13 Olive

Silvia Doveri[1] and Luciana Baldoni[2]

[1] NIAB, Molecular Research Group, Huntingdon Road, Cambridge CB3 0LE, UK,
e-mail: silvia.doveri@gmail.com
[2] National Research Council – Institute of Plant Genetics, Via Madonna Alta, 130, 06128, Perugia, Italy

13.1 Introduction

13.1.1 Brief History of the Crop

The tree species olive (*Olea europaea* L.) is among the most ancient of crops of the Mediterranean region (Zohary and Spiegel-Roy 1975) (Figs. 1 and 2). In the archaeological records, woods of cultivated olive from Eastern Spain and Southern France have been dated up to the Neolithic age (Terral 2000). Before its domestication, wild olive was endemic across the Mediterranean region, but particularly in the Middle East. Wild olive grows abundantly in thick forest, and is believed to be indigenous to the Mediterranean Basin (Green 2002). The domestication process is thought to have involved the selection of trees of large fruit size and/or high oil content, and their vegetative propagation, either directly planted via cuttings or grafted onto indigenous oleasters. There is evidence for contemporaneous starting of olive domestication at both ends of the Mediterranean. In the Near East it occurred in the Early Bronze Age (second half of the 5th millennium BCE), as has been demonstrated both by the discovery of olive oil presses and by the presence of pollen grains, stones and wood remains (Zohary and Spiegel-Roy 1975; Liphschitz et al. 1991); while analysis of archaeological charcoal and olive stones have dated domestication to the end of the Bronze Age in the north-western Mediterranean area (Terral 2000; Terral et al. 2004). From the 6th century BC, cultivated olive spread throughout the Mediterranean, reaching Tunisia and Sicily, and later Northern Italy. With the European settlement of America after the XV century, olives arrived in the New World, but only in recent times has its cultivation extended significantly beyond the Mediterranean area. Today, it is grown commercially in Australia, South America (Argentina and Chile) and South Africa.

13.1.2 Botanical Description

Olive belongs to the *Oleaceae* family, sub-family *Oleideae*. The family includes about 30 genera (Johnson 1957), accounting ornamental shrub species such as jasmine (*Jasminum fruticans* L.), lilac (*Syringa vulgaris* L.) and forsythia (*Forsythia* × *intermedia* Zabel); and tree species, such as ash (*Fraxinus excelsior* L. and *F. angustifolia* Vahl.), privet (*Ligustrum vulgare*

Fig. 1. An ancient olive tree (*Olea europaea* L.)

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L.) and phyllirea (*Phyllirea angustifolia* L., *P. media* L. and *P. latifolia* L.). The genus *Olea*, sub-family *Oleideae,* includes two sub-genera*: Olea* and *Paniculatae.* The former is divided in two sections: *Olea*, which contain only *O. europaea* (including both cultivated and wild forms), and *Ligustroides*. According to recent revisions of *O. europaea* taxonomy (Green and Wickens 1989; Green 2002), this species is divided into six sub-species, based on morphology and geographical distribution:

1) subsp. *europaea*, with the two botanical varieties *europaea* (cultivated olive) and *sylvestris* (wild olive), widely distributed throughout the Mediterranean Basin;
2) subsp. *cuspidata,* distributed from SE Asia to SW China, as well as from the Arabian peninsula through East and South Africa;
3) subsp. *laperrinei*, restricted to the Sahara region;
4) subsp. *maroccana*, restricted to Morocco;
5) subsp. *cerasiformis*, restricted to the island of Madeira;
6) subsp. *guanchica*, restricted to the Canary Islands.

Wild olive fruits are smaller in size and have lower mesocarp oil content than do cultivars (Terral and Arnold-Simard 1996). Populations of wild olive are restricted to a few isolated areas of native Mediterranean forest, where pollen/stones may be wind/bird-distributed (Lumaret et al. 2004). Molecular analysis, using both nuclear and cytoplasmic markers, has shown that the eastern and western Mediterranean populations are strongly differentiated from one another (Besnard et al. 2001a, 2001b, 2002b; Lumaret et al. 2004). On the contrary, cultivated olives do not show such geographical structure, even though their variability is quite high. It has been repeatedly shown evidence for the multilocal selection of most cultivars (Besnard et al. 2001b; Rotondi et al. 2003), empirically undertaken by olive growers from naturally cross-bred genotypes. At least 1,275 cultivars have been described (Bartolini et al. 1998), but many other local varieties and ecotypes contribute to the richness of the olive germplasm. Few cultivars are dispersed over a widespread area; rather, the majority is highly localized.

Fig. 2. Ripening olive fruits of cultivar Frantoio

The olive is a long-living evergreen tree, which can attain a mature height of up to 15 m and a spread of 9 m; its life span is typically more than 500 years, but trees older than 2,000 years have been recorded. Mature leaves are elliptic and characteristically gray-green in color, as a result of the presence of star-hairs. Flowers are wind pollinated, and although most cultivars are self-incompatible, some are self-compatible. The flowers are generally hermaphroditic, but certain cultivars are male-sterile (Besnard et al. 2000), while others are purely staminate. The fruit is a drupe, with a thick, fleshy oil-accumulating mesocarp. When pulped, the mesocarp is made up of oil (22%), water (50%), proteins (1.6%), carbohydrates (19.1%), cellulose (5.8%) and minerals (1.5%).

Green olives destined for canning are usually harvested when the fruit is completely developed and the skin color starts to change from green to reddish, while olives used either as a source of oil, or for processing as black olives, are picked later in the ripening process, when oil accumulation is completed and the skin has become black. The characteristic compound oleuropein, which confers a strong bitter taste to the fruit, makes the fruit unpalatable, so that pretreatment is necessary before table consumption.

Olive trees grow in semi-arid to temperate climates, on almost any well-drained soil with a pH below 8.5, and are reasonably tolerant of mild soil salinity. They show cold winter hardiness, tolerating temperatures as low as −12 °C. Even though olive has the ability to initiate vegetative shoots from the base of the trunk, productivity may be compromised for several years following episodes of severe cold-induced die-back.

Significant pests and diseases include the olive-specific pathogens *Spilocaea oleagina* Cast., causing olive leaf spot, and the olive fruit fly (*Bactrocera oleae*). Major non-specific pathogens cause *Verticil-*

lium wilt (*Verticillium dahliae* Kleb.) and olive knot (*Pseudomonas syringae* subsp. *savastanoi*). *B. oleae* directly attacks the fruit mesocarp, and can have serious consequences on production, by inducing early fruit fall or causing total disruption of the pulp. Plant propagation is generally by cutting or grafting onto seedling rootstocks. Cultivars are mostly diploid ($2n = 2x = 46$) (Falistocco and Tosti 1996; Minelli et al. 2000), but tetraploid plants have been reported (Rugini et al. 1996). The DNA content is 2.2 pg per 1C nucleus (Rugini et al. 1996), equivalent to a genome size of 2.2 Gbp (De la Rosa et al. 2003).

13.1.3 Economic Importance

Olive is one of the most important crops as a source of oil, and for table consumption. Olive oil has favorable nutritional properties, and as a result, its consumption, traditionally restricted to the Mediterranean area (77% of the world production area), is increasing worldwide (mainly United States, Canada, Australia and Japan). Some varieties are cultivated specifically for table consumption, but the majority is used for oil extraction.

Virgin olive oil is mechanically extracted from pressed or centrifuged pulped fruit. In the commonest process (the continuous extraction system), two centrifugations generate three fractions: oil, pomace and vegetable water. Olive production is concentrated in Southern Europe, mainly Spain and Italy, followed by Greece, Portugal and France, which together account for about the 85% of world production. Turkey, Syria, Lybia, Morocco, Algeria and Tunisia are also important producers. Over the last ten years olive cultivation has extended around the world, from South Africa to Latin America (Argentina and Chile), California, New Zealand and Australia and, since the late 1990s, there has been a strongly rising production trend in these countries; nevertheless, the major producers remain in Europe. Olive oil production in Europe in 2003 was 2.3 Mt, while competitor oil-producing crops such as rapeseed and sunflower generated, respectively, 4.2 Mt and 4.9 Mt (data from FAOSTAT database).

Olive oil is a relatively expensive vegetable oil due to its high cultivation costs and limited production. Fruit production can start 3–5 years after planting, if properly cultivated, but generally optimal yields are not attained before trees are 10 years old. Mean production per tree (15–50 kg fruit) and per unit area (about 2 t/ha) are low in comparison with other oil crops, and extractability rarely exceed 24% of fresh weight (depending on variety, agro-climatic conditions and extraction method). Yield is unpredictable from year to year, but the source of much of this variation remains unclear. Oil accumulates in the fruit mesocarp, and to a lesser extent, also in the seed (Harwood and Sanchez 2000).

Virgin olive oil is overwhelmingly made up of triglycerides (98–99%), along with a small proportion of other compounds. The dominant triglyceride fatty acid species are the mono-unsaturated acids oleic (18:1) (57–78%), palmitic (16:0), and stearic (18:0), and the poly-unsaturated acids linoleic (18:2) (7–19%) and linolenic (18:3) (0.6–0.8%) (Salas et al. 2000). The minor compounds (alcohols, polyphenolic compounds, chlorophyll, carotenoids, sterols, tocopherols and flavonoids) contribute to the organoleptic qualities, taste, flavor, and nutritional value (Servili and Montedoro 2002; Garcia-Gonzalez et al. 2004), which may distinguish olive oils originating from different production regions. Recent studies have shown that olives contain antioxidants in abundance (up to 16 g/kg), represented by acteosides, hydroxytyrosol, tyrosol and phenilpropionic acids. Olive oil, especially extra virgin, contains smaller amounts of hydroxytyrosol and tyrosol, but also contains secoiridoids and lignans, as well as other compounds deemed to be anticancer agents (e.g. squalene and terpenoids) (Fabiani et al. 2002; Owen et al. 2004).

The European Union has developed a PDO (Protected Designation of Origin) assignation to olive oils with important regional traditional origins. Oil quality is strongly cultivar-dependent, but is also affected by agro-climatic factors and agronomic practices.

Various categories of olive oil have been defined (Reg. CEE 1513/01):

- *virgin oil:* oil produced by mechanical or other physical means under conditions (e.g. temperature) that do not lead to any chemical alteration in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation and filtration. Within this category is included the 'extra virgin olive oil': virgin olive oil which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams, and the other character-

istics of which correspond to those fixed for this category in this standard;
- *refined oil:* oil obtained from virgin oil by refining methods which do not lead to an alteration in the initial glyceric structure;
- *olive oil*: oil consisting of a blend of refined and virgin olive oil;
- *olive-pomace oil*: oil obtained by treating olive pomace with solvents, to the exclusion of oil obtained by re-esterification processes and of any mixture with oils of other kinds.

Two types of adulteration have been identified: blending of virgin olive oils with olive oils of lower grade, and mixing olive oils with other vegetable oils. Mislabeling of olive oils is of considerable concern, as this results in the product not being of the claimed grade (Lai et al. 1994; Yoke et al. 1994; Spangenberg and Ogrinc 2001). The International Olive Oil Council (1993) and the Codex Alimentarius Commission (1993) have therefore produced standards for virgin, refined and olive-pomace oils. Instruments such as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Guaranteed (TSG) are all important for quality control in this context.

13.1.4 Breeding Objectives

Primary goals in olive breeding are directed towards overcoming current limiting factors for production. These include: shortening the juvenile stage; increasing fruit number and size; increasing oil content and quality (fatty acid composition, polyphenol content, etc.); stabilising yield; dwarfing, and other manipulations of tree architecture to facilitate mechanical pruning and harvesting; improving resistance to pests (in particular olive fruit fly, *Bactrocera oleae*) and diseases (leaf peacock spot, caused by *Spilocaea oleagina*; Verticillium wilt, *Verticillium dahliae*; and olive knot, *Pseudomonas savastanoi*). Other important objectives relate to improvement in cold tolerance (to allow cultivation in more northerly areas) and to the promotion of self-fertility (to reduce reliance on pollinators). Tree architecture and vigour are particularly important because the height of the tree prevents mechanical harvesting and pruning, thereby increasing the costs of cultivation. Although the olive is generally considered to be a drought-tolerant species, its productivity is strongly reduced under drought conditions, and thus there is interest in the possibility of tolerant cultivars, as well as those that can thrive on saline and heavy soils. Rootstock selection is focused on the ability to control scion vigour, and to improve the level of resistance to biotic and abiotic stresses.

13.1.5 Breeding Achievements

In spite of its economic importance to all Mediterranean countries, there has been little directed olive breeding to date, despite the pressing need to improve productivity and agronomic performance. Most selection programs have so far relied on clonal selection, on the assumption that in a long-living plant such as olive, natural mutations generating any positive alteration in a trait of agronomic interest, can be maintained by vegetative propagation (Rallo 1995; Belaj et al. 2004). Exploration of phenotypic variability in agronomic characters has led to the identification of valuable clones within numerous olive cultivars (Suárez et al. 1990; Lavee et al. 1995; Bartolini et al. 2002; Grati-Kammoun et al. 2002). However, in spite of the significant efforts made towards clonal selection, very few clones have outstanding performance (Loussert and Berrichi 1995; Tous et al. 1998). Similarly, induced mutagenesis has not been encouraging, and so far has succeeded in producing only a compact mutant of the cv. Ascolana Tenera (Roselli and Donini 1982). The evaluation of minor local cultivars, present in every cultivation area, has recently been exploited to identify individuals highly adaptive to extreme environmental conditions (Pannelli et al. 2003; Rotondi et al. 2003). Clonal rootstocks with high rooting ability have been identified from crossbred populations (Baldoni and Fontanazza 1990), and other selected rootstocks have shown ability to control scion vigor and resistance to frost injury (Pannelli et al. 2002). The use of the cvs. Souri, Muhasan and Barnea as rootstocks under dry conditions, after 10 years from planting, did not show any significant effect on tree vigor, shape and fruit production (Lavee and Schachtel 1999).

Experiments of genetic transformation are in progress with the aim to select disease resistant cultivars or to introduce key genes involved in important metabolic pathways (Rugini et al. 2000; Rugini and Baldoni 2004).

The long generation time has severely hindered both classical breeding and genetic studies (De la Rosa et al. 2003). It is possible to greatly reduce the length of the juvenile phase by using forcing protocols, but the evaluation of the agronomic performance of mature plants still requires at least five years of experimentation (Santos Antunes et al. 1999). Furthermore, the genetic control of the major traits is unknown (De la Rosa et al. 2003). Vigor, leaf size and fruit shape seem controlled by major genes showing dominance (Bellini 1993), while the inheritance of other characters, such as fruit size, flowering intensity, fruit set, ripening time and yield remains uncertain (Bellini 1993; Parlati et al. 1994). Very few cultivars have been emerged from formal breeding programs.

A new cultivar (Maalot) resistant to *Spilocea oleagina* has been selected from the selfed F_1 progeny of a semi resistant seedling probably of Chemlali (Lavee et al. 1999). From seedling populations obtained by unknown parents two other cultivars were selected: 'Barnea', with vigorous and upright growth, and 'Kadesh', as a table olive (Lavee 1978; Lavee et al. 1986).

Three new olive cultivars (Arno, Tevere and Basento) were released from the progeny of the cross 'Picholine × Manzanilla' (Bellini et al. 2002) and their performance is still under evaluation.

The University of Adelaide has recently established a selection program utilizing the plant olives locally reproduced from cultivars previously introduced in Australia and well adapted to that environment. The aim of the project is the identification of new improved olive cultivars showing superior morphological and oil characteristics (Sedgley 2000).

13.2 Construction of Genetic Maps

The first linkage map of the olive genome was based on RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) dominant markers, along with a small number of codominant RFLPs (restriction fragment length polymorphisms) and SSRs (simple sequence repeats) (De la Rosa et al. 2003). The mapping population consisted of a progeny derived from two highly heterozygous cultivars, Leccino and Dolce Agogia. The Leccino map covered 2,765 cM and comprised 249 markers, falling into 22 major and 17 minor linkage groups (the latter each involving less than four markers). The Dolce Agogia map was of similar length (2,445 cM) and comprised 236 markers arranged in 27 major and three minor linkage groups. Mean inter-marker distances were similar in both maps (13.2 cM in Leccino and 11.9 cM in Dolce Agogia). AFLP and RAPD markers were homogeneously distributed across all of the linkage groups. Based on the olive genomic size, estimated around 3,000 cM (Wu et al. 2004), the Leccino × Dolce Agogia map is thought to have covered about 80% of the genome. A second linkage map was constructed by Wu et al. (2004), based on RAPDs, SCARs and SSRs exploiting the progeny of a cross between the cultivars Frantoio and Kalamata. The greater use of codominant markers allowed the integration of the two parental maps to generate 15 linkage groups, covering 101 loci and 879 cM with a mean inter-marker distance of 10.2 cM.

In situ hybridization using tandem repeated sequences has allowed most of the olive chromosomes to be distinguished, and has also revealed structural heterozygosity in three chromosome pairs (Minelli et al. 2000).

At present, no further olive genome mapping data are available, and as yet, no QTL have been detected, neither is there any detailed analysis on genome organization.

13.3 Gene Mapping

Mapping of gene sequences has concentrated on orthologous genes characterized in other species (Table 1). Particular attention has focused on genes encoding key enzymes involved in fatty acid biosynthesis, modification, triacylglycerol synthesis and storage. These include enoyl-ACP reductase (*ear*), stearoyl-ACP desaturase, omega 6 plastidial desaturase (*fad6*), omega 3 plastidial desaturase (*fad7*), cytochrome b5 (*cyt b5*), omega 6 cytoplasmic desaturase (*fad2*), omega 3 cytoplasmic desaturase (*fad3*), acyl-CoA:diacylglycerol acyltranferase (DGAT) and oleosin (Hatzopoulos et al. 2002). The temporal and transient expression of stearoyl-ACP desaturase (a key enzyme for the conversion of 18:0 stearic acid to 18:1 oleic acid, the main component of olive oil) has been studied during fruit development (Haralampidis et al. 1998). Expression of a cDNA encoding an ω-3 fatty acid desaturase

Table 1. Genes and expressed sequences identified in olive

GenBank Accession Number	Gene encoding for	Authors (Year of publication)	Length (bp)
AJ536118	Partial putative copia retrotransposon RNaseH gene and gene encoding retrotranscriptase	Natali L, Giordani T, Maestrini P, Cavallini A (2005)	1,164
AJ536119	Partial putative copia retrotransposon RNaseH gene and putative LTR	Natali L, Giordani T, Maestrini P, Cavallini A (2005)	499
AJ536120	Partial putative gypsy retrotransposon RNase gene, and genes encoding retrotranscriptase and integrase	Natali L, Giordani T, Maestrini P, Cavallini A (2005)	1,930
AY772187	Fatty acid desaturase 6 (fad6)	Moressis A, Banilas G, Hatzopoulos P (2005)	1,597 (complete cds)
AJ810085	Beta-1,3-glucanase (glu-4 gene)	Caliente R, Barea J, Azcon C, Ferrol N (2004)	1,032
AJ810086	Beta-1,3-glucanase (glu-5 gene)	Caliente R, Barea J, Azcon C, Ferrol N (2004)	643 (partial cds)
AY445635	Acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1)	Giannoulia K, Hatzopoulos P (2004)	1,836 (complete cds)
AY738639	Phenylalanine ammonia-lyase (PAL) gene	Tosti N, Baldoni L (2004)	713 (partial cds)
AY788899	Actin	Tosti N, Baldoni L (2004)	427 (partial cds)
AY083161	Oleosin	Giannoulia K, Haralampidis K, Milioni D, Hatzopoulos P (2002)	792 (complete cds)
AY083162	Beta-glucosidase (bglc)	Gazis F, Hatzopoulos P (2002)	1,902 (complete cds)
AY083163	Fatty acid desaturase 2 (fad2)	Nikoloudakis N, Hatzopoulos P (2002)	1,452 (complete cds)
AY083164	Enoyl ACP reductase (ear)	Poghosyan Z, Hatzopoulos P (2002)	1,674 (complete cds)
AJ428575	Cu/Zn super-oxide dismutase	Butteroni C, Afferni C, Tinghino R et al. (2002)	459
AY095446	Photosystem II protein D1 (PSBA)	Muleo R, Proietti C, Paolucci I et al. (2002)	942 (partial cds)
AF492010	Monosaccharide transporter (MST)	Oliveira JM, Geros HV, Tavares RM (2002)	726 (partial cds)
AF479171	26S ribosomal RNA gene	Soltis DE, Senters A, Kim S et al. (2002)	1,603 (partial sequence)
AF428256	Acyl carrier protein (ACP)	Guerrero CM, Valpuesta V, Baldoni L (2001)	763
AJ416434	Ty1-copia-like retrotransposon, Toe21 gene	Stergiou G, Katsiotis A (2001)	264
L49289	18S ribosomal RNA gene	Johnson LA, Soltis DE, Soltis PS (2001)	1,729 (partial sequence)
AF426829	Cu/Zn-superoxide dismutase	Corpus FJ, Barroso JB, Romero-Puertas MC et al. (2001)	312 (partial sequence)
AY040811	trnT-trnL intergenic chloroplast spacer	Baldoni L, Guerrero CM, Abbott AG et al. (2001)	658
AF384051	Expansin	Ferrante A, Hunter DA, Reid MS (2001)	486 (partial cds)
AF384050	Anthocyanidin synthase	Ferrante A, Hunter DA, Reid MS (2001)	789 (partial cds)
AF384049	Chalcone synthase	Ferrante A, Hunter DA, Reid MS (2001)	571 (partial cds)
AY059387	Putative cullin protein	Butowt R, Rodriguez-Garcia MI (2001)	2,637
AF429429/AF429430	Polyubiquitin OUB1 and OUB2	Butowt R, Rodriguez-Garcia MI (2001)	1,184/1,666
AF427107	Manganese superoxide dismutase	Corpus FJ, Barroso JB, Romero-Puertas MC et al. (2001)	435 (partial cds)
AY036055	Hexose transporter pGlT	Butowt R, Rodriguez-Garcia MI (2001)	2,039

Table 1. (continued)

GenBank Accession Number	Gene encoding for	Authors (Year of publication)	Length (bp)
AF130163	NADH dehydrogenase subunit F (ndhF)	Olmstead RG, Kim KJ, Jansen RK, Wagstaff SJ (2000)	2,217
AF288707	Cytochrome c oxidase subunit I (cox1) mitochondrial gene	Zilhao IT, Tenreiro RP, Fevereiro PS (2000)	447 (partial cds)
AF225275	Ribosomal protein S16 (rps16) chloroplast gene	Wallander E, Albert VA (2000)	853 (partial intron)
AF191342	Copper/zinc superoxide dismutase (SOD1)	Alche JD, Castro AJ, Rodriguez-Garcia MI (1999)	276 (partial cds)
AB025343	Lupeol synthase	Shibuya M, Zhang H, Endo A et al. (1999)	2,546
AB025344	Cycloartenol synthase	Shibuya M, Zhang H, Endo A et al. (1999)	1,983 (partial cds)
AJ236163	ATP synthase beta subunit	Albach DC, Soltis PS, Soltis DE, Olmstead G (1998)	1,493
Z70240/Z70241	Cytochrome oxidase; subunit 3, cox3 gene	Perrotta G, Cavallotti A, Quagliariello C (1997)	1,817/1,818
AJ001766	Chloroplast ribulose 1,5-bisphosphate carboxylase large subunit (rbcL) gene	Oxelman B, Backlund M, Bremer B (1997)	1,402 (partial cds)
AJ001369/AJ001370	Cytochrome b5 genes 1 and 2	Martsinkovskaya AI, Poghosyan ZP, Haralampidis K et al. (1997)	688/752
AF027288	NADH dehydrogenase (ndhF) chloroplast gene	Oxelman B, Backlund M, Bremer B (1997)	2,193 (partial cds)
U58141	Stearoyl-ACP desaturase	Baldoni L, Georgi LL, Abbott AG (1996)	1,493
AF511041	Retrotransposon	Muleo R, Intrieri MC (2002)	484 (partial sequence)
AY095446	PSBA gene	Muleo R, Proietti C, Paolucci I et al. (2002)	942 (partial cds)

has been studied in leaves, anthers and embryos (Poghosyan et al. 1999), and two cytochrome b_5 genes and their spatial and temporal patterns of expression have been characterized during flower and fruit development (Martsinkovskaya et al. 1999). The differential expression of other genes such as diacylglycerol acyltransferase (DGAT) and oleate desaturase has been evaluated in various tissues (Giannoulia et al. 2000; Banilas et al. 2005). Finally, a candidate stearoyl-ACP desaturase was mapped on linkage group 4 of cv. Leccino (De la Rosa et al. 2003).

13.4 Marker-Assisted Breeding

The very preliminary works performed on olive genomics are far before producing effective results toward the selection of new cultivars by the use of molecular tools.

For that reason and considering the lack of knowledge on the real useful variability already present in the cultivated and wild olive germplasm, attention has been focused in the last ten years mainly on the evaluation of such germplasm. The large number of cultivars and wild populations, in fact, positions olive as a crop species with a very extensive germplasm. The geographic distribution of variability within the *Olea* genus and the genetic relationships among the different species have been studied using various molecular methods, including cpDNA profiles (Lumaret et al. 2000; Baldoni et al. 2002), AFLPs (Angiolillo et al. 1999; Baldoni et al. 2000), and rDNA and mtDNA polymorphisms (Besnard and Bervillé 2002; Besnard et al. 2002a, 2002b). The wild relatives of cultivated olive (oleasters) have been widely analysed using RFLP markers derived from mitochondrial, chloroplast and nuclear DNA, which, in addition to allozyme markers, provide evidence for the survival of indigenous oleaster populations, particularly in the western Mediterranean (Lumaret and Ouazzani 2001; Lumaret et al. 2004). Within wild populations, a clear distinction between the eastern and western Mediterranean has been noted (Besnard and Bervillé 2000; Besnard et al. 2002b; Bronzini de Caraffa et al. 2002).

Internal transcribed spacer 1 (ITS-1) sequences, RAPD and inter-SSR (ISSR) markers have been deployed to evaluate the colonization history of *O. europaea* (Hess et al. 2000). Some *Olea europaea* retroelements have also been identified (Hernandez et al. 2001) and their copy number has been estimated (Stergiou et al. 2002).

The development of SCAR markers has been attempted from RAPDs (Hernandez et al. 2001), and one such has been reported by Mekuria et al. (2001) to be linked to tolerance to leaf peacock spot.

DNA fingerprinting is a powerful aid for the identification of olive oil provenance, since it can be used to generate a profile specific for any given plant genotype. Over the last decade, molecular markers have been widely applied also to characterize and identify olive cultivars. These analyses have utilised RAPDs (Fabbri et al. 1995; Belaj et al. 1999; Mekuria et al. 1999; Barranco et al. 2000; Gemas et al. 2000; Belaj et al. 2001; Besnard et al. 2001c; Belaj et al. 2002; Guerin et al. 2002), AFLPs (Angiolillo et al. 1999; Rotondi et al. 2003; Owen et al. 2005; Montemurro et al. 2005), ISSRs (Hess et al. 2000; Pasqualone et al. 2001; Vargas and Kadereit 2001) and SSRs (Rallo et al. 2000; Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; Bandelj et al. 2004). The same methods have also been applied to trace the geographic origin of batches of olive oil (Muzzalupo and Perri 2002; Busconi et al. 2003; Breton et al. 2004; Pasqualone et al. 2004; Testolin and Lain 2005).

Single Nucleotide Polymorphisms (SNPs) are currently under development (Reale et al. 2006) in order to clearly distinguish inter-cultivar variability and characterize the clonal variants.

13.5 Future Scope of Works

Projects are currently under development in order to address gaps in genetic mapping and molecular breeding in olive. Three main areas of interest can be resumed:

1) completing the research on the evaluation, characterization and utilization of the available genetic resources, both on cultivated varieties and wild relatives;
2) continuing the project on genomic, functional and physical mapping;

3) establishing new breeding programs and completing those in progress by the extended application of marker-assisted selection.

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