Metadata of the chapter that will be visualized online

Series Title		
Chapter Title	Olea	
Chapter SubTitle		
Copyright Year	2011	
Copyright Holder	Springer Berlin Heidelberg	

Corresponding Author	Family Name	Rugini
	Particle	
	Given Name	**E.**
	Suffix	
	Division	Dipartimento di Produzione Vegetale
	Organization	Università degli Studi della Tuscia
	Address	01100, Viterbo, Italy
	Email	rugini@unitus.it
Author	Family Name	Pace
	Particle	**De**
	Given Name	**C.**
	Suffix	
	Division	
	Organization	
	Address	
	Email	
Author	Family Name	Gutiérrez-Pesce
	Particle	
	Given Name	**P.**
	Suffix	
	Division	
	Organization	
	Address	
	Email	
Author	Family Name	Muleo
	Particle	
	Given Name	**R.**
	Suffix	
	Division	
	Organization	
	Address	
	Email	

Abstract	The genus *Olea* contains about 30 species split into three subgenera, *Tetrapilus*, *Paniculatae*, and *Olea* (cultivated olive and wild relatives), found in Asia, Australia and Asia, Africa and Europe, respectively. The species O. *europaea* L. includes six subspecies: *Olea europaea* L. ssp. *europaea* (the Mediterranean

olives); *O. e. laperrinei* (distributed in Saharan massifs of Hoggar, Aïr, Jebel Marra in Algeria); *O. e. cuspidata* (which moved from South Africa to Egypt, East Australian areas and Hawaii, and from Arabia to northern India and Southwest China); *O. e. guanchica* (Canary Islands); *O. e. maroccana* (southwestern Morocco); and *O. e. cerasiformis* (Madeira).

Using molecular markers, it has been ascertained that the Mediterranean olives include the cultivated types (*O. europaea* L. ssp. *europaea* var. *sativa*), the true wild *oleaster* (*O. e. e.* var. *sylvestris*), and the feral form *olevaster* from seedlings born from seeds of the cultivated types. The *oleaster* has a narrow range of distribution and it is often mistaken for *olevaster*. Recolonization of *Oleaster* of the Mediterranean basin occurred after the last glacial event, from refuges located in both eastern and western Mediterranean basin areas toward southern Europe. *Oleaster* is a source of rootstock for propagating new improved cultivated varieties. Cultivated and wild forms have the same diploid chromosome number ($2n = 46$) and are fully interfertile. Triploid and tetraploid genotypes have been isolated from cultivated *O.e.e.*, but polyploid forms have been found in endangered natural populations of *O. e. guancica* (tetraploid) and *O. e. maroccana* (hexaploid).

Individual oleaster trees showing superior performance for size and/or oil content of fruit were selected empirically during olive domestication and propagated vegetatively as clones using cuttings that were planted directly or, more recently, grafted onto indigenous *oleasters*.

Genetic markers linked to the most important agronomic traits, such as size of the tree, content of secondary products of fruit, flowering induction, oil quality, and biotic and abiotic resistance, will help introgression by conventional breeding of oleaster trait-enhancing genes into cultivated olive. Successful results were difficult to achieve due to both the complex genetic basis of the traits to be improved and the long juvenile period of the progenies that delays the expression of the target traits. In vitro techniques to regenerate doubled haploids from hybrids or somaclonal variation induction may complement classical breeding procedures. Genetic transformation could speed up the development of new genotypes, and transgenic olive plants with modified growth habit and putative induced disease resistance are being tested under filed conditions. However, the development of an efficient regeneration method from mature tissue is the limiting factor for the routine application of this technology to olive genetic improvement.

Chapter 5
Olea

E. Rugini, C. De Pace, P. Gutiérrez-Pesce, and R. Muleo

5.1 Basic Botany of the Species

5.1.1 Taxonomic Position

The genus *Olea* belongs to the Oleaceae family, which comprises approximately 30 genera with 600 species (Cronquist 1981), distributed in every continent as ornamental plants or in productive orchards (Fig. 1).

Although the classification is not well defined yet, according to several modern authors, the genus *Olea* splits into three subgenera, *Tetrapilus*, *Paniculatae*, and *Olea* (cultivated olive and wild relatives), found in Asia, Australia and Asia, Africa and Europe, respectively. The subgenus *Olea* is divided into two sections: *Ligustroides* (about 10 species) and *Olea* (one species: *europaea*). Both these sections thrive in the mountains of East Africa and in the Pacific Islands. In particular, *Olea* is also found in west of the Sahara, in the Macaronesian Islands (Canary and Madeira), and the Mediterranean basin (Green 2002) (see Sect. 5.1.2). The section *Olea* includes the complex of *O. europaea* L, the Mediterranean olive tree, the only species cultivated for oil extraction and table consumption, which accounts more than 1,000 of cultivars, although many of these might be just different landraces stemmed from the same original genetic stock or different named varieties derived from the same original genetic stock. It has been reported that the cultivated olive is not a species, but rather a group of forms, which originated from mutations and natural hybridizations. Tropical and subtropical Afro-Asiatic species, such as *Olea chrysophilla* Lam. and *Olea excelsa* Ait (old nomenclature for Ligustroides species), probably contributed to the evolution of the Euro-Mediterranean olive (*O. europaea* L.) (Mazzolani and Altamura Betti 1976, 1977; Green and Wickens 1989; Zohary 1994). The Euro-Mediterranean olive (*O. europaea* L. ssp. *europaea*) comprises the wild *oleaster* (var. *sylvestris*) and the cultivated (var. *sativa*) types (Fig. 2). The var. *sylvestris* has a narrow range of distribution and it is often mistaken for *olevaster* (Fig. 3), which is a feral form derived from seeds of the cultivated type *O. europaea* ssp. *europaea* var. *sativa*. The feral form *olevaster* remains at a constant juvenile stage and never produces flowers unless it is transferred to standard cultivation (Rugini and Lavee 1992). The var. *sylvestris* with its very small fruits and small and almost round leaves, thorny shoots, and quadrangular branch section was used in the past as rootstock for the var. *sativa*. According to Green and Wickens (1989), the *O. europaea* L. would include also its wild relatives, i.e., the Afro-Asiatic subspecies *cuspidata*, *laperrinei*, and *cerasiformis*. Nonetheless, the systematic classification of the genera is still far to be defined. While some authors recognize six subspecies in *O. europaea* (Sect. 5.1.2), Green and Wickens (1989) distinguished four subspecies according to their morphology and their geographical distribution: *O. europaea* ssp. *europaea* of the Mediterranean Basin; *O. europaea* ssp. *laperrini* (Batt. and Trab.) Ciferri of the Sahara Massifs; *O. europaea* ssp. *cerasiformis* (Webb. and Berth.) Kunk. and Sund. of the Canary Islands and Madeira; *O. europaea* ssp. *cuspidata* (Wall. Ciferri) of Asia (China, India, Pakistan, Nepal, Iran, South Arabia) and Southeast Africa (Fig. 4). A complete review of the history of classification of olive germplasm is reported by Bartolini et al. (2002) and Ganino et al. (2006).

E. Rugini (✉)
Dipartimento di Produzione Vegetale, Università degli Studi della Tuscia, Viterbo 01100, Italy
e-mail: rugini@unitus.it

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources*, *Temperate Fruits*,
DOI 10.1007/978-3-642-16057-8_5,

Fig. 1 World distribution of the main *Olea* species and subspecies

Fig. 2 Cultivated olives tree (*Olea europea* ssp. *europaea* var. *sativa*)

Phylogenetic reconstruction based on plastid DNA data sustained a maternal origin of *O. e. laperrini* populations in South Algeria, where a higher allele richness was observed (Besnard et al. 2007b). Based on nuclear microsatellite data, two levels of structure were revealed: first, individuals from Niger and Algeria were separated into two distinct groups; second, four less-differentiated clusters corresponded to the four studied mountain ranges (Hoggar, Tassili n' Ajjer, Bagzane, and Tamgak). These results provide support to the fact that desert barriers have greatly limited long distance gene flow.

Phylogenetic congruence of both ITS (ribosomal internal transcribed sequence) and plastid lineages suggested an evolutionary scenario of predominant isolation during the Plio-Pleistocene in Macaronesia, the Mediterranean, southern Africa, eastern Africa, and Asia.

The clear-cut geographical distribution of chloroplast DNA haplotypes supports an early differentiation between populations from southern Africa to China (subsp. *cuspidata*) and the Mediterranean, Macaronesia, and the Sahara (all other subspecies) (Besnard et al. 2007a).

Fig. 3 One of the oldest *olevaster* genotypes located in central Italy has a trunk circumference of about 7.2 m, and it is able to produce up to 1 ton of olives per year

Fig. 4 *Olea europaea* ssp. *cuspidata* is one of the numerous wild *Olea europaea* species, which grows also on the mountains of Nepal

Both ITS-1 and plastid DNA data indicate that most populations of O. *europaea* are differentiated phylogenetically in five geographical areas (1) equatorial and southern Africa (ssp. *laperrini*); (2) eastern Africa and southern Asia (ssp. *cuspidata*); (3) the eastern Mediterranean (ssp. *europaea* est); (4) the western Mediterranean (ssp. *europaea* west); and (5) Macaronesia and Northwest Africa (ssp. *guancica*, *cerasifomis*, *maroccana*). However, unexpected incongruences between plastid DNA and ITS-1 analyses,

associated with ITS-1 intraindividual polymorphism, led Besnard et al. (2007a) to propose a more dynamic biogeographical pattern.

Populations of ssp. *cuspidata* are present across a large area (from Ethiopia to China) and may have diverged from an ancient common ancestor that colonized in this geographical area. We hypothesize that populations from African and Arabian coasts of the Red Sea may reflect a secondary divergence from populations from eastern Africa and southern Asia.

Most of the African individuals of subsp. *cuspidata* display haplotypes from African and Arabian coasts of the Red Sea and eastern Africa and southern Asian populations except for one population from southern Egypt, which forms part of a new sublineage together with eastern Mediterranean (subsp. *europaea*) and Saharan (subsp. *laperrinei*) populations (Besnard et al. 2007a).

This suggested an ancient hybridizone from the Sahara to northeastern African mountains, where divergent plastid and nuclear lineages still coexist. Other past hybridization events imply to all the subspecies. In fact, populations of ssp. *europaea* form part of three western sublineages plus a sublineage (E1), including Mediterranean and Saharan ssp. (*europaea*, *laperrini* and *cuspidata*), and a further lineage (M), which included the three subspecies (spp. *maroccana*, *guanchica*, *cerasiformis*) of northwest Africa and Macaronesia (Besnard et al. 2007a). Therefore, there are more plastid lineages than recognized taxa, whereas two lineages (E1, M) contain the six subspecies. Cooccurrence of two divergent ITS-1 copies in an intersubspecies hybrid reported in an invasive Australian population of O. *europaea* (Besnard et al. 2007a) provides further evidence that mixture of divergent copies in a single lineage is the result of hybridization between genetically distant genomes.

Secondary contacts in a potential, large hybrid zone consisting of the Saharan mountains (from the Hoggar to southern Egypt) when climatic conditions were favorable, and gene exchange in the Kenyan and Ethiopian highlands (Rift Valley) through a corridor connecting eastern and southern Africa, may account for limited morphological differentiation and incongruence between taxonomy, nuclear sequences, and plastid haplotypes in the olive tree complex.

Overall, the differentiation of isolated populations (ssp. *laperrinei*, *maroccana*, *guanchica*, and *cerasiformis*) is considered a more recent event occurring after the mentioned pattern of hybridization already occurred.

In the late 6,000 years, the continuous olive domestication through local hybridization of cultivated trees with natural populations has brought the remarkably high genomic diversity among cultivated trees in the Mediterranean basin.

Since its formation (ca. 7 Mya), the Saharan desert may have been an effective barrier, limiting significant reproductive contacts between the two major plastid lineages, which are separated in all molecular analyses (Vargas and Kadereit 2001). However, a succession of humid transitional events in the Saharan mountains may have facilitated recurrent contacts between *O. europaea* populations from the Mediterranean basin and Tropical Africa. Long-distance gene flow via pollen dissemination might have occurred.

Palaeobotanical and palaeoclimatic evidence agrees with a deeper genetic and geographical isolation of the Saharan populations of subsp. *laperrinei* on high mountains as a result of aridification, particularly after the last deglaciation (Quézel 1978; Gasse et al. 1990).

The ssp. *europaea* originated from a pre-Quaternary Mediterranean ancestor, with no evidence for a recent hybrid origin. Common ancestry of *O. europaea* in Africa may have spawned new lineages in Macaronesia, Asia, and the Mediterranean long before the Quaternary (Palamarev 1989). Evolution of the Mediterranean climate in the past 3 Mya may have caused differentiation of subsp. *europaea* populations in the thermophilous vegetation of the Mediterranean Basin (Palamarev 1989). The occurrence of several glacial refugia in Macaronesia, Morocco, southern Iberia, and the eastern Maghreb, coupled with the introduction of multiple cultivars across the Mediterranean bringing about gene flow with local populations, may account for recurrent contacts of olive genomes resulting in a higher genetic diversity observed in the western Mediterranean basin (Besnard et al. 2002a, b). AU2

5.1.2 Geographical Distribution and Genetic Diversity

Several evidences demonstrated the presence of some forms of olive during the last glaciation in the western and eastern Mediterranean regions 18,000 years BC (Carrion and Dupre 1996; Watts et al. 1996). Leaf

fossils have been found in Pliocene deposits at Mongardino in Italy (between ending of Miocene 5.3 millions of years and beginning of the Pleistocene 1.8 millions years before present). Fossilized remains have been discovered in strata from the Upper Paleolithic at the Relilai snail hatchery in North Africa, and pieces of wild olive trees and stones have been uncovered in excavations of the Chalcolithic period and the Bronze Age in Spain. The existence of the olive tree, therefore, dates back to the twelfth millennium BC. Numerous studies confirmed that olives have been present for several thousands of years before its domestication in the Mediterranean Basin, particularly in the Middle East. Olive was probably domesticated in the Jordan River valley ca. 5700–5500 years BC (Zohary and Spiegel-Roy 1975), and more precisely, according to Liphschitz et al. (1991) domestication occurred during the bronze period (5200 years BC).

Wild olive trees are extremely abundant and grow in thick forests in Asia Minor. It appears to have spread from Syria to Greece via Anatolia (De Candolle 1883) although other hypotheses point to Lower Egypt, Nubia, Ethiopia, the Atlas Mountains, or certain areas of Europe as its source area.

Mediterranean olives comprise genuinely wild olive trees, wild-looking forms (feral olives) that are secondary derivatives produced by sexual reproduction among cultivated olives, and varieties. Past climatic conditions suitable for oleaster growth such as an increase from 1 to 1.5°C recorded in southern France at the beginning of the Subatlantic chronozone of Holocene could be related to the expansion of thermophilous vegetation including *Olea* (Terral and Mengüal 1999). Using genetic markers (alleles for isozyme) associated with characters (prolonged juvenile phase) that render plants unsuitable for domestication, Lumaret and Ouazzani (2001) showed that genuinely wild olive trees, which cannot be distinguished morphologically from feral forms, still survive in a few Mediterranean forests in southern France and Spain, North Morocco and Tunisia, Corsica, Sicily and Cyprus islands, and Palestine. In the eastern part of the Mediterranean Basin (i.e., Egypt, Syria, Turkey, Crete, or Greece), where olive trees have been extensively cultivated for longer periods, no candidate forests containing genuinely wild olive trees were found.

The precise relationships of the Mediterranean olives (*O. europaea* L. subsp. *europaea*) to the other subspecies have remained elusive (Besnard et al. 2007a, b, c). Relatives of the Mediterranean olive tree are circumscribed in five subspecies (Green 2002): *O. e.* ssp. *laperrinei*, distributed in Saharan massifs (Hoggar, Aïr, Jebel Marra in Algeria); *O. e.* ssp. *cuspidata*, from Egypt to South Africa (from here it was introduced in East Australian areas and Hawaii), and from Arabia to northern India and Southwest China; *guanchica* in the Canary Islands; *O. e.* ssp. *maroccana* in Agadir mountains, southwestern Morocco; and *O. e.* ssp. *cerasiformis* in Madeira island (Besnard et al. 2007a).

The spread of the cultivated olive to other countries of the Mediterranean region accompanied migrations of different civilizations (Egyptian, Phoenician, Greek, Etruscan, Roman, and Arab). It is considered to be indigenous to the entire Mediterranean Basin and Asia Minor, which have been the birthplaces of the cultivated olive some six millennia ago. The Assyrians and Babylonians were the only ancient civilizations that were not familiar with the olive tree. The original home of the olive tree is the area from the southern Caucasus to the Iranian plateau, while in the Mediterranean coasts of Syria and Palestine, its cultivation developed considerably, spreading from there to the island of Cyprus and on toward Anatolia or from the island of Crete toward Egypt.

When archeological olive stones are compared with stones of modern cultivars, an early and autochthonous olive domestication in northwestern Mediterranean areas is suggested. The appearance of cultivated forms at the Chalcolithic/Bronze Age seems to corroborate that farming and selective practices have been operated at least since that time. These results support hypotheses from previous bioarcheological and paleoenvironmental studies that evidence for the emergence of cultivation practices from the Neolithic and the Bronze in Spain (Terral et al. 2004).

In the sixteenth century BC, the Phoenicians started disseminating the olive throughout the Greek islands, later introducing it into Greek mainland between the fourteenth and twelfth centuries BC, where its cultivation increased and gained importance in the fourth century BC after Solon issued decrees regulating olive planting during the Athenian democracy (600 BC), in the first written legislation of the world, prohibiting the cutting down of olive trees.

From the sixth century BC onward, *O. europaea* ssp. *europaea* spread throughout the Mediterranean countries reaching Tripoli, Tunis, and the island of

Sicily. From there, it moved to southern Italy. It is believed that the introduction of olive trees in Italy dates back to three centuries before the fall of Troy (1200 BC). Another Roman annalist (Penestrello) defends the traditional view that the first olive tree was brought to Italy during the reign of Lucius Tarquinius Priscus the Elder (616–578 BC), possibly from Tripoli or Gabes (Tunisia). Cultivation moved upwards from south to north, from Calabria to Liguria. When the Romans arrived in North Africa, they found evidences on the use, from the Berbers of the wild gene pool as scion to graft domesticate olives; the Romans really developed the grafting technology and spread its adoption throughout the territories they occupied (Zohary and Hopf 1994; Besnard et al. 2001b; Spennemann and Allen 2000; Green 2002; Terral et al. 2004).

The Romans continued the expansion of the olive tree cultivation area to the countries bordering the Mediterranean, using it as a peaceful weapon in their conquests to settle the people. It was introduced in Marseilles around 600 BC and spread from there to the whole of Gaul. The olive tree made its appearance in Sardinia in Roman times, while in Corsica, the traditional belief dated the beginning of olive cultivation to a period after the fall of the Roman Empire, using specimen introduced from Liguria. Olive was introduced into Spain during the maritime domination of the Phoenicians (1050 BC) but did not develop to a noteworthy extent until the arrival of Scipio (212 BC) and Roman rule (45 BC). After the third Punic War, olives occupied a large stretch of the Baetica valley and spread toward the central and Mediterranean coastal areas of the Iberian Peninsula including Portugal. The Arabs brought their varieties with them to the south of Spain and influenced the spread of cultivation so much that the Spanish words for olive (aceituna), oil (aceite), and wild olive tree (acebuche) and the Portuguese words for olive (azeitona) and for olive oil (azeite) have Arabic roots. With the discovery of America, olive farming spread beyond its Mediterranean confines. The first olive trees were carried from Seville to the West Indies and later to the American Continent. By 1560, olive groves were cultivated in Mexico, and in the last few years, growth of olive production has expanded throughout the world to countries such as Australia, Canada, China, Peru, Chile, Argentina, and the United States (Reale et al. 2006).

5.1.3 Genetic Diversity and Gene Flow Between Wild and Cultivated Olive

The above-mentioned results of Lumaret and Ouazzani (2001) provide evidence of the survival of indigenous oleaster populations, particularly in the western part of the basin. Genetic diversity values over cultivars, feral olives, and the wild olives in ten forest areas around the Mediterranean basin were 0.286, 0.414, and 0.506, respectively, which is consistent with the interpretation that the domesticated olive represents a sample of the genetic variation in genuinely wild olive populations that persist today. Owing to their very long lifespan, these wild trees should be closely related to the Neolithic olives recognized as the crop progenitor.

Other authors, using a sample of 166 oleasters taken from 20 groves of modern populations, and 40 cultivars to represent molecular diversity in the cultivated olive, evidenced that oleaster genetic diversity can be traced to the geographical diversity among seven regions that could overlay past glacial refuges (Breton et al. 2006). The gradient, or cline, of genetic diversity revealed by chloroplast and microsatellite or simple sequence repeat (SSR) molecular markers was explained by oleaster recolonization of the Mediterranean basin from refuges after the last glacial event, located in both eastern and western regions. It is likely that gene flow has occurred in oleasters mediated by cultivars spread by human migration or through trade.

The observed patterns of genetic variation for amplified fragment length polymorphism (AFLP) markers suggested a clear distinction of the wild populations from the cultivated landraces and continental from insular regions (Baldoni et al. 2006). Island oleasters were highly similar to each other and were clearly distinguishable from those of continental regions. Ancient cultivated material from one island clustered with the wild plants, while the old plants from the continental region clustered with the cultivated group.

A general picture (Fig. 5) on the past events that shaped the current oleaster distribution has emerged from mitochondrial DNA variation and RAPD marker (Bronzini de Caraffa et al. 2002a, b; Besnard and Bervillé 2002), chloroplast and SSR variation (Breton et al. 2006), and isozyme analysis of Lumaret et al. (2004). Oleasters from ancestors that during the last ice-age glaciation strived in the western Mediterranean

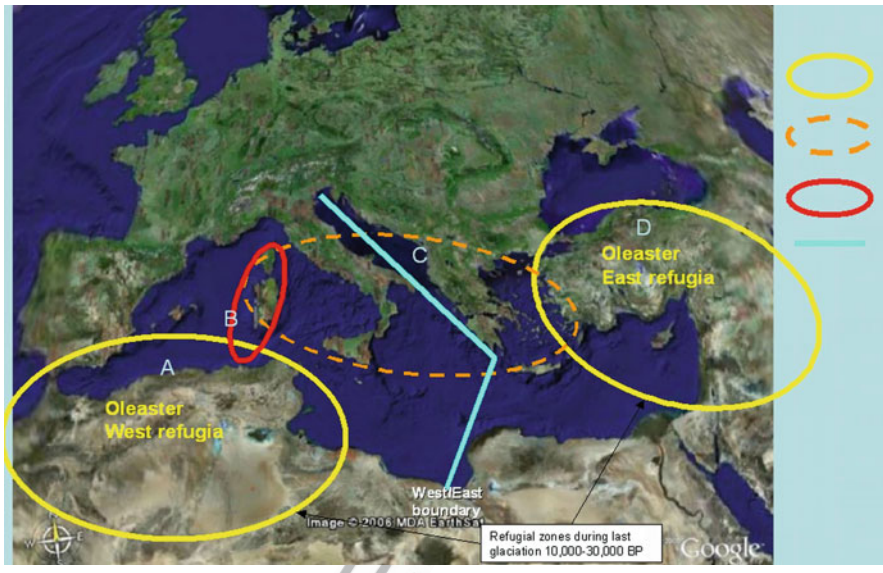

Fig. 5 Oleaster distribution based on mitochondrial DNA variation and RAPD marker (Bronzini de Caraffa et al. 2002a, b; Besnard and Berville 2000), chloroplast and SSR variation (Breton et al. 2006), and isozyme analysis of Lumaret et al. (2004). (**a**, **d**) Glacial refugia of oleaster. In the west refugia, oleaster with MOM and MCK mitotype, COM and CCK chlorotype, and western RAPD pattern were found. In the east refugia, oleaster with ME1 and ME2 mitotype, CE1 chlorotype, and eastern RAPD pattern were found. Molecular diversity is much higher in the west than in the east, supporting the hypothesis that western oleaster are genuine and differentiated earlier (Breton et al. 2006). (**b**) True oleasters in Sardinia and Corsica are only those plants with MOM and MCK mitotype and west RAPD pattern; oleaster with ME1 mitotype might be feral form derived by hybridization of cultivated olives with ME1 mitotype (female) × true oleaster (male) with western RAPD pattern. The reciprocal hybridization also produced feral form with MOM mitotype and east RAPD pattern. Oleaster with ME1 mitotype and east RAPD are considered feral form of the cultivated varieties and not true oleasters. Therefore, some western cultivars in those islands might have originated from western oleaters (Breton et al. 2006). (**c**) Cultivated olives show mostly ME1 mitotype and eastern RAPD pattern. The model support the eastern domestication of olives and the east to a west diffusion of varieties (Loukas and Krimbas 1983; Zohary and Hopf 1994), including some restricted domestication events from local genuine western oleaster occurring in Corsica and south of Spain

refugia (corresponding to the territory of Algeria, Morocco, Tunisia), share the MOM and MCK mitotype and COM and CCK chlorotype that are absent in oleasters from the ancestral populations occupying the eastern refugia (Turkey, Palestine, Syria–Iran boundary). Oleasters in East Mediterranean share the ME1 and ME2 mitotype, CE1 chlorotype, and eastern RAPD pattern. Molecular diversity is much higher in the west than in the east, supporting the hypothesis that western oleaster are genuine and differentiated earlier (Breton et al. 2006). True oleasters in Sardinia and Corsica are only those plants with MOM and MCK mitotype and west RAPD pattern; oleaster with ME1 mitotype might be the feral form derived by hybridization of cultivated olives with ME1 mitotype (female) × true oleaster (male) with western RAPD pattern. The reciprocal hybridization also produced feral form with MOM mitotype and east RAPD pattern. Oleaster with ME1 mitotype and east RAPD are considered the feral form of the cultivated varieties and not true oleasters. Therefore, some western cultivars in those islands might have originated from western oleaters (Breton et al. 2006). Cultivated olives show mostly ME1 mitotype and eastern RAPD pattern, supporting the eastern domestication of olives and the east to a west diffusion of varieties (Loukas and Krimbas 1983; Zohary and Hopf 1994).

5.1.4 Morphology

Wild and domesticated olives grow in the same areas but the wild type shows some morphological differences compared to cultivated forms, such as smaller fruit size and lower oil content in the mesocarp (Terral

and Arnold-Simard 1996). Two distinct wild types of olives have been recognized: *oleaster* and feral forms. *Oleaster* occupies primary niches in undisturbed areas as a constituent of evergreen plant associations. Wild and cultivated varieties, representing two genetically separated complexes, have been *interconnected* as a result of occasional hybridizations that could have allowed the introgression of genes from the wild forms into the cultivated varieties. Due to their long life span, there has been relatively little selection and the cultivated olive gene pool is assumed to be very similar to the gene pool of their wild progenitors (Liphschitz et al. 1991).

Baali-Cherif and Besnard (2005) mentioned that *O. e. laperrinei* in the Hoggar (Algeria) was a small multi-stemmed tree, thus suggesting that it may use a vegetative strategy for its reproduction and persistence in arid environments (Anthelme et al. 2008). In contrast, in the wetter western Darfur, *O. e. laperrinei* was described as a tree reaching up to 15 m high without mention of a multistemmed shape (Quézel 1969). The rarity of sexual reproduction is one of the most remarkable life-traits of *O. e. laperrinei* in the Hoggar (Quézel 1969; Baali-Cherif and Besnard 2005).

The olive is a long-lived evergreen plant that adapts quite easily to many and varied environmental conditions. Its characteristic basitony gives the plants a shrub-like appearance, when allowed to grow without any pruning or training. The branches may vary from upright to pendulous according to the cultivar, and their vigor depends upon position, productivity, and the nutritional status of the tree. To develop olive trees with compact growth habit is an important breeding objective, since small olive plants would contribute to lower costs of manual labor for harvesting and pruning and may allow the use of more efficient machines than the current shaking ones.

Flower buds and, less frequently, mixed buds differentiate in the same year of flowering, although flower induction occurs already by the end of the preceding summer (Proietti and Tombesi 1996). Flowers gathered during inflorescence normally are hermaphroditic, although flower anomalies frequently occur. Depending on the cultivar, flowers may be partially self-fertile or self-fertile (Fontanazza et al. 1990).

The olive fruit is a drupe that contains a bitter component (oleuropein), a low sugar content (2.6–6%) compared with drupes of other species (12% or more), and high oil content (12–30%) depending on the time of year and variety.

These characteristics make it a fruit that cannot be consumed directly from the tree, and it has to undergo a series of processes that differ considerably from region to region and from one cultivar to another. Some olives are, however, an exception to this rule because they sweeten right on the tree as they ripen in most cases due to fermentation. One case in point is the Thrubolea variety in Greece.

Fruit-set is very low-ranging from 1 to 5% of flowers. The most crucial phases of fruit morphogenesis are the cell division and cell enlargement in the pericarp and then lipid synthesis and accumulation. They show a maximum rate, whose pattern may be strongly influenced by stresses and nutritional deficits. Apparent parthenocarpy is frequent in peculiar environmental conditions and for given cultivars, leading to very small (3–4 mm in diameter) and round drupes, which often ripen. Overcoming self-sterility, enhancing fruit-setting ability, and assuring a constant high productivity are further objectives in the improvement of the species.

5.1.5 Karyotype and Genome Size

Olea is considered a genus containing diploid species, with the basic chromosome number $x = 23$. The nuclear DNA content of olive cultivars was determined for the first time by Rugini et al. (1996), who used Feulgen cytophotometry to estimate the 2C nuclear DNA content of cvs. "Frantoio" and "Leccino." The 1C DNA content has been estimated to be approximately 2.2 pg corresponding to a genome size of 2,200 Mb. Evidences for the presence of triploid and tetraploid mutants from the "Frantoio" and "Leccino" cultivars were given. More recently and using the same technique, Bitonti et al. (1999) estimated the genome size of cvs. "Dolce Agogia" and "Pendolino." The results of these studies indicated a high intraspecific variation for genome size among the studied Italian cultivars. Bitonti et al. (1999) also analyzed the genome size of other *Olea* species and verified that it was considerably lower than the genome size of O. *europaea* cultivars. On the other hand, using flow cytometry, Loureiro et al. (2007) determined that the nuclear DNA content of O. *europaea* cultivars ranged between 2.90 ± 0.020 pg/2C and 3.07 ± 0.018 pg/2C and the genome size of wild olive was estimated

as 3.19 ± 0.047 pg/2C DNA. These results suggest a low intraspecific variation at least among the studied cultivars and between them and wild olive. The presence of polyploid plants in natural populations of *O. e.* ssp. *cuspidata* (from Iran) and *maroccana* was inferred from microsatellites data by Rallo et al. (2003) and Besnard et al. (2008) using flow cytometry, and nuclear microsatellite analyses provided strong evidences for polyploidy in ssp. *cerasiformis* (tetraploid) and *maroccana* (hexaploid), whereas the other subspecies appeared to be diploids. Because polyploidy is found in narrow endemic subspecies from Madeira (subsp. *cerasiformis*) and the Agadir Mountains (subsp. *maroccana*), it has been hypothesized that polyploidization has been favored to overcome inbreeding depression (Besnard et al. 2008). Based on previous phylogenetic analyses, Besnard et al. (2008) hypothesize also that tetraploid subsp. *cerasiformis* resulted after hybridization between ancestors of subspp. *guanchica* and *europaea*.

5.1.6 Agricultural Status

Cultivated olive is one of the most important tree crop species of the Mediterranean basin, representing not only the 90% of the olive groves of the world but also the 90% of the olive world production. Only Spain, Italy, and Greece produced around 75% of the world's olive oil, and together with Turkey and Tunisia are the five largest producers in the world. In 2005, world production was approximately 15,500 Mt, while in 2007, it increased to 17,500 Mt, destined to both oil and fresh consumption (FAOSTAT 2007).

The world production and consumption trend of olive oil in the last 30 years have increased significantly and will continue, considering the recent introduction of its cultivation in Japan, USA, Australia, China, South America, and South Africa.

An increasing interest in olive products in Australia occurred during the late 1940s and 1950s coinciding with the immigration from the Mediterranean basin. By 1959, 2,929 ha were under cultivation in Australia (Hartmann 1962), and by 1998, plantings of *O. europaea* had increased to more than 5,000 ha, with plants equivalent to another 7,000 ha of trees on order (Spennemann and Allen 2000). Olive orchards are now found in all the six Australian states. Today, there are over 100 known varieties of olives in Australia (Sweeney and Davies 1998). Australia could have the opportunity to produce organic olive oil because of the absence of the major diseases prevalent in the traditional olive-growing countries.

The world olive oil production is estimated to be three million tons, ranking sixth in the world production of fluid vegetable fats exceeded by soybean, cottonseeds, peanuts, and sunflower. The olive oil production is not constant over the years, owing to the proverbial alternate-bearing tendency of this species that is genetically and environmentally determined.

O. europaea ssp. *cuspidata* does not have great economic importance, but its wood can be used for furniture in South Africa, can be cultivated as an ornamental tree in China, and can be utilized as a rootstock and hedge plant (Spennemann and Allen 2000; Starr et al. 2003).

The past history of domestication, demography (geographic isolation and ecotypic adaptation to a vast array of environmental conditions), and gene flow within and among subspecies have shaped and genetically structured the gene pool of *Olea*. The primary gene pool (GP1) of *O. europaea* ssp. *europaea* includes all the diploid and interfertile Mediterranean forms of *O. europaea*. The wild subprimary gene pool incorporates populations of the genuine *oleaster* and of the *olevaster*, and the cultivated subprimary gene pool comprises the orchard varieties. Although at the end of the last glaciations, hybridization among the ancestors of the current species might have occurred, as it is suggested by Besnard et al. (2008) for the *guanchica* and *europaea* hybridization that gave rise to the species *cerasiformis*, subsequent geographic and genetic (polyploidization) isolation events occurred, riding biological barriers for the gene flow among the six *O. europaea* species. Therefore, those species contribute to the formation of the secondary gene pool (GP2) of *O. europaea* ssp. *europaea* (Fig. 6). The tertiary gene pool (GP3) accounts for most of the *Ligustroides* species from which the gene transfer to *O. europaea* ssp. *europaea* could occur only through in vitro culture of hybrid embryos and of somatic hybrids. The quaternary gene-pool of *O. europaea* ssp. *europaea* so far includes those bacteria and fungi from which the *rol* and *osmotin* genes have been transferred to *O. europaea* ssp. *europaea* (Fig. 6) (Rugini et al. 2008).

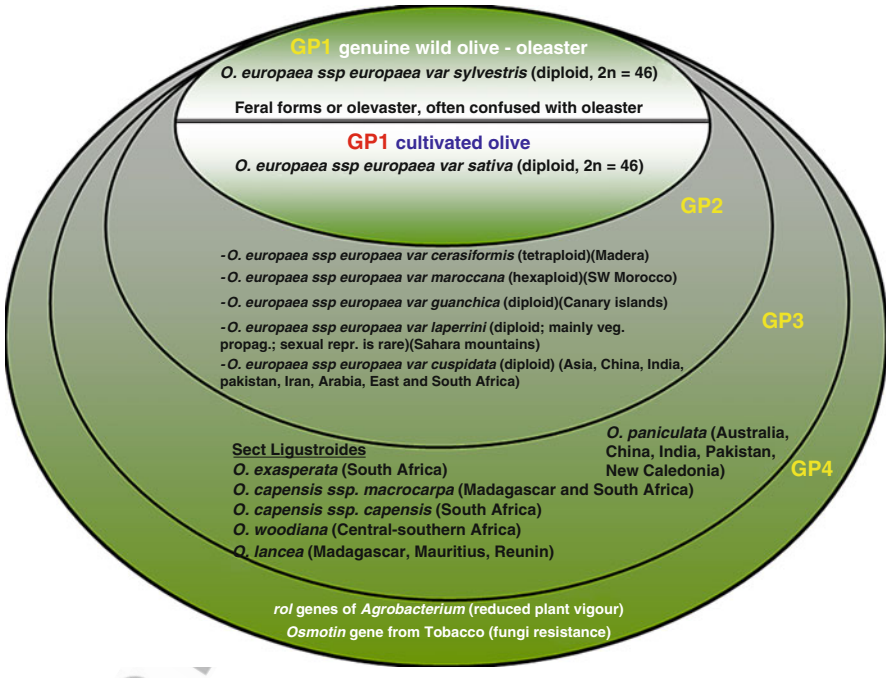

Fig. 6 An example of Gene pools of *Olea* useful in a program of genetic improvement: *GP1* Primary gene pool; *GP2* Secondary gene pool; *GP3* Tertiary gene pool; *GP4* Quaternary gene pool

5.2 Conservation Initiatives

5.2.1 Evaluation of Genetic Erosion

The discovery of indigenous oleaster populations (Lumaret and Ouazzani 2001) along the western Mediterranean basin will encourage in those areas the preservation of environmental conditions that favor the survival of large populations of oleasters that are properly isolated from cultivated olive trees. For *O. e. laperrinei*, population on Mt. Hoggar (Algeria) acts as an important genetic reservoir that has to be taken into account in future conservation programs. Moreover, very isolated endangered populations (for example, Bagzane) displaying evident genetic particularities have to be urgently considered for their endemism (Besnard et al. 2007b).

5.2.2 Germplasm Banks

Olive (*O. europaea* L.) germplasm is normally preserved in clonal collections, located in different sites of several countries. There is not a centralized service for conservation, but several institutions carry on, on their own, the recovery, propagation, and maintenance of a number of accessions, mainly autochthonous ones. Most recently, the International Olive Oil Council promoted a campaign aimed at retrieving and also preserving accession from distant locations and countries where preservation is not provided (Fabbri et al. 2004). These field banks (ex situ) are located mainly in traditional countries including Spain, Iran (Amiri 2008), and Italy (Sicily; G. Fontanazza personal communication). Since few varieties are cultivated compared to a large number of known accessions, it is very important to have a large clonal collection replicated in several countries to reduce the risk of loss of valuable germplasm and at the same time to facilitate their use for genetic improvement.

5.2.3 Modes of Preservation and Maintenance

During the last decades, extensive work has been done using in vitro techniques aiming at a rapid "true-to-type" propagation, the production of disease-free plants, the cryopreservation of elite germplasm, and genetic improvement. In vitro propagation of the olive cultivar by axillary bud stimulation has successfully been used and is now a commercial reality in the

nursery production in Italy. In addition, in Spain, thousands of rootstocks from seedling origin are also produced. This technique allows producing high quality and rapid growing plants. Recently, agronomic information on micropropagated plants show that the micropropagated plants flower at the same time as propagated plants by cutting. The in vitro culture for the olive is, therefore, being used in the breeding programs for the improvement of the species. In vitro culture techniques applied are discussed below.

5.2.3.1 Axillary Bud Stimulation

Up to now, the major difficulties encountered for in vitro micropropagation of mature tissues of olive cultivar was the establishment of axenic cultures and subsequent initially growth of shoots. Different attempts to establish sterile cultures with meristems or shoot-tips from field-grown or greenhouse plants were unsuccessful due to the rapid oxidation of tissues after collection, even with high doses of active antioxidants. Vigorous node explants, possibly hiding a subepidermal vegetative buds and inflorescence sprouted from mixed buds, which both are easy to surface sterilize without damaging the buds and the secondary buds are advisable to use for starting tissue to get sterile shoots. The rapid growth is supported by OM medium (Rugini 1984, DUCHEFA catalog) with the addition of natural cytokinin, zeatin, or a mixture of them (zeatin, BAP, TDZ and metatopolin) and GA_3 (20–40 mg/l), and mannitol, in place of sucrose, induced the production of tender, longer shoots more suitable for the subsequent rooting phase. Some cultivars showed beneficial effect on shoot proliferation with the use of Dikegulak (Mendoza-De Gyves et al. 2008), which increases lateral bud sprout. Reinvigoration of shoots in vitro may be essential. It can be achieved by forcing the basal lateral buds of the new shoots still attached to initial woody nodal explant, for several subcultures, by cutting the apical part at each subculture (E. Rugini unpublished); furthermore, grafting the buds on juvenile seedling in vitro should be advisable (Revilla et al. 1996). Rooting of explants benefits by basal etiolation (Rugini et al. 1987) or by keeping 5–7 days in the dark and by addition of 1 mM of putrescine to the medium (Rugini and Fedeli 1990). Putrescine contributes to increase total peroxidase activity in the basal part of the shoots in the first hours after explant preparation, allowing promoting earlier root protrusion than with untreated ones (Rugini et al. 1997). The micropropagation allows propagating difficult-to-root cultivars by cutting as Nocellara etnea (Briccoli Bati et al. 1999). In some cultivars, it is possible to root the explants in vivo by supplying both auxin and putrescine at the basal end of the explant.

5.2.3.2 Shoot Organogenesis

From Embryonal Material

Organogenesis was observed under different conditions and in different tissue origin. Direct shoot organogenesis was obtained from olive hypocotyl sections (Bao et al. 1980). Explants from mature embryos are suitable material for shoot regeneration (Rugini 1986). High shoot regeneration has also been shown by callus derived from mature cotyledon fragments of the cvs. Tanche and Picual. The regeneration was higher in calli from cotyledon segments proximal to the embryo axes than distal ones (Canas and Benbadis 1988).

From Mature Tissue of Cultivars

Adventitious shoots were induced only in the dark from petioles of in vitro-grown shoots of cultivars Canino, Moraiolo, Dolce Agogia, and Halkidikis. The petioles-forming shoots ranged from 10 to 40% depending on the leaf position on the shoot and the medium used (Mencuccini and Rugini 1993). The quality of shoots used to collect the petioles is essential for the subsequent success in regeneration, and the addition of gibberellic acid (GA_3) in proliferation medium enhances regeneration rate. Currently, the shoot organogenesis from olive mature tissues is not an efficient method to regenerate plants from transgenic cells; however, it seems to be essential as the first necessary step to achieve somatic embryogenesis.

5.2.3.3 Somatic Embryogenesis

In olive, somatic embryogenesis (SE) has been successfully achieved from both embryonal (immature and mature zygotic embryos) and somatic (seedling tissues and leaf petioles of cultivars) explants.

SE from Embryonal Explants

The first report on induction of somatic embryogenesis in olive was achieved by wounding the roots still attached to the seedlings, originated from mature embryos in vitro; the callus produced in the wounded zone produced embryos, which converted into plantlets (Rugini and Tarini 1986). However, high yield of somatic embryos can be recovered from immature (60–75 days after fertilization) zygotic embryos (Rugini 1988; Leva et al. 1995). This high capacity may be extended for at least 2 months by storing at 14–15°C the whole fruitlets, collected at 75 days after fertilization. Under these conditions, although the small embryos continue to develop, they maintain the embryogenic capacity, contrary to the corresponding embryos collected from fruits left on the plants (Rugini 1995).

Callus from segments of non-germinated mature embryos both of wild (Orinos and Mitrakos 1991) and cultivated olive (Mitrakos et al. 1992) could produce somatic embryogenesis. Calli derived from rootlets give the highest somatic embryogenesis. The difference in the amount of embryogenesis depends on the origin of the explant (proximal, medium, and distal part of the embryo) and on the last of the period of permanence of explants on callus induction medium. Somatic embryogenesis is not influenced by the salt concentration of the medium or by the light or dark conditions (Orinos and Mitrakos 1991).

SE from Somatic Mature Tissues of Cultivars

SE from mature tissues is still difficult to achieve. However, for two cultivars, Canino and Moraiolo, it has been done using one strategy, named later in a similar work on apple by Rugini and Muganu (1998) as the "double regeneration system." It consists of regenerating first adventitious buds from leaf petioles of in vitro grown shoots, then subculturing the small leaflets in a proper medium until pro-embryo masses appear. The embryos differentiate from its surface, recognizable by porous and smooth epidermis and yellowish in color. Cyclic embryogenesis both from normal embryos and from teratoma can be obtained indefinitely (Rugini and Caricato 1995). The embryos differentiate mainly from epidermal surface with mainly unicellular origin (Lambardi et al. 1999). The continuous production of long-term cycles of embryos from epidermal cells could be a great advantage in regenerating plants from transgenic cells, because it avoids the callus formation, which could be a wide source of undesirable genetic variability. In addition, it avoids wounding of the tissues that could be the cause of browning and reducing the efficiency of *Agrobacterium*-mediated transformation.

The aim with somatic embryos is to use in transformation work but at the same time to produce "synthetic seeds." A first successful attempt of encapsulation was done by Micheli et al. (1998) but using in vitro-proliferated short apical shoots in place of embryos.

Capelo et al. (2010) described a protocol for SE-induction from an adult wild olive tree (*O. europaea* ssp. *europaea* var. *sylvestris*). The protocol used confirmed for the first time that there is no need to use juvenile or rejuvenated material for SE induction. It opens perspectives for using this strategy in SE protocols both for the wild and commercial genotypes.

For SE induction, petiole and leaf (proximal, intermediary, and distal zones) explants were grown on Murashige and Skoog (MS) medium or Olive medium (OM) with different combinations of plant growth regulators (PGR): α-naphthaleneacetic acid (NAA), Zeatin (Zea), indole-3-butyric acid (IBA), 2-isopentyl adenine (2iP), thidiazuron (TDZ), and 6-benzylaminopurine (BAP).

5.3 Elucidation of Origin and Evolution of *Olea* Species

5.3.1 Related Species

Recently, three taxons have been described as new allied species of *O. europea*. *Chionanthus greenii* Lombardi, a new species of Oleaceae, is distinguished by the unique combination of strigose indument, short congested inflorescences, small corolla lobes, and reniform anthers, which do not match to any described South American species (Lombardi 2006). *Chionanthus amblirrhinus* sp. nov. is described from Thailand, and *C. decipiens* sp. nov. from Burma and Thailand. The following new combinations are made: *C. eriorachis* (from Thailand), *C. malaelengi* subsp. *linocieroides* (from S. India) and subsp. *terniflorus*

(from NE India, Burma, Thailand and Indo-China), *C. microbotrys* (from Thailand), *C. microstigma* (from Thailand and Indo-China), *C. sutepensis* (from Thailand), *C. thorelii* (from Thailand and Indo-China), and *C. velutina* (from Thailand) (Green 1996).

5.3.2 Biochemical and Molecular Markers Employed for Phylogenetic and Polymorphism Studies Within Olea

Several genes encoding for key enzymes in fatty acid and antioxidant biosynthesis, modification, and triacylglycerol storage have been isolated (Hatzopoulos et al. 2002). The gene expression during fruit growth and seed development as well as their transient and temporal expression in different tissues allowed identification of those most important for storage of fatty acids and to the provision of signaling molecules important in plant defense mechanisms and reproduction.

5.3.2.1 Molecular Markers to Infer Evolutionary and Domestication Events

Patterns of Evolution and Domestication of Olive

The olive is one of the most ancient cultivated fruit tree species in the Mediterranean basin (Zohary and Hopf 1994).

Palynological and fossil charcoal evidences support the occurrence of wild olive forms (*O. europaea* ssp. *sylvestris* (Miller) Hegi var. *oleaster* (Hoffmanns and Link) DC) in forests of the Mediterranean Basin during the Paleolithic age (Liphschitz et al. 1991). According to anthracological studies, during the Neolithic period (10,000–7,000 years ago), use of wild olive (oleasters) persisted in several parts of the basin (Liphschitz et al. 1991; Zohary and Hopf 1994).

Cultivated and wild forms have the same chromosome number ($2n = 46$) (Green and Wickens 1989) and are fully interfertile.

It was hypothesized that during the last glaciations (30,000–10,000 BP), at least two Mediterranean refugial zones would have existed: one in the east (Israel, Syria, Turkey) and another in the west (Sicilia, Maghreb) (Zohary and Hopf 1994; Terral and Arnold-Simard 1996; Besnard and Berville 2000). Subsequently, the wild olives recolonized the Mediterranean Basin, and many crossings between olive trees of the east and the west probably occurred. The cultivated forms have been selected from the wild local forms in several eastern places (Zohary and Spiegel-Roy 1975). The domestication of the olive tree started in the Near East about 6,500 years ago and olive has been disseminated all around the Mediterranean Basin (Zohary and Spiegel-Roy 1975; Loukas and Krimbas 1983).

It is now believed that two wild forms exist: the true oleasters (corresponding to the wild forms present in natural areas) and the feral forms (which escaped from cultivation or resulted from hybridizations between the true oleasters and varieties) (Zohary and Hopf 1994; Angiolillo et al. 1999; Baldoni et al. 2000; Besnard and Berville 2000).

Clear signs of olive domestication come from olive oil presses' remnants dated to the Early Bronze Age (second half of the fifth millennium BP) and found in the Near East (Zohary and Spiegel-Roy 1975; Liphschitz et al. 1991). It has been assumed that individual oleaster trees showing superior performance for size and/or oil content of fruit were selected empirically and propagated vegetatively as clones, using cuttings that were planted directly or, more recently, grafted onto indigenous oleasters. Olive varieties were then developed and distributed by various successive human migrations throughout the Mediterranean Basin, especially from east to west, although the selection of olive cultivars was multilocal and included originally western plant material.

Substantial genetic differentiation was observed between the eastern oleaster populations genetically close to most olive clones cultivated in the Mediterranean Basin and the western populations that are related to the wild Canarian populations. As matter of fact, the wild subspecies *O. europaea* subsp. *guanchica*, endemic to the Canary Islands, is closely related to oleasters.

Studies on olive pollen flow by wind (Griggs et al. 1975), on seed dispersal of wild olive by birds in Spain (Alcantara et al. 2000), and from local reports about the extensive use of seeds from olive groves for forestation in several Mediterranean countries (Chevalier 1948) suggested that genuinely wild olive populations may be restricted to very few isolated forest areas.

Consequently, at the present time, oleasters are absent from many regions (Ouazzani et al. 1993) and are limited to restricted areas along the shores of the Mediterranean (Zohary and Spiegel-Roy 1975). However, the study of oleasters appears of real interest since it might constitute a gene pool useful for olive improvement programs, i.e., for disease and stress resistance (Fig. 5).

Using Molecular Markers to Validate the Patterns of Evolution and Domestication of Olive

Genetic diversity within and between plant populations results from a combination of geographical distance, population size, type of mating system (selfing or outcrossing), mode of dispersal of pollen and seed, and rate of gene flow (Loveless and Hamrick 1984). The mating system of plants appears to play a major role in genetic diversity by determining the rate of exchange of genes (Darmency 1997). Species that are predominantly outcrossing are reported to show lower interpopulation and higher intrapopulation differences in genetic variation compared to species where self-fertilization predominates (Maguire and Sedgley 1997).

O. europaea L. is a predominantly allogamous species showing a high degree of outcrossing. Most pollen transfer is by wind with insect involvement occurring to a small extent (Lavee 1996). Progenies are readily derived from crosses between cultivated varieties (cultivars) as well as between cultivars and both feral (escaped) and wild (oleaster) olives (Angiolillo et al. 1999). The degree of outcrossing varies between cultivars and environments (Lavee 1996).

Different techniques have been used to evaluate olive diversity. Cantini et al. (1999) used morphological characters such as leaf, fruit, pit, and growth form to evaluate genetic variation within and between different accessions of known and unknown olive cultivars. Isozyme analysis has also been used to examine the genetic diversity in wild and cultivated olives (Ouazzani et al. 1993). Random amplified polymorphic DNA (RAPD) technique successfully identifies olive cultivars (Weisman et al. 1998; Mekuria et al. 1999; Gemas et al. 2000) and, together with the analysis of molecular variance (AMOVA) (Excoffier et al. 1992), it has been used to study population genetics in many plants (Gillies et al. 1997; Maguire and Sedgley 1997). This technique was used to evaluate the level of genetic variation within an isolated feral olive population to better understand the dynamics of spread of *O. europaea* L. in an isolated population of 45 trees within an area of about 1 km^2 by using RAPD technique. Based on visual observation, three putative groups were identified: nine trees that appeared to be an original grove, 12 trees that were assumed to be planted progenies of the original grove, and 24 feral trees in the surrounding hills and valleys. The AMOVA showed high genetic variation within each of the three putative groups of the population, but there were no significant genetic differences among them. A dendrogram showed the presence of three clusters significantly different from one another that persisted when the data were subjected to multidimensional scaling. Each cluster consisted of at least one or more trees from the original grove and others from the population. The overall trend supported the derivation of feral trees from the original grove. The distribution of these clusters within the population suggests that the predominant mode of feral spread was by fruit drop close to the trees and in lesser extent by animals or birds (Mekuria et al. 2002).

Informative Molecular Markers

Genetic studies based on allozyme polymorphism (Lumaret et al. 1997), DNA tandem repeated sequences (Katsiotis et al. 1998), AFLP (Angiolillo et al. 1999), RAPD profiles and mitochondrial restriction fragment length polymorphism (RFLP) (Besnard and Berville 2000; Claros et al. 2000; Besnard et al. 2001a, b; Bronzini de Caraffa et al. 2002a), SSR (Rallo et al. 2000), as well as inter simple sequence repeat (ISSR) markers (Vargas and Kadereit 2001) helped to validate the patterns of domestication envisaged on the basis of palynological and archeological remnants. Molecular analyses are extremely useful when the morphological distinction between wild and feral olive forms cannot be determined because they have similar characters, such as a smaller fruit size, lower oil content in the drupe, and a typical wild aspect. They are also important diagnostic tools for the correct identification of chromosomes (Minelli et al. 2000) and cultivars and, because of their high polymorphism and discerning power, SSR or microsatellite markers have been developed and used with success for discrimination of olive

cultivars (Rallo et al. 2000; Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; Sabino et al. 2006).

5.3.2.2 Species Differentiation Using Molecular Markers

Tandem-repeat sequences isolated from an olive *Sau*3AI partial genomic library have been used as molecular markers to provide new insights in genome organization and in identifying superior olive tree genotypes. These repeated sequences were detected at DAPI-stained heterochromatic regions having a subtelomeric or interstitial location in *Olea chrysophylla*, *O. e. e.* var. *sylvestris* (syn. *O. oleaster*), and *O. e. cuspidata* (syn. *O. africana*) but were absent from other *Oleaceae* genera (Katsiotis et al. 1998). Sequence analysis of these repeated elements from different cultivars could also contribute to cultivar identification and discrimination.

Angiolillo et al. (1999), using AFLP profile data, argue that *Olea* accessions of the same species but from different places of East Africa and Asia may be assigned to different species.

Because of early olive cultivation, the cultivated forms, which have extended considerably over the natural populations, have caused the regression of oleasters (Bronzini de Caraffa et al. 2002b). Consequently, at the present time, oleasters are absent from many regions (as first deduced from isozyme analyses by Ouazzani et al. 1993) and are limited to restricted areas along the shores of the Mediterranean (Zohary and Spiegel-Roy 1975).

The nuclear genetic variation of oleasters was analyzed over the Mediterranean Basin using isoenzymes (Lumaret et al. 2004), RAPD markers (Besnard et al. 2001a, b), and in more restricted areas, using RAPDs (Bronzini de Caraffa et al. 2002a, b), AFLPs (Angiolillo et al. 1999), and allozyme polymorphism (Lumaret et al. 1997; Lumaret and Ouazzani 2001).

Allozymes at several independent loci analyzed in numerous cultivated olive trees (Ouazzani et al. 1993, 1996; Lumaret et al. 1997) were considered as appropriate codominant markers to characterize oleaster genetic variation.

Using isozyme markers on multiple samples from wide areas over the Mediterranean Basin, a clear nuclear genetic differentiation was observed between oleasters from the eastern and western parts of the Mediterranean Basin. Substantial genetic differentiation was observed between the eastern oleaster populations (genetically close to most olive clones cultivated in the Mediterranean Basin) and the western oleaster populations that are related to the wild Canarian populations (Lumaret et al. 2004).

Lumaret et al. (2004) studied allozyme variation at ten loci in 31 large and 44 small oleaster populations distributed in various habitats of the Mediterranean Basin and in two populations of the wild subspecies *O. europaea* ssp. *guanchica*, endemic to the Canary Islands and closely related to oleasters. Three isozyme alleles were shared by the Canarian populations of *O. europaea* ssp. *guanchica* and by the most occidental oleaster populations (more particularly those from the selected forests of Andalusia and Morocco), suggesting that the populations growing in the most westerly part of the species distribution have a common origin. The most western populations of the Mediterranean Basin are genetically close to the Canarian populations of ssp. *guanchica*, which supports previous results obtained from plant material collected in the same areas and analyzed using RAPD and ISSR markers (Hess et al. 2000).

Genetic evidence that non-domesticated oleasters still survive locally was provided by the occurrence of four and one isozyme alleles shared exclusively by the eight western and two eastern oleaster populations, respectively, which were collected in forests potentially containing genuinely wild forms. Except for those four very rare isozyme alleles, oleaster populations possess all the alleles already identified in cultivars. This result is consistent with the interpretation that the domesticated olive represents a subsampling of the genetic variation in genuinely wild olive, which persists today in the Mediterranean Basin (Lumaret et al. 2004).

The oriental populations are very close genetically to the two groups of olive clones cultivated in the eastern and in the western parts of the Basin, respectively, suggesting that most cultivars of the Mediterranean Basin originated from plants selected in the Middle East, as envisaged previously from the analyses of cpDNA, mtDNA, and RAPD (Besnard and Berville 2000; Besnard et al. 2001a, 2002a, b). However, in the western group, a few cultivars (particularly from Sicily, Corsica, and Andalusia) showed allozyme characteristics similar to those found in the western oleaster populations, supporting the previous

evidence of a multilocal selection of cultivars in olive (e.g., Besnard and Berville 2000; Besnard et al. 2001a; Bronzini de Caraffa et al. 2002a, b; Contento et al. 2002).

Feral olive-trees display an oleaster phenotype and are characterized by very close molecular relationships with varieties and not with oleasters (Angiolillo et al. 1999; Besnard and Berville 2000). Therefore, the identification of the feral forms is done a posteriori with molecular markers since generally they display close relationships with the varieties from which they are derived (Angiolillo et al. 1999; Besnard and Berville 2000). The presence of feral forms, resulting from cultivars escaping cultivation and also hybridizations between varieties and oleasters, have also been suggested by molecular marker analyses in Corsica and Sardinia accessions and other geographic centers in eastern places.

RAPD polymorphism in oleaster displayed a gradient between east and west of Mediterranean Basin. This gradient was less visible in cultivars due to diffusion and selection (Besnard and Berville 2000).

The analysis of 290 polymorphic AFLP bands allowed the detection of two major clusters of similarity: one cluster consisted mainly from cultivars, wild olives, and Northwest African species, while the other included species from East Africa and Asia. The first major group was further subdivided into (a) cultivars originating mostly from Italy, (b) cultivars dispersed in different Mediterranean countries, (c) wild olives, and (d) *Olea* species from Morocco (Angiolillo et al. 1999). The authors argued that *Olea* accessions of the same species but from different places of East Africa and Asia might be assigned to different species.

RFLP of chloroplast DNA was first applied to differentiate 70 olive cultivars, about 90 old trees, and 101 oleasters. The analysis showed five distinct chlorotypes. The type I was predominant in both cultivated olive trees and oleasters, and types II, III, and IV were observed exclusively in oleasters and were restricted mainly to isolated forest populations (Amane et al. 1999). Chlorotype V was more widely distributed in both cultivated and wild olives even on distant populations. This chlorotype was correlated exclusively to male-sterile trees, suggesting that it may be related to the high yields of fruits usually observed on male-sterile trees (Amane et al. 1999).

Fifty-six olive genotypes from the Malaga province in Spain using RAPD analysis were distinguished into 22 varieties and clustered into three main groups. Group I contains both wild type and introduced varieties, group II consists of mostly native olive trees, and group III is a heterogeneous cluster including varieties originating from Andalusia (Claros et al. 2000).

Bronzini de Caraffa et al. (2002a) showed that most of oleasters in Corsica and Sardinia carried either the MOM or MCK mitotype, characteristic of olives in the western Mediterranean, whereas most of the varieties carried the ME1 mitotype, characteristic of olives in the East Mediterranean.

The true oleasters are characterized by a western mitotype and a western RAPD pattern. Feral forms originate either from varieties or from hybridization between a variety and an oleaster. Consequently, as expected, some of them aggregated with the varieties from which they were derived. The other feral forms are clustered with the oleasters and were detected only by their mitotype determination.

These results showed that the majority of the varieties displayed an eastern mitotype. This is in agreement with the history of olive domestication, which suggests an east to a west diffusion of varieties. The mtDNA RFLPs provide, thus, a sensitive and reliable method to assess intraspecific cytoplasmic diversity within the economically important olive tree cultivars in Italy and provide new insights into the maternal lineages involved in the evolution of the Euro-Mediterranean olive trees.

5.3.2.3 Molecular Markers for Differentiating Within Cultivated Forms

The pioneering work on distinction of olive cultivars using RAPD molecular markers was published by Fabbri et al. (1995). In that work, olive cultivars were clustered into two main groups, one consisting of small-fruited cultivars grown mainly for oil production and the other including large-fruited cultivars.

Using RAPD analyses, Hatzopoulos et al. (2002) showed that most of the Greek cultivars were clustered into two main groups and a third minor group. The first major group included mainly table olive cultivars while the other mostly of olive oil cultivars. The third minor group consisted mainly of the wild type olive cultivars. Using the same methodology to discriminate olive oil genotypes from Cyprus, it was possible to demonstrate that these genotypes could be grouped

according to their fruit size into large or small fruit clusters (Hatzopoulos et al. 2002).

RAPD methodology could provide an acceptable resolving power for many cultivar identification projects (Belaj et al. 2001).

Bronzini de Caraffa et al. (2002a) were able to differentiate, using a combination of mitochondrial and nuclear RAPD markers, two populations of cultivated olives in Corsica: one with close relationships with the Italian varieties (influenced by the east) and one selected from local oleasters probably due to a better local adaptation than foreign varieties. All of the varieties from mainland Italy and Sicily displayed the ME1 eastern mitotype. The ME2 mitotype was revealed only in three Sicilian varieties.

Substantial polymorphism has been detected at SSR loci developed from a genomic library of *O. europaea* L. (Sabino Gil et al. 2006). Twelve out of 19 loci revealed to be polymorphic, each showing from 2 to 14 alleles. Those loci were informative for characterizing genetic differentiation among 33 cultivars from the Cordoba Germplasm Bank (9 Italian, 4 Portuguese, 2 Tunisian, 9 Spanish, 3 Greek, 3 French, 1 Moroccan, 2 Turkish, and 1 Algerian).

Structural details of genetic diversity within cultivated olive gene pool has been revealed from analysis of 119 polymorphic AFLP fragments in olive germplasm, including 65 economically important accessions cultivated in the eastern Mediterranean Basin (Owen et al. 2005). A factorial correspondence analysis (FCA) plot indicated that the cultivars clustered into two relatively modestly defined groups. The first broad group was not only dominated by cultivars from Turkey but also included genotypes originating from the Middle East (Syria and Lebanon) that collectively formed a tight subcluster. The second group comprised the Greek cultivars and those originating from the western Mediterranean. A significant genetic distance value between the Greek and Turkish cultivars was provided by an AMOVA.

There was also evidence of substructure here, with an apparent separation of most Spanish and Italian clones.

These findings are, in general, in accordance to previous suggestions of an east–west divergence of olive cultivars, although the dichotomy is less extensive than reported previously and complicated by regional variation within each group.

Assessment of genetic variability of olive varieties by microsatellite and AFLP markers has been performed by Bandelj et al. (2004). The results of clustering analysis with both molecular systems showed the common genetic background of the Tuscan varieties (Frantoio, Leccino, Leccione, Maurino, and Pendolino) and genetic divergence within the Slovene olive germplasm. The Slovenian varieties, "Buga," "Štorta," and "Samo," might represent regionally selected olives, while "Zelenjak" and "Črnica" are probably derived from the Central Italian region. The predominant local "Istrska belica" was introduced to Slovenia independently from the other regional varieties and showed the lowest genetic similarity with the other regional varieties. Recently, applying the AFLP technology on the most relevant and old varieties cultivated in Abruzzo Region (Central Italy), Albertini et al. (2010) have clearly distinguished eight cultivars within seven clusters. The obtained data suggest that both sexual and clonal propagation have played an important role in the evolution of olive cultivars, and the authors hypothesized that some ancestral population spread in Central Italy with a relevant role of seed propagation followed by a selection of superior clones from which more traditional varieties originated.

The mtDNA, generally maternally inherited in dicotyledonous species, has been studied in olive in order to determine the geographical origin of the cultivated accessions. Four mitotypes have been distinguished in the Mediterranean olives: ME1 (the mitotype from the eastern Mediterranean, no. 1) and ME2 (the mitotype from the eastern Mediterranean, no. 2) characteristic of olives in the eastern Mediterranean (Egypt, Greece, Near East and Turkey), and MOM (the mitotype from the western Mediterranean) and MCK (the mitotype characteristic of the cultivar Chemlal de Kabylie from the western Mediterranean) found in olives from the west (France, Italy, Maghreb, Spain, Yugoslavia). However, an eastern influence on some western regions, namely Italy and Libya, as well as France and Maghreb, has been shown for cultivated olives (Besnard and Berville 2000).

The mtDNA RFLPs provide, thus, a sensitive and reliable method to assess intraspecific cytoplasmic diversity within the economically important olive tree cultivars in Italy. Our results also provide new insights into the maternal lineages involved in the evolution of the Euro-Mediterranean olive trees.

Analysis of the Italian *O. europaea* L. population by mtDNA RFLPs allowed the identification of three diagnostic probe cyclooxygenase (*cox3*)/enzyme (*Eco*RI and *Hind*III) combinations that can be used as markers of cytoplasmic diversity to detect additional significant mtDNA RFLPs in the Euro-Mediterranean *O. europaea* L. complex (Cavallotti et al. 2003). The mtDNA RFLPs allowed the assignment of the analyzed 37 olive tree mitochondrial genomes to three distinct mitotypes: MIT1, MIT2, and MIT3. MIT1 mitotype is the most frequently represented and comprises 33 elite cultivars currently grown for oil, edible fruits, or both purposes all over the Italian continental territory and islands without any geographical dependence. Most of these elite cultivars are reported as self-sterile and some like the French Lucques are totally male-sterile (Besnard et al. 2000). The MIT2 mitotype comprises two Italian olive trees cultivated for oil (Canino and Vallanella) and the wild *O. sylvestris*. The MIT3 mitotype consists of just the cultivar Cerasòla from West Sicily showing the greatest diversity detectable with a single probe (*cox3*) and two different restriction enzymes (*Eco*RI and *Hind*III). It has been hypothesized that a duplication event of the *cox3* locus occurred in the progenitor cytoplasm, leading to the cytoplasm observed in the cultivar Cerasòla (MIT3).

In 1952, Baldini and Guccione reported the cultivar Cerasòla as a male-sterile cultivar for its inability to develop pollen on the basis of microscopic observations, mtDNA RFLPs, sequencing data, and expression behavior of the *cox3* locus in the olive cultivar Cerasòla that suggest a possible correlation between the presence of duplicated sequences from the *cox3* locus within the mitochondrial genome of this cultivar with the male-sterile phenotype. Besnard et al. (2000) mention two major mtDNA RFLPs detected with the *cox3*/*Hind*III combination: one with only one hybridizing fragment and the other one with two fragments pattern detected in the French cultivar Olivière. Interestingly, this polymorphic *Hind*III fragment is associated with the so-called MCK mitotype, which is found strictly related to a male-sterile phenotype. Although no size of these *Hind*III fragments is given, it is presumed that the *Hind*III pattern observed in cultivar Olivière is the same as that observed with the *cox3* probe in the cultivar Cerasola.

The mtDNA RFLPs seems to provide a sensitive and reliable system in probing genetic diversity and identifying cytoplasms possibly associated with a CMS phenotype. The mtDNA RFLP markers can be also helpful for the management of the genetic and phenotypic resources in conservation and breeding programs.

The redundancy level of tandem repeated DNA sequences can be used to differentiate and identify cultivars. Three tandem repeated DNA sequences have been described in *O. europaea* ssp. *sativa* (the cultivated olive tree) until now: the pOSE218 family isolated from the cultivar "Koroneiki" (Katsiotis et al. 1998) also present in other species of the genus *Olea;* the new family described here, present in the cultivars "Picual," "Koroneiki," "Hojiblanca," "Manzanilla," "Arbequina," "Frantoio," and "Mastoides"; and the 81 bp family (Katsiotis et al. 1998) with significant similarity with OetTaq80 repeats (Bitonti et al. 1999) and with the second family of repetitive DNA described here (Lorite et al. 2001). This last repetitive DNA is present in several cultivars and other species of the genus *Olea,* although with very different frequencies even between the olive cultivars studied (Bitonti et al. 1999). The authors suggest that the redundancy levels of the given repeated DNA sequences might provide suitable parameters for varietal identification within cultivated olives.

5.3.2.4 Molecular Markers for Differentiating Cultivated Forms from Diverse Geographic Origin

RAPD markers have been used for cultivar identification and identity typing of olive trees (Wiesman et al. 1998; Barranco et al. 2000; Bandelj et al. ~~2001~~).

In some studies, no apparent clustering of olive cultivars according to their geographic origin was evident (Fabbri et al. 1995). Using the same methodology, other workers could discriminate nine olive genotypes from Central Italy (Cresti et al. 1996), or 11 genotypes from Italy that can be hardly distinguished on the basis of morphological traits and are thus easily mistaken for each other (Vergari et al. 1996).

Cultivars from restricted areas were grouped according to geographical origin within Valencia, and there was no apparent clustering according to fruit size or other morphological characters using RAPD primers (Sanz-Cortez et al. 2001).

Nuclear ribosomal internal transcribed spacer 1 (ITS-1) sequences and ISSR and RAPD analyses were conducted to describe the colonization history of *O. europaea* in Macaronesia, showing a strong support for two independent dispersal events following an east to west direction (Hess et al. 2000). ISSR polymorphisms were also used in determining the wild status of isolated populations of this species in the Euro-Siberian north of the Iberian Peninsula (Vargas and Kadereit 2001).

One hundred and two RAPD profiles from around the Mediterranean Basin were correlated with the use of the olive fruits and the country or region of origin, suggesting a selection scheme from different genetic pools in different areas (Besnard et al. 2001a).

Fifty-one cultivars could be distinguished using 46 random primers into three major groups (1) Cultivars from eastern or northern Spain, (2) Turkish, Syrian, and Tunisian cultivars, and (3) the common olive cultivars in Spain (Belaj et al. 2001).

Besnard and Berville (2000), using mitochondrial DNA, were able to distinguish four mitotypes, ME1, ME2, MOM, and MCK. From these, only ME2 was unique to some cultivars while the predominant mitotype ME1 marks the Near Eastern origin of olive. The authors also argue that these different mitotypes mark independent cultivated olive origins.

The spread of olive from a few centers of origin and further multilocal selection (Besnard et al. 2001a; Terral et al. 2004), together with the continuous interchange of plant material between the different regions, have contributed to the confused pattern of geographic distribution. With SSR markers, it was possible to discriminate 96% of the pairwise comparisons among 118 cultivars from Italy (61 samples), Spain (22 samples), Greece (ten samples), Turkey (six samples), France (five samples), and two samples each from Croatia, Syria, Egypt, Israel, Algeria, Tunisia, and Morocco (Sarri et al. 2006). A combination of banding patterns for three SSR loci practically allowed distinction of all the entries, and a selection of six loci allowed correct reassignment of 75.4% of the entries to their country of origin.

Hannachi et al. (2008) compared cultivars and oleasters from North Tunisia to determine their relationships based on morphological traits, oil composition, and SSR genotyping at seven loci. Gas chromatography was used to determine fatty acid composition of 30 cultivar trees and 13 oleaster trees. Based on morphology, oleaster trees from agroecosystems clustered broadly in an intermediate position between cultivars and oleasters from natural ecosystems. SSR revealed that the feral and genuine oleasters plus cultivars are always overlapping. Oil composition was similar between cultivar and oleaster trees. Oil composition as fruit descriptors and drupe size appeared inefficient to discriminate between olive and oleaster trees, in comparison to SSR.

5.4 Use of the Wild Gene-Pool for Olive Improvement Through Traditional and Advanced Tools

The domestication process that led to the current varieties can be traced to a small number of desirable genotypes for fruit size identified in oleaster population by our ancestors few millennia ago. It is, therefore, expected that the available cultivated gene pool underrepresent the alleles at functional loci of the original wild population used for selection. Therefore, the necessary alleles for further improving the mentioned traits might not be present in the genome of the cultivated forms. In this case, efforts should be devoted to (a) identification of the desired alleles in the wild forms using the genomic tools described in Sect. 5.5 and (b) developing efficient and rapid gene transfer strategies of the selected alleles from the wild form to the defective cultivars. For this second strategy, sexual gene transfer is well known to be efficient but not rapid unless the offsprings have suitable genetical response to the management practices that reduce juvenility period.

Breeding in olive is difficult to carry out not only due to the very long juvenile period (>10 years) but also to the different flower fertility expressed in the breeding populations, which range from full male-sterility (Tombesi 1978; Fontanazza 1993; Bartolini and Guerriero 1995) to partial self-fertility, up to self-fertility (Fontanazza et al. 1990). Therefore, while no emasculation is necessary before hybridization when the female plant is male-sterile, the labor-intensive and costly emasculation process is required for the tiny olive floret, which expresses also a low percentage (1–2%) of fruit-setting.

Genes controlling the length of juvenility period by anticipating the flower initiation phase have been

identified in *Arabidopsis* (Yanotsky 1995; Simpson et al. 1999), and two of them, *LEAFY* (*LFY*) and *APETALA1* (*AP1*), have been used to transform *Citrus* (Pena et al. 2001) with the resulting effect of flower induction in 1-year-old plantlets. However, similar experiments have not been performed in olive and other fruit tree species, and in those cases, the juvenile period can be shortened by attempting methods such as plant training by continuous illumination and the choice of right cultivars as pollinators. It has been observed that the length of juvenility is genotype-dependent. In fact, the progenies obtained from cvs. Leccino (used as mother plant) compared to the progenies of cvs. Coratina, Picholine, and Tanche showed a reduced juvenility period (Bellini 1990; Fontanazza and Bartolozzi 1998). A similar effect was observed in the offspring from cv. Verdale.

Up to now, only a few cultivars have been obtained by dedicated breeding programs, producing interesting plants in F_2 generation. Two cultivars, one for table and one for oil extraction, the latter being resistant also to peacock spot, have been selected. It seems that some characters as vigor, leaf size, and fruit shape are dominant in F_1 progenies (Rugini and Lavee 1992; Bellini et al. 1995). Other characters such as carpological traits of fruit, blossoming intensity, fruit set, period of fruit ripening, and yield have been considered (Bellini 1993; Parlati et al. 1994; Bellini et al. 1995). Other interesting genotypes have been selected by Prof. G. Fontanazza and coworkers such as *O. europaea* cultivars FS17, Don Carlo, and Giulia. Those cultivars exhibit medium plant vigor, erect branch structure, grayish green fruit, and superior olive oil. The cultivars FS17 (Patent IRO-CNR 1165/nv) and Don Carlo (US Patent PP13077) demonstrated satisfactory oil composition too (Cipriani et al. 2006).

Nowadays, there is an increasing demand in terms of quantitative and qualitative olive production, and the olive crop improvement can have a key role in guiding production agriculture toward sustainability. Modern objective for olive breeding point to a new plant model in which reduced size, reduced apical dominance, and constant and high productivity in terms of fruit and oil are the main features. In addition, it is desired that cultivar express multiple innovative traits such as self-fertility, fruit with a suitable composition of fatty acids, high content of polyphenol and compounds with therapeutic property, a correct pattern of degradation of phenolic and pectic compounds during fruit ripening as well as tolerance to cold, salinity, drought, diseases, and pests, low plant vigor, high fruit-set capacity, and canopy architecture should be attempted. Actually, although there are more than 1,000 cultivars under cultivation originated mostly from selections made by growers over many centuries, none of them possesses all the desirable traits that could be introgressed into cultivars by conventional breeding or by gene transfer.

For these reasons, to reach the mentioned goals, (see Point b before), it will be necessary to develop suitable procedures to find proper breeding materials with the promising alleles and for their transfer. This will require research-intensive analysis of the olive genetic resources to study inheritance of traits and identifying the target breeding materials.

On the other end, conventional and biotechnology procedure based on in vitro culture applied to the wild gene pool could help breeding programs. These methods will be applied at different gene pool levels. They will be useful in inducing new genetic variation by mutation breeding, in transferring genes from the wild to cultivated primary gene pool by hybridization and clonal selection, to induce somaclonal variation, to change the ploidy level, and to obtain homozygous plants (dihaploids) for producing mapping populations and inbreds for hybrid programs.

The embryo rescue will be the biotechnological tool to be chosen when the gene transfer implies a wild species of the secondary gene pool of the cultivated olive.

Protoplast technology and genetic transformation will help transferring genes from more distant wild genera to olive cultivars. In fact, the role of wild gene pool in fruit breeding is promising, particularly when wild plants are used as rootstocks, with the aim to reduce growth of the scion and improve resistance to biotic and abiotic stresses of the root system. In this case, gene transfer by *Agrobacterium* to the wild *Olea* spp. will be of great importance.

Studies in the above directions have been carried out so far in cultivated olive, and the methods and objectives are reviewed briefly for the strong implication on the use of genetic resources of the wild *Olea* gene pool.

5.4.1 Mutation Breeding

Stable mutation rarely occurs in olive, while chimeric plants of cv. Frantoio (Roselli and Donini 1982) and Canino (Parlati et al. 1994) have been observed. On the contrary, dwarf plants from artificially irradiated rooted cuttings of cv. Ascolana Tenera have been obtained by Roselli and Donini (1982) and they are used as ornamental. Other compact mixoploid mutants have been obtained following irradiation of "Frantoio" and "Leccino" plantlets, resulting in the selection of dwarf "Leccino," which is self-sterile and late-blooming and seems to be a promising rootstock (Pannelli et al. 1990). The mixoploid mutants allowed us to select triploid and tetraploids plants (Rugini et al. 1996).

5.4.2 Hybridization and Clonal Selection

The selection of new clones has not been completely explored, so it is important to search new "accessions" to solve the present lack of suitable cultivars (Morettini 1972; Berenguer 1978; Fontanazza 1987). More recently, clonal rootstocks have been selected with high rooting ability and the ability to control scion vigor (Baldoni and Fontanazza 1990) and many accessions, particularly for small tree size are under observation (Pannelli et al. 1993). The criteria for the selection of rootstocks include good rooting potential, control of scion vigor, resistance to drought, saline, and heavy soils, resistance to root diseases, and graft-compatibility with the scion. Very few studies have addressed the selection of clonal rootstocks. Preliminary work was concerned with the influence of rootstocks on scion performance. Some rootstocks are able to control scion vigor and confer cold tolerance (Pannelli et al. 2002).

5.4.3 Somaclonal Variation

In order to increase the spontaneous somaclonal variation, it should be advisable to create a selective pressure conditions with a selective agent (biotic or abiotic origin, such as fungal filtrate, purified toxin of some pathogens, high osmotic pressure, etc.) or additional mutation with ionizing radiation. Reports on somaclonal variation in olive have not been reported yet following plant production through the three methods of plant production. Some phenotypic variations of in vitro grown shoots or whole plants have been observed, but they were only of temporary effect of juvenility induced by the in vitro culture. Today, this technique can be exploited by using the potent cyclic somatic embryogenesis of some cultivars as Canino, since other efficient methods of regeneration from mature tissues of valuable cultivar has not reported yet.

5.4.4 Changes in Ploidy Level

Tetraploid and triploid plants of the cultivar Leccino and Frantoio have been produced (Rugini et al. 1996). First, mixoploid shoots were obtained by in vitro axillary bud stimulation of mixoploid buds collected from adult mixoploid plants raised from rooted cuttings previously treated with γ-rays. Then, the group of in vitro mixoploid shoots spontaneously split into two cytotypes: $2n$ and $4n$. Tetraploid shoots were recognizable by a wider, longer, and thicker leaves than diploid ones. Subsequently, the $2n$ and $4n$ shoots have been rooted and transferred to the field. In field trial, $4n$ plants compared to $2n$ plants, showed smaller plant size, more flexible branches, absence of a juvenile phase, and larger leaves and fruits, while the intensity of blossom resulted scarce in some years. Both cytotypes, when tested as rootstocks, reduce the growth of the scions. The original mixoploid plants produced both large and small fruits. Triploid seedlings were obtained from seeds of the largest fruits.

5.4.5 Haploids and Homozygotic Plants

Production of homozygous olive plants by self-fertilization seems very improbable considering the self-sterility characteristic and the long juvenile phase of the species. Homozygous plants would be of great interest for isolation of mutants and recessive traits. Anthers and ovary or pollen and ovule cultures should be explored with the aim to produce dihaploid plants

by doubling the chromosome number. Much effort has been taken in the past to recover haploids of olive but not with clear results (Mulè et al. 1992; Perri et al. 1994b). Recently, promising results were obtained employing a new method of isolated microspore culture, which led to cell division and pro-embryos formation in the cultivar Arbequina (Bueno et al. 2006).

5.4.6 Embryo Rescue

Recently, the embryo rescue technique has been applied with successful results in hybrid plants between a cultivar Mary and an Iranian local variety (Hossein Ava and Hajnajari 2006). In Spain, several crossing among the main Spanish cultivars, by using different methodologies for the reduction of the juvenile period of seedlings, revealed interesting characteristics of the offspring regarding oil content and yield efficiency (Leòn et al. 2008). In Italy, field evaluation of selections derived from crossing among several local varieties showed a different vegetative and productive behavior of the genotypes (Bartolini et al. 2006; Pannelli et al. 2006; Ripa et al. 2006).

5.4.7 Protoplast Technology

Protoplast techniques are useful for several studies including fusion of them in an attempt to produce hybrids from cross-incompatible varieties and species. Furthermore, protoplasts allow genetic modifications by introducing exogenous DNA by liposome or cellular organelles. Viable olive protoplasts from hypocotyls, cotyledons, and leaves of micropropagated shoots were isolated and cultured, and in some cases, microcalli have been achieved (Rugini 1986; Cañas et al. 1987; Mencuccini 1991; Perri et al. 1994a). Since regeneration is not possible yet in olive from derived protoplast callus, this technology cannot be applied successfully at the moment. In addition, one should not expect good results, since somaclonal variation and chromosome rearrangements heavily change the expected results substantially, but anyway asymmetric fusion should be attempted.

5.4.8 Genetic Transformation for Higher Yield, Biotic and Abiotic Stresses Tolerance, and Quality Attributes

Very few reports have been presented on biotechnological approaches for using genetic resources of *Olea* taxa. Genetic studies in olive have been targeted to change some traits including self-fertility, oil content and composition, parthenocarpy, abiotic stress tolerance, plant habit, fruit ripening, regular cropping, and resistance to pathogens and pests. However, the low efficiency of both conventional and modern techniques for genetic improvement suggests that genetic transformation by alien gene transfer could be a promising technique to speed up the development of new cultivars. Several genes of different origins that govern these characters have been identified; however, development of efficient regeneration methods from mature tissue of elite selections remains the limiting factor for using this technology. In olive, two procedures have been used to accomplish gene transfer into plant cells: *Agrobacterium*-mediated gene transfer (Rugini et al. 2000) and bombardment of particles coated with DNA (Lambardi et al. 1999).

Most of the reports on transformation and isolation of transgenic plants of olive have involved *Agrobacterium tumefaciens* or *A. rhizogenes*. These bacteria transfer a segment of the T-DNA known as tumor-inducing plasmid Ti and root-inducing plasmid Ri into the nuclear genome of the plants (Chilton et al. 1982). The efficiency of transformation depends on several factors such as bacterial strains, plant cell competence, transgenic cell selection, and plant regeneration methods. In olive, the best strain of *A. rhizogenes* is "1855" (Rugini Eddo personal communication).

Large availability of genes is essential to start a good program of genetic improvement by this technology, both from *Olea* taxa and from other species or genera.

The transformation experiments enlisted in the following paragraphs have been carried out by using somatic embryos of cultivars because an efficient protocol for regeneration via cyclic somatic embryogenesis is available. Both an efficient in vitro regeneration method and the availability of suitable genes are essential factors.

5.4.8.1 Increasing Rooting Ability

In vitro grown shoots can be rooted by inoculating in the middle or in the basal part of the stem, with a scalpel infected with *A. rhizogenes* (Rugini 1986, 1992); the roots emerging from both the inoculation points on the shoots rarely resulted in transformed roots (Rugini and Fedeli 1990; Rugini 1992; Rugini and Mariotti 1992). Putrescine, added to the hormone-free medium, in combination with the agrobacteria, promoted an earlier root differentiation and an increase of the rooted explant frequency. Although we have no data yet on the growth habit in the field of the rooted plants with agrobacteria, with transformed root system, we expect, on the basis of observations done on plum trees rooted with the same agrobacterium treatment, a drastic reduction of plant size. Plant size reduction has been observed also in cherry plants when grafted on transgenic for *ri*-T-DNA rootstock (Biasi et al. 2003).

5.4.8.2 Modifications of Vegetative Habits and Plant Architecture

Dwarf and semi-dwarf new varieties are required in the high-density olive groves. Overexpression of *rol* (root loci) genes in plants has demonstrated a drastic effect on the plant architecture (Rugini et al. 1991; van der Salm et al. 1997, 1998; Pérez and Ochoa 1998; Gutiérrez-Pesce et al. 1998; Mercuri et al. 2001; Zhu et al. 2001), although reproduction system in some woody plants is modified (Edo Rugini personal communication), and scarce is the knowledge on the interaction of these genes with the genome of the plants. Moreover, the development of plants with an extensive root system and/or with reduced water consumption as demonstrated in kiwi plants transgenic for *rolABC* genes (Gutiérrez-Pesce and Rugini 2008) could be an advantage in areas with scarce water availability. The *rolABC* genes of *A. rhizogenes* and the marker gene *npt*II were transferred to somatic embryos of the cv. Canino by using the *A. tumefaciens* strain LBA4404. Transgenic somatic embryos selected in liquid medium under light conditions converted into plantlets, which were transferred to the experimental field (Rugini and Caricato 1995; Rugini et al. 1999). Those transgenic plants still show by RT-PCR analysis the stable integration of the *rol* genes and their correct transcription. In addition, those plants showed a different expression of each gene in the organs studied (stem, leaf, root), although they are under the same promoter. The genes influenced plant architecture: leaves had a wider angle of insertion on the stem and they showed changes in leaf blade shape with a reduction of internode length. In some cases, (in transgenic clone-5) showed an AU20 increase of chlorophyll content in the leaves. The transgenic clones showed different plant development and growth patterns as revealed by the significant increase of the number of internodes, wider angle of insertion of the leaves with a reduced area and less apical dominance as demonstrated by the outgrowth of lateral shoots giving them their bushy structure. An overexpression of glycosyl-transferase elongation factor, *psb*B, *psb*C, and *psb*D genes in the transgenic clones compared to control plants were also observed by molecular analysis. Furthermore, molecular analysis showed that not all the transcript of the *rol* genes is polyTail. The levels of polyTail transcript of all *rol* genes are different in the diverse tissue of plantlets. The less amount of transcript, independently of the *rol* gene, has been detected in the leaf tissue. This behavior is not related with the culture conditions because the same trend has been found both in vitro and in vivo. Total amount of transcript did not differ among the tissue, as seen by *rolA* transcript, but it is different in *rol*B and *rol*C (Miano et al. 2004). Untransformed cultivars have been grafted on transgenic olive rootstocks, and the preliminary results seem to modify the architecture of scion.

The modification of plant receptors in order to change the light perception is another possibility for modifying the growth and reproductive behavior. *Phytochrome* genes (*phy*A, and/or *phy*B, sense or antisense), which, together with other photoreceptors, control plant development such as circadian rhythms, apical dominance, blossom, growth and fruit ripening, photosynthesis product distribution, development of photosynthetic systems, transpiration control and hormone synthesis, may contribute to develop plants with high agronomic value and suitable for very high density planting (Tucker 1976; Vince-Prue and Canham 1983; Baraldi et al. 1992; Muleo and Morini 2008; Muleo et al. 2009).

5.4.8.3 Increasing Resistance to Biotic Stress

The challenge of using biotechnology in this area is to generate broad resistance mechanisms that have been difficult to achieve with classical breeding approaches. The use of antifungal genes or a pool of genes may be effective for improving resistance to fungal diseases, particularly *Verticillum* wilt and *Spilocea oleagina*.

Recently, studies of a plant defense PR-5 protein called osmotin, which belongs to a large, diverse family of proteins that defend plants from fungal pathogens, revealed its expression only under stress conditions, including pathogenic attacks (Zhu et al. 1993, 1995; Grillo et al. 1995).

Osmotin gene as well as *stilbene synthase* gene (Hain et al. 1993), hydrolytic enzymes such as *chitinase* and *glucanase* (Broglie et al. 1991; Yoshikawa et al. 1993), *glucose oxidase* gene (Wu et al. 1995), and polygalacturonase that inhibit the activity of endopolygalacturonases released by fungi on invasion of the plant cell wall could improve the olive defense system.

The tobacco *osmotin* gene has been extensively employed for plant transformation in some species including grape (Salzman et al. 1998) and potato (Liu et al. 1994; Zhu et al. 1995). Four days of exposure to *Phythophthora infestans* induces the accumulation of both mRNA and its protein (Zhu et al. 1995), while a longer exposure seems to be necessary to induce the response to low temperature or osmotic stress (NaCl) as elicitors. Moreover, in transgenic potato and tobacco plants osmotin, overexpression is able to delay the development of *Phythophthora* disease symptoms (Liu et al. 1994). However, its mRNA induction did not always lead to protein accumulation (Grillo et al. 1995), suggesting transcription and post-translation controls.

In olive, transgenic plants for the *osmotin* gene under CaMV35S promoter have been recovered from somatic embryos derived from petioles of cultivar Canino (Rugini et al. 1999).

The in situ localization of *osmotin* was performed by using a polyclonal primary antibody against osmotin jointed with a secondary antibody with alkaline phosphatase (AP) activity. The AP reaction in presence of NBT/BCIP substrate caused a purple color into the plant tissues. In transections of 1-year-old stems, coming from the apical internodes of field-grown plants, osmotin labeling was observed in epidermal and subepidermal tissues and, rarely, also in the most superficial layers of the cortical parenchyma. At cellular level, the protein was mainly localized around the vacuole. The signal was also present in the phloem and, mainly, in the cambium, but to a lesser extent than in the superficial tissues. In some cases, immature deuteroxylem cells resulted were also labeled (D'Angeli et al. 2001).

Inoculation tests for the presence of *Spilocea oleagina* spots showed that one transgenic genotype seems to possess higher susceptibility than control plants whereas the other ones show a lower susceptibility. Both *osmotin*-transgenic self-rooted plants and grafted onto non-transgenic rootstock are under evaluation in field trials for their level of resistance to *Spilocea oleagina* (Rugini et al. 2000).

In unstressed plants grown in the field, osmotin labeling was observed in the epidermal and subepidermal tissues and, rarely, also in the most superficial layers of the cortical parenchyma of the apical third internode of 1-year-old twigs. At cellular level, the protein was mainly localized around the vacuole. The signal was also present in the phloem and, mainly, in the cambium but at lower levels than in the superficial tissues. In some cases, immature deuteroxylem cells were also labeled. No signal was ever observed in the unstressed leaves. Our results confirm the organ specificity of the protein accumulation controlled by a post-translation pathway able to negatively interfere with the gene constitutive expression of 35S promoter (Gutiérrez-Pesce and Rugini 2008).

Regarding bacterial diseases, it is not so easy to introduce this kind of resistance into plants because the product of the gene should act in the intercellular space; however, some species with enhanced resistance to bacteria have been obtained by introducing genes encoding bactericidal polypeptide, i.e., thionin (Carmona et al. 1993), attacin E (Norelli et al. 1994), the synthetic analog MB39 of cecropine (Mills et al. 1994), and cecropine (Huang et al. 1997). Particular attention should be paid to human lysozyme, which confers resistance to both fungi and to bacterium *P. syringae* in tobacco plants (Nakaijma et al. 1997). AU21

The isolation of homologous genes from olive cultivars with low susceptibility to the fruit-fly (*Bactrocera oleae*) is a potential way to achieve resistance to this pest in susceptible cultivars. Although, in the battle against insect attack, the *Bt* gene from *Bacillus thuringensis* (Vaeck et al. 1987) has

successfully been introduced in other species with encouraging results and could be an alternative way to prevent olive fly damage. In addition, gene or gene isolation from more tolerant genotype, such as "Bianca di Tirana" should be attempted.

Against olive fly (*Bactrocera oleae*), a group of researchers have developed a Minos-based transposon vector carrying a self-activating cassette, which overexpresses the enhanced green fluorescent protein (EGFP). The self-activating gene construct combined with transposase mRNA present a system with potential for transgenesis in very diverse species (Koukidou et al. 2006).

In the following paragraphs are summarized the main objectives and studies on genes useful for improving oil quality in *Olea* taxa by applying genetic transformation.

5.4.8.4 Fruit Ripening and Oil Quality

Pattern of fruit ripening and the increase of oil content and quality are of much interest in an attempt to delay fruit ripening and to reduce early fruit drop. This pattern may be corrected by generating transgenic plants with a reduced ethylene biosynthesis, by using an antisense gene (Oeller et al. 1991) or for reducing polygalacturonase activity (Smith et al. 1988).

With regard to the oil quality, two molecular strategies can be used to modify the oil composition and content (1) the alteration of the major fatty acid levels by suppressing or expressing a specific key enzyme in lipid biosynthesis and/or (2) by creating an unusual fatty acid. The oleic acid is the main component of olive oil and is responsible for its high dietary value (Baldoni et al. 1996). Since the stearoyl-acyl carrier protein denaturase is the key enzyme for the conversion of saturated stearic acid (C18:0) to monounsaturated oleic acid (C18:1), by antisense suppression or cosuppression of oleate denaturase, it is possible to increase oleic acid (C18:1) from 24% to 80% in the transgenic soybean (Kinney 1995, 1996a, b, 1997; Yadav 1996) and in *Brassica* seed by the antisense expression of a stearoyl-ACP denaturase gene (Knutson et al. 1992). Temporal and transient expression of the *S-ACP* denaturase gene has been studied during fruit development (Haralampidis et al. 1998), and its expression is developmentally regulated with earlier expression in embryos than in mesocarp. The same strategy was adopted to increase stearate acid (C18:0) by up to 30% both in canola and rapeseed (Auld et al. 1992; Falco et al. 1995).

Unusual fatty acids can be produced in one plant by transferring a gene encoding the specific biosynthetic enzyme. An example can be seen in canola that naturally does not produce laurate (C12:0), while a new transgenic genotype does contain laurate. The oxidative stability of the oleic acid in soybean oil can be also improved as reported by Ellis et al. (1996).

5.4.8.5 Parthenocarpy

The alternate bearing and often the lack of pollination are the main causes of low yield in olive; particularly, the second problem happens often in areas with less intensive olive cultivation. Under some environmental conditions, olive shows some tendency to natural parthenocarpy, but the fruits remain very small. Genes inducing parthenocarpy would permit the development of parthenocarpic fruits with regular size and would overcome the problem related to self- and intersterility among olive cultivars. Self-sterility is under the control of the placental-ovule-specific *defh9* gene regulator sequence, expressed during early flower development. Production of transgenic olive plants with the parthenocarpy gene of *Arabidopsis* may allow olive fruit development as already successfully experienced in eggplant (Rotino et al. 1997). However, in olive, attention should be paid because it produces hundred thousand flowers per plant, and normally fruit set is around 1%, so parthenocarpy could represent a problem, if fruits are produced from most of the flowers.

5.4.8.6 Abiotic Stress Tolerance

Olive is a species sensitive to chilling; at −7°C is subject to frost damage in leaves. For this reason, genetic improvement for cold-resistance is one of the main important objectives for olive, since few genotypes are slightly tolerant to frost. Genetic transformation with the antifreeze protein (Hightower et al. 1991) or the overexpression of the superoxide dismutase gene (McKersie et al. 1993; Van Camp et al. 1994), and the overexpression of the *Arabidopsis CBF1* gene (Jaglo-Ottosen et al. 1993), which enhances freezing

tolerance by inducing genes associated with cold acclimatization, could also be attempted in olive. Recent studies on transgenic olive plants for the osmotin gene obtained by Rugini et al. (2000) reported that osmotin is positively involved in (a) the acclimation-related programmed cell death (PCD), (b) blocking the cold-induced calcium signaling, and (c) affecting cytoskeleton in response to cold stimuli (D'Angeli and Altamura 2007). Additionally, osmotin, a pathogenesis-related (PR5) protein that exhibits cryoprotective functions, is a potential target to enhance cold tolerance. This has been proven by a study done by D'Angeli and Altamura (2007), which showed that osmotin is positively involved in the acclimation-related programmed cell death (PCD), in blocking the cold-induced calcium signaling, and in affecting cytoskeleton in response to cold stimuli (D'Angeli and Altamura 2007).

Cansev et al. (2008) found that patterns of antioxidative enzymes catalase (CAT: EC 1.11.1.6), ascorbate peroxidase (APX: EC 1.11.1.11) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase varied depending on the cold-acclimation stage and the cold-hardiness level of the cultivars. Domat and Lecquest were found to have the highest cold-hardiness, and Ascolona, Gemlik, and Hojoblanca had moderate cold-hardiness, while Samanli, Meski, Uslu, and Manzanilla were more sensitive. Furthermore, total soluble proteins were higher in the cold-acclimated stage than in the non-acclimated stage. The tissues of cvs. Domat, Lecquest, Ascolona, Hojoblanca, and Gemlik enhanced the structural stability of cellular membranes in the cold-acclimated stage by increasing both the activity of CAT, APX, and NADPH oxidase to activate the antioxidative systems and the expression of 43 kDa dehydrins.

5.4.8.7 Other Useful Genes

Potentially useful genes have already been isolated from several species and could be introduced into olive. The selection procedure should be improved by replacing traditional selection markers (*npt*II) with other markers (*gfp, lecI, ipt, pmi, xyla*), although the percentage of escapes is quite high (>40%) in fruit crops (May et al. 1995; Pena et al. 1995, 1997; Mourgues et al. 1998; Perl and Eshdat 1998). Endo et al. (2001) suggested that the *ipt* gene from *A. tumefaciens* might be a suitable selectable gene. Moreover, a multi-auto-transformation (MAT) vector, which combines the use of genes that stimulate growth and morphogenesis for positive selection of transformed cells with an excision mechanism to remove the markers and allow recovery of plants with normal phenotypes, could be very useful. The vector *ipt* and maize transposable element *Ac* for removing the *ipt* gene seem to be promising (Ebinuma et al. 1997). Other approaches might involve *xyla* (xylose isomerase) (Haldrup et al. 1998) and *pmi* (phosphomannose isomerase) genes that confer the capacity to use non-metabolizable substrates. For highly regenerative cultures, it could be possible to eliminate the marker gene and select on the basis of physiological or morphological parameters, such as the response to toxins, culture filtrates, and salt/drought resistance (Graham et al. 1996).

5.5 Development of Genomic Resources

To develop proper genomic resources, it is necessary to include all the gene pools that represent the genetic diversity of all the olive species, and hence the wild gene pools became an integral part of all the materials to be explored in order to answer questions related to the evolution, domestication, natural selection effects, and other driving forces that shaped the genetical structure of the olive population. In general, the available datasets and information on functional genomic, proteomic, and metabolomic tools for studying olive tree development, tolerance to biotic and abiotic stresses, and fruit development are still scarce. Therefore, it should be necessary to enlarge both the representativeness of the current population in the olive genomic databases and the molecular tools available for approaching the mentioned aspects. Currently, olive genome has not been sequenced yet, and functional genomics of the most important traits have been restricted to single genes. For this reason, it should be given priority to all genomic resources that, in absences to the sequenced genome, could allow innovative molecular studies for the olive species.

Different genomic approaches, therefore, have been used to increase the information concerning plant signaling, transcriptional networks, and regulatory circuits involved in important physiological and developmental processes. In particular, fruit development

was the most biological process studied with different innovative molecular technology as expressed sequence tags (ESTs), large-scale microarrays, deep transcriptome profiling, etc. Currently, only few olive genes have been identified and annotated, and as of the fifth of May 2010, just 6,504 as a total of nucleotide sequences (4,860 ESTs, 130 genes, 1,514 nucleotide sequences) have been deposited in the public genome databases (i.e., The National Center for Biotechnology Information, NCBI) as compared to 376,006 nucleotide sequences for apple (335,398 ESTs, 38,091 genes, 2,517 nucleotide sequences), 204,214 for *Prunus* (98,536 ESTs, 103,153 genes, 2,625 others), 606,348 for *Citrus* (594,172 ESTs, 252 genes, 11,924 nucleotide sequences), 547,064 for grape (395,454 ESTs, 33,948 genes, 117,662 nucleotide sequences), and 506,332 for poplar (307,985 ESTs, 45,032 gene, 153,315 nucleotide sequences).

Recently, a website named *Olea* ESTdb (http://140.164.45.140/oleaestdb/), containing over 60,000 transcripts that were isolated from olive fruit, covering all the developmental stages from the beginning to the end, has been launched (Alagna et al. 2009). The EST reads were generated through 454 pyrosequencing technology. The database is constructed by tentative consensus (TC) sequences and singletons (sESTs).

All the 130 genes were generated from complete sequencing of chloroplast genome of the cultivar Bianchera. ESTs were generated from three differential libraries, two of them from fruitlets and leaves and the third from infected leaves with the fungus *Spilocaea oleagina*. Most of 30% of the nucleotide sequences are non-coding sequences (e.g., microsatellite 190, SRAP 26, intergenic space 102) that are used as molecular markers. A large amount of chloroplast and mitochondrial genes are also deposited, together with a very high number of ribosomal RNA genes, up to 109 sequences. Most of the olive ESTs are of the allergen coding sequences (208), while the number of ESTs of all other biological plant process is reduced (164). Many genes encoding key enzymes for fatty acid biosynthesis and modification, triacylglycerol synthesis, and storage have been isolated. These are enoyl-Acyl carrier protein reductase (*ear*), stearoyl-ACP desaturase, ω6 plastidial desaturase (*fad6*), ω3 plastidial desaturase (*fad7*), cytochrome b5 (*cyt b5*), ω6 cytoplasmic desaturase (*fad2*), ω3 cytoplasmic desaturase (*fad3*), acyl CoA diacylglycerol acyltranferase (DGAT), and oleosin (Giannoulia et al. 2007). Stearoyl-ACP desaturase (Baldoni et al. 1996) is the key enzyme for the conversion of saturated stearic acid (C18:0) to mono-unsaturated oleic acid (C18:1), the main component of olive oil and responsible for its high dietary value. A cDNA has been isolated encoding a fatty acid desaturase, which is responsible for biosynthesis of a trienoic acid, linolenic acid, a major component of chloroplast membranes, and a precursor of the oxylipins i.e., methyl jasmonate. The latter are important in signal transduction pathways relating to plant development and responses to stress and have been studied in leaves, anthers, and embryos (Poghosyan et al. 1999). Two cytochrome b_5 genes and their spatial and temporal patterns of expression have been characterized during floral and fruit development (Martsinkovskaya et al. 1999). The expression of oleosin gene is strongly embryonic-stage-dependent and the transcript reaches maximum levels at mid-torpedo stage and thereafter declines, coinciding the stages of most oil accumulation in embryo tissues, while in mesocarp tissues oleosin gene is not expressed (Giannoulia et al. 2007). A triterpene synthase cDNA, cloned from olive leaves by PCR amplification using primers designed from oxidosqualene cyclases, codes for the lupeol synthase protein (Shibuya et al. 1999). The differential expression of diacylglycerol acyltransferase (DGAT) genes has been evaluated in different olive tissues (Giannoulia et al. 2000). The sequence of a partial cDNA clone (1,402 bp) of the chloroplast ribulose 1,5-bisphosphate carboxylase large subunit (*rbc*L) gene of olive has been compared with that of other genera in order to establish the systematic position of the Oleaceae family (Wallander and Albert 2000). Similarly, the chloroplast *ndhF* gene has been sequenced for phylogenetic studies (Olmstead et al. 2000).

Olive trees are persistent (up to 1,000 years) evergreens, tolerate drought and salinity (Bongi and Palliotti 1994), and are generally cultivated in areas where water is the main limiting factor in agricultural production. Recently, the molecular bases of water transport in olive have been studied, and three aquaporin (AQPs) genes have been isolated from tissues of the cv. Leccino (Secchi et al. 2007a). In plants, AQPs are classified in different groups according to their sequence identity or to their structural features (e.g., sizes or N- and C-termini). The main groups include plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic

proteins (found in the symbiosome membrane surrounding nitrogen-fixing bacteria of soybean), and small basic intrinsic proteins, a recently formed group that includes various AQPs whose subcellular localization is generally unknown (Johanson and Gustavsson 2002). The plant PIP group is divided into two groups (PIP1 and PIP2), which are characterized by specific amino acid residues at the N- and C-terminals and around the conserved NPA motifs (Schaffner 1998). A phylogenetic analysis of the corresponding polypeptides, and expression assays in *Xenopus laevis* oocytes, confirmed that they were part of water channel proteins localized in the plasma membrane and in the tonoplast. In well-hydrated plants, the highest expression level of *OeTIP1.1* was detected in twigs, while *OePIP2.1* mRNAs were very abundant in roots and moderate in twigs, and at lower levels were detectable in leaf extracts. The accumulation of *OeTIP1.1* mRNA was detected in twigs, while in roots and leaves, the expression was low. In plants subjected to drought stress, the three AQPs expression genes, in root, shoot, and leaf, were downregulated (Secchi et al. 2007b), evidencing that lower gene expression in drought shoots is associated with higher hydraulic resistance. The downregulation of AQP genes can be considered a negative effect of water stress (Smart et al. 2001), but can also play the role of protecting the plant from excessive water loss in conditions of severe water stress and intense transpiration requirements (North et al. 2004; Vandeleur et al. 2005). During recovery after drought, the *OePIP2.1* gene expression was upregulated. This biological process would imply an increase in cell conductivity and a high water availability in vessel-surrounding living cells that may enhance the xylem refilling capacity of parenchyma cells (Secchi et al. 2007b). The expression of AQP genes in olive resulted also regulated by intrinsic plant factors dependent from development of plant architecture. Dwarfing genotypes of cv. Leccino (D), inducing strongly reduced growth when used as rootstock (Rugini et al. 1996), probably due to its lower root hydraulic conductance (Nardini et al. 2006), have higher expression levels of *OePIP1.1* and *OePIP2.1* aquaporins in root, stem, and leaf than in Leccino plants carrying a vigorous (M) phenotype when used as rootstock. This expression behavior is in agreement with a higher hydraulic conductance of D plant roots and leaf plants than that of M plants, when these physiological parameters are scaled by unit root DW and unit leaf surface area (Lovisolo et al. 2007).

Aquaporin genes are members of a large family of genes coding for membrane intrinsic protein (MIP). Plant genomes include a large number of MIP genes, e.g., in *Arabidopsis thaliana* 35 different AQP transcripts are found (Johanson et al. 2001), the genome of *Zea mays* encodes 33 AQP homologs (Chaumont et al. 2001), and in *Oryza sativa*, 33-like AQP-like genes have been identified (Sakurai et al. 2005); therefore, most of the olive AQP genes should be identified.

Large-scale EST sequencing projects are in progress to obtain better insight into the molecular mechanisms of flower induction and development, fruit growth, and ripening processes in olives, and to prepare probes for the identification of several transcription factors and genes related to defense and stress response.

In the last 2 years, a high abundance of transcripts have been analyzed using two main approaches (1) suppression subtractive hybridization (SSH) for identifying differentially expressed genes (Galla et al. 2009) and (2) 454 pyrosequencing technology for high-throughput DNA sequencing, allowing gene discovery and evaluation of expression patterns in olive fruit (Alagna et al. 2009). The investigation of the expression pattern for a large set of genes is one of the most important objectives of functional genomics; accordingly, in our laboratory, several cDNA libraries underlying both the induction and development of flower in Leccino and Frantoio olive cultivar have been developed.

5.6 Therapeutic Properties and Compounds Used as Herbal Drugs

Fruits and oil from semi-domesticated olive trees were widely used in nutrition and medicine, during the Etruscan and Roman civilizations, Middle Ages, and Renaissance. Because most of wild *Olea* spp. contain small amount of extractable oil, and samples of their fruits are difficult to collect from the original growing sites (often inaccessible or remote), and scientific knowledge about chemical compounds with therapeutical properties have been acquired from the widely available fruits, leaves, and oil of *O. europaea* subsp *europaea*. Through these studies, it has been discovered

that the well-known healthy effects of olive oil, which delay aging, must be attributed to all its metabolite components and not to a single compound. They act through the reduction of the risk factor leading to coronary heart disease and several types of cancer (Colomer et al. 2007) and to the modification of immune and inflammatory responses. Among the various important chemicals, particular relevance assume oleic acid (essential for its nutritional effect and stability during cooking at high temperature) and polyphenols (with their antioxidant properties; Boskou 1996; Servili et al. 2004). Some olive oil polyphenols are rare in other plant species, such as hydrophilic phenols (Shahidi 1996), and some others, such as biophenols and secoiridoids (oleuropein) (Iwai et al. 2005), are present only in the species of the *Oleaceae* family. Oleuropein, the most abundant biophenols, protects membrane lipid oxidation for preventing cardiac disease (Mercier 1997), acts on coronary dilation through antirhythmic action (Petkov and Manolov 1978), improves lipid metabolism and obesity-related problems (Iwai et al. 2005), prevents hypertensive cell death in cancer patients (Bonoli et al. 2004), and exhibits antiviral properties (Kubo et al. 1995; Uccella 2000).

In addition, olive oil contains high content α-tocopherol (Psomiadou et al. 2000; Rotondi et al. 2004) and many other compounds; some of them are mentioned below.

Verbascoside (hydroxycinnamic acid derivative) has been tested as a repairing substance in oxidative damage caused by heroin consumption (Qiusheng et al. 2005). Hydroxytyrosol (derivative of oleuropein and verbascoside) shows better antioxidant capacity than vitamins C and E or 2,6-di-tertbutyl-4-methylphenol (BHT) (Visioli et al. 1998; Fabiani et al. 2002), providing protective effects from cardiac diseases and cancer (Luque de Castro and Japòn Lujàn 2006). Oleocanthal has shown to be a COX inhibitor (Beauchamp et al. 2005). The various health benefits exerted by the olive oil compounds are part of large benefit endowed by the Mediterranean diet (Tulp et al. 2006).

Leaves of *Olea* taxa have been used for the treatment of wounds, fever, diabetes, gout, arterioscleroses, and hypertension since ancient times (Jänicke et al. 2008), and olive leaf extract (EFLA®943) has been demonstrated to be a prophylactic for lowering blood pressure in humans (Perrinjaquet-Moccetti et al. 2008). Only recently, it has been scientifically demonstrated that leaf extracts of *Oleaceae* family, particularly *O. europaea* subsp. *europaea* and *O. lancea* (syn. O. *europaea* (subsp. *cuspidata*, syn. *O. africana*)) and *Ligustrum vulgare*, have the expected antimicrobial activity (Pereira et al. 2007) due to the presence of oleuropein, and the positive effect in reducing blood pressure due to the presence of oleacin (secoiridoid 2-(3,4-dihydroxyphenyl)ethyl 4-formyl-3-(2-oxoethyl)-4E-hexenoate), which is an ACE (angiotensin converting enzyme)-inhibitor (Perrinjaquet-Moccetti et al. 2008).

The total oil content of the fruit (rarely exceeding 25% of fresh weight), the peculiar concentration of oleic acid (C18:1 mono-unsaturated fatty acid, 40–80% of the total triacylglycerides) and linoleic acid (about 10%), and total amount of polyphenols (50–800 mg/kg) could change according to variety and environmental conditions (Cahoon and Shanklin 2000; Bruner et al. 2001; Rahman et al. 2001). All these parameters of quality in virgin olive oils make the studies of gene regulation of oil metabolism and oil accumulation of principal interest. Leòn et al. (2008) suggested that new olive cultivars with fatty acid composition fulfilling consumer and market demands could be obtained through cross-breeding because a quite different fatty acid composition in the oil of 15 advanced selections and their three progenitors was observed with the percentages of C18:1, C18:2, and saturated fatty acids being the main contributors to the total variation.

5.7 The Dark Sides of *Olea* and Ways to Address Them

The problems related with the wild olive genetic resources, as deduced from the information in the previous paragraphs, can be synthesized in the following three aspects (a) release of pollen-causing allergies, (b) colonization events leading to invasiveness, and (c) extinction of populations due to a narrow geographical distribution.

Pollen from *Olea* taxa produce respiratory allergy in the human settlings nearby where those plants are widely present, especially in the Mediterranean countries, Australia, and North and South America (Wheeler 1992; Liccardi et al. 1996), due to the content of up to 10 allergens (Ole e 1 to Ole e 10)

(Rodríguez et al. 2002; Barral et al. 2004). These proteins have been indexed like members of the denominated "pollen proteins of the Ole e 1 family" (Bateman et al. 2004). *Olea* taxa display wide differences in the expression levels of many allergens and in the number and molecular characteristics of the allergen isoforms expressed. These differences are maintained over the years and are dependent on the genotype of each taxa. Quantitative differences in the content of Ole e 1 have been described in the pollen of several *Olea* taxa (Castro et al. 2003). The presence of nucleotide substitutions at the locus encoding Ole e 1 proteins result in many cases in amino acid changes (Villalba et al. 1993). The molecular variability of Ole e 1 allergen was studied throughout a number of *Olea* taxa, and it was concluded that the varietal origin of olive pollen is a major factor determining the diversity of Ole e 1 variants. The presence of an extremely wide germplasm in the *Olea* genus (Bartolini et al. 1994) clearly points to this genetic variability as a putative cause of polymorphism for Ole e 1 sequence (Alché et al. 2007). This information is useful for the identification of the Ole e 1 genetic variants and for future genetic engineering applications for modifying pollen allergen composition in *Olea* ssp. pollen (Hamman-Khalifa et al. 2008).

The wide range of geographical sites, where the different wild olive subspecies originated (Green 2002), is frequently considered as an explanation for the invasive performance in some places where they have been introduced, such as in the Pacific islands and East and South Australia (West 2002; Starr et al. 2003; Cooke et al. 2005; Bass et al. 2006; Breton et al. 2006).

In the nineteenth century, both *O. europaea* L. subsp. *europaea* and subsp. *cuspidata* were first introduced in Sydney territory for economic purposes, and naturalized populations (i.e., self-reproducing trees not planted for domestic or commercial use) were first recorded in the Norfolk Island, Adelaide, and across a wide range of habitats, predominantly within the 400–600 mm average annual rainfall range (Spennemann and Allen 2000; Mekuria et al. 2002; Bass et al. 2006). Since the 1960s, *cuspidata* naturalized populations have been found in Hawaii Archipelago (Starr et al. 2003). In the sites where *Olea* taxa have been introduced, the olive fruit is highly attractive to avian fauna dispersal agents and have few predators. Spennemann and Allen (2000) suggest that foxes may disperse *O. europaea* seed for 40–50 km from the fruit source.

Since 1836, hundreds of *Olea* taxa have been introduced into South Australia, mainly from olive-growing regions in the Northern Hemisphere. In the 1870s, olive production in South Australia was considered to be a more viable enterprise than wine production. This led to the establishment of a 1.2 ha orchard in 1874, which was expanded to over 40.5 ha by 1882 (Reichelt and Burr 1997). By the early 1900s, however, the olive industry in South Australia was in decline. Cross-breeding from abandoned groves over 160 years has resulted in populations of feral olives that grow in all Australian regions (Spennemann and Allen 2000; Bass et al. 2006) and now feral olive trees are found in western Australia, Victoria, New South Wales, Southeast Queensland, and Tasmania (Spennemann and Allen 2000; Bass et al. 2006), and in northern New Zealand (Heenan et al. 1999). These feral populations may present ecological problems, as the olive is thought to compete successfully with native Australian vegetation, particularly in disturbed habitats. Diversity of native flora was found to be at least 50% lower in eucalypt woodland heavily invaded by *O. europaea*, when compared to similar woodland relatively free of *O. europaea* (Crossman 2002). This view has led some local councils to proclaim the olive tree as a weed and instigate eradication programs. Removal of feral forms of olive requires careful planning before doing so in order to avoid problems in fragile ecosystems (Crossman et al. 2002).

The phylogeographic approach (Amsellem et al. 2000; Milne and Abbott 2004) was used by Besnard et al. (2007c) for reconstructing a detailed distributional range of the *O. europaea* complex based on DNA polymorphism from the nuclear (bi-parentally inherited) and plastid (maternally inherited) genomes. This study provided powerful data to test hypotheses about the origins of naturalized olive populations and evidenced that subspecies *europaea* and *cuspidata* is likely to hybridize or introgress when in sympatry to other *Olea* taxa (Besnard et al. 2001a; Rubio de Casas et al. 2006). This phenomenon could on one side alleviate the loss of genetic diversity due to bottlenecks arising from small initial founder populations during colonization events (Husband and Barrett 1991; Lee 2002), but on the other side contribute to the ecological success of colonizing populations. Using the mentioned molecular tools, it was determined

that East Australian and Hawaiian populations of subsp. *cuspidata* have originated from southern Africa, while South Australian populations of subsp. *europaea* have mostly derived from western or central Mediterranean cultivars. Invasive populations of subsp. *cuspidata* showed significant loss of genetic diversity in comparison to a putative source population, and a recent bottleneck was evidenced in Hawaii. Conversely, invasive populations of subsp. *europaea* did not display significant loss of genetic diversity in comparison to a native Mediterranean population. One hybrid (*cuspidata* × *europaea*) was identified in East Australia. The importance of hybridizations in the future evolution of the olive invasiveness remains to be investigated (Besnard et al. 2007a, b, c).

A relationship between polyploidy and narrow endemic taxa is observed for *O. europaea* ssp. *maroccana* (hexaploid) and *O. europaea* ssp. *cerasiformis* (tetraploid) (Besnard et al. 2008). As stated earlier, these two taxa are endemic to subtropical areas of the Agadir Mountains and Madeira, respectively, and their populations are endangered (Besnard et al. 2008). Particularly, *O. europaea* subsp. *maroccana* is considered to be one of the ten most threatened trees in the Mediterranean Basin (Médail et al. 2001). Also, the current populations of *O. e. laperrinei* in the Aïr mountain range of Niger are endangered due to the absence of an efficient sexual reproductive strategy coupled with the high fragmentation of very small populations and a narrow altitudinal range of distribution (Anthelme et al. 2008).

To ensure adequate protection for the three *Olea* ssp. and their habitat, recommendations to facilitate appropriate mitigation in the form of revegetation and restoration for enhancing territorial stability and conservation of habitat complexity, while reducing long-term maintenance costs, should be addressed.

References

Alagna F, D'Agostino N, Torchia L, Servili M, Rao R, Pietrella M, Giuliano G, Chiusano ML, Baldoni L, Perrotta G (2009) Comparative 454 pyrosequencing of transcripts from two olive genotypes during fruit development. BMC Genomics 10:99–414

Albertini E, Torricelli R, Bitocchi E, Raggi L, Marconi G, Pollastri L, Di Minco G, Battistini A, Papa R, Veronesi F (2010) Structure of genetic diversity in *Olea europaea* L. cultivars from Central Italy. Mol Breed (in press)

Alché JD, Castro AJ, Jiménez-López JC, Morales S, Zafra A, Hamman-Khalifa AM, Rodríguez-García MI (2007) Differential characteristics of olive pollen from different cultivars: biological and clinical implications. J Invest Allerg Clin Immunol 17:69–75

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

Amiri ME (2008) The status of genetic resources of deciduous, tropical, and subtropical fruit species in Iran. Acta Hortic 769:159–167

Amsellem L, Noyer JL, Le Bourgeois T, Hossaert-Mckey M (2000) Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (*Rosaceae*) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers. Mol Ecol 9:443–455

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

Anthelme F, Abdoulkader A, Besnard G (2008) Distribution, shape and clonal growth of the rare endemic tree *Olea europaea* subsp. *laperrinei* (Oleaceae) in the Saharan mountains of Niger. Plant Ecol 198:73–87

Auld DL, Heikkinen MK, Erickson DA, Sernyk JL, Romero JE (1992) Rapeseed mutants with reduced levels of polyunsaturated fatty acids and increased levels of oleic acid. Crop Sci 32:657–662

Baali-Cherif D, Besnard G (2005) High genetic diversity and clonal growth in relict populations of *Olea europaea* subsp. *laperrinei* (Oleaceae) from Hoggar, Algeria. Ann Bot 96:823–830

Baldoni L, Fontanazza G (1990) Preliminary results on olive clonal rootstocks behaviour in the field. Acta Hortic 286:37–40

Baldoni L, Abbott AG, Georgi LL (1996) Nucleotide sequence of a cDNA clone (U58141) from *Olea europaea* encoding a stearoyl-acyl carrier protein desaturase (PGR 96-052). Plant Physiol 111:1353

Baldoni L, Angiolillo A, Pellegrini M, Mencuccini M (1999) A linkage genome map for olive as an important tool for marker-assisted selection. Acta Hortic 474:111–115

Baldoni L, Pellegrini M, Mencuccini M, Angiolillo A, Mulas M (2000) Genetic relationships among cultivated and wild olives revealed by AFLP markers. Acta Hortic 521: 275–284

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

Bao ZH, Ma YF, Liu JF, Wang KJ, Zhang PF, Ni DX, Yang WQ (1980) Induction of plantlets from the hypocotyl of *Olea europaea* L. in vitro. Acta Bot Sin 2:96–97

Baraldi R, Cristoferi G, Facini O, Lercari B (1992) The effect of light quality in *Prunus cerasus*. I. Photoreceptors involved in internode elongation and leaf expansion in juvenile plants. Photochem Photobiol 56:541–544

Barral P, Batanero E, Palomares O, Quiralte J, Villalba M, Rodríguez R (2004) A major allergen from pollen defines a novel family of plant proteins and shows intra and interspecies cross-reactivity. J Immunol 172:3644–3651

Barranco D, Trujillo I, Rallo P (2000) Are 'Oblonga' and 'Frantoio' olives the same cultivar? HortScience 35:1323–1325

Bartolini S, Guerriero R (1995) Self-compatibility in several clones of oil olive cv. Leccino. Adv Hortic Sci 9:71–74

Bartolini G, Prevost G, Messeri C (1994) Olive tree germplasm: descriptor lists of cultivated varieties in the world. Acta Hortic 365:116–118

Bartolini G, Petruccelli R, Tindall HD (2002) In: Tindal HD, Menini UG (eds) Classification, origin, diffusion and history of the olive. FAO, Rome, Italy

Bartolini S, Andreini L, Guerriero R, Gentili M (2006) Improvement of the quality of table olives in Tuscany through cross-breeding and selection: preliminary results of Leccino × Konservolia hybrids. In: Proceedings of 2nd international seminar on biotechnology and quality of olive tree products around the Mediterranean basin, Marsala, Italy, 5–10 Nov 2006, pp 143–146

Bass DA, Crossman ND, Lawrie SL, Lethbridge MR (2006) The importance of population growth, seed dispersal and habitat suitability in determining plant invasiveness. Euphytica 148:97–109

Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Griffiths-Jones S, Khanna A, Marshall M, Moxon S, Sonnhammer ELL, Studholme DJ, Yeats C, Eddy SR (2004) The Pfam protein families database. Nucleic Acids Res 32(Database Issue):D138–D141

Beauchamp GK, Keast RSJ, Morel D, Lin J, Pika J, Han Q, Lee CH, Smith AB, Breslin PAS (2005) Ibuprofen-like activity in extra-virgin olive oil. Nature 437:45–46

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

Bellini E (1990) Behaviour of some genetical characters in olive seedlings obtained by cross-breeding. In: Proceedings of 23rd international horticulture congress, Florence, Italy, Poster 3062 (Abstract)

Bellini E (1993) Genetic variability and heritability of some characters in cross-bred olive seedlings. Olivae 49:21–34

Bellini E, Pariah MY, Pandolfi S, Giordani E, Perri E, Silletti A (1995) Miglioramento genetico dell' olivo: prime osservazioni su selezioni ottenute da incrocio. Ital Hortic 2:3–12

Berenguer AG (1978) Seleccion clonal en Olivo (*Olea europaea* L.). Olea 6:7–15

Besnard G, Berville A (2000) Multiple origins for mediterranean olive (*Olea europaea* L. ssp. *europaea*) based upon mitochondrial DNA plymorphisms. Life Sci 323:173–181

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

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

Besnard G, Baradat P, Bervillé A (2001a) Genetic relationships in the olive (*Olea europaea L.*) reflect multilocal selection of cultivars. Theor Appl Genet 102:251–258

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

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

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

Besnard G, Christin PA, Baali-Cherif D, Bouguedoura N, Anthelme F (2007b) Spatial genetic structure in the Laperrine's olive (*Olea europaea* subsp. *laperrinei*), a long-living tree from the central Saharan mountains. Heredity 99: 649–657

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

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

Biasi R, Gutiérrez Pesce P, Muganu M, Magro P, Bernabei M, Rugini E (2003) Modifications of growth pattern in kiwifruit and cherry induced by the T-DNA genes of *Agrobacterium rhizogenes*. In: Proceedings of XLVII Italian society of agricultural genetics – SIGA annual congress, Verona, Italy, 24–27 Sept 2003, ISBN 88-900622-4X

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

Bongi G, Palliotti A (1994) Handbook of environmental physiology of fruit crops. CRC, New York, NY

Bonoli M, Bendini A, Cerretani L, Lercker G, Toschi TG (2004) Qualitative and semiquantitative analysis of phenolic compounds in extra virgin olive oils as a function of the ripening degree of olive fruits by different analytical techniques. J Agric Food Chem 52:7026–7032

Boskou D (1996) Olive oil chemistry and technology. AOCS, Champaign, IL

Breton C, Tersac M, 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

Broglie K, Chet I, Holliday M, Cressman R, Biddle P, Knowlton S, Mauvais CJ, Broglie R (1991) Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. Science 254:1194–1197

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

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

Bruner AC, Jung S, Abbott AG, Powell GL (2001) The naturally occurring high oleate oil character in some peanut varieties results from reduced Oleoyl-PC Desaturase activity from

mutation of Aspartate 150 to Asparagine. Crop Sci 41: 522–526

Bueno MA, Pintos B, Martin A (2006) Induction of embryogenesis via isolated microspore culture in *Olea europaea* L. In: Proceedings of 2nd international seminar on biotechnology and quality of olive tree products around the Mediterranean basin, Marsala, Italy, 5–10 Nov 2006, pp 19–26

Cahoon EB, Shanklin J (2000) Substrate-dependent mutant complementation to select fatty acid desaturase variants for metabolic engineering of plant seed oils. Proc Natl Acad Sci USA 97:12350–12355

Cañas LA, Wyssmann AM, Benbadis MC (1987) Isolation, culture and division of *olive* (*Olea europaea* L) protoplasts. Plant Cell Rep 5:369–371

Cansev A, Gulen H, Eris A (2008) Cold-hardiness of olive (*Olea europaea* L.) cultivars in cold-acclimated and non-acclimated stages: seasonal alteration of antioxidative enzymes and dehydrin-like proteins. J Agric Sci 147:51–61

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

Capelo A, Silva S, Brito G, Santos C (2010) Somatic embryogenesis induction in leaves and petioles of a mature wild olive. Plant Cell Tiss Org Cult. doi:10.1007/s11240-010-9773-x

Carmona JJ, Molina A, Fernandez JA, Lopez-Fando JJ, Garcia-Olmedo F (1993) Expression of the a-thionin gene from barley in tobacco confers enhanced resistance to bacterial pathogens. Plant J 3:457–462

Carnés-Sánchez J, Iraola VM, Sastre J, Florido F, Boluda L, Fernández-Caldas E (2002) Allergenicity and immunochemical characterization of six varieties of *Olea europaea*. Allergy 57:313–318

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

Carrion JS, Dupre M (1996) Late quaternary vegetational history at Navarrès. Eastern Spain. A two core approach. New Phytol 134:177–191

Castro AJ, Alché JD, Cuevas J, Romero PJ, Alché V, Rodríguez-García MI (2003) Pollen from different olive tree cultivars contains varying amounts of the major allergen Ole e 1. Int Arch Allerg Immunol 131:164–173

Cavallotti A, Regina TMR, Quagliariello C (2003) New sources of cytoplasmic diversity in the Italian population of *Olea europaea* L. as revealed by RFLP analysis of mitochondrial DNA: characterization of the cox3 locus and possible relationship with cytoplasmic male sterility. Plant Sci 164:241–252

Chaumont F, Barrieu F, Wojcik E, Chrispeels MJ, Jung R (2001) Aquaporins constitute a large and highly divergent protein family in maize. Plant Physiol 125:1206–1215

Chilton MD, Tepfer DA, Petit A, Casse-Delbart F, Tempe J (1982) *Agrobacterium rhizogenes* inserts T-DNA into the genomes of the host plant root cells. Nature 295:432–434

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

Cipriani M, Cerretani, L, Bendini A, Fontanazza G, Lercker G (2006) Study of phenolic compounds in *Olea europea* L. fruits of cvs. Frantoio, FS17 and Don Carlo during olive's growth. In: Proceedings of 4th EuroFed lipid congress on oils, fats and lipids for a healthier future. The need for interdisciplinary approaches, Madrid, Spain, 1–4 Oct 2006, p 449

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

Colomer R, Moreno-Nogueira JM, Garcia-Luna PP, Garcia-Peris P, Garcia-de-Lorenzo A, Zarazaga A, Quecedo L, del Llano J, Usan L, Casimiro C (2007) Fatty acids, cancer and cachexia: a systematic review of the literature. Br J Nutr 97:823–831

Contento A, Ceccarelli M, Gelati, MT, Maggini F, Baldoni L, Cionini PG (2002) Diversity of *Olea* genotypes and the origin of cultivated olives. Theor Appl Genet 104: 1229–1238

Cooke J, Willis T, Groves R (2005) Impacts of woody weeds on Cumberland Plain Woodland biodiversity. In: The ecology and management of Cumberland Plain habitats. University of West Sydney, Campbelltown, Australia

Cresti M, Linskens HF, Mulcahy DL, Bush S, Di Stilio V, Xu MY, Vignani R, Cimato A (1996) Preliminary communication about the identification of DNA in leaves and in olive oil of *Olea europaea*. Adv Hortic Sci 10:105–107

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

Crossman ND (2002) The impact of the European olive (*Olea europaea* L. subsp. *europaea*) on grey box (*Eucalyptus microcarpa* Maiden) woodland in South Australia. Plant Protect Q 17:140–146

Crossman ND, Bass DA, Virtue JG, Jupp PW (2002) Feral olives (*Olea europaea* L.) in southern Australia: an issue of conservation concern. Adv Hortic Sci 16:17583

D'Angeli S, Altamura MM (2007) Osmotin induces cold protection in olive trees by affecting programmed cell death and cytoskeleton organization. Planta 225:1147–1163

D'Angeli S, Gutiérrez-Pesce P, Altamura MM, Biasi R, Ruggier, B, Muganu M, Bressan R, Rugini E (2001) Genetic transformation of olive tree (*Olea europaea* L.) with *osmotin* gene and in situ protein localisation in the transgenic tissues. In: Proc Società Italiana di Genetica Agraria, Salsomaggiore Terme, 26–29 Sept 2001. ISBN 88-900622-1-5

De Candolle A (1883) Origine des Plantes Cultivées, 2nd edn. Germer Baillière, Paris, France

Ebinuma H, Sugita K, Matsunaga E, Ymakado M (1997) Selection of marker free transgenic plants using the isopentenyl transferase gene. Proc Natl Acad Sci USA 94:2117–2121

Ellis SKB, Kelly EF, Knowlton S (1996) Improved oxidative stability of high oleic acid soybean oil. In: 87th Annual meeting of the American oil chemists' society, Indianapolis, Indiana, USA, 22–24 June 1996, p 273

Endo S, Kasahara T, Sugita K, Matsunaga E, Ebinuma H (2001) The isopentenyl transferase gene is effective as a selectable marker gene for plant transformation in tobacco (*Nicotiana tabacum* cv. Petite Havana SRI). Plant Cell Rep 20:60–66

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

Fabbri A, Bartolini G, Lambardi M, Kailis S (2004) Olive propagation manual. CSIRO, Collingwood, Australia

Fabiani R, De Bartolomeo A, Rosignoli P, Servili M, Montedoro GF, Morozzi G (2002) Eur J Cancer Prev 11:351

Falco SC, Guida T, Locke M, Mauvais J, Sanders C, Ward RT, Weber P (1995) Transgenic canola and soybean seeds with increase lysine. Biotechnology 13:577–582

FAOSTAT (2007) http://faostat.fao.org

Fontanazza G (1987) Presentiamo la cultivar 1-77. Terra e Vita 46:10–11

Fontanazza G (1993) Olivicoltura Intensiva Meccanizzata. Edagricole, Bologna, Italy, 312 p

Fontanazza G, Bartolozzi F (1998) Olive. In: Scarascia-Mugnozza GT, Pagnotta MA (eds) Italian contribution to plant genetics and breeding. University of Tuscia, Viterbo, Italy, pp 723–748

Fontanazza U, Baldoni L, Corona C (1990) Osservazioni preliminari sul valor agronomico di una nuova cultivar da olio: "Fs-17". In: Proc Problematiche qualitative dell'olio d'oliva. Accademia Nazionale dell'Olivo, Spoleto, Italy, pp 69–75

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

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

Gasse F, Téhet R, Durand A, Gibert E, Fontes JC (1990) The arid–humid transition in the Sahara and the Sahel during the last deglaciation. Nature 346:141–146

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

Giannoulia K, Haralampidis K, Poghosyan Z, Murphy DJ, Hatzopoulos P (2000) Differential expression of diacylglycerol acyltransferase (DGAT) genes in olive tissues. Biochem Soc Trans 28:695–697

Giannoulia K, Banilas G, Hatzopoulos P (2007) Oleosin gene expression in olive. J Plant Physiol 164:104–107

Gillies ACM, Cornelius JP, Newton AC, Navarro C, Hernández M, Wilson J (1997) Genetic variation in Costa Rica populations of the tropical timber species *Cedrela odorata* L, assessed using RAPDs. J Mol Ecol 6:1133–1145

Graham J, Greig K, McNicol RJ (1996) Transformation of blueberry without antibiotic selection. Ann Appl Biol 128:557–564

Green PS (1996) Taxonomic notes on asiatic Chionanthus (Oleaceae). Kew Bull 51:765–770

Green PS (2002) A revision of *Olea* L. (*Oleaceae*). Kew Bull 57:91–140

Green PS, Wickens GE (1989) The *Olea europaea* complex. In: Tan K (ed) The Davis and Hedge Festschrift. Edinburgh Universitty Press, Edinburgh, UK, pp 287–299

Grillo S, Leone A, Xu Y, Tucci M, Arancione R, Hasegawua PM, Monti L, Bressan RA (1995) Control of osmotin gene expression by ABA and osmotic stress in vegetative tissue of wild type and ABA-deficient mutants of tomato. Physiol Plant 93:498–504

Gutiérrez-Pesce P, Rugini E (2008) Kiwifruit. In: Kole C, Hall TC (eds) Compendium of transgenic crop plants, vol 5, Transgenic tropical and subtropical fruits and nuts. Blackwell, Oxford, UK, pp 185–212

Gutiérrez-Pesce P, Taylor K, Muleo R, Rugini E (1998) Somatic embryogenesis and shoot regeneration from transgenic roots of the cherry rootstock "Colt" (*Prunus avium* × *P. pseudocerasus*) mediated by pRi 1855 T-DNA of *Agrobacterium rhizogenes*. Plant Cell Rep 17:574–580

Hain R, Reif HJ, Krause E, Langebartels R, Kindl H, Vornam B, Wiese W, Schmelzer E, Schreicher PH, Stocker RH, Stenzel K (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361:153–156

Haldrup A, Petersen SG, Okkels FT (1998) Positive selection: a plant selection principle based on xylose isomerase, an enzyme used in the food industry. Plant Cell Rep 18:76–81

Hamman-Khalifa A, Castro AJ, Jiménez-López JC, Rodríguez-García MI, Alché JD (2008) Olive cultivar origin is a major cause of polymorphism for Ole e 1 pollen allergen. BMC Plant Biol 8:10

Hannachi H, Breton C, Msallem M, El Hadj SB, El Gazzah M, Bervillé 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 Hortic 116:280–290

Haralampidis K, Milioni D, Sanchez J, Baltrusch M, Heinz E, Hatzopoulos P (1998) Temporal and transient expression of stearoyl-ACP carrier protein desaturase gene during olive fruit development. J Exp Bot 49:1661–1669

Hartmann HT (1962) Olive growing in Australia in 1962. Econ Bot 16:31–44

Hatzopoulos P, Banilas G, Giannoulia K, Gazis F, Nikoloudakis N, Milioni D, Haralampidis K (2002) Breeding, molecular markers and molecular biology of the olive tree. Eur J Lipid Sci Technol 104:574–586

Hess J, Kadereit W, 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

Hightower R, Baden C, Penzes E, Lund P, Dunsmuir P (1991) Expression of antifreeze proteins in transgenic plants. Plant Mol Biol 17:1013–1021

Hossein Ava S, Hajnajari H (2006) Obtaining embryo rescued hybrid plants of olive (Mary × Roghani M.) in cross breeding for precocity induction. In: Proceedings of the second international seminar on biotechnology and quality of olive tree products around the Mediterranean Basin, Marsala, Italy, 5–10 Nov 2006, pp 27–29

Huang Y, Nordeen RO, Di M, Owens LD, McBeath JH (1997) Expression of an engineered cecropin gene cassette in transgenic tobacco plants confers disease resistance to *Pseudomonas syringae* pv. *tabaci*. Mol Plant Pathol 87:494–499

Husband BC, Barrett SCH (1991) Colonisation history and population genetic structure of Eichornia paniculata in Jamaica. Heredity 66:287–296

Iwai K, Oi Y, Koyama F, Watanabe K, Hiraoka M, Sekiguchi T (2005) Patent Application: JP 2004-342612 20041126, Jap Kokai Tokkyo Koho

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1993) *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. Science 280:104–106

Jänicke C, Grünwald J, Brendler T (2008) Food supplementation with an olive (*Olea europaea* L.) leaf extract reduces

blood pressure in borderline hypertensive monozygotic twins. Phytother Res 22:1239–1242

Johanson U, Gustavsson S (2002) A new subfamily of major intrinsic proteins in plants. Mol Biol Evol 19:456–461

Johanson U, Karlsson M, Johansson I, Gustavsson S, Sjovall S, Fraysse L, Weig AR, Kjellbom P (2001) The complete set of genes encoding major intrinsic proteins in *Arabidopsis* provides a framework for a new nomenclature for major intrinsic proteins in plants. Plant Physiol 126:1358–1369

Katsiotis A, Hagidimitriou M, Douka A, 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

Kinney AJ (1995) Improving soybean seed quality. In: Proceedings of symposium on induced mutations and molecular techniques for crop improvement, Vienna, Austria, 19–23 June 1995, pp 101–103

Kinney AJ (1996a) Designer oils for better nutrition. Nat Biotechnol 14:946

Kinney AJ (1996b) Development of genetically engineered soybean oils for food applications. J Food Lipids 3:273–292

Kinney AJ (1997) Genetic engineering of oilseeds for desired traits. Genet Eng 19:149–166

Knutson DS, Thompson GA, Radke SE, Johnson WB, Knauf VC, Kridl JC (1992) Modification of *Brassica* seed oil by anti-sense expression of a stearoyl-acyl carrier protein desaturase gene. Proc Natl Acad Sci USA 89:2624–2628

Koukidou M, Klinakis A, Reboulakis C, Zagoraiou L, Tavernarakis N, Livadaras I, Economopoulos A, Savakis C (2006) Germ line transformation of the olive fly *Bactrocera oleae* using a versatile transgenesis marker. Insect Mol Biol 15:95–103

Kubo A, Lunde CS, Kubo I (1995) Antimicrobial activity of the olive oil flavor compounds. J Agric Food Chem 43:1629–1633

Labat JN, Pignal M, Pascal O (1999) Three new species of Oleaceae and note on the presence of *Olea* capensis in the Comoro Archipelago [Trois especes nouvelles d'Oleaceae et note sur la presence d'*Olea* capensis dans l'Archipel des Comores]. Novon 9:66–72

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

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

Leòn L, De la Rosa R, Gracia A, Barranco D, Rallo L (2008) Fatty acid composition of advanced olive selections obtained by crossbreeding. J Sci Food Agric 88:1921–1926

Lesage-Meessen L, Navarro D, Maunier S, Sigoillot JC, Lorquín J, Delattre M, Simon JL, Esther M, Labat M (2001) Simple phenolic content in olive oil residues as a function of extraction systems. Food Chem 75:501–507

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

Liccardi G, D'Amato M, D'Amato G (1996) *Oleaceae* pollinosis: a review. Int Arch Allergy Immunol 111:210–217

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

Liu D, Raghothama KG, Hasegawa PM, Bressan RA (1994) Osmotin overexpression in potato delays development of disease symptoms. Proc Natl Acad Sci USA 91:1888–1892

Lombardero M, Barbas JA, Moscoso del Prado J, Carreira J (1994) cDNA Sequence analysis of the main olive allergen, Ole e I. Clin Exp Allergy 24:765–770

Lombardi JA (2006) *Chionanthus greenii* (Oleaceae), a new species from Minas Gerais, Brazil. Kew Bull 61:179–182

Lorite P, Garcia MF, Carrello JA, Palomeque T (2001) A new repetitive DNA sequence family in the olive (*Olea europaea* L.). Hereditas 134:73–78

Loukas M, Krimbas B (1983) History of olive cultivars based on their genetic distances. J Hortic Sci 58:121–127

Loureiro J, Rodriguez E, Costa A, Santos C (2007) Nuclear DNA content estimations in wild olive (*Olea europaea* L. ssp. *europaea* var. sylvestris Brot.) and Portuguese cultivars of O. *europaea* using flow cytometry. Genet Resour Crop Evol 54:21–25

Lovisolo C, Secchi F, Cardini A, Salleo S, Buffa R, Schubert A (2007) Expression of PIP1 and PIP2 aquaporins is enhanced in olive dwarf genotypes and is related to root and leaf hydraulic conductance. Physiol Plant 130:543–551

Lumaret R, Ouazzani N (2001) Ancient wild olives in Mediterranean forests. Nature 413:700

Lumaret R, Ouazzani N, Michaud H, Villemur P (1997) Cultivated olive and oleaster: two closely connected partners of the same species (*Olea europaea* L.): evidence from allozyme polymorphism. Bocconea 7:39–42

Lumaret R, Amane M, Ouazzan N, Baldoni L (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, Di Giusto F (2004) Allozyme variation of oleaster populations (wild olive tree) (*Olea europaea* L.) in the Mediterranean Basin. Heredity 92:343–351

Maguire TL, Sedgley M (1997) Genetic diversity in Banksia and Dryandra (Proteaceae) with emphasis on Banksia cuneata, a rare and endangered species. Heredity 79:396–401

Martsinkovskaya AI, Poghosyan ZP, Haralampidis K, Murphy DJ, Hatzopoulos P (1999) Temporal and spatial gene expression of cytochrome b5 during flower and fruit development in olives. Plant Mol Biol 40:79–90

McKersie BD, Chen Y, de Beus M, Bowley SR, Bowler C, Inzé D, D'Hallewin K, Botterman J (1993) Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (*Medicago sativa* L). Plant Physiol 103:1155–1163

Médail F, Quézel P, Besnard G, Khadari B (2001) Systematics, ecology and phylogeographic significance of *Olea europaea* L. subsp. *maroccana* (Greuter & Burdet) P. Vargas et al., a relictual olive tree in south-west Morocco. Bot J Linn Soc 137:249–266

Mekuria GT, Collins GG, Sedgley M (1999) Genetic variability between different accessions of some common commercial olive cultivars. J Hortic Sci Biotechnol 74:309–314

Mekuria GT, Collins G, Sedgley M (2002) Genetic diversity within an isolated olive (*Olea europaea* L.) population in relation to feral spread. Sci Hortic 94:91–105

Mencuccini M (1991) Protoplast culture isolated from different tissues of olive (*Olea europoea* L.) cultivars. Physiol Plant 82:14 (Abstract)

Mencuccini M, Rugini E (1993) *In vitro* shoot regeneration from Olive cultivar tissue. Plant Cell Tissue Org Cult 32: 283–288

Mendoza-De Gyves E, Rosana Mira F, Ruiu F, Rugini E (2008) Stimulation of node and lateral shoot formation in Micropropagation of olive (*Olea europea* L.) by using dikegulac. Plant Cell Tissue Org Cult 92:233–238

Mercier J (1997) Phytochemistry of fruits and vegetables. Clarendon, Oxford, UK

Mercuri A, Bruna S, De Benedetti L, Burchi G, Schiva T (2001) Modification of plant architecture in *Limonium* spp. induced by *rol* genes. Plant Cell Tissue Org Cult 65:247–253

Miano D, Gutiérrez Pesce P, Lupi R, Dradi G, Roncasaglia R, Taratufolo C, Rugini E, Muleo R (2004) *rolABC* genes are differently regulated in the organs and modify gene expression and morphology of olive cultivar 'Canino'. In: Proceedings of XLVIII Italian society of agricultural genetics SIFV-SIGA, Joint Meeting, Lecce, Italy, 15–18 Sept 2004, Poster Abstract

Mills D, Hammerschlag FA, Nordeen RO, Owens LD (1994) Evidence for the breakdown of cecropin B by proteinases in the intercellular fluid of peach leaves. Plant Sci 104:17–22

Milne RI, Abbott RJ (2004) Geographic origin and taxonomic status of the invasive Privet, *Ligustrum robustum* (Oleaceae), in the Mascarene Islands, determined by chloroplast DNA and RAPDs. Heredity 92:78–87

Minelli S, Maggini F, Gelati MT, Angiolillo A, Cionini PG (2000) The chromosome complement of *Olea europaea* L.: characterization by differential staining of the chromatin and in situ hybridization of highly repeated DNA sequences. Chrom Res 8:615–619

Mitrakos K, Alexaki A, Papadimitriou P (1992) Dependence of olive morphogenesis on callus origin and age. J Plant Physiol 139:269–273

Morettini A (1972) Olivicoltura. Reda, Roma, Italy

Mourgues F, Brisset MN, Chevreau E (1998) Strategies to improve plant resistance to bacterial diseases through genetic engeneering. Trends Biotechnol 16:203–210

Muleo R, Morini S (2008) Physiological dissection of blue and red light regulation of apical dominance and branching in M9 apple rootstock growing in vitro. J Plant Physiol 165:1838–1846

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

Nardini A, Gascò A, Raimondo F, Gortan E, Lo Gullo MA, Caruso T, Salleo S (2006) Is rootstock-induced dwarfing in olive an effect of reduced plant hydraulic efficiency? Tree Physiol 26:1137–1144

Norelli JL, Aldwinckle HS, Destefano-Beltran L, Jaynes JM (1994) Transgenic 'Malling 26' apple expressing the attacin E gene has increased resistance to *Erwinia amylovora*. Euphytica 77:123–128

North GB, Martre P, Nobel PS (2004) Aquaporins account for variations in hydraulic conductance for metabolically active root regions of *Agave deserti* in wet, dry, and rewetted soil. Plant Cell Environ 27:219–228

Oeller PW, Ming-Wong T, Taylor LP, Pike DA, Theologis A (1991) Reversible inhibition of tomato fruit senescence by antisense RNA. Science 254:437–439

Olmstead RG, Kim KJ, Jansen RK, Wagstaff SJ (2000) The phylogeny of the Asteridae *sensu lato* based on chloroplast *ndhF* gene sequences. Mol Phylogenet Evol 16:96–112

Orinos T, Mitrakos K (1991) Rhizogenesis and somatic embryogenesis in calli from wild olive (*Olea europaea* var. *sylvestris* (Miller) Lehr mature zygotic embryos. Plant Cell Tissue Org Cult 27:183–187

Ouazzani N, Lumaret R, Villemur P, Di Giusto F (1993) Leaf allozyme variation in cultivated and wild olive trees (*Olea europaea* L.). J Hered 84:34–42

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

Palamarev E (1989) Paleobotanical evidences of the tertiary history and origin of the Mediterranean sclerophyll dendroflora. Plant Syst Evol 162:93–107

Pannelli G, Famiani F, Rugini E, Bignami D, Natali S (1990) Preliminary characteristics of olive somatic mutants from gamma irradiated 'Frantoio' and 'Leccino' plantlets. Acta Hortic 286:77–80

Pannelli G, Volpe D, Preziosi P, Famiani F (1993) Comparison of the vegetative and reproductive characteristics of traditional olive cultivar and selected low vigorous accessions in central Italy. Acta Hortic 356:123–126

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

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

~~Patil SC, Singh VP, Satyanarayan PSV, Jain NK, Singh A, Kulkarni SK (2003) Protective effect of flavonoids against aging and lipopolysaccharide-induced cognitive impairment in mice. Pharmacology 69:59–67~~

Pena L, Cervera M, Juarez J, Navarro A, Pina JA, Duran-Vila N, Navarro L (1995) *Agrobacterium*-mediated transformation of sweet orange and regeneration of transgenic plants. Plant Cell Rep 14:616–619

Pena L, Cervera M, Juarez J, Navarro A, Pina JA, Navarro L (1997) Genetic transformation of lime (*Citrus aurantifolia* Swing.): factors affecting transformation and regeneration. Plant Cell Rep 16:731–737

Pena L, Martin-Trillo M, Juarez J, Pina JA, Navarro L, Martinez-Zapater JM (2001) Constitutive expression of *Arabidopsis* LEAFY or APETALA1 genes in citrus reduces their generation time. Biotechnology 19:263–267

Pereira AP, Ferreira ICFR, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA (2007) Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules 12:1153–1162

Perl A, Eshdat Y (1998) DNA transfer and gene expression in transgenic grapes. Biotechnol Genet Eng Rev 15:365–386

Perri E, Parlati MV, Rugini E (1994a) Isolation and culture of olive (*Olea europaea* L.) cultivar protoplasts. Acta Hortic 356:51–53

Perri E, Parlati MV, Mulé R, Fodale AS (1994b) Attempts to generate haploid plants from *in vitro* cultures of *Olea europaea* L. anthers. Acta Hortic 356:47–50

Perrinjaquet-Moccetti T, Busjahn A, Schmidlin C, Schmidt A, Bradl B, Aydogan C (2008) Food supplementation with an olive (*Olea europaea* L.) leaf extract reduces blood pressure in borderline hypertensive monozygotic twins. Phytother Res 22:1239–1242

Petkov V, Manolov P (1978) Pharmacological studies on substances of plant origin with coronary dilatating and antiarrhythmic action. Comp Med East West 6:123–130

Poghosyan ZP, Haralampidis K, Martinkovskaya AI, Murphy DJ, Hatzopoulos P (1999) Developmental regulation and spatial expression of a plastidial fatty acid desaturase from *Olea europaea*. Plant Physiol Biochem 37:109–119

Proietti P, Tombesi A (1996) Translocation of assimilates and source-sink influences on productive characteristics of the olive tree. Adv Hortic Sci 1:11–14

Psomiadou E, Tsimidou M, Boskou D (2000) α- Tocopherol content of Greek virgin olive oils. J Agric Food Chem 48: 1770–1775

Qiusheng Z, Yuntao Z, Rongliang Z, Dean G, Changling L (2005) Effects of verbascoside and luteolin on oxidative damage in brain of heroin treated mice. Pharmazie 60: 539–543

Quézel P (1969) Flore et végétation des plateaux du Darfur Nord-occidental et du Jebel Gourgeil. Dossiers de la RCP, vol 45. CNRS, Marseille

Quézel P (1978) Analysis of the flora of Mediterranean and Saharan Africa. Ann MO Bot Gard 65:479–534

Rahman SM, Kinoshita T, Anai T, Takagi Y (2001) Combining ability in loci for high oleic and low linolenic acids in soybean. Crop Sci 41:26–29

Rallo P, Dorado G, Martín 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, Martín A (2003) Transferability of olive microsatellite loci across the genus Olea. Theor Appl Genet 107:940–946

Reale S, Angiolillo A, Baldoni L, D'Andrea M, Lima G, Scarano MT (2006) Olive authochthonous germplasm of Molise: molecular characterization by means of SSRs and SNPs. In: Proceedings of second international seminar on biotechnology and quality of olive tree products around the Mediterranean Basin, Marsala, Italy, 5–10 Nov 2006, pp 195–198

Reichelt K, Burr M (1997) Extra virgin: an Australian companion to olives and olive oil. Wakefield, Adelaide, Australia

Ripa V, De Rose F, Tucci A, Scalercio S, Tucci P, Pellegrino M (2006) Preliminary observations on the agronomical behaviour of olive cross breedings cultivated in Rossano Calabro. In: Proceedings of second international seminar on biotechnology and quality of olive tree products around the Mediterranean Basin, Marsala, Italy, 5–10 Nov 2006, pp 139–142

Rodríguez R, Villalba M, Monsalve RI, Batanero E, González EM, Monsalve RI, Huecas S, Tejera ML, Ledesma A (2002) Allergenic diversity of the olive pollen. Allergy 57:6–15

Roselli U, Donini B (1982) Briscola. Nuova cultivar di olivo a sviluppo compatto. Riv Ortoflorofrutticolt Ital 66:103–114

Roselli G, Petruccelli R, Polsinelli L, Cavalieri D (2002) Variability in five Tuscan olive cultivars (*Olea europaea* L.). J Genet Breed 56:51–60

Rotino GL, Perri E, Zottini M, Sommer H, Spena A (1997) Genetic engineering of parthenocarpic plants. Nat Biotechnol 15:1398–1401

Rotondi A, Bertazza G, Magli M (2004) Effect of olive fruits quality on the natural antioxidant compounds in extravirgin olive oil of Emilia-Romagna region. Prog Nutr 6:139–145

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

Rugini E (1984) In vitro propagation of some olive (*Olea europaea* L.) cultivars with different root-ability, and medium development using analytical data from developing shoots and embryos. Sci Hortic 24:123–134

Rugini E (1986) Olive. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 10. Springer, Berlin, pp 253–267

Rugini E (1988) Somatic embryogenesis and plant regeneration in olive (*Olea europaea* L.). Plant Cell Tissue Org Cult 14:207–214

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

Rugini E (1995) Somatic embryogenesis in olive (*Olea europaea* L.). In: Jain SM, Gupta PK, Newton RJ (eds) Somatic embryogenesis in woody plants, vol 2. Kluwer, Dordrecht, The Netherlands, pp 171–189

Rugini E, 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, Fedeli E (1990) Olive (*Olea europaea* L.) as an oilseed crop. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 10: Legumes and oilseed crops I. Springer, New York, pp 593–641

Rugini E, Lavee S (1992) Olive biotechnology. In: Hammerschlag FA, Litz R (eds) Biotechnology of perennial fruit crops. CABI, Wallingford, UK, pp 371–382

Rugini E, Mariotti D (1992) *Agrobacterium rhizogenes* T-DNA genes and rooting in woody species. Acta Hortic 300: 301–308

Rugini E, Muganu M (1998) A novel strategy for the induction and maintenance of shoot regeneration from callus derived from established shoots of apple [*Malus* × *domestica* BorkH.] cv. golden delicious. Plant Cell Rep 17:581–585

Rugini E, Tarini P (1986) Somatic embryogenesis in olive (*Olea europaea* L.). In: Proceedings of conference on fruit tree biotechnology, Montecarlo, Italy, 14–15 Oct 1986, p 62

Rugini E, Bazzoffia A, Jacoboni A (1987) A simple in vitro method to avoid the initial dark period and to increase rooting in fruit trees. Acta Hortic 227:438–440

Rugini E, Pellegrineschi A, Mencuccini M, Mariotti D (1991) Increase of rooting ability in the woody species Kiwi (*Actinidia deliciosa* A. Chev.) by transformation with *Agrobacterium rhizogenes* T-DNA *rol* genes. Plant Cell Rep 10:291–295

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

Rugini E, Caricato G, Muganu M, Taratufolo C, Camilli M, Cammilli C (1997) Genetic Stability and agronomic

evaluation of six-year-old transgenic kiwi plants for *rolABC* and *rolB* genes. Acta Hortic 447:609–610

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

Rugini E, Biasi R, Muleo R (2000) Olive (*Olea europaea* var. *sativa*) transformation. In: Jain SM, Minocha SC (eds) Molecular biology of woody plants, vol 2. Kluwer, Dordrecht, Netherlands, pp 245–279

Rugini E, Gutiérrez-Pesce P, Muleo R (2008) Olive. In: Kole C, Hall TC (eds) Compendium of transgenic crop plants, vol 4, Transgenic temperate fruits and nuts. Blackwell, Oxford, UK, pp 233–258

Sabino Gil F, Busconi M, Da Câmara MA, Fogher C (2006) Development and characterization of microsatellite locifrom *Olea europaea*. Mol Ecol Notes 6:1275–1277

Sakurai J, Ishikawa F, Yamaguchi T, Uemura M, Maeshima M (2005) Identification of 33 rice aquaporin genes and analysis of their expression and function. Plant Cell Physiol 146: 1568–1577

Salzman RA, Tikhonova I, Bordelon BP, Hasegawa PM, Bressan RA (1998) Coordinate accumulation of antifungal proteins and hexoses constitutesa developmentally controlled defence response during fruit ripening in grape. Plant Physiol 117:465–472

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

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

Schaffner AR (1998) Aquaporin function, structure, and expression: are there more surprises to surface in water relations? Planta 204:131–139

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

Secchi F, Lovisolo C, Schubert A (2007b) Expression of OePIP2.1 aquaporin gene and water relations of *Olea europaea* twigs during drought stress and recovery. Ann Appl Biol 150:163–167

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

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

Shahidi F (1996) Natural antioxidants chemistry, health effect and applications. AOCS, Champaign, IL

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

Simpson GG, Gendall AR, Dean C (1999) When to switch to flowering. Annu Rev Cell Dev Biol 15:519–550

Smart LB, Moskal WA, Cameron KD, Bennett AB (2001) MIP genes are down-regulated under drought stress in *Nicotiana glauca*. Plant Cell Physiol 42:686–693

Smith CJS, Watson CF, Morris PC, Bird CR, Morris PC, Schuch W, Grierson D (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature 334:724–726

Spennemann DHR, Allen LR (2000) Feral olives (*Olea europaea*) as future woody weeds in Australia: A review. Aust J Exp Agric 40:889–901

Starr F, Starr K, Loope L (2003) Plants of Hawaii. http://www.hear.org/starr/hiplants/reports

Sweeney S, Davies G (1998) The Olive industry. In: Hide K (ed) The new rural industries: a handbook for farmers and investors. RIRDC, Barton, USA, pp 405–411

Terral J-F, Arnold-Simard G (1996) Beginnings of olive cultivation in eastern Spain in relation to Holocene bioclimatic changes. Quaternary Res 46:176–185

Terral J-F, Mengüal X (1999) Reconstruction of Holocene climate in southern France and Eastern Spain using quantitative anatomy of olive wood and archaeological charcoal. Palaeogeogr Palaeoclimatol Palaeoecol 153:71–92

Terral J-F, Alonso N, Buxó I, Capdevila R, Chatti N, Fabre L, Fiorentino G, Marinval P, Jordá GP, Pradat B, Rovira N, 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

Tombesi A (1978) La fertilità nell'olivo. Riv Ortoflorofrutticolt Ital 62:435–450

Tucker KJ (1976) Effect of far-red light of the hormonal control of side shoot growth in the tomato. Ann Bot 40:1033–1042

Uccella N (2000) Olive biophenols: biomolecular characterization, distribution and phytoalexin histochemical localization in the drupes. Trends Food Sci Technol 11:315–327

Vaeck M, Raynaerts A, Hofte H, Jansens S, DeBeukeleer M, Dean C, Zabeau M, van Montagu M, Leemans J (1987) Transgenic plants protected from insect attack. Nature 328: 33–37

Van Camp W, Willekens H, Bowler C, Van Montagu M, Inze D, Reupold-Popp P, Sandermann H, Langebartels C (1994) Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. Biotechnology 12:165–168

van Der Salm TPM, Van Der Toorn CJG, Bouwer R, Ten Cate ChHH, Dons HJM (1997) Production of *rol* gene transformed plants of *Rosa hybrida* L. and characterization of their rooting ability. Mol Breed 3:39–47

van der Salm TPM, Bouwer R, Van Dijk AJ, Keiser LCP, Ten Cate ChHH, Van der Plas LHW, Dons JJM (1998) Stimulation of scion bud release by *rol* gene transformed rootstocks of *Rosa hybrida* L. J Exp Bot 49:847–852

Vandeleur R, Niemietz C, Tilbrook J, Tyerman SD (2005) Roles of aquaporins in root responses to irrigation. Plant Soil 274:141–161

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

Vergari G, Patumi M, Fontanazza G (1996) Utilizzo dei marcatori RAPDs nella caratterizzazione del germoplasma di olivo. Olivae 60:19–22

Villalba M, Batanero E, López-Otín C, Sánchez LM, Monsalve RI, González de la Peña MA, Lahoz C, Rodríguez R (1993) The amino acid sequence of Ole e I, the major allergen from olive tree (*Olea europaea*) pollen. Eur J Biochem 216:863–869

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

Vince-Prue D, Canham AE (1983) Horticultural significance of photomorphogenesis. In: Shropshire W, Mohr H (eds) Encyclopedia of plant physiology. Springer, Berlin, Germany, pp 518–544

Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247:60–64

Wallander E, Albert VA (2000) Phylogeny and classification of *Oleaceae* based on *rps* 16 and *trnL-F* sequence data. Am J Bot 87:1827–1841

Watts WA, Allen JRM, Huntley B (1996) Vegetation history and palaeoclimate of the last glacial period at Lago Grande di Monticchio, Southern Italy. Quaternary Sci Rev 15:133–153

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

West CJ (2002) Eradication of allien plants on Raoul Island, Kermadec Islands, New Zealand. In: Veitch CR, Clout MN (eds) Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group, Cambridge, pp 365–373

Wheeler AW (1992) Hypersensitivity to the allergens of the pollen from olive tree (*Olea europaea*). Clin Exp Allergy 22:1052–1057

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

Wu G, Shott BJ, Lawrence EB, Levine EB, Fitzsimmons KC, Shah DM (1995) Disease resistance conferred by expression of a gene encoding H2O2-generating glucose oxidase in trangenic potato plants. Plant Cell 7:1357–1368

Yadav NS (1996) Genetic modification of soybean oil quality. In: Verma DPS, Shoemaker RC (eds) Soybean genetics: molecular biology and biotechnology. CABI, New York, pp 165–188

Yanotsky M (1995) Floral meristems to floral organs: genes controlling early events in Arabidopsis flower development. Annu Rev Plant Physiol Mol Biol 46:167–188

Yoshikawa M, Tsuda M, Takeuchi Y (1993) Resistance to fungal diseases in transgenic tobacco plants expressing the phytoalexin elicitor-releasing factor, -1, 3-endoglucanase from soybean. Naturwissenschaften 80:417–420

Zhu B, Chen TH, Li PH (1993) Expression of an ABA-responsive osmotin-like gene during the induction of freezing tolerance in *Solanum commersonii*. Plant Mol Biol 21:729–735

Zhu B, Chen TH, Li PH (1995) Expression of three osmotin like protein genes in response to osmotic stress and fungal infection in potato. Plant Mol Biol 28:17–26

Zhu LH, Holefors A, Ahlman A, Xue ZT, Welander M (2001) Transformation of the apple rootstock M.9/29 with the *rolB* gene and its influence on rooting and growth. Plant Sci 160:433–439

Zohary D (1994) The wild genetic resources of the cultivated olive. Acta Hortic 356:62–65

Zohary D, Hopf M (1994) Olive: *Olea europaea*. In: Domestication of plants in the old world, 2nd edn. Oxford Claredon, Oxford, UK

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

Author Queries

Chapter No.: 5

Query Refs.	Details Required	Author's response
AU1	'Mazzolani and Altamura Betti 1976' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Mazzolani G. and Altamura Betti M.M (1976) Elementi per la revisione del genere *Olea* (Tourn.). Nota introduttiva. Ann. Bot. (Roma) 35-36: 463-469.
AU2	Please clarify whether the reference is Besnard et al. (2002a or b) throughout the text.	The citation is already indicated in the text
AU3	Please clarify whether the citation is 'Besnard et al. 2007a or b or c' throughout the text.	The citation is already indicated in the text
AU4	Please clarify whether the reference is Bronzini de Caraffa et al. 2002a or b throughout the text.	The citation is already indicated in the text
AU5	Please check if the edit in the sentence "Depending on the cultivar....or self-sterile." is appropriate.	It is ok
AU6	'Revilla et al. 1996' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Revilla MA, Pacheco J, Casares A, Rodríguez R (1996) In vitro reinvigoration of mature olive trees (*Olea europea* L.) through micrografting. In Vitro Cell Dev Biol Plant 32: 257 -261
AU7	'Briccoli Bati et al. 1999' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Briccoli Bati C. Fodale A., Mulé R., Trombino T (1999) Trials to increase in vitro rooting of *O*lea *europaea* L. cuttings. Acta Horticulturae 474: 91-94.
AU8	'Canas and Benbadis 1988' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Canas LA, Benbadis A. (1988) In-vitro plant regeneration from cotyledon fragments of the olive tree (*Olea euro*paea L.). Plant Sci 54: 65 -74
AU9	'Micheli et al. (1998)' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Micheli, M., Mencuccui M. and Standardi A (1998)Encapsulation of in vitro proliferated buds of olive. Adv. Hort. Sci., 12: 163-168.
AU10	'Griggs et al. 1975' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	**GriggsWH, Hartmann HT, Bradley MV, Iwakiri BT, Whisler JE** (1975) Olive pollination in California. California Agricultural Experimental Station Bulletin 869.
AU11	'Alcantara et al. 2000' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Alcantara JM, Rey PJ, Valera F, Sanchez-Lafuente M (2000). Factors shaping the seedfall pattern of a bird-dispersal plant. Ecology 81: 1937 -1950.
AU12	'Chevalier 1948' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Chevalier A (1948) L'origine de l'olivier cultivé e ses variations. Rev. Bot. Appl. 303-303: 1-25
AU13	'Loveless and Hamrick 1984' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Loveless, M. D., and J. L. Hamrick (1984) Ecological determinants of genetic structure of plant populations. Ann. Rev. Ecol. Syst. 15:65-95.
AU14	'Darmency 1997' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Darmency H (1997) Gene flow between crops and weeds: Risk for new herbicide resistance weeds? In: De Prado R, Jorrín J, García-Torres L. Weed and crop resistance to herbicides. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp.239-248.
AU15	'Lavee 1996' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Lavee, S (1996) Biology and physiology of the olive. p. 59 - 110 & 180 - 182. In: IOOC (ed.). World Olive Encyclopaedia. Plaza & Janés Editorial, Barcelona.
AU16	'Excoffier et al. 1992' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Excoffier L, Smouse P and Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.
AU17	'Bandelj et al. (2001, 2004)' are cited in text but not given in the reference list. Please provide details in the list or delete the citations from the text.	Bandelj D, Jakse J and Javornik B (2002) DNA fingerprinting of olive varieties by microsatellite markers. Food Technol. Biotechnol. 40: 185-190. Bandelj D, Jakse J. and Branka J (2004) Assessment of genetic variability of olive varieties by microsatellite and AFLP markers. Euphytica 136(1): 93-102. DOI: 10.1023/B:EUPH.0000019552.42066.10

AU18	'Mulè et al. 1992' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Mulé R., Fodale A.S., Parlanti M.V.M., Tucci A (1992) ¨Tonda dolce Partanna': nuova varietà di olivo da mensa a maturazione precocissima. Riv Frutt11: 25-29.
AU19	'Pannelli et al. 2006' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Pannelli G., Rosati A., Pandolfi S., Padula G., Mennone C., Giordani E., Bellini E (2006) *Field evaluation of olive selections derived from a breeding program.* - Proceedings Second International Seminar Olivebioteq 2006, "Biotechnology and Quality of Olive Tree Products around the Mediterranean Basin", Mazara del Vallo (TP), 5-10 November, vol. 1, pp. 95-102.
AU20	Please check the sentence "In some cases....leaves" for missing words.	
AU21	'Nakaijma et al. 1997' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Nakajima H., Muranaka, T., Ishige, F., Akutsu, K., Oeda, K (1997) Fungal and bacterial disease resistance in transgenic plants expressing human lysozyme. Plant Cell Reports 16: 674-679
AU22	'May et al. 1995' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	May GD, Afza R, Mason HS, Wiecko A, Novak FJ, Arntzen CJ (1995). Generation of transgenic banana (*Musa acuminata*) plants via Agrobacterium-mediated transformation. Biotechnology 13: 486-492.
AU23	AQ: Please check if the edits in the sentence "Recently, a website...to the end." are appropriate and convey the intended meaning.	It is appropriate
AU24	Please check the sentence "The expression of AQP genes....architecture." for clarity.	
AU25	'Luque de Castro and Japòn Luján 2006' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Japon-Lujan, R. and Luque de Castro, M.D (2006) **Superheated liquid extraction of oleuropein and related biophenols from olive leaves.** Journal of Chromatography 1136(2): 185-191.
AU26	'Tulp et al. 2006' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	**Tulp M., Bruhn J.B. and Bohlin L (2006)** Food for thought. Drug Discovery Today 11(23-24): 1115-1121.
AU27	'Heenan et al. 1999' is cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	Heenan PB, de Lange PJ, Glenny DS, Breitwieser I, Brownsey PJ, Ogle CC (1999) Checklist of dicotyledons, gymnosperms, and pteridophytes naturalised in New Zealand: additional records 1997 -1998. NZ J Bot 37: 629 -642.
AU28	Following references are not cited in text: Baldont et al. (1999), Carnés-Sánchez et al. (2002), Labat et al. (1999), Lesage-Meessen et al. (2001), Lombardero et al. (1994), Lumaret et al. (2000), Patil et al. (2003), Roselli et al. (2002), Villalba et al. (1994). Please cite these references in text or delete them from the list.	done
AU29	Please update the reference Albertini et al. (2010) if possible.	not published yet