly *rol* genes of *A. rhizogenes* T-DNA cloned in *A. tumefaciens* LBA4404. Transformation with the entire T-DNA of *A. rhizogenes* may increase rooting efficiency, but only chimeric plants have been obtained (Rugini 1992). The *rol*ABC genes allow morphological plant characteristics to be modified, but somatic embryos and plants have been obtained only from cv. Canino (Rugini et al. 1999), whereas in all other cases no regeneration has been obtained. Transformation with the *osmotin* gene increases defence against fungal pathogens, and olive transgenes have been regenerated and evaluated in field trials (Rugini et al. 1999). Olive plants expressing the *osmotin* gene under the 35S promoter have shown reduced growth. Experiments are in progress to transform 3 genes from tobacco (o*smotin* + *chitinase* + *PR1*) in one construct.

Neomycin phosphotransferase II (*npt II*), which encodes resistance to kanamycin, has been most widely used as a selective marker. To increase public acceptance of transgenic olives, the procedures of selection should be improved, possibly by replacing traditional selection markers. The development of methods avoiding the use of antibiotic-dependent selection or allowing elimination of marker genes from transformed plants is a research priority in coming years.

Other methods of genetic modification include induced mutations. By irradiating rooted cuttings of cv. Ascolana Tenera with gamma rays, dwarf plants have been obtained (Roselli and Donini 1982). Irradiation of 'Frantoio' and 'Leccino' plantlets has produced mixoploid mutants showing dwarf habit, self-sterility and late blooming (Rugini et al. 1996).

13.8.3 Other In Vitro Technologies

Haploid recovery. Unlike the great interest in obtaining homozygous plants to isolate mutants, identify recessive alleles and facilitating whole genome sequencing, little work has been devoted to generating haploid plants (to be used for self-fertilization) from in vitro cultured anthers, and no regeneration has been obtained (Perri et al. 1994).

Polyploids. Tetraploid plants have been produced by irradiation of 'Leccino' and 'Frantoio' plantlets. Triploid plants have been obtained by pollinating mixoploid or tetraploid plants with regular haploid pollen.

Protoplast culture. Viable protoplasts have been isolated from hypocotyls, cotyledons, and leaves of micropropagated shoots, but it has been impossible to obtain regeneration (Canas et al. 1987).

Somaclonal variation. Somaclonal variation has been observed in mature olive plants regenerated through somatic embryogenesis of the 'Frangivento' cultivar obtained from embryogenic tissue induced on immature cotyledon portions. Plants have shown two types of variation: BOS (bushy olive somaclone), characterized by reduced plant height, leaf, inflorescence and fruit size, and COS (columnar olive somaclone), characterized by increased plant height, canopy volume and fruit size. The causes of this variation remain unknown (Leva and Petruccelli 2007).

Cryopreservation. With respect to medium- and long-term conservation, promising results have been obtained by slow growth storage and cryopreservation. In 'Arbequina' 30% survival of shoot tips has been obtained following their desiccation to 30% moisture content and direct immersion into liquid nitrogen (Martinez et al. 1999). Utilizing vitrification and one-step freezing in liquid nitrogen for shoot tips excised from in vitro 'Frantoio' shoot cultures, Lambardi et al. (2000) have achieved 15% survival, following re-warming to 40°C and plating on re-growth medium. A higher percentage (38%) of cryopreserved embryogenic cultures was obtained when using embryogenic cultures such as proembryonal masses and somatic embryos, and recovered embryogenic tissues showed enhanced proliferative and morphogenic activity. The encapsulation-dehydration procedure proved ineffective for cryopreservation (Martinez et al. 1999) but has potential applications in olive propagation (Micheli et al. 1998). Generally, cryogenic methods can be applied for long-term conservation of olive germplasm and the establishment of in vitro repositories, which could safeguard olive biodiversity.

13.9 Concluding Remarks

Studies on olive genetic resources have been intensively carried out in recent years, while a serious gap is envisaged for what concerns breeding activities and genomic research. Efforts are currently put on by many research groups to rapidly cover these areas.

References

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

Arsel, H. and Cirik, N. (1994) A general picture of the olive breeding activities in Turkey. Olivae 52, 25–27.

Baldoni, L. and Fontanazza, G. (1990) Preliminary field results on olive clonal rootstocks behaviour. Acta Hort. 286, 37–40.

Baldoni, L., Cultrera, N.G.M., Mariotti, R., Ricciolini, C., Arcioni, S., Vendramin, G.G., Buonamici, A., Porceddu, A., Sarri, V., Ojeda, M.A., Trujillo, I., Rallo, L., Belaj, A., Perri, E., Salimonti, A., Muzzalupo, I., Casagrande, A., Lain, O., Messina, R. and Testolin, R. (2009) A consensus list of microsatellite markers for olive genotyping. Mol. Breed. DOI:10.1007/s11032-009-9285-8 (in press).

Baldoni, L., Guerrero, C., Sossey-Aloui, K., Abbott, A.G., Angiolillo, A. and Lumaret, R. (2002) Phylogenetic relationships among *Olea* species, based on nucleotide variation at a non-coding chloroplast DNA region. Plant Biol. 4, 346–351.

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

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

Bandelj, D., Jakse, J. and Javornik, B. (2002) DNA fingerprinting of olive varieties by microsatellite markers. Food Technol. Biotechnol. 40, 185–190.

Barranco, D. and Rallo, L. (2000) Olive cultivars in Spain. Hortechnology 10, 107–110.

Bartolini, G., Prevost, G., Messeri, C., Carignani, G. and Menini, U. (1998) *Olive Germplasm: Cultivars and World-wide Collections*. FAO, Rome.

Bartolini, S., Guerreiro, R. and Loreti, F. (2002) Two new clones of cv. Leccino. Acta Hort. 586, 225–228.

Beauchamp, G.K., Keast, R.S.J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.H., Smith, A.B., Breslin, P.A.S. (2005) Ibuprofen-like activity in extra-virgin olive oil. Nature 437, 45–46.

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

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

Belaj, A., Satovic, Z., Rallo, L. and 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.

Bellini, E., Giordani, E., Parlati, M.V. and Pandolfi, S. (2002a) Olive genetic improvement: thirty years of research. Acta Hort. 586, 105–108.

Bellini, E., Parlati, M.V. and Giordani, E. (2002b) Three new olive cultivars obtained by cross breeding. Acta Hort. 586, 221–223.

Benelli, C., Fabbri, A., Grassi, S., Lambardi, M. and Rugini, E. (2001) Histology of somatic embryogenesis in mature tissue of olive (*Olea europaea* L.). J. Hort. Sci. Biotech. 76, 112–119.

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

Besnard, G., Garcia-Verdugo, C., Rubio De Casas, R., Treier, U.A., Galland, N. and 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., Khadari, B., Baradat, P. and Bervillé, 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. and Bervillé, A. (2002b) *Olea europaea* (Oleaceae) phylogeography based on chloroplast DNA polymorphism. Theor. Appl. Genet. 104, 1353–1361.

Bottalico, G., Saponari, M., Campanale, A., Mondelli, G., Gallucci, C., Serino, E., Savino, V. and Martelli, G.P. (2004) Sanitation of virus-infected olive trees. J. Plant Pathol. 86(4), 311.

Breton, C., Claux, D., Metton, I., Skorski, G. and Bervillé, A. (2004) Comparative study of methods for DNA preparation from olive oil samples to identify cultivar SSR alleles in commercial oil samples: possible forensic applications. J. Agric. Food Chem. 52, 531–537.

Breton, C., Tersac, M. and Bervillé, A. (2006) Genetic diversity and gene flow between the wild olive (oleaster, *Olea europaea* L.) and the olive: several Plio-Pleistocene refuge zones in the Mediterranean basin suggested by simple sequence repeats analysis. J. Biogeogr. 33, 1916–1928.

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

Canas, L.A., Wyssmann, A.M. and Benbadis, M.C. (1987) Isolation, culture and division of olive (*Olea europaea* L) protoplasts. Plant Cell Rep. 5, 369–371.

Carriero, F., Fontanazza, G., Cellini, F. and Giorio, G. (2002) Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theor. Appl. Genet. 104, 301–307.
Carrion, J.S. and Dupre, M. (1996) Late quaternary vegetational history at Navarrès, Eastern Spain. A two core approach. New Phytol. 134, 177–191.
Cipriani, G., Marrazzo, M.T., Marconi, R., Cimato, A. and 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.
Clara, M.I.H., Rei, F.T., Felix de R., Leitao, F.A., Serrano, J.F. and Potes, M.F. (1997) Virosis que afectan a *Olea europaea* L. y técnicas de diagnóstico. Olivae 66, 56–60.
Consolandi, C., Palmieri, A., Doveri, S., Maestri, E., Marmiroli, N., Reale, S., Lee, D., Baldoni, L., Tosti, N., Severgnini, M., de Bellis, G. and Castiglioni, B. (2007) Olive variety identification by ligation detection 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., De Bellis, G. and 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.
De la Rosa, R., Angiolillo, A., Rallo, L., Guerrero, C., Pellegrini, M., Besnard, G., Bervillé, A., Martin, A. and Baldoni, L. (2003) A first genetic linkage map of olive (*Olea europaea* L.) cultivars using RAPD and AFLP markers. Theor. Appl. Genet. 106, 1273–1282.
De la Rosa, R., James, C. and Tobutt, K.R. (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., James, C.M. and Tobutt, K.R. (2004) Using microsatellites for paternity testing in olive progenies. HortScience 39, 351–354.
De la Rosa, R., Kiran, A.I., Barranco, D. and León, L. (2006) Seedling vigour as a pre-selection criterion for short juvenile period in olive breeding. Aust. J. Agric. Res. 57, 477–481.
Díaz, A., De la Rosa, R., Martín, A. and 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 relationship studies. Tree Genet. Gen. 2, 165–175.
Díaz, A., De la Rosa, R., Rallo, P., Muñoz-Diéz, C., Trujillo, I., Barranco, D., Martín, A. and Belaj, A. (2007) Selection of an olive breeding program identified by microsatellite markers. Crop Sci. 47, 2317–2322.
Díaz, A., Martín, A., Rallo, P., Barranco, D. and De la Rosa, R. (2006b) Self-incompatibility of 'Arbequina' and 'Picual' olive assessed by SSR markers. J. Am. Soc. Hort. Sci. 131, 250–255.
Doveri, S., O'Sullivan, D.M. and 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.
Faggioli, F., Ferretti L., Albanese, G., Sciarroni, R., Pasquini, G., Lumia, V. and Barba, M. (2005) Distribution of olive tree viruses in Italy as revealed by one-step RT-PCR. J. Plant Pathol. 87(1), 49–55.
Fontanazza, G. and Baldoni, L. (1990) Proposal for a program of olive breeding. Olivae 34, 32–40.
Fontanazza, G., Bartolozzi, F. and Vergari, G. (1998) Fs-17. Riv. Frutticoltura 5, 61.
Galli, C. (2006) Bioactive minor compounds of extra-virgin olive oil, the ibuprofen-like activity of oleocanthal. Eur. J. Lipid Sci. Technol. 108, 95–96.
Gemas, V.J., Rijo-Johansen, M.J., Tenreiro, R. and Fevereiro, P. (2000) Inter- and intravarietal analysis of three *Olea europaea* L. cultivars using the RAPD technique. J. Hort. Sci. Biotechnol. 75, 312–319.
Giamarellos-Bourboulis, E.J., Geladopoulos, T., Chrisofos, M., Koutoukas, P., Vassiliadis, J., Alexandrou, I., Tsaganos, T., Sabracos, L., Karagianni, V., Pelekanou, E., Tzepi, I., Kranidioti, H., Koussoulas, V. and Giamarellou, H. (2006) Oleuropein: a novel immunomodulator conferring prolonged survival in experimental sepsis by *Pseudomonas aeruginosa*. Shock 26, 410–416.

González-Lamothe, R., Segura, R., Trapero, A., Baldoni, L., Botella, M.A. and Valpuesta, V. (2002) Phylogeny of the fungus *Spilocaea oleagina*, the causal agent of peacock leaf spot in olive. FEMS Microb. Lett. 210, 149–155.

Gonzalo-Claros, M., Crespillos, R., Aguilar, M.L. and Canovas, F.M. (2000) DNA fingerprinting and classification of geographically related genotypes of olive-tree (*Olea europaea* L.). Euphytica 116, 131–142.

Grati-Kammoun, N., Khilf, M., Ayadi, M. and Karray, B. (2002) Clonal selection of olive cv. Chemlali de Sfax: preliminary results. Acta Hort. 586, 147–150.

Green, P.S. (2002) A revision of *Olea* L. Kew Bull. 57, 91–140.

Green, P.S. and Wickens, G.E. (1989) The *Olea europaea* complex. In: K. Tan (Ed.), *The Davis and Hedge Festschrift*. Edinburgh University Press, Edinburgh, UK, pp. 287–299.

Guerin, J.R., Sweeney, S.M., Collins, G.G. and Sedgley, M. (2002) The development of a genetic database to identify olive cultivars. J. Am. Soc. Hort. Sci. 127, 977–983.

Hamdi, H.K. and Castellon, R. (2005) Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. Biochem. Biophys. Res. Commun. 334, 769–778.

Hernandez, P., De la Rosa, R., Rallo, L., Martin, A. and Dorado, G. (2001) 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, W. and 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 sequence repeats (ISSR). Mol. Ecol. 9, 857–868.

Katsiotis, A., Hagidimitriou, M., Douka, A. and Hatzopoulus, P. (1998) Genomic organization, sequence interrelationship, and physical localization using in situ hybridization of two tandemly repeat DNA sequences in the genus *Olea*. Genome 41, 527–534.

Lambardi, M., Benelli, C., De Carlo, A., Fabbri, A., Grassi, S. and Lynch, P.T. (2000) Medium- and long-term *in vitro* conservation of olive germplasm (*Olea europaea* L.). Acta Hort. 586, 109–112.

Lavee, S. (1978) 'Kadesh' table olive. HortScience 13, 62–63.

Lavee, S. (1990) Aims, methods and advances in breeding of new olive (*Olea europaea* L.) cultivars. Acta Hort. 286, 23–36.

Lavee, S. and Schachtel, J. (1999) Interaction of cultivar rootstocks and water availability on olive tree performance and fruit production. Acta Hort. 474, 399–414.

Lavee, S., Avidan, N., Haskal, A. and Ogrodovich, A. (1996) Shortening the juvenil phase of olive seedlings: a tool useful for olive breeding. Olivae 60, 33–41.

Lavee, S., Avidan, N. and Meni, Y. (2003) 'Askal', a new high-performing oil variety for intensive and super-intensive olive orchards. Olivae 97, 53–59.

Lavee, S., Harshemesh, H., Haskat, A., Meni, V., Wodner, M., Ogrodovich, A., Avidan, B., Wiesman, Z., Avidan, N. and Traper-Casa, A. (1999) 'Maalot' a new orchard-resistant cultivar to peacock eye leaf spot (*Spilocaea oleagina* Cast.). Olivae 78, 51–59.

Lavee, S., Haskal, A. and Vodner, M. (1986) 'Barnea', a new olive cultivar from first breeding generation. Olea 17, 95–99.

Lavee, S., Wiesman, Z. and Avidan, N. (1995) Clonal selection and fingerprint identification in the east Mediterranean autochthonous olive cultivars 'Souri' and 'Nabali'. Olea 23, 46.

León, L., De la Rosa, R., Barranco, D. and Rallo, L. (2004a) Ten years of olive breeding in Córdoba (Spain). Acta Hort. 663, 747–750.

León, L., De la Rosa, R., Barranco, D. and Rallo, L. (2007) Breeding for early bearing in olive. HortScience 42, 499–502.

León, L., Martín, L.M. and Rallo, L. (2004b) Phenotypic correlations among agronomic traits in olive progenies. J. Am. Soc. Hort. Sci. 129, 271–276.

Leva, A.R. and Petruccelli, R. (2007) Field performance of olive somaclones derived from somatic embryos. Acta Hort. 748, 181–189.

Leva, A.R., Muleo, R. and Petruccelli, R. (1995) Long-term somatic embryogenesis from immature olive cotyledons. J. Hort. Sci. 70, 417–421.

Liphschitz, N., Gophna, R., Hartman, M. and Biger, G. (1991) The beginning of olive (*Olea europaea*) cultivation in the Old World: a reassessment. J. Archaeol. Sci. 18, 441–453.

Loureiro, J., Rodriguez, E., Costa, A. and 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. Gen. Res. Crop Evol. 54, 21–25.

Loussert, R. and Berrichi, M. (1995) Evaluation of olive varieties by a network of experimental fields in the main Moroccan areas of olive cultivation. Olivae 58, 43–45.

Lumaret, R., Amane, M., Ouazzani, N., Baldoni, L. and Debain, C. (2000) Chloroplast DNA variation in the cultivated and wild olive taxa of the genus *Olea* L. Theor. Appl. Genet. 101, 547–553.

Lumaret, R., Ouazzani, N., Michaud, H., Vivier, G., Deguilloux, M.F. and Di Giusto, F. (2004) Allozyme variation of oleaster populations (wild olive tree) (*Olea europaea* L.) in the Mediterranean Basin. Heredity 92, 343–351.

Martelli, J.P. (1998) Enfermedades, infecciones y certificación de olivo: panorama general. Phytoma 102, 180–186.

Martinez, D., Arroyo-Garcia, R. and Revilla, A.M. (1999) Cryoconservation of in vitro grown shoot-tips of *Olea europaea* L. var. Arbequina. CryoLetters 20, 29–36.

Mastrangelo, N. (1982) The dark ages and a thousand years. In: *The Olive*. Ed. Calderini Edagricole, Bologna, pp. 46–91.

Mekuria, G., Collins, G., Lavee, S. and Sedgley, M. (2001) Identification of a RAPD marker linked to tolerance to peacock disease (*Spilocaea oleaginea*) in olives (*Olea europaea*). Acta Hort. 546, 565–567.

Mekuria, G.T., Graham, G.C. and Sedgley, M. (1999) Genetic variability between different accessions of some common commercial olive cultivars. J. Hort. Sci. Biotechnol. 74, 309–314.

Mencuccini, M. and Rugini, E. (1993) In vitro shoot regeneration from olive cultivar tissue. Plant Cell Tiss. Org. Cult. 32, 283–288.

Micheli, M., Mencuccini, M. and Standardi, A. (1998) Encapsulation of *in vitro* proliferated buds of olive. Adv. Hort. Sci. 12, 163–168.

Montemurro, C., Simeone, R., Pasqualone, A., Ferrara, E. and Blanco, A. (2005) Genetic relationships and cultivar identification among 112 olive accessions using AFLP and SSR markers. J. Hort. Sci. Biotechnol. 80, 105–110.

Mookerjee, S., Guerin, J., Collins, G., Ford, C. and Sedgley, M. (2005) Paternity analysis using microsatellite markers to identify pollen donors in an olive grove. Theor. Appl. Genet. 111, 1174–1182.

Mulas, M., Fadda, A. and Cauli, E. (2004) First observations on oleaster (*Olea europaea* var. *sylvestris* Hoff. E Link) clones selected as forestry resources. It. Hort. 4, 214–217.

Muzzalupo, I., Pellegrino, M. and Perri, E. (2007) Detection of DNA in virgin olive oils extracted from destoned fruits. Eur. Food Res. Technol. 224, 469–475.

Newmark, H.L. (1999) Squalene, olive oil, and cancer risk: review and hypothesis. Ann. New York Acad. Sci. 889, 193–203.

Obied, H.K., Prenzler, P.D., Ryan, D., Servili, M., Taticchi, A, Esposto, S. and Robards, K. (2008) Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids from *Olea europaea* L. Nat. Prod. Rep. 25, 1167–1179.

Owen, C.A., Bita, E.C., Banilas, G., Hajjar, S.E., Sellianakis, V., Aksoy, U., Hepaksoy, S., Chamoun, R., Talhook, S.N., Metzidakis, I., Hatzopoulos, P. and 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. and Marmiroli, N. (2005) Traceability of plant contribution in olive oil by AFLPs. J. Agric. Food Chem. 53, 6995–7002.

Pannelli, G., Baldioli, M., Servili, M. and Montedoro, G.F. (2003) *Caratteristiche dei frutti e dell'olio di germoplasma olivicolo in Umbria*. Ed. ARUSIA, Perugia.

Pannelli, G., Rosati, S. and Rugini, E. (2002) The effect of clonal rootstocks on frost tolerance and on some aspects of plant behaviour in Moraiolo and S. Felice olive cultivars. Acta Hort. 586, 247–250.

Parlati, M.V., Bellini, E., Perri, E., Pandolfi, S., Giordani, E. and Martelli, S. (1994) Genetic improvement of olive: initial observations on selections made in Florence. Acta Hort. 356, 87–90.

Pasqualone, A., Caponio, F. and 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., Caponio, F. and Blanco, A. (2004) Identification of virgin olive oil from different cultivars by analysis of DNA microsatellites. J. Agric. Food Chem. 52, 1068–1071.

Perri, E., Parlati, M.V., Mulé, R. and Fodale, A.S. (1994) Attempts to generate haploid plants from in vitro cultures of *Olea europaea* L. anthers. Acta Hort. 356, 47–50.

Pritsa, T.S. and Voyiatzis, D.G. (1999) The *in vitro* morphogenetic capacity of olive embryos, as affected by their developmental stage and the L-arginine and L-glutamine concentration in the nutrient substrate. Acta Hort. 474, 87–90.

Pritsa, T.S., Voyiatzis, D.G., Voyiatzi, C.J. and Sotiriou, M.S. (2003) Evaluation of vegetative growth traits and their relation to time to first flowering of olive seedlings. Austral. J. Agric. Res. 54, 371–376.

Rallo, L. (1995) Selection and breeding of olive in Spain. Olivae 59, 46–53.

Rallo, P., Dorado, G. and Martin, A. (2000) Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). Theor. Appl. Genet. 101, 984–989.

Rallo, L., Barranco, D., De la Rosa, R. and León, L. (2008) 'Chiquitita' olive. HortScience 43, 529–531.

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

Rey, P.J. and Alcántara, J.M. (2000) Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. J. Ecol. 88, 622–633.

Rodríguez-Ariza, M.O. and Montes Moya, E. (2005) On the origin and domestication *of Olea europaea* L. (olive) in Andalucía, Spain, based on the biogeographical distribution of its finds. Veget. Hist. Archaeobot. 14, 551–561.

Roselli, G. and Donini, B. (1982) 'Briscola':Nuova cultivar di olivo a sviluppo compatto. Riv. Ortoflorofrutt. It. 66, 103–114.

Rotondi, A., Magli, M., Ricciolini, C. and Baldoni, L. (2003) Morphological and molecular analyses for the characterization of a group of Italian olive cultivars. Euphytica 132, 129–137.

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

Rugini, E. (1992) Involvement of polyamines in auxin and *Agrobacterium rhizogenes*-induced rooting of fruit trees in vitro. J. Am. Soc. Hort. Sci. 117, 532–536.

Rugini, E. and Caricato, G. (1995) Somatic embryogenesis and plant recovery from mature tissues of olive cultivars (*Olea europaea* L.) 'Canino' and 'Moraiolo'. Plant Cell Rep. 14, 257–260.

Rugini, E., Biasi, R. and Muleo, R. (2000) Olive (*Olea europaea* var. *sativa*) transformation. In: S.M. Jain and S.C. Minocha (Eds.), *Molecular Biology of Woody Plants*. Vol. 2, Kluwer Acad. Publ., Dordrecht, pp. 245–279.

Rugini, E., Gutierrez-Pesce, P., Spampanato, P.L., Ciarmiello, A. and D'Ambrosio, C. (1999) New perspective for biotechnologies in olive breeding: morphogenesis, in vitro selection and gene transformation. Acta Hort. 474, 107–110.

Rugini, E., Pannelli, G., Ceccarelli, M. and 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. and Fogher, C. (2006) Development and characterization of microsatellite loci from *Olea europaea*. Mol. Ecol. Notes 6, 1275–1277.
Salas, J.L., Sanchez, J., Ramli, U.S., Manaf, A.M., Williams, M. and Harwood, J.L. (2000) Biochemistry of lipid metabolism in olive and other oil fruits. Progr. Lipid Res. 39, 151–180.
Santos Antunes, A.F., Léon, L., De la Rosa, R., Alvarado, J., Mohedo, A., Trujillo, I. and Rallo, L. (2005) The length of juvenile period in olive as influenced by vigor of the seedlings and the precocity of parents. HortScience 40, 1213–1215.
Sanz-Cortés, F., Badenes, M.L., Paz, S., Iñiguez, A. and Llácer, G. (2001) Molecular characterization of olive cultivars using RAPD markers. J. Am. Soc. Hort. Sci. 126, 7–12.
Sanz-Cortés, F., Parfitt, D.E., Romero, C., Struss, D., Llácer, G. and Badenes, M.L. (2003) Intraspecific olive diversity assessed with AFLP. Plant Breed. 122, 173–177.
Sarri, V., Baldoni, L., Porceddu, A., Cultrera, N.G.M., Contento, A., Frediani, M., Belaj, A., Trujillo, I. and Cionini, P.G. (2006) Microsatellite markers are powerful tool for discriminating among olive cultivars and assigning them to geographically defined populations. Genome 49, 1606–1615.
Sedgley, M. (2004) *Wild Olive Selection for Quality Oil Production*. RIRDC Publication No. 318 04/101, Barton, Australia.
Sefc, K.M., Lopes, M.S., Mendonça, D., Rodrigues Dos Santos, M., Laimer Da Câmara Machado, M. and Da Câmara Machado, A. (2000) Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. Mol. Ecol. 9, 1171–1173.
Sensi, E., Vignani, R., Scali, M., Masi, E. and Cresti, M. (2003) DNA fingerprinting and genetic relatedness among cultivated varieties of *Olea europaea* L. estimated by AFLP analysis. Sci. Hort. 97, 379–388.
Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G.F. and 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. Chromat. 1054, 113–127.
Suárez, M.P., López-Rivares, E.P., Cantero, M.L. and Ordovás, J. (1990) Clonal selection on 'Manzanilla de Sevilla'. Acta Hort. 286, 117–119.
Terral, J.F. (2000) Exploitation and management of the olive tree during prehistoric times in Mediterranean France and Spain. J. Arch. Sci. 27, 127–133.
Terral, J.F., Alonso, N., Capdevila, R.B., Chatti, N., Fabre, L., Fiorentino, G., Marinval, P., Jordá, G.P., Pradat, B., Rovira, N. and Alibert, P. (2004) Historical biogeography of olive domestication (*Olea europaea* L.) as revealed by geometrical morphometry applied to biological and archaeological material. J. Biogeogr. 31, 63–77.
Terral, J.F. and Arnold-Simard, G. (1996) Beginnings of olive cultivation in eastern Spain in relation to Holocene bioclimatic changes. Quart. Res. 46, 176–185.
Testolin, R. and Lain, O. (2005) DNA extraction from olive oil and PCR amplification of microsatellite markers. J. Food Sci. 70, 108–112.
Tous, J., Romero, A. and Plana, J. (1998) Selección clonal de la variedad de olivo 'Arbequina'. Presentación del clon IRTA-I-18. Phytoma 102, 154–158.
Trigui, A. (1996) Improving the quantity and quality of olive production in Tunisia: unavoidable need and outlook for olive identification and breeding. Olivae 61, 34–40.
Trujillo, I., Rallo, L. and Arus, P. (1995) Identifying olive cultivars by isozyme analysis. J. Am. Soc. Hort. Sci. 120, 318–324.
Vargas, P. and Kadereit, J.W. (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.
Watts, W.A., Allen, J.R.M. and Huntley, B. (1996) Vegetation history and palaeoclimate of the last glacial period at Lago Grande di Monticchio, Southern Italy. Quart. Sci. Rev. 15, 133–153.
Wu, S.B., Collins, G. and Sedgley, M. (2004) A molecular linkage map of olive (*Olea europaea* L.) based on RAPD, microsatellite and SCAR markers. Genome 47, 26–35.