Chapter 14
Strategies for Olive (*Olea europaea* L.) Breeding: Cultivated Genetic Resources and Crossbreeding

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Abstract Olive cultivars represent an invaluable heritage of genetic variability selected over more than 5500 years of cultivation. This high diversity of local cultivars is a common feature in traditional olive-producing countries. Most cultivars are old and continue to be cultivated around areas where they have likely been selected. Crossbreeding in olives was only initiated in the second half of the twentieth century and currently represents the most promising strategy to provide farmers with new cultivars that are well adapted to the new high density olive plantations spreading

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in traditional and new olive-growing countries. This chapter focuses on cultivated genetic resources and crossbreeding strategies in olive. Exploration, cataloguing and authentication for the conservation and sustainability of true-to-type cultivars by morphological and DNA markers in the Network of Germplasm Banks promoted by the International Olive Council, is the most extensive and worldwide initiative to date. The strategies, methodologies and advances in crossbreeding programs worldwide are reviewed. Shortening the juvenile period, early selection and other strategies for the evaluation of valuable agronomical traits are integrated into the framework of alternative protocols that also provide information regarding the variability and heritability of these traits. In addition, the possibilities provided by new genomics tools to shorten the protracted crossbreeding process are also presented. Finally, new developments on in vitro culture and genetic transformation as well as the feasibility of using these tools in breeding programs are discussed.

Keywords *Olea europaea* · Clonal selection · Cryopreservation · Biotechnology Genomics · In vitro regeneration · Morphological descriptors · Molecular markers

14.1 Introduction

Olive (*Olea europaea* L.) is a Mediterranean tree species that was probably domesticated in the Middle East and in Central Mediterranean approximately 5500 years B.P. Since that time, the crop has expanded along both shores of the Mediterranean Sea. Olive growing was well-established in Roman times, as witnessed by the agricultural treatises of Pliny and Columela. Beginning in the fifteenth century, the transoceanic voyages of Christopher Columbus, Ferdinand Magellan and Juan Sebastián Elcano helped olives reach and spread throughout the New World. They are currently also grown in South Africa, China, Japan and Australia.

Empiric local selection within wild olives and crosses between the previous selected or introduced cultivars, and other local cultivars or wild olives, in all growing areas have yielded a huge number of local cultivars. This high diversity of local cultivars is therefore a common trait in traditional olive-producing countries. Most cultivars are old and are cultivated around areas where they were likely selected. In most cases, cultivars are self-rooted. Grafted trees are found only in the case of difficult-to-root cultivars or due to top grafting onto wild olives or onto other obsolete cultivars (Díez et al. 2015). Most cultivars are exclusively used for oil production. However, certain cultivars are used for table olives, and others may be used for both oil and table olives.

According to data published by the International Olive Council (IOC); (www.international.oliveoil.org), the world's olive-growing area reached approximately 11.4 million ha in 2015, which was mostly located (>96%) in the Mediterranean Basin. Seventy-eight percent of this area is rainfed, and 22% is irrigated. Nonbearing trees represent 13% of the total orchards. The average production and consumption per year for the periods 2012/2013 to 2016/2017 was approximately 2,760,000 and

2,951,700 mt of olive oil, respectively. For table olives, the average production and consumption for the same period were 2,632,100 and 2,542,300 mt, respectively. Production and consumption are currently balanced; the large price variations between years and seasons are usually due to the biennial bearing habit of olive crops.

Olives have traditionally been a long-lived rainfed crop that is well adapted to the Mediterranean climate, which is characterized by a low and irregular yield, a high demand for labor for harvesting, empirical technology and low investment. However, since World War II, olive growing has been changing in both Northern Mediterranean countries and in new olive-growing regions. Human migration from rural to urban areas has required more productive and mechanized orchards. The accumulated changes since that time have led to new olive plantations (Fig. 14.1). These new olive orchards bear earlier; however, their overall life span is shorter than in the past. Improved agricultural practices to avoid soil erosion and contamination of the environment are increasingly being employed. Annual cultural practices are being simplified, and pruning and harvesting have become more mechanized. Yield has increased through the implementation of irrigation and high-density plantations (Fig. 14.1). In many countries, traditional and local practices of selection and propagation of cultivars by farmers are being replaced by a few cultivars multiplied by nurseries. Finally, olive oil is now considered an important agricultural product for health, and its consumption has steadily increased. Extensive and comprehensive books on olive growing have been published in Italy (Fiorino 2003) and Spain (Barranco et al. 2017). An earlier edition of the latter book has been translated into English (Barranco et al. 2010).

During this time of change, the conservation and sustainable use of genetic resources and breeding have become necessary. For the first time in history, olive growers face an increasing risk of genetic erosion, and coordinated efforts to establish an International Network on Olive Germplasm Banks have been developed by the IOC since 1994 (Rallo et al. 2011). Additionally, the need for new improved cultivars has prompted the first olive-breeding programs in the world (Bellini et al. 2002a; Lavee 1990). Furthermore, recent advances in genomics and biotechnology (Rugini et al. 2016) will allow early genetic selection of progenies and the development of transformation protocols. This review focuses on the state of the art of the conservation of cultivated germplasm and breeding with a particular focus on crossbreeding and the pervasive concern of how to reduce the time needed to create and release new cultivars.

14.2 The Genetic Resources: First Strategy for Breeding

Exploration, conservation, evaluation and sustainable use of genetic resources have become a priority in most plant species of interest for agriculture and food. The diversity of cultivars in olive-growing countries represents a legacy of genetic variability from more than 5500 years of cultivation for the breeding and future growth of olives.

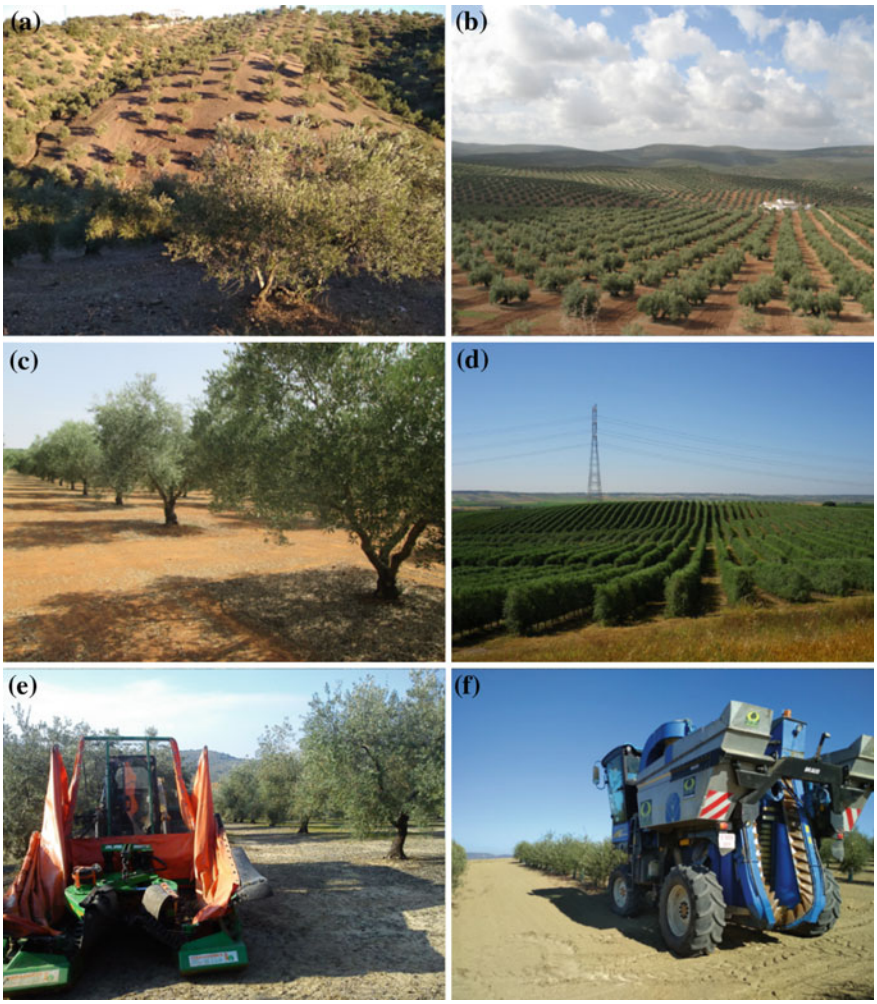

Fig. 14.1 Traditional and new olive plantations. **a** Traditional rainfed orchard on high-slope soil, **b** Traditional rainfed orchard on low-slope soil, **c** Intensive high density irrigated orchard, **d** Narrow hedgerow (super intensive) orchard, **e** Mechanical harvest in intensive orchard, **f** Mechanical harvest in super intensive orchard. Photos **a**, **c**, **e**, **f** by P. Valverde; **b** by D. Barranco; **d** by D. Cabello

A recent, extensive and updated review of olive genetic resources (Belaj et al. 2016) stated, as a first conclusion, that "*A better knowledge, management, and exploitation of cultivated, wild, and ancient trees are still needed, establishing common protocols for the molecular identification and for cultivar naming*,". This section will focus on the works exploring the conservation of true-to-type cultivars, which have been historically used as the first strategy for breeding. However, this critical aspect is still missing in olive cropping. Furthermore, true-to-type cultivars repre-

sent an urgent and compulsory requirement for olive growing in the world due to the global exchange of plant material. Within the requirement of trueness to type, we also review the works on *clonal selection* in olive.

For other aspects of the management of genetic resources, we refer the reader to the above-cited review (Belaj et al. 2016).

14.2.1 Exploring and Cataloguing Cultivars

To date, efforts to explore and catalogue true-to-type olive cultivars in different countries are incomplete. Until the beginning of the twentieth century, only a few scattered studies on olive cultivars had been published. At the VII Congreso Internacional de Olivicultura (olive growing) held in Seville in 1924, Coupin proposed a world catalogue of cultivars, their agronomic evaluation, and the need to breed new cultivars. However, catalogues of true-to-type cultivars are still pending in many countries; the common drawback of establishing synonyms and homonyms has not yet been solved in most cases. Furthermore, agronomic evaluation of cultivars is fragmentary and confusing because traits are assigned to varietal denominations (accessions) rather than to specific cultivars (Bartolini et al. 1998). As such, olive breeding is still in its infancy.

14.2.2 Olive World Germplasm Bank (OWGB) of Córdoba, Spain

The case of exploring and cataloguing true-to-type olive cultivars in Spain represent a long-lasting work on the exploration, cataloguing and conservation of true-to-type cultivars (Rallo et al. 2005). Initial works before the 1970s (Priego 1935) demonstrated incomplete sampling and a lack of representativeness of the characterized plant material, insufficient description methodologies and confusion in cultivar naming.

The Olive World Germplasm Bank of Cordoba (OWGB) was established in 1970 on the Alameda del Obispo Farm of INIA (National Institute for Agrarian Research), as a first attempt toward the conservation of olive cultivated genetic resources through an FAO-INIA project. Since the beginning, this collection has been cared for by INIA and the University of Córdoba (UCO). The collection was enlarged through prospecting surveys and cataloguing of cultivars in Andalucía (Barranco and Rallo 1984), Spain (Barranco et al. 2005a) and other international prospecting surveys and by exchanges with other germplasm banks (Caballero et al. 2006). The Alameda del Obispo collection was incorporated into the Andalusia Institute for Agricultural and Fishing Research (IFAPA in 2003 (Fig. 14.2a) and new exchanges with other banks have increased its accessions (Belaj et al. 2016). In 2011, a duplicate of true-to-type

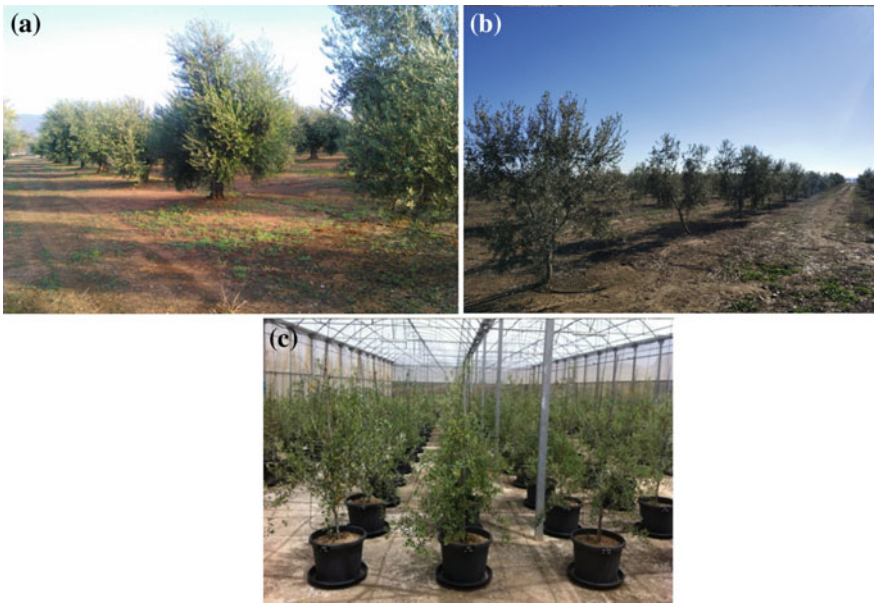

Fig. 14.2 The Olive World Germplasm Bank (OWGB) of Córdoba, Spain. **a** Collection in Alameda del Obispo Farm (IFAPA), **b** Collection in Rabanales Farm (UCO), **c** Isolated repository of true-to-type and pathogen-free cultivars (CAP-UCO-IFAPA). See text above. Photos: **a** by P. Valverde; **b**, **c** by P. Morello

cultivars of this collection was established in the UCO experimental Rabanales Farm in a soil free from *Verticillium dahliae* to guarantee the conservation of the cultivated genetic resources in the OWGB (Fig. 14.2b). In 2009, UCO, in cooperation with the CAP (Andalusia Agricultural and Fishing Administration) and IFAPA, established an isolated repository to conserve true-to-type and pathogen-free authenticated commercial accessions of the Alameda del Obispo collection (Fig. 14.2c). An agreement between the CAP, UCO and IFAPA for the management of the OWGB was signed in 2013. The OWGB was acknowledged by the International Olive Council (IOC) as an International Bank of Reference of its Network of Germplasm Banks in 2015.

14.2.3 Identification and Authentication

The identification of existing cultivars represents the first step in their cataloguing. Naming is the main difficulty in olive-cultivar identification because it has been historically based on common morphological traits (particularly of the fruit), toponyms or practical uses of varieties (Barranco and Rallo 1984; Barranco et al. 2005a). Until the 1980s, only morphological descriptors were used for identification purposes. The effect of environment on the expression of these traits was the main shortcom-

ing for the use of these characteristics. However, morphological descriptors have been a useful tool for naming true-to-type cultivars, synonyms, homonyms and inaccurate denominations for the most explored and sampled genotypes in Andalusia and Spain (Barranco and Rallo 1984; Barranco et al. 2005a), also establishing criteria for unambiguously naming genotypes carrying the same name (homonyms). Both catalogues represent the most complete morphological characterization of Spanish cultivars listing true-to-type cultivar names, homonyms, synonyms and inaccurate denominations. To distinguish among different genotypes carrying the same name, these authors proposed to add to the generic name of the cultivar the site of main diffusion (e.g. Manzanilla de Sevilla, Manzanilla de Jaén and Manzanilla Cacereña). True-to-type cultivars catalogued in this work were planted in the ex situ collection at Alameda del Obispo in Córdoba.

The use of molecular markers for genotyping olive cultivars started with isozymes in the 1980s (Pontikis et al. 1980; Trujillo et al. 1995). Molecular markers have provided a powerful tool to manage ex situ germplasm and distinguish between genotypes. The advent of DNA markers and their use for genotyping olives started in the mid-1990s with random amplified polymorphic DNA (RAPD) (Bogani et al. 1994; Fabbri et al. 1995).

Belaj et al. (2016) reviewed the use and DNA markers for molecular identification, the most used tool for studies of genetic variability worldwide. Their use has facilitated the management of the OWGB (CAP-UCO-IFAPA) (Table 14.1), evidencing duplications, synonyms and homonyms (Atienza et al. 2013; Belaj et al. 2003a, c; Fendri et al. 2010; Noormohammadi et al. 2007; Trujillo et al. 2014). The complementary use of SSRs and morphological characteristics of the endocarp have proved to be a powerful and discriminant method to authenticate accessions (true-to-type cultivars), duplications, homonyms, synonyms and inaccurate denominations in the OWGB of Córdoba (Trujillo et al. 2014). Comparison of endocarps between genotypes and true-to-type control samples allows the discrimination of molecular variants of a cultivar (i.e. genotypes with very high SSR similarity and undistinguished by endocarp morphological traits) and different cultivars (genotypes with a very high SSR similarity and very different endocarp characteristics) as shown in Fig. 14.3.

14.2.4 The International Olive Council (IOC) Network

Prospecting surveys in many countries and the exchanges of cultivars between countries have contributed to the very high number of conserved accessions in ex situ collections. Bartolini et al. (1998, 2008) reviewed for FAO the accessions conserved in approximately 100 regional and national collections in 53 countries, which include more than 4000 accessions. This inventory is indicative of the cultivated accessions conserved worldwide. However, the criteria for the choice of accessions are diverse, and their identification and authentication are still lacking in most collections. We currently do not know whether such accessions correspond to true-to-type cultivars

Table 14.1 Works on the exploration, cataloguing, conservation and authentication of olive cultivars jointly developed by the University of Córdoba and the Andalusia Institute for Agricultural and Fishing Research (IFAPA)

Years	Description	References
From 1971	*Exploration, cataloguing and conservation* Exploration (511 sampled trees in 83 localities), cataloguing by morphological descriptors (55), establishment of differentiated cultivars (156) and of synonyms and homonyms between the initial names (197) and conservation of Andalusia cultivars in the OWGB (CAP-UCO-IFAPA) of Córdoba	Barranco and Rallo (1984)
	Exploration in the rest of Spain (489 sampled trees in 196 localities) cataloguing by morphological descriptors (28), establishment of differentiated cultivars (262) and of synonyms and homonyms between the initial names (501) and conservation of Spain cultivars in the OWGB (CAP-UCO-IFAPA) of Córdoba	Barranco et al. (2005a)
	Exploration and genetic diversity of monumental olives in Andalusia and wild olives in Spain	Belaj et al. (2007, 2010, 2011), Diez et al. (2011, 2015)
From 1996	*Use of Molecular Markers for studies on genetic variability* Isozymes. First study on the genetic variability of the current accessions of the OWGB of Córdoba (CAP-UCO-IFAPA)	Trujillo et al. (1995)
	RAPDs. Further studies on the genetic variability of the current accessions of the OWGB of Córdoba (CAP-UCO-IFAPA)	Belaj et al. (2001, 2002, 2004b, c)
	RAPDs and AFLPs. Intra-cultivar variability in Manzanilla de Sevilla and Arbequina	Belaj et al. (2004a)
	SSRs Studies on the management of genetic variability in accessions of the OWGB of Córdoba (CAP-UCO-IFAPA)	Atienza et al. (2013), Belaj et al. (2012), Muñoz-Diez et al. (2012), Rallo et al. (2000a)
	Authentication 332 different cultivars identified from 823 trees representing 499 accessions from 21 countries of the OWGB of Córdoba (CAP-UCO-IFAPA) were characterized with 33 SSRs and 11 morphological characteristics of the endocarp. 200 cultivars were authenticated by comparison of SSRs and endocarp profiles with authentic control samples. 130 SSRs genotypes were considered molecular variants because they showed minimal molecular differences, but the same morphological profile, compared with 48 catalogued cultivars. 15 previously described and 37 new cases of synonyms as well as 26 previously described and seven new cases of homonyms were reported	Trujillo et al. (2014)
	Core collections SSRs, DARTs SNPs and morphological traits. Accurate definition of genetic variability and definition of core collections in the OWGB	Atienza et al. (2013), Belaj et al. (2012), Muñoz-Diez et al. (2012)

True-to-type cultivars are conserved in the Olive World Germplasm Bank (OWGB) of Córdoba

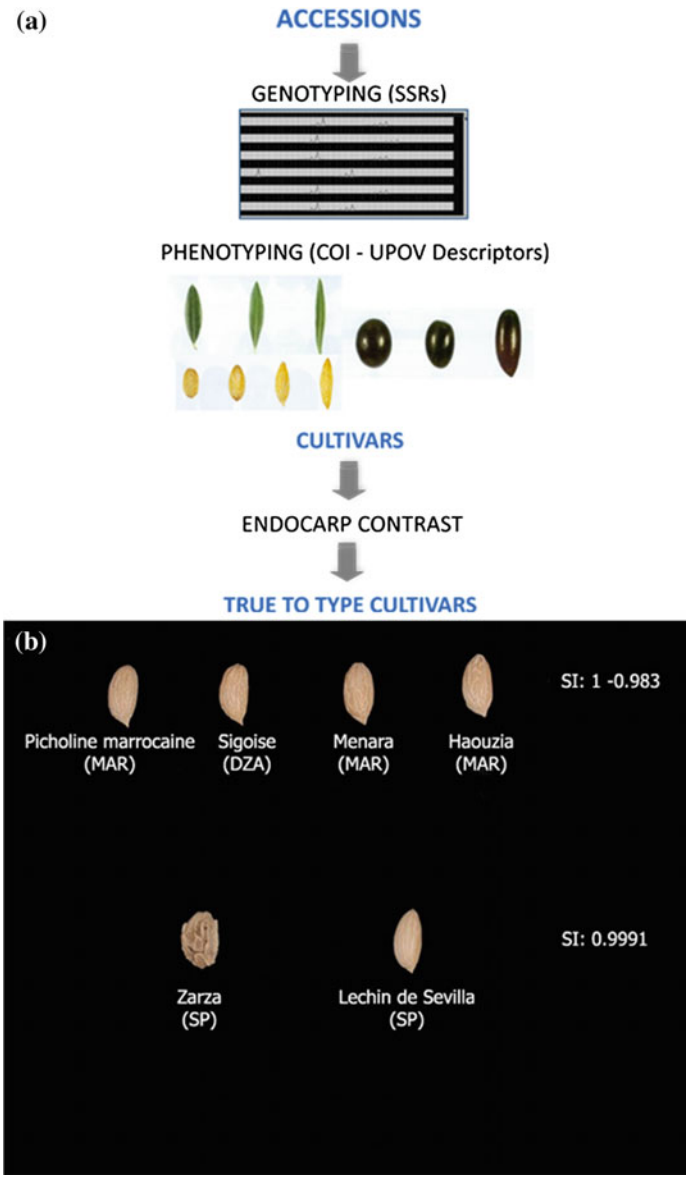

Fig. 14.3 Identification **(a)** and authentication **(b)** in the Olive World Germplasm Bank (OWGB) of Córdoba. Photo **b** from: Trujillo et al. (2014) used by permission of Springer

and both corresponding homonyms, synonyms and inaccurate denominations. Consequently, data on the phenotyping of many agronomic traits in these collections are not concordant for the same denominations, thus generating confusion. This finding

demonstrated a need to authenticate the accessions as a first step in any collection to avoid inconsistent phenotyping data in many publications based on the names of the accessions in any collection.

Since 1994, the IOC has promoted a network that currently includes National Germplasm Banks in 22 countries focusing on the exploration and conservation and cataloguing of local cultivars. The network conserves more than 1100 accessions, which were collected and have been partially characterized http://www.internationaloliveoil.org/resgen/index.html). The IOC published a first partial World Catalog of Cultivars (Barranco et al. 2000), an incentive publication of some additional national catalogues (Barranco et al. 2005a; Hosseini-Mazinani et al. 2013; Mendil and Sebai 2006; Moutier et al. 2004; Muzzalupo et al. 2010; Trigui et al. 2002, 2006).

The IOC has recognized three International Olive World Germplasm Banks in Córdoba, Spain, Marrakech, Morocco and Izmir, Turkey. These banks are enriched by the interchange of material with other banks, particularly those of the IOC Network. Currently, the OWGB of Córdoba conserves more than 1000 accessions from 25 Countries (Belaj and Barranco, pers comm), the OWGB of Marrakech conserves 591 accessions that have been partially identified (El Bakkali et al. 2013; Haouane et al. 2011) and the OWGB of Izmir has established 183 identified cultivars from 13 different countries to date (Gurbuz, pers comm).

Currently, the IOC aims to authenticate the accessions of all collections and to conserve them true-to-type and pathogen-free in all the acknowledged National Germplasm Bank of its network. Thus, the IOC has recently proposed a project to the UCO to cooperatively authenticate the accessions of the National Germplasm Banks by the morphological and SSR protocol proposed by Trujillo et al. (2014). This initiative will also guarantee the absence of devastating effects by diseases such as verticillium wilt (Jiménez-Díaz et al. 2012) and *Xylella fastidiosa* (Saponari et al. 2013). Thus, this project will ensure, over the short term, the conservation of true-to-type and pathogen-free of: (a) main commercial cultivars of the world in a first step. Afterwards other projects will progressively authenticated accessions in the IOC Networks.

14.2.5 Clonal Selection

Most of the traditional olive varieties grown today are the result of the selection of singular seedlings from wild, feral or cultivated trees with outstanding characteristics such as a larger fruit size, greater production, higher oil content or adaptation to certain climatic zones, among others, and the clonal propagation of those trees (Barranco and Rallo 1984). All current cultivars are in fact selected clones.

There is evidence that cultivars often contain somatic mutations. This phenomenon has been described in several fruit crops, such as grape, apple, peach, pear and plum (Badenes and Byrne 2012). Somatic mutations might not have phenotypical consequences but they might also become the basis for new varieties, as it has been

the case for citrus and grape, among others (Moore 2001; Pelsy et al. 2010; This et al. 2006).

Somatic mutations have also been reported in olives by using different molecular markers such as RAPD, AFLP and SSR (Charafi et al. 2008; Cipriani et al. 2002; Diez et al. 2011; Garcia-Diaz et al. 2003; Nikoloudakis et al. 2003; Trujillo et al. 2014). These mutations were responsible for subtle genetic differences among several genotypes and their closest cultivar (Diez et al. 2011; Trujillo et al. 2014). No morphological differences were observed among these genotypes and they were considered *molecular variants* of the standard genotype (Trujillo et al. 2014).

Variability within a cultivar due to somatic mutations should not be confused with a case of homonym; this is the existence of different cultivars sharing the same name. Clonal mutations happen at a very low rate and normally affect few loci (Heinze and Fussy 2008; Sarkar et al. 2017) leading to very close genetic similarity indexes (similarity index ~0.8 to 0.9) between the clones and the cultivar from which they derived. Because of this reason, the description of extensive genetic variation within a putative cultivar (Gemas et al. 2004; Martins-Lopes et al. 2009) should be treated with caution. In these cases, an accurate genetic, morphological and agronomical characterization of the accessions involved (Ben-Ari et al. 2014) should be required to corroborate the clonal origin of the observed variability.

Sampling surveys aimed to look for phenotypic diversity within olive cultivars showed that intra-cultivar phenotypic diversity seems to be an unusual phenomenon in olive. Since the 1970s, clonal selections within the main olive cultivars have been performed, such as cvs. Manzanilla de Sevilla, Picual, Arbequina and Arbosana in Spain (Garcia Berenguer 1988; Suarez et al. 1990; Tous et al. 1999); Chemlali de Sfax in Tunisia (Kamoun et al. 2002; Khlif and Trigui 1990); Picholine Marrocaine in Morocco (Boulouha 1986); Moraiolo, Canino, Biancolilla, Giarraffa Moresca, Cresuola, Tonda Iblea and Nera in Italy (Caruso et al. 2014; D'Hallewin et al. 1990); Souri in Israel (Ben-Ari et al. 2014; Lavee et al. 2008); and Cobrançosa, Santulhana, Verdeal Trasmontana, Azeitera, Blanqueta, Carraquenha, Redondil, Galega Vulgar and Macanilha Algarvia in Portugal (Fernandes Serrano 1990; Gomes et al. 2008; Leitao et al. 1999; Martins et al. 1998).

All these works were based on the selection of outstanding trees for some agronomic traits, usually the production or fruit size, within major cultivars in various locations. Comparison of these phenotypic selections in one comparative field trial with few replications did not provide a consistent genetic basis for selection.

To date these works have provided few new commercial selected clones. To our knowledge, only Arbequina IRTA i.18^{R} (Tous et al. 1999, 2005) selected from cv. Arbequina trees in Catalonia and Haouzia, Menara and Dhabia cvs. selected from cv. Picholine Marocaine trees in three distinct locations (Amal Hadiddou, pers comm) have been commercially propagated. However, Arbequina i.18^{R} did not show any significant difference, with the standard Arbequina in long-term experiments at 7 and 14 years after planting (De la Rosa et al. 2007; Diez et al. 2016). Additionally, Amal Hadiddou (pers comm) did not observe significant differences between Haouzia, Menara Dhabia and Picholine Marocaine cvs. in yield and oil content in 5-year crops in a comparative field trial in Marrakech. In both cases, only minor differences

for DNA markers leading to very close similarity indexes were detected. These differences were not associated with any morphological difference (Belaj et al. 2004a; Trujillo et al. 2014) (Fig. 14.3).

14.3 Crossbreeding

The first olive crossbreeding programs in the world were initiated between 1960 and 1971 in Israel (Lavee 1990) and Italy (Bellini et al. 2002a), respectively. Since the 1980s, new crossbreeding programs have been developed in different countries (Arias-Calderon et al. 2014; Bellini 1992; Dabbou et al. 2012; Lavee 2012, 2013; Lavee and Avidan 2011; Ozdemir et al. 2016; Rallo 1995; Rallo et al. 2008b; Trapero et al. 2013a; Zeinanloo et al. 2009). Most of the programs aim to obtain cultivars for olive oil, and only a few for table olives or both, with a special focus on early bearing, high yielding and adaptability to the new planting systems designed for mechanical harvesting (Rallo 2014a; Rallo et al. 2013). Recently, breeding programs are underway for resistance to verticillium wilt (Arias-Calderón et al. 2015a, b; Trapero et al. 2013a, b, 2015). Pre-breeding evaluation of cultivars and new genotypes from breeding programs for resistance to the bacteria *Xylella fastidiosa* are also under development (Landa B pers comm).

A previous revision of the olive-breeding protocol has been published (Rallo et al. 2011). Recently, Rugini et al. (2016) and Rugini and De Pace (2016) published two protocols focusing on biotechnological approaches.

14.3.1 Strategies for Crosses

The design of crosses requires previous knowledge of the genetics of the desired traits and information on pollen pistil compatibility. In their absence, the initial crosses of the current programs include progenitors with the desired phenotypic traits to incorporate or complement the other selected progenitors. In these programs, the progenitors are cultivars derived from the same or different regions of origin. Recently, crosses of cultivated olive (*Olea europaea* ssp. *europaea* var. *europaea*) with wild olive (*O. europaea* ssp. *europaea* var. *sylvestris*) and with other subspecies (*O. europaea* ssp. *cuspidata* and *O. europaea* ssp. *laperrini*) have been reported (Caceres et al. 2015; Klepo et al. 2014; Trapero et al. 2015), with the aim of enlarging the basis of genetic variability for olive breeding.

Olive is an allogamous species, in which self-incompatibility is the general rule with some cases of inter-incompatibility (Díaz et al. 2007; Koubouris et al. 2014; Rodriguez-Castillo et al. 2009; Seifi et al. 2011; Selak et al. 2014; Wu et al. 2002). The pollen tube of alien pollen grows faster than the pollen of the same genotype, triggering an earlier growth of both the seed and the fruit in cross-pollination than in self-pollination (Rallo et al. 1990). Therefore, emasculation it is usually not nec-

essary for performing crosses. However, there may be some problems related to contamination by undesirable alien pollen due to the fast and long distance airborne transport of pollen that may have contaminated the limbs before they were bagged (De la Rosa et al. 2004; Díaz et al. 2007; Rallo 2000b). Currently, paternity testing by different SSRs is being used in breeding programs (De Rosa et al. 2013; Díaz et al. 2007), representing a compulsory step for any genetic study.

14.3.2 Strategies to Shorten the Juvenile Phase

The time elapsed from seed germination to first flowering of a seedling is known as the *juvenile phase* (JP). This phase is usually very long in woody species and represents one of the major drawbacks for olive tree breeding by crossings since the JP in this species can reach up to 15–20 years (Bellini 1992; Fontanazza and Baldoni 1990). For example, in an olive crossbreeding program initiated in the early 1970s in Italy, many seedlings were still juveniles 25 years after planting (Bellini et al. 2002a). The cost of maintaining in the field a large number of trees that will take many years to flower is extremely high. Consequently, many efforts have been undertaken in different olive-breeding programs to shorten the JP to initiate evaluations of fruit traits in the progenies as soon as possible (Lavee et al. 1996).

The first strategies to shorten the JP consisted of forcing the growth of the seedlings to achieve a certain size in a short time, since the onset of first flowering in many woody species is reported to be related to the size of the plant (Hackett 1985). Santos-Antunes et al. (1999, 2005) established a forced-growth protocol in which seedlings are grown under continuous light and fertigation in the greenhouse for approximately 7–8 months, after which the plants are transplanted to field conditions. This protocol has been shown to be very efficient, obtaining approximately 50% of flowering seedlings by the third year (Fig. 14.4). Other management techniques to reduce JP have been studied regarding plant canopy height (Moreno-Alías et al. 2010a) or soil solarization (El Riachy et al. 2011), among other traits.

The relationship between seedling growth traits and the time of first flowering has been widely studied (De la Rosa et al. 2006; Pritsa et al. 2003; Rallo et al. 2008b; Santos-Antunes et al. 2005) to establish preselection criteria to cull seedlings with a long juvenile period in early stages, before transplantation to the field. Seedling vigor traits, mainly plant height and stem diameter, measured after the forced growth period in the greenhouse are strongly correlated to the earliness of first flowering and, thus, are currently used in breeding programs to preselect the seedlings to be planted. Threshold values based on a specific seedling height (approximately 100 cm; Moreno-Alías et al. 2010b) have been proposed, whereas Rallo et al. (2008b) suggest culling up to 35–40% of the shortest seedlings since growth performance may vary among progenies and years.

Differences in the earliness of first flowering among progenies have been reported, indicating a strong influence of the genitors on the length of the JP of the progenies (Bellini et al. 2002a; De la Rosa et al. 2006; Moral et al. 2013; Rallo et al. 2008b;

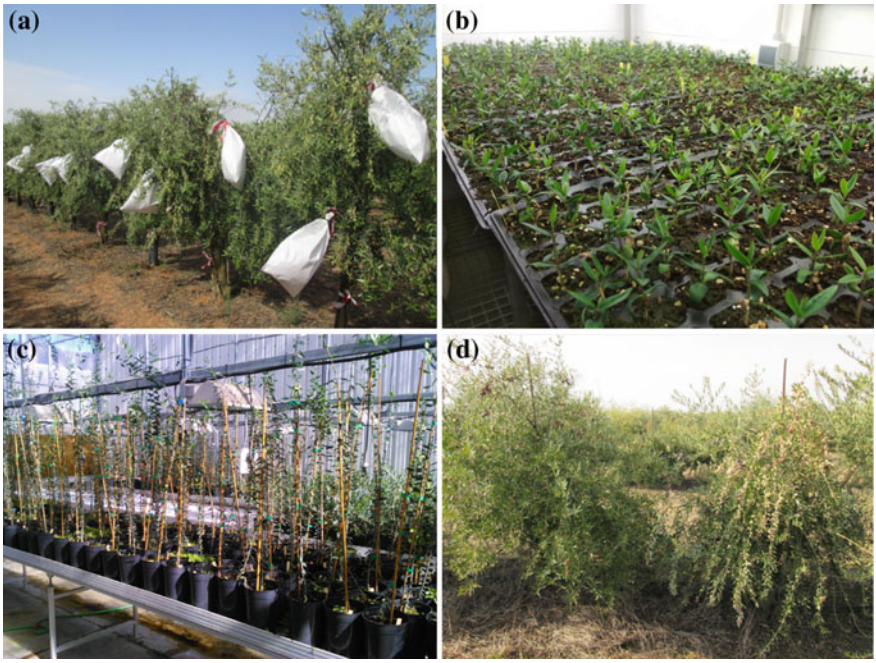

Fig. 14.4 Steps to shorten the juvenile period. **a** Cross, **b** Seed germination, **c** Forcing seedlings growth in greenhouse under continuous light, **d** Forcing growth in field until first flowering and fruiting. Photos by P. Valverde

Santos-Antunes et al. 2005; Suárez et al. 2011). Thus, parental selection of certain genitors is a good strategy to accelerate first flowering in progenies. Furthermore, the unproductive period of genotypes in the adult stage has been positively associated with the JP of the same genotypes (Leon et al. 2007). Therefore, early bearing is enhanced when selection for a short JP is performed, and similarly, the use of early-bearing parents will help to reduce the length of the JP of progenies.

The transition from the juvenile to the adult stage is not yet well understood in the olive tree. As described by Hackett (1985) and Hartmann et al. (2002), phase changes in woody species follow a cone-like pattern, known as the juvenility cone, during which the basal and inner parts of the adult seedling remain juvenile. In the case of the olive tree, the position of the first flower in seedlings has been studied as a marker of the end of the JP, and a juvenile cone has been confirmed in this species (Moreno-Alías et al. 2010a; Suárez et al. 2011). The attainment of a certain distance from the root to the meristem is required to flower, which was determined at approximately 200 cm for olive by Moreno-Alias et al. (2010a), although significantly different values among progenies were reported by Suarez et al. (2011). The latter authors also found correlations with the length of the juvenile period such that early flowering genotypes required a lower minimum distance to the ground to overcome juvenility.

In addition to the onset of flowering, other markers of the phase change from the juvenile to the adult stage have been studied, including proteins, morphological and anatomical traits and NIR (near-infrared) spectra of leaves, and rooting ability (Casanova et al. 2014; Garcia et al. 2000; Leon and Downey 2006; Moreno-Alías et al. 2009). Recently, much effort is being focused on the discovery of genes involved in the transition from juvenile to the adult stages and in flowering in olive species (Fernández-Ocaña et al. 2010; Garcia-Lopez et al. 2014; Haberman et al. 2017; Jiménez-Ruiz et al. 2015; Muñoz-Mérida et al. 2013; Sgamma et al. 2014). A better understanding of the complex genetic control of the juvenile-adult phase change will elucidate new avenues for the development of molecular tools for early transcriptomic selection of short JP seedlings.

14.3.3 Strategies for the Evaluation of Genotypes: Crossbreeding Steps

Crossbreeding in fruit trees is an extended process due to the length of the JP and the required multistep protocol to evaluate seedling progenies and their successive clonally-propagated selections (Hancock 2008). After crossbreeding pollination, seed germination and forced seedling growth to shorten the JP, selected genotypes are submitted to several selection steps for further evaluation. In each step, the number of genotypes is reduced and the number of replications per genotype and characters under evaluation increases up to the final release of new cultivars. The total number of evaluated genotypes and selection steps are usually determined by the available human and financial resources and facilities.

Figure 14.5 shows a general overview of the selection steps followed at the Spanish olive-breeding programs: UCO-IFAPA (University of Córdoba- Andalusia Institute for Agricultural and Fishing Research) and US (University of Sevilla).

Two alternative protocols are used. Two steps of evaluation in the first crosses (Rallo et al. 2016a; Fig. 14.5 blue arrow): (1) for progenies, and (2) a final step for selected genotypes. As an example, 15 of 748 genotypes were selected in the first step of evaluation, and one new cultivar (Sikitita/Chiquitita) was registered after a comparative trial, 15 years after plantation of the progenies (Rallo et al. 2008a). After some additional comparative trials, two selections will soon be registered. In the USA's table olive breeding program, 40 of 1800 genotypes were selected in the first step, 20 of which are currently in field trials for final evaluation (Rallo P, pers comm).

The second protocol proposes three steps (Leon et al. 2015; Figure 14.5 (red arrows): (1) initial progenies, (2) intermediate for preliminary selections and (3) final for advanced selection. Following this protocol, 108 of 1548 genotypes were selected in the first step, 14 of which remained after the intermediate step (15 years after plantation of the progenies). The final evaluation of the 14 advanced selections in comparative field trials ended approximately 20 years after planting of the seedlings.

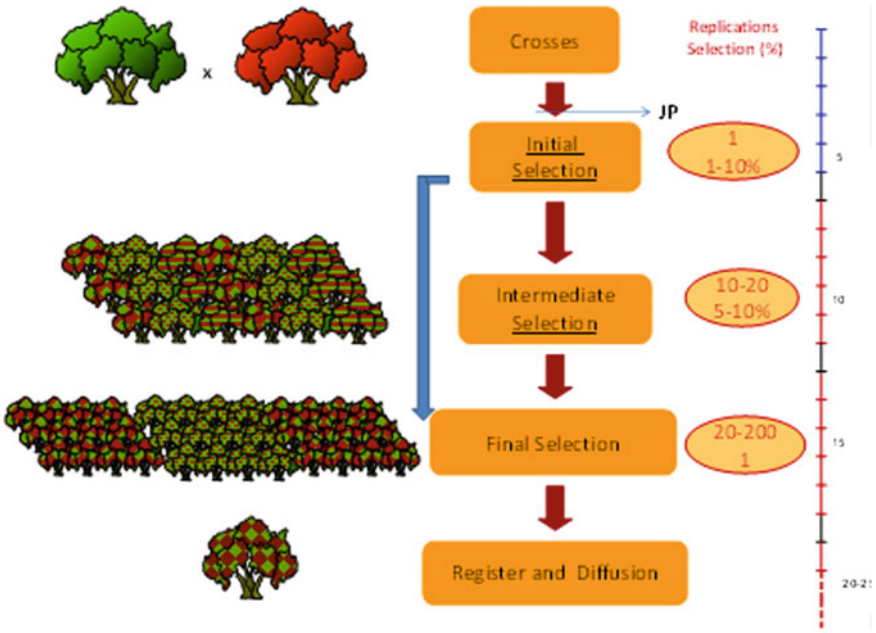

Fig. 14.5 Proposed breeding scheme in olive breeding programs in two (blue arrow) or three (red arrows) selection steps. JP: Early selection for the short juvenile phase

These data indicate that the choice between both protocols will depend on available human, financial and logistic resources. Cooperation with stakeholders may facilitate a two-step protocol and accelerate the breeding process (Fig. 14.7c).

14.3.3.1 Step 1: Initial (Evaluation of Progenies)

Initially, the breeder has to manage a large number of non-replicated genotypes per cross. Rigorous criteria and techniques for early or indirect selection according to the main objectives of the program must drastically reduce the initial population of genotypes in this first step to save time, labor and money. The earliness of bearing, oil content and composition and adaptation to new plantation systems are systematically evaluated in all progenies in all crosses of the UCO-IFAPA Breeding Program (Rallo et al. 2016a).

Early criteria for selection in the first period of seedling growth must be a general concern in any olive breeding program. Early selection tests have also been an excellent and general strategy to shorten the JP and to eliminate late flowering genotypes in any cross (see Sect. 14.3.2). Additionally, early selection for *Verticillium dahliae* resistance has be conducted in 2-month-old seedlings, eliminating more than 90% of them for later evaluation in field plantations in highly infested soils (Trapero

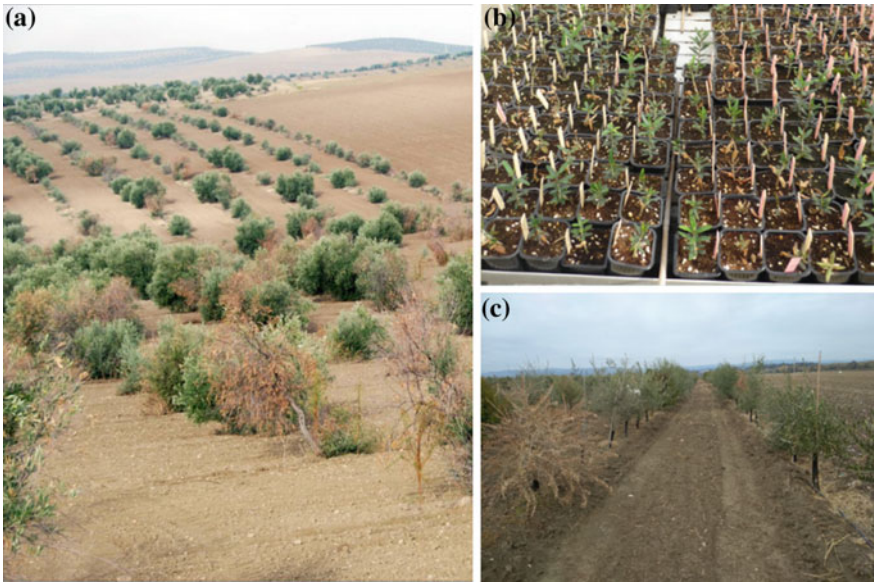

Fig. 14.6 Two-step selection for verticillium wilt resistance. **a** Young orchard devastated by this disease, **b** Evaluation of inoculated seedlings in greenhouse, **c** Evaluation of pre-selected seedlings in field. Photos:**a** by C. Trapero; **b**, **c** by P. Valverde

et al. 2013a) (Fig. 14.6). Currently, newly-developed genomic tools are providing some efficient tests for early genetic-based selection of relevant agronomic traits (see Sect. 14.4).

The evaluation of progenies usually persists for 5 years, i.e. the three first crops after overcoming the JP. Many traits have been evaluated in the initial step. Earliness of bearing (see Sect. 14.3.2), plant architecture, oil content and composition (see Sect. 14.3.6) of breeding oil cultivars are usually assessed in the initial step based on values correlated with those in subsequent steps and on moderate to high heritability values (Ben Sadok et al. 2013, 2015; Leon et al. 2015). Additionally, different fruit traits have also been accurately evaluated during this initial step for breeding table olives (see Sect. 14.3.6).

The simplification of recording and the use of suitable methods to evaluate small samples have been used in the initial step. Categorical data for the number of flower seedlings have demonstrated a fast, easy and reliable record for earliness of bearing that has been routinely adopted for seedling evaluation (Leon et al. 2015). Non-destructive and multicomponent trait evaluation by NIR spectrometry has allowed the evaluation of oil content, fruit traits, ripening index and oil composition in a fast and accurate manner (Bellincontro et al. 2013; Leon et al. 2003, 2004a; Morales-Sillero et al. 2011) in small samples, particularly in fruits, allowing an efficient way to evaluate the progeny variability in any segregating population for multiple traits.

The evaluation of progenies also represents a basic step for genetic and genomic studies that are undergoing active development (see below and Sect. 14.4).

14.3.3.2 Step 2. Intermediate (Evaluation of Preliminary Selections)

Vigor and yield are critical traits for final selection. Intermediate steps attempt to evaluate these traits with some replicates to first obtain and compare records for preliminary selections that cannot be accurately evaluated in the initial step. Additionally, the intermediate step may confirm the consistency of previously evaluated traits in the initial selection step.

Correlations between data recorded for seedling and intermediate steps indicate that selection for the earliness of bearing, fruit size and oil content can be efficiently performed at the seedling stage, whereas evaluation at the intermediate step would be necessary to select for yield and vigor. Similar conclusions were obtained from analyses of heritability estimated for these characteristics at the intermediate step of selection. Additionally, a high number of genotypes was retained in the intermediate step, allowing large variability for both previously evaluated and non-evaluated traits such as fatty acid composition, tocopherol and phytosterol contents and the phytosterol profile (Leon et al. 2015).

14.3.3.3 Step 3. Final (Evaluation of Advanced Selections)

Genotype × environment (G × E) evaluation is the main objective of the final evaluation of advanced selections before new cultivars are registered. In addition to vigor and yield, the most important traits in any new cultivar, other traits related to the breeding objectives must be re-evaluated at this step in several locations. Therefore, cooperation with growers in different sites provides information about the selection performance for different environments to other growers from those zones, thus promoting new potential registered cultivars. The UCO-IFAPA program has developed this strategy since the first crosses. Currently, more than a dozen stakeholders, including nurseries, participate in this step of evaluation.

Different field experiments corresponding to initial, intermediate and final evaluations are shown in Fig. 14.7.

14.3.3.4 The Components of Variability

Research on the genetics of agronomic traits in olive is in its infancy. Nevertheless, studies examining the components of genetic variability in cultivars and genotypes from crossbred progenies have been increasing in recent years. Several patterns have been observed in these studies.

A large variability among genotypes has been a general pattern for any evaluated trait in cultivars from germplasm banks and in genotypes from any crossbred progeny.

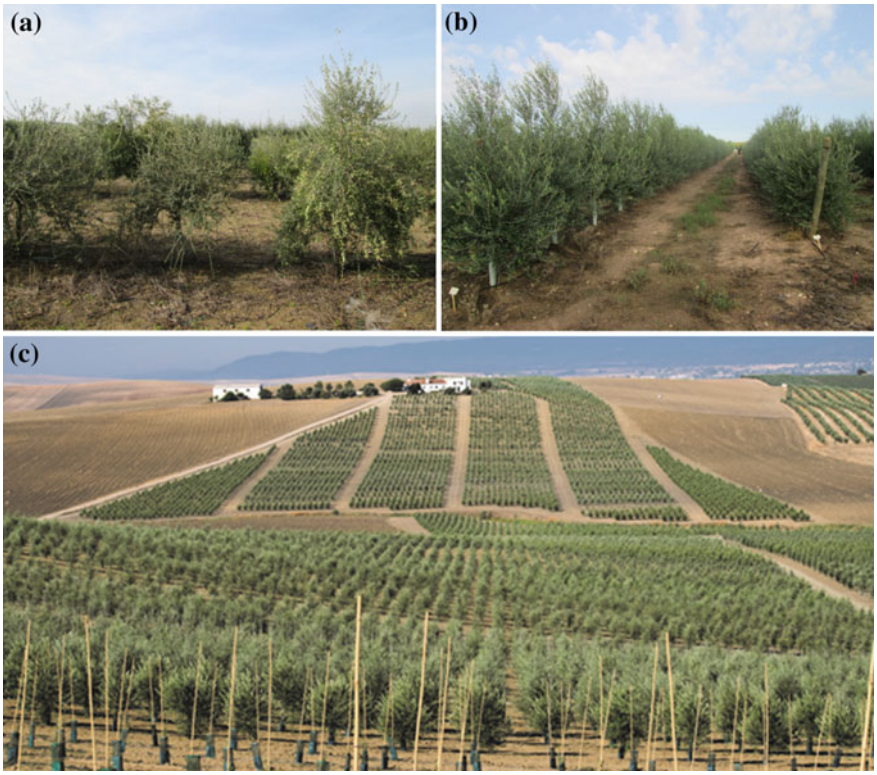

Fig. 14.7 Field evaluation steps for crossbreeding. **a** Initial evaluation of unreplicated genotypes from progenies, **b** Intermediate evaluation of preliminary soft selection, many genotypes with some replications, **c** Final selections of genotypes in a comparative precommercial field trial. Photos: **a**, **b** by P. Valverde; c courtesy of TODOLIVO

This is the case for all reported traits in this review (Sects. 14.3.2, 14.3.4–14.3.6). As a rule, the values of the descendants transgress the genitor values in the segregating progeny. In all analyzed models, the genetic factor within crosses is the main component of the variability of the evaluated traits, as expected due the extensive heterozygosity of this species. This is a favorable situation from a clonally-propagated species such as the olive.

Few studies have examined heritability in olive. Hassani and Tombesi (2008) measured the growth characteristics in the greenhouse of a full diallel cross among nine cultivars. The heritability of characters such as dry weight and internode length in progenies indicates high values, (0.75 and 0.97), respectively. Zeinanloo et al. (2009) designed one-way diallel crosses with six genotypes for nine quantitative characteristics. Broad sense heritability ranged from 0.31 to 0.86 and narrow-sense heritability from 0.17 to 0.28. Zard and Roghani cvs. were good combiners for fruit weight, percentage of dry matter, fruit length, fruit width, and percentage of oils.

Architectural traits have attracted attention due to the search for compact and early bearing trees for new high-density systems. Hammami et al. (2011, 2012) proposed five traits (main vertical axis, preferential distribution of lateral shoots, dominant length of lateral shoots, branch orientation and branch bending) as the most relevant descriptors for the olive seedling architecture based on the high capacity to indicate diversity, a strong influence of the parent genotype, a lack of correlation with each other, and a demonstrated value for agronomic performance. They also showed that cvs. Picual, Arbosana and Sikitita were promising cultivars for use as genitors because of their tendency to produce offspring with desirable growth habit traits.

Ben Sadok et al. (2013, 2015) studied the heritability of tree architecture and reproductive traits in a progeny of 120 trees from the Oliviere × Arbequina cross in two contrasting growing sites. After selection of a model, broad sense heritabilities were estimated. Despite strong environmental effects on most traits, no G × E interaction was found. Moreover, the internal structure of the trait covariation was similar in both sites. Ontogenetic growth variation, related to (i) the overall tree form and (ii) the growth and branching habit at growth unit scale was not altered by the environment. Finally, moderate to strong genetic control was identified for traits at the whole tree scale and at the internode scale. Among all studied traits, the maximal internode length exhibited the highest heritability ($H2 = 0.74$).

A detailed variance analysis of oil content components and fruit morphology traits in an olive progeny issued from the cross Oliviere × Arbequina (Leon et al. 2016) showed the lowest values of heritability for fruit moisture (0.40) and the highest values for oil content in fruit fresh weight (0.94). An estimation of heritability and environmental variation over varying numbers of years and tree replications revealed that the inclusion of more annual replications could be more effective than the addition of tree replications. Finally, this study provides a global view of adequate spatial and temporal replications required to accurately develop criteria for both conventional and marker-assisted selection on fruit traits from olive crossing progenies.

An evaluation of resistance to the defoliating pathotype of *Verticillium dahliae* Kleb was performed in 12 cultivars and 52 genotypes previously selected from a wider initial population based on their agronomic performance (Arias-Calderon et al. 2014) and 6017 genotypes derived from 48 crosses obtained by open pollination and crosses between olive cultivars, wild olive genotypes and other *Olea* species and *Olea europaea* subspecies (Trapero et al. 2015). In both studies, high genetic variability and wide segregation of resistance were observed. The estimates of heritability suggest that it is possible to breed for verticillium wilt resistance in the olive.

14.3.4 Strategies for Tolerance to Abiotic Stress Selection

Environmental adaptation is a cornerstone to successfully grow a crop in a specific region. Olive cultivars that are able to tolerate many abiotic limiting factors such as cold, heat, water stress or nutrient-deficient soils can be found due to uninten-

tional selection over thousands of years. However, these cultivars often lack other desired agronomic characteristics. Although the combination of both characteristics seems possible, the development of new cultivars with increased tolerance to abiotic stresses is not a major objective in current olive breeding programs worldwide, and research studying the genetic variability of tolerance to abiotic stresses is generally not extensive. Rootstock breeding also appears to be an opportunity to overcome some of these abiotic stresses.

Olive growth and productivity can be seriously limited by low temperatures, especially temperatures below −7 °C (Palliotti and Bongi 1996). Physiological responses related to cold damage in olive have been identified (Ruiz et al. 2006), and methods to quickly assess tolerance to damaging temperatures have also been established (Azzarello et al. 2009). This screening method, combined with the existence of genetic diversity regarding tolerance to cold conditions (Barranco et al. 2005b; Bartolozzi and Fontanazza 1999; Gómez-del-Campo and Barranco 2005), suggests that breeding for this trait should be feasible.

Chilling requirements in olive is an increasing limiting factor because of the expansion of olive plantations to new areas (Rapoport 2014), as well as the global temperature increase in established olive growing areas (Gabaldón-Leal et al. 2017). This trait is genetically dependent on field observations (Hartmann and Porlingis 1957; Zouari et al. 2017) and modeling (Garcia-Mozo et al. 2009). A method to determine the dormancy period has been recently developed (Ramos et al. 2018) and could accelerate the identification of previously initiated olive genotypes with low-chilling requirements (Cabello, unpublished data).

Olive is quite tolerant to drought stress in comparison to other tree species (Fernández and Moreno 1999). Some of the physiological factors that control drought tolerance are known (Sofo et al. 2004), and an important array of studies have reported genetic differences between olive cultivars in terms of tolerance to drought (Bacelar et al. 2009; Guerfel et al. 2007; Tugendhaft et al. 2016). Wild germplasm has also been identified as a possible source of tolerance to this abiotic stress (Besnard et al. 2012). Prospects for the breeding of this trait in the future are positive due to the development of a technique using chlorophyll fluorescence to screen for this factor (Faraloni et al. 2011) and the identification of a gene that enhances drought tolerance (Chiappetta et al. 2015). Tolerant genotypes may be used as rootstocks to alleviate this problem.

Chlorosis due to iron deficiency can result in important yield losses. This condition is common in plants growing in calcareous soils. Although olive species are considered quite tolerant in comparison to other plant species (De la Guardia and Alcántara 2002), they are grown in calcareous soils throughout the world and are therefore affected by iron chlorosis. Olive cultivars present notable genetic differences in their tolerance to this abiotic stress (Alcántara et al. 2003). As in other species, tolerance can be achieved by the use of rootstocks. A few other studies have reported genetic variability in the tolerance to other abiotic factors such as salinity (Marin et al. 1995) or heat stress (Mancuso and Azzarello 2002), although sources of tolerance have not been deeply investigated.

14.3.5 Strategies for Resistance to Biotic Agents

Diseases and pests have always been limiting factors for olive growing. Olive genotypes with a higher level of resistance to major pests and diseases have been selected intentionally and unintentionally for a long time, and therefore sources of resistance to a range of pests and diseases are available, as shown in Table 14.2. However, their use in breeding programs is limited due to other agronomic and adaptive traits that normally comprise the main breeding objectives (Leon et al. 2007). Breeding for disease or pest resistance aims to combine the best agronomic and adaptation traits with resistance to the desired disease or pest in a single genotype. It is a slow process due to the time necessary for plant generation evaluations, as well as the lack of knowledge about the mechanisms and genetics underlying the resistant expression (Johnson and Jellis 1992).

Verticillium wilt of olive is a vascular wilt caused by the soil-borne fungus *Verticillium dahliae*. It has recently become the main disease in many olive-producing countries and is threatening olive production in many areas (Jiménez-Díaz et al. 2012; López-Escudero and Mercado-Blanco 2011). Factors such as the inefficacy of chemical compounds to control the disease make the use of plant material that is resistant to verticillium wilt especially important. However, most of the olive cultivars evaluated to date have been identified as susceptible or extremely susceptible to this disease in the field (Trapero et al. 2013b) and under controlled conditions (López-Escudero and Mercado-Blanco 2011). For many years, efforts to develop new olive material that is resistant to *V. dahliae* have been quite limited (Hartmann et al. 1971; Wilhelm and Taylor 1965). However, due to recent increases in the importance of the disease in the last 10 years, verticillium-wilt resistance has been incorporated into breeding programs, sometimes as a major objective. Mass screening methodologies (Trapero et al. 2013a) have allowed the evaluation of large numbers of genotypes generated by open pollination and targeted crosses in an attempt to combine high levels of resistance and positive agronomic traits (Arias-Calderón et al. 2015b; Colella et al. 2008; Trapero et al. 2015). According to these studies, complete resistance to verticillium wilt in olive is unlikely to exist, although high levels of resistance have been identified in olive cultivars, wild populations and related *Olea* species (Table 14.2). New varieties with higher level of resistance are likely to be generated in the upcoming years, once they have been assessed for a number of years in fields infested by the pathogen. The selection of undomesticated material that is resistant to disease for use as rootstock should first be evaluated for efficacy for controlling the disease. Verticillium wilt resistance is believed to be highly polygenic (Leyva-Pérez et al. 2017) and molecular tools to select for this trait are currently not available.

The main foliar diseases attacking olive are peacock spot (caused by *Venturia oleginea*), anthracnose (*Colletotrichum* spp.), and cercospora leaf spot (*Pseudocercospora cladosporioides*). These pathogens cause tree defoliation, premature fruit drop and fruit rot, which can be devastating under favorable weather conditions (Viruega et al. 2011). Although traditionally managed by cultural and chemical methods, genetic resistance has been demonstrated to be highly effective, especially when

Table 14.2 Sources of resistance available against the major diseases and pests that affect olive and their current situation in breeding programs

Disease/Pest	Sources of resistance	Level of resistance	Breeding stage	References
Verticillium wilt	Cultivars	Moderate/high	Advanced phases	Arias-Calderón et al. (2015a), Trapero et al. (2015), Wilhelm and Taylor (1965)
	Wild genotypes	High	Rootstock (early phase)	Colella et al. (2008), Jiménez-Fernández et al. (2016)
	Related species	High	Rootstock (early phase)	Arias-Calderón et al. (2015b), Trapero et al. (2015)
Defoliating diseases	Cultivars	Moderate/high	Released/ advanced phase	Lavee et al. (1999), Moral et al. (2015)
	Wild genotypes	High		Xavier (2015)
Olive knot	Cultivars		Not applied	Penyalver et al. (2006), Young et al. (2004)
Xylella fastidiosa	Cultivars	Moderate/unknown	Not applied	Frisullo et al. (2014), Luvisi et al. (2017)
Olive fly	Cultivars	Moderate	Not applied	Garantonakis et al. (2017), Gonçalves et al. (2012), Iannotta et al. (2007), Malheiro et al. (2015)

disease pressure is high. The identification of sources of resistance has been based on both field assessment (Moral and Trapero 2009) and artificial inoculation methods (López-Doncel et al. 1999). Genotypes generated from crossings between varieties with different levels of resistance have been selected in several breeding programs (Ciccarese et al. 2002; Moral et al. 2015; Rhouma et al. 2013) and one cultivar has been released with a reported high level of resistance to peacock spot generated by the self-pollination of a moderately resistant cultivar (Lavee et al. 1999). The inheritance of resistance to these diseases is likely to be mostly polygenically controlled according to the cited studies.

Olive knot is a disease characterized by the formation of cankers on the olive trunk, branches and shoots. It is caused by the bacteria *Pseudomonas savastanoi* pv.

savastanoi. Although it is not a major objective in olive breeding programs, susceptible genotypes are usually identified in the field and culled. By using inoculation methods under controlled conditions, (Marcelo et al. 1999; Penyalver et al. 2006) identified a high level of resistance among olive cultivars (Young et al. 2004) that could be useful for breeding.

Xylella fastidiosa is a bacterium that infects and colonizes the xylem of a broad range of plant species and causes leaf scorch and *quick decline syndrome* in the olive. This disease was of little importance to olive until the recent outbreak in southern Italy (Saponari et al. 2013). Currently, the level of resistance of different cultivars is largely unknown, apart from recent field observations and studies using a small number of cultivars (Frisullo et al. 2014; Luvisi et al. 2017), which suggest a potential variability in resistance to the pathogen. *Xylella fastidiosa* is currently a major concern in Europe as it is likely to spread to other growing regions (Stokstad 2015). Therefore, breeding efforts are likely to arise in the near future.

The olive fly (*Bactrocera oleae*) is the major pest affecting olive worldwide, causing severe damage to fruits and reducing their quantity and quality (Daane and Johnson 2010). Differences in the resistance level of a number of olive cultivars have been reported in several studies using preference or oviposition experiments (Garantonakis et al. 2017; Gonçalves et al. 2012; Iannotta et al. 2007; Malheiro et al. 2015). Fruit traits such as size, maturity date and hardness usually explain a great proportion of the level of resistance, although Grasso et al. (2017) have shown that resistance to olive fly can also be induced. To date, resistance to this pest has not been targeted as a major objective by any olive breeding program.

14.3.6 Strategies for Quality

Quality is becoming a prime target for plant breeders since consumer demand is moving toward food products with improved sensorial, nutritional and nutraceutical value. Table olives and virgin olive oil, the two main products obtained from olive fruits, are staple foods of the Mediterranean diet, and the benefits of their consumption in human health have been extensively documented. Additionally, their extraordinary organoleptic properties are also responsible for their increasing demand worldwide (IOC 2017).

Olive quality is a wide and complex concept involving many traits that may be important for table olives, olive oil or both (Rallo et al. 2011, 2017). For instance, fruit appearance is essential for table olives, which encompass traits such as fruit size, shape, symmetry, color or absence of bruising, whereas oil content components and fatty acid profiles are particularly important for olive oil. Recently, a special emphasis has been placed on both products with respect to minor bioactive compounds such as tocopherols, phenolics, squalene, sterols or triterpenic acids, among others. Table 14.3 lists quality traits that have been evaluated in olive-breeding programs based on fresh unprocessed fruits, oil or processed table olives. High levels of variability have been observed for most of these traits, with cases of transgressed seg-

Table 14.3 Examples of quality traits evaluated in olive breeding programs

Product	Traits	References
Fresh olives	*Fruit attributes* Fruit weight, width and length Stone weight, width and length Fruit shape and circularity Flesh/stone ratio Fruit bruising Skin and flesh color Flesh texture Flesh detachment *Fruit compounds* Oil content and moisture Fatty acids profile Phenolic compounds Pigments Sugars Sterols Squalene Tocopherols	Arias-Calderon et al. (2014), Avidan et al. (2012), Bellini (1993), Bellini et al. (2002a, b), De la Rosa et al. (2013, 2014, 2016), Ersoy et al. (2008), Fourati et al. (2002a), Jimenez et al. (2011), Klepo et al. (2014), Lavee and Avidan (2011), Leon et al. (2004b, 2015, 2016), Ozdemir and Kurultay (2015), Ozdemir et al. (2016), Padula et al. (2008), Rallo et al. (2008b, 2012), Rjiba et al. (2010), Roca et al. (2011), Velasco et al. (2014), Zeinanloo et al. (2009)
Olive oil	*Oil composition* Fatty acids profile Omega3 fatty acid Phenolic compounds Pigments Sterols Tocopherols Volatile compounds *Legal quality parameters* Free acidity Peroxide value K232, 270 Oxidative stability	Baccouri et al. (2007), Bellini et al. (2002c, 2004), Dabbou et al. (2012), De la Rosa et al. (2013), El Riachy et al. (2012a, b, c), Fourati et al. (2002a, b), Garcia-Gonzalez et al. (2010), Hernandez et al. (2017), Klepo et al. (2014), Leon et al. (2008, 2011, 2015), Ozdemir et al. (2016), Perez et al. (2014, 2016), Ripa et al. (2008), Rjiba et al. (2010), Roca et al. (2011), Sanchez de Medina et al. (2015a, b, c), Velasco et al. (2014)
Table olives	Bruising Oil and water content Salt content pH, acidity Phenolic compounds Triterpenic acids Sensory attributes	Jimenez et al. (2011), Medina et al. (2012), Ozdemir and Kurultay (2015), Sorrentino et al. (2016)

regation and significant differences among crosses and/or genotypes within crosses (Arias-Calderon et al. 2014; El Riachy et al. 2012a; Lavee and Avidan 2011; Medina et al. 2012; Perez et al. 2014, 2016).

Breeding for quality in olive is more complex than in other fruit species since the olive drupe may not be consumed directly but may need to undergo industrial processing to obtain table olives or olive oil. Although some of the abovementioned

quality traits may be evaluated in fresh unprocessed fruits, most should be assessed in the final elaborated products. This procedure hampers olive breeder work, especially in the early stages of evaluation, because processing a large number of genotypes is time-consuming and expensive. Furthermore, the low yield of genotypes in this stage limits the amount of fruit required for processing. In this regard, the strategy applied to correlate traits in fresh fruit with quality traits of the final product, for indirect selection based solely on fresh olive evaluation, has been studied. High correlations have been found between fruit flesh and extracted oils for the main fatty acids, tocopherols, sterols and squalene (De la Rosa et al. 2016; Velasco et al. 2014), and between oleuropein content in fresh fruit and different quality traits of Spanish-style green olives and black olives (Rallo et al. 2018 P, pers comm).

The complex methodologies needed to assess many of the abovementioned quality traits, along with the large number of genotypes to be screened, have encouraged olive breeders to explore the use of alternative fast and non-destructive methods, such as NIR spectrometry, to evaluate both olive oil and table olive quality traits (Giovenzana et al. 2015; Leon et al. 2003, 2012; Mailer 2004; Morales-Sillero et al. 2011). Good calibration models have been obtained for fruit weight, diameter and volume, flesh texture, oil content, moisture content, chlorophyll pigment, oleic and linoleic fatty acids.

The recent detection of loci associated with interesting quality traits (fruit weight, flesh/stone ratio, oil traits, fatty acid composition, among others) via classical QTL mapping approaches (Atienza et al. 2014; Ben Sadok et al. 2013; Hernandez et al. 2017) or through genome-wide association studies (GWAS) (Kaya et al. 2016) will soon enable marker-assisted breeding for olive quality, allowing very early selection of outstanding genotypes at the juvenile seedling stage.

14.4 Genomic Tools for Olive Breeding Programs

Despite the remarkable recent development of olive breeding programs, breeders lack the capacity to generate cultivars quickly in response to new growing systems, evolving consumer preferences and crises. Olive breeding is still based on classic methods using directed crosses between suitable cultivars followed by selection within the progenies, and finally the cloning of outstanding individuals. Non-conventional breeding approaches, such as protoplast technology or the selection of mutants from induced in vivo mutagenesis, have also been applied to olive with variable levels of success (Rugini et al. 2016). However, to our knowledge only cv. Briscola (Roselli and Donini 1982) obtained by gamma rays mutation of cv. Ascolana Tenera was released for its ornamental value. Thus, this section will be focused on genetic and genomic approaches that could accelerate classical breeding methods in the near future.

The olive tree is an extremely heterozygous species with high genetic variability. Thus, seedlings from breeding programs have been shown to undergo segregation for all the agronomical characters evaluated to date (Rallo 2014a, b), even the frequent

presence of individuals showing transgressive segregation (seedlings with trait values exceeding the values of their genitors) (de la Rosa et al. 2016; Leon et al. 2004b; Trapero et al. 2015). Given this remarkable variability, breeding new olive cultivars with improved characteristics requires the germination and evaluation of thousands of seedlings. For example, more than 10,000 genotypes have been evaluated to obtain a new cultivar, two promising advanced selections and more than 30 preselections with high production, early bearing, and adaptation to mechanical harvesting and high-density at the UCO-IFAPA breeding program (Rallo et al. 2016a). In addition, olive-breeding cycles are extremely long (>12 years), mostly due to the length of the JP of long-lived perennials (van Nocker and Gardiner 2014). From an agronomic perspective, JP is the period between seed germination and first flowering, which are influenced by both environmental and genetic factors. As mentioned in Sect. 14.3.2, the length of the JP is inversely correlated with plant vigor. This association has led to the culling of approximately 40% of plants with a predictably long JP a few months after their germination (De la Rosa et al. 2006; Rallo et al. 2008b). Thus, the height of seedlings is currently the most effective early selection marker for the short juvenile phase in olive (Rallo 2014a, b). New biotechnological tools that are able to produce precocious flowering of juvenile plants via the action of the viral vector might also help to accelerate the breeding process of perennial crops in the near future (Haberman et al. 2017; Velázquez et al. 2016). Therefore, understanding the mechanisms controlling the JP transition in perennials and the detection of additional early selection markers related to other agronomic traits are important requirements to advance olive breeding.

The main use of genomics in breeding is marker-assisted selection (MAS) for traits controlled by major genes or quantitative trait loci (QTLs). By MAS, genetic markers that either are known to cause a phenotype or are strongly linked to the causal genetic variant can be genotyped at the seedling stage, allowing a prediction of the phenotype of the adult plant. The construction of a high-density linkage map is a necessary prior step to localize QTLs and genes controlling agronomic traits. Several olive linkage maps have been developed using the following molecular markers and cultivar progenies: RAPD, AFLP, RFLP and SSR—Leccino × Dolce Agogia (De la Rosa et al. 2003); RAPD, SSR and SCAR—Frantoio × Kalamata (Wu et al. 2004); AFLP and SSR Picholine Marocaine × Picholine du Languedoc (Aabidine et al. 2010); AFLP, ISSR and SSR Oliviere × Arbequina (Aabidine et al. 2010); DArT and SSR—Picual × Arbequina (Domínguez-García et al. 2012); SNP—F2 from the selfing of Koroneiki (Marchese et al. 2016); and SNP, CAP and SSR—Gemlik × Edincik Su (İpek et al. 2017). These mapping approaches, combined with the phenotypical characterization of genitors and progenies, have allowed the location of QTLs related to flowering and fruiting traits (Ben Sadok et al. 2013), oil content, moisture, ratio pulp to stone, fruit weight and trunk diameter (Atienza et al. 2014) (see also Sect. 14.3.4).

The development of new high-throughput sequencing techniques (also known as next-generation sequencing) has resulted in an increase in studies focused on olive transcriptomics. These studies have led to the identification of expressed sequence tags (ESTs) (Alagna et al. 2009), the assembly and annotation of the olive transcrip-

tome (Muñoz-Mérida et al. 2013), the assessment of differential expression patterns among tissues and plant treatments (Bazakos et al. 2012, 2015; Carmona et al. 2015; Guerra et al. 2015; Jiménez-Ruiz et al. 2015) and the generation of saturated linkage maps (İpek et al. 2017; Marchese et al. 2016). Many of these studies have ultimately pursued the identification of candidate genes for agronomic traits such as the length of the juvenile period (Fernández-Ocaña et al. 2010; Jiménez-Ruiz et al. 2015).

Despite these diverse and numerous initiatives, the development of early markers to increase the efficiency of olive-breeding programs is still a pending task. The application of MAS to complex traits, such as yield or biennial bearing behavior, is not straightforward. Difficulties in manipulating these traits are derived from their genetic complexity, principally the number of genes involved, interactions between genes (epistasis) and environment-dependent expression of genes.

A recent publication concerning the reference genome of the cv. Farga (Cruz et al. 2016) and a wild olive (Unver et al. 2017) provides a landmark that can steer the generalization of genomic tools for the characterization of genetic resources and the selection of candidate genotypes. According to Cruz et al. (2016) and Unver et al. (2017), the olive tree, with $2n = 46$ and a genome size ~1.3 to 1.4 Gb, has approximately 50,000 protein-coding genes and an evolutionary history marked by polyploidization events. The existence of these reference genomes along with the decreasing cost of sequencing will: (a) increase the number of sequenced cultivars, allowing the possibility of performing genome-wide association mapping and, ultimately, developing early markers to accelerate the breeding process; (b) allow an accurate evaluation of the germplasm variability for the classification of cultivars into gene pools to design crossbreeding strategies (Rugini et al. 2016) shed light on the controversial domestication history of olive (Besnard et al. 2013; Díez et al. 2015) by applying methodologies that have been previously used for annual plants with reference genomes (Gaut et al. 2015).

14.5 Biotechnology Applications

Olive is a species difficult to manipulate in vitro; however, up to date, significant progress has been made in the understanding of its culture requirements under controlled conditions. In this section, the state of the art of micropropagation of important cultivars as well as key factors involved in adventitious regeneration, genetic transformation and in vitro storage of juvenile and adult tissues are reviewed, with indications regarding the possibilities of using these technologies as breeding tools.

14.5.1 Micropropagation

In olive micropropagation, explants of juvenile origin generally show high morphogenetic capacity (Cañas et al. 1992); however, working with this type of material is

not of interest since agronomical traits are not known. Attempts to establish in vitro explants of adult origin has encountered several problems such as explant oxidation and necrosis, difficulties of disinfection, poor growth as well as a strong influence of the genotype (Lambardi et al. 2013; Rugini and Baldoni 2005). The obtainment of responsive material has required the use of explants from specific sources: (a) new growth obtained after severe pruning of field-grown trees (Peixe et al. 2007; Roussos and Pontikis 2002) shoots from rooted cuttings grown in the greenhouse (Sghir et al. 2005) suckers sprouting at the tree base (Rugini and Fontanazza 1981; Vidoy-Mercado et al. 2012), (d) new growth obtained after forcing ovules (Rama and Pontikis 1990) or hardwood cuttings (Vidoy-Mercado et al. 2012) to sprout in a controlled environment and (e) shoots from in vivo (Garcia-Férriz et al. 2002) or in vitro grafts (Revilla et al. 1996).

Explants used for culture establishment are generally nodal sections with lateral buds forced to elongate in vitro and subsequently propagated by segmentation of elongated shoots (Lambardi et al. 2013). This method is considered very reliable in terms of genetic stability of the obtained material (George 1993). Regarding nutritional requirements, Rugini (1984) developed the OM formulation, which, in comparison to the widely used MS (Murashige and Skoog 1962), contains higher levels of Ca, Mg, S, Cu and Zn, lower NH_4 as well as an additional supplement of reduced nitrogen in the form of glutamine. The OM formulation has been widely used in olive culture (Brhadda et al. 2003b; Chaari et al. 2002; Lambardi et al. 2013; Sghir et al. 2005; Vidoy-Mercado et al. 2012), although others mineral nutrient mixtures developed for woody plants have also shown to be adequate for olive; e.g. WPM (Lloyd and McCown 1980) in cvs. Chondrodia and Chalkidikis (Grigoriadou et al. 2002) and cv. Kalamon (Dimassi-Theriou 1994) or a modified DKW (Driver and Kuniyuki 1984) in cvs. Koroneiki (Roussos and Pontikis 2002) and Arbequina (Vidoy-Mercado et al. 2012). In terms of carbon source, either sucrose (Grigoriadou et al. 2002; Rugini 1984; Sghir et al. 2005) or mannitol (Lambardi et al. 2013; Peixe et al. 2007; Roussos and Pontikis 2002) have been used; however, in a comparative study with cv. Maurino, Leva et al. (2013) showed that mannitol promoted shoot sprouting and growth more than sucrose.

Shoot elongation and proliferation require the presence of a cytokinin in the culture media, zeatin or zeatin riboside (4.6–13.6 μM) being the most widely used (Grigoriadou et al. 2002; Lambardi et al. 2013; Roussos and Pontikis 2002; Rugini 1984; Sghir et al. 2005); however, due to the high cost of these hormones, attempts have been made to replace them with other cytokinins; along this line, Peixe et al. (2007) in cv. Galega Vulgar obtained good results with benzyladenine and a coconut milk supplement, while Peyvandi et al. (2009a) used 2-isopenteniladenine (2iP) in cv. Rowghani. In some cases, a GA_3 supplement has been used for shoot elongation either in combination with zeatin (Grigoriadou et al. 2002; Vidoy-Mercado et al. 2012) or as a separate treatment prior to rooting (Lambardi et al. 2013).

Rooting of olive microcuttings is generally carried out in two phases, e.g. Rugini (1984) recommended a 2-week exposure to 5.4 μM naphthaleneacetic acid (NAA) followed by transfer to another medium devoid of auxin but supplemented with zeatin and activated charcoal; however, in most cases, shorter exposures to auxin in

liquid medium with subsequent transfer to either basal solid medium (Peixe et al. 2007; Sghir et al. 2005) or directly to the acclimatization substrate (peat moss/coco fiber/perlite, at different ratios, or jiffy pots) (García-Férriz et al. 2002; Peyvandi et al. 2009a) are preferred. In several cases, incubation in darkness and/or incorporation of putrescine to root induction medium have given positive results (Grigoriadou et al. 2002; Rugini et al. 1993). In addition, Peyvandi et al. (2009a) indicated that shoots which had proliferated in the presence of mannitol, rooted better than those previously multiplied in the presence of sucrose did. Acclimatization, a critical phase of the micropropagation process, is generally carried out on mist benches within a greenhouse with controlled light and temperature. Cozza et al. (1997) showed that survival of micropropagated plants of cv. Nocellara Etnea was linked to a higher level of vascular differentiation in comparison to those of Nocellara Belice. After hardening, Rugini (1984) recommends the use of GA_3 sprays to speed up regrowth of acclimatized plantlets.

Genetic stability of micropropagated olive plants has been evaluated through RAPDs analysis and conflicting results have been obtained, e.g. while García-Férriz et al. (2002), in cvs. Arbequina, Picual and Empeltre and Leva and Petruccelli (2012) in cv. Maurino, observed similar banding patterns in micropropagated material and the corresponding mother plants, Peyvandi et al. (2009b) and Farahani et al. (2011a, b) obtained opposite results in several Iranian cultivars; in addition and, according to these authors, observed variations increased with the number of subcultures. Regarding field performance, while Leva (2009) found no differences between plants micropropagated through axillary buds and self-rooted controls of cv. Maurino in terms of vegetative and reproductive growth patterns, Briccoli-Bati et al. (2006) obtained different results depending on genotype, e.g. while plants of cv. Nocellara Etnea showed higher production and lower average fruit size than control-grafted plants, micropropagated material of cv. Carolea showed very low production throughout the 8-year evaluation period, leading these authors to indicate that further research is needed to elucidate whether epigenetic variations could have occurred during the long time the plants had spent in culture.

14.5.2 Organogenesis

Early studies on adventitious shoot formation in olive were carried out using juvenile explants; e.g. Cañas and Benbadis (1988) evaluated the in vitro organogenic capacity of basal and apical sections of cotyledons, observing a better response in the former. Shoot regeneration required a 3-week culture period on OMc medium ((OM formulation with BN macroelements (Bourgin and Nitsch 1967) and 1 g/l casein hydrolysate)) supplemented with 25 μM indole-3-butyric acid (IBA), followed by subsequent transfer to basal medium supplemented with the cytokinin 2iP. Petioles from apical and basal nodes of adult micropropagated shoots of cvs. Moraiolo and Dolce Agogia were used by Mencuccini and Rugini (1993) obtaining better response in apical nodes of Moraiolo. No response was observed when using petioles derived

from adult plants grown either in the field or in the greenhouse. A hormonal supplement containing either 10 μM 2iP and 2.2-μM benzyladenine or 5–40 μM thidiazuron (TDZ) was recommended (Mencuccini and Rugini 1993). Interestingly, when regeneration took place in the presence of TDZ, MS formulation at full strength was required, although in cvs. Canino and Moraiolo adventitious shoots could also be obtained in ½ MS supplemented with 30 μM TDZ and 0.54 μM NAA (Rugini and Caricato 1995).

14.5.3 Somatic Embryogenesis

Somatic embryogenesis in olive was firstly observed when using immature zygotic embryos for culture establishment (Rugini 1988). Embryogenic callus could be induced in the absence of growth regulators although a cytokinin supplement was beneficial (Table 14.4). Orinos and Mitrakos (1991) in wild, as well as Mitrakos et al. (1992) and Cerezo et al. (2011) in domestic olives, recommended the use of isolated radicles from mature embryos to induce the process (Fig. 14.8a). In these cases, a medium with high auxin/cytokinin ratio was required to induce formation of embryogenic callus (Fig. 14.8b) followed by transfer to a medium without growth regulators or a very low auxin concentration, to enhance embryo differentiation (Fig. 14.8c). Similar requirements were found when using cotyledon (Brhadda et al. 2003a; Leva et al. 1995; Trabelsi et al. 2003) or root segments, from in vitro germinated seedlings (Rugini 1995; Rugini et al. 1995; Shibli et al. 2001), as explants (Table 14.4). Wounding the root surface and placing the explants in horizontal position enhanced callus formation (Rugini 1995).

Somatic embryogenesis has also been observed in adult material. Rugini and Caricato (1995) used petioles from shoots of adventitious origin of cvs. Canino and Moraiolo and cultured them in a medium with 2iP (0.5 μM), BA (0.44 μM), IBA (0.25 μM) and 0.42 mM cefotaxime. Resulting embryogenic structures could be maintained proliferating in the dark under the same conditions or in hormone-free medium supplemented with 0.1% activated charcoal; in the last case, proliferation of secondary embryos took place directly from epidermal or subepidermal cells of primary embryos (Benelli et al. 2001). In the Moroccan cv. Dahbia, Mazri et al. (2013) were able to induce embryogenesis following a 4-day exposure of leaf sections from in vitro grown shoots to liquid medium supplemented with 30 μM TDZ and 0.54 μM NAA followed by transfer to solid hormone-free medium for 8 weeks and final culture in the hormonal combination recommended by Rugini and Caricato (1995) (Table 14.4).

Mineral requirements for somatic embryogenesis seem to depend on explant used and genotype; e.g. for radicles, the OMc formulation (Mitrakos et al. 1992; Orinos and Mitrakos 1991) has been used, while in the case of roots from seedlings, MS was preferred (Rugini 1995; Rugini et al. 1995; Shibli et al. 2001) (Table 14.4). For cotyledons, adult leaf fragments or petioles, either OMc (Rugini and Caricato 1995;

Table 14.4 Somatic embryogenesis in olive

Cultivar	Explant	Induction/Callus proliferation	Embryo development	Conversion	References
Frantoio/Moraiolo/Dolce Agogia	75-day old embryos	½ MS + (0.5–2.5) μM BA ± 0.5 μM NAA	½ MS basal medium	OM + (0.5–2.5) μM zeatin	Rugini (1988, 1995), Rugini et al. (2005)
Wild olive	Mature embryo radicle/cotyledon	OMc + 2.5 μM 2iP + 25 μM IBA	OMc + 0.5 μM IBA	½ MS basal medium	Orinos and Mitrakos (1991)
Koroneiki	Mature embryo radicle	OMc + 2.5 μM 2iP + 25 μM IBA	OMc ± 2.5 μM IBA	-	Mitrakos et al. (1992)
San Agostino	Roots of seedlings	MS + 25 μM NAA + 0.5 μM BA	1/2MS + 0.5 μM NAA + 0.5 μM BA	¼ MS + 2.5 μM zeatin	Rugini (1995), Rugini et al. (1995)
Frangivento/Picholine/Frantoio	Immature cotyledons	SH + 2.5 μM NAA ± 0.5 μM 2iP	SH basal medium	SH basal medium	Leva et al. (1995)
Canino/Moraiolo	Leaflets/petioles from adult shoots of adventitious origin	OMc + 0.2 μM 2iP + 0.44 μM BA + 0.25 μM IBA	Basal liquid OMc or solid OMc + 0.1% AC	OMc + 1.3 μM zeatin	Benelli et al. (2001), Rugini and Caricato (1995), Rugini et al. (2005)
Nabali	Roots of seedlings	MS + 5.0 μM 2,4D + 0.5 μM Kin + 5.0 μM NAA	MS + 10 μM 2iP	MS basal medium	Shibli et al. (2001)
Picholine Maroccaine	Cotyledons	MS + 2.3 μM zeatin + 10.7 μM NAA	MS + 2.3 μM zeatin	MS + 2.3 μM zeatin	Brhadda et al. (2003a)
Chetoui/Chemlali/Arbequina	Cotyledons	OMc + 25 μM IBA + 2.5 μM 2iP	OMc basal medium	OM + 4.6 μM zeatin	Trabelsi et al. (2003)
Chondrolia Chalkidikis	Immature cotyledons Mature cotyledons	½ MS + 4.44 μM BA + 2.26 μM 2,4 D ½ MS + 4.44 μM BA	½ MS + 4.44 μM BA + 2.26 μM 2,4D ½ MS+4.44 μM	– –	Pritsa and Voyiatzis (2004)
Wild olive	Adult leaf/Petiole	MS + 12.25 μM IBA + 4.56 μM zeatin	MS basal	–	Capelo et al. (2010)
Picual	Mature embryo radicle	OMc + 2.5 μM 2iP + 25 μM IBA/OMc + 0.5 μM IBA/ ECO + 0.2 μM 2iP + 0.44 μM BA + 0.25 μM IBA	ECO+0.1% AC	1/3 MS macros +10 g/l sucrose	Cerezo et al. (2011)
Dahbia	Adult leaves	½ MS + 30 μM TDZ + 0.5 μM NAA/1/2MS basal/ ECO + 0.2μM2iP + 0.44 μM BA + 0.25 μM IBA	–	–	Mazri et al. (2013)

AC (activated charcoal); BA (6-benzylaminopurine); 2,4-D (2-4 dichlorophenoxyacetic acid); ECO (¼ OM macroelements, ¼ MS microelements, ½ OM vitamins and 550 mg/l glutamine); IBA (indole-3-butyric acid); 2iP (6-dimethylaminopurine); MS (Murashige and Skoog 1962); NAA (naphthaleneacetic acid); OM (Olive medium, Rugini 1984); OMc (OM in which macroelements were replaced by those of Bourgin and Nitsch (1967) with addition of 1 g/l casein hydrolysate and omitting glutamine); SH (Schenk and Hildebrand 1972); TDZ (thidiazuron)

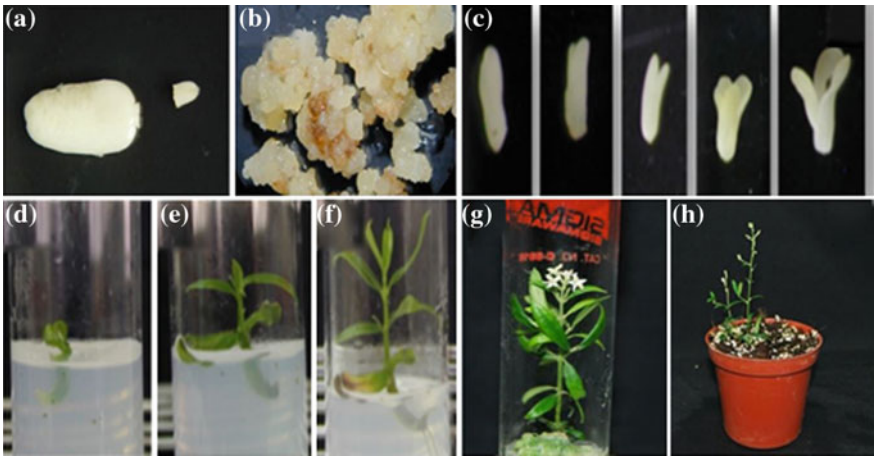

Fig. 14.8 Olive regeneration and transformation via somatic embryogenesis. **a** Radicle from mature embryo used for initiation of cultures, **b** Embryogenic callus, **c** Somatic embryos at torpedo and cotyledonary stages, **d–f** Germination of somatic embryo, **g**, **h** Transgenic plants, obtained after inoculation of globular somatic embryos with *Agrobacterium tumefaciens*, overexpressing the *Mt*FT gene developed flowers in vitro (**g**) or after the acclimatization phase (**h**). Photos by S. Cerezo

Trabelsi et al. 2003), MS (Brhadda et al. 2003a; Capelo et al. 2010) or ½ MS (Mazri et al. 2013; Pritsa and Voyiatris 2004) have shown to be adequate (Table 14.4).

In a comparative study between the basal formulations OMc (Sect. 14.5.2) and ECO (¼ OM macroelements, ¼ MS microelements and 550 mg/l glutamine), Cerezo et al. (2011) observed similar callus proliferation rates, although embryogenic structures grown in ECO formulation, of lower mineral strength, showed a higher capacity to form mature embryos. The same authors also found that culture of embryogenic callus for 4 weeks in liquid medium followed by sieving though a 3 × 3 mm mesh, could help to synchronize cultures prior to undergo embryo maturation in the presence of semipermeable cellulose acetate membranes. Embryos under this treatment showed a much lower water potential and germinated at 37.8% rate in a modified MS basal medium with ½ macroelements and 10 g/l sucrose (Fig. 14.8d–f) (Cerezo et al. 2011).

14.5.4 Somaclonal Variation

It is widely known that phenotypic variations can appear in plants regenerated in vitro, with the chances to occur being higher when adventive regeneration protocols, including a callus phase, are used (Bairu et al. 2011; Larkin and Scowcroft 1981). In olive, Cañas and Benbadis (1988) found that plants regenerated from cotyledon fragments showed morphological alterations, such as dichotomy or presence of double leaves.

In attempts to evaluate the effect of time in culture on regeneration capacity of olive embryogenic cells, Bradai et al. (2016a) studied the in vitro behavior of several embryogenic lines which had been kept in culture for 2 and 8 years. A strong genotypic effect was found, although embryos of aged lines showed a general decrease in maturation capacity and a reduced germination rate in comparison to younger ones; however, plants could be regenerated from material of all ages. Phenotypic evaluation of plants showed several alterations in vegetative traits (plagiotropic growth, fasciated stem and the appearance of 3 axillary shoots per whorl), phyllotaxy (4 leaves per verticil), leaf morphology (double leaves) and reproductive traits (flowers with 5–7 petals and 3–5 stamens). Again, genotype had a strong influence although, as expected, the frequency of variant phenotypes (19.78 vs. 4.0) and the percentage of plants showing these anomalies (37.36 vs. 10.0) were higher in aged than in younger lines (Bradai et al. 2016b). In plants obtained via somatic embryogenesis from immature cotyledons of cv. Frangivento, Leva (2009) characterized two types of somaclones: columnar and dwarf, differing in plant height, canopy projection, canopy volume and reproductive traits.

14.5.5 Genetic Transformation

Genetic transformation has been attempted in olive through biolistics and via *Agrobacterium tumefaciens*. Using the PDS-1000/He system to compare the efficiency of two promoters, Lambardi et al. (1999) found that the sunflower ubiquitin yielded higher *Gus* gene expression than the cauliflower 35S, in torpedo stage somatic embryos of cv. Canino, although opposite results were obtained with embryos at more advanced stages. Pérez-Barranco et al. (2009) using globular embryos derived from a mature embryo of cv. Picual reported similar results. These authors established a 6 cm target distance and a 900-psi bombardment pressure as optimum conditions for transformation. Gus expression could be observed 12 weeks after bombardment; however, as reported by Lambardi et al. (1999), no transgenic plants could be regenerated. Pérez-Barranco et al. (2009) also evaluated the response of olive cells to different antibiotics, pointing out the relatively high tolerance to kanamycin and paromomycin in solid medium while in liquid, antibiotics impaired cell growth at much lower concentrations.

Rugini et al. (2000) first attempted genetic transformation via *Agrobacterium tumefaciens* in somatic embryos of cv. Canino. In this protocol, after an initial 48 h exposure to the bacterial suspension, somatic embryos were transferred for 30 days to a medium deprived of antibiotics for recovery and subsequently exposed to 0.21 mM kanamycin in the dark; afterwards, embryogenic material was cultured in liquid medium in the light and only those embryos that turned green were selected. Finally, embryos were returned to the dark for secondary embryo production; these embryos were then induced to germinate in liquid medium deprived of antibiotics and supplemented with 1.3 μM zeatin. A different approach was undertaken by Torreblanca et al. (2010), using the highly virulent strain AGL1 for inoculation of globular stage

embryos, with continuous exposure to the antibiotic paromomycin 2 days after the co-culture phase. To avoid selection of chimeric tissues, a 3-week culture in liquid medium in the presence of antibiotic was used. For transgenic plant recovery the protocol of Cerezo et al. (2011), previously described (Sect. 14.5.3), was used.

Transformation via *Agrobacterium tumefaciens* has been used in attempts to improve agronomic traits as well as to undertake functional genomic studies. In an attempt to modify growth habit, somatic embryos of cv. Canino were transformed with rol ABC genes from *A. rhizogenes*; resulting transgenic plants showed hairy root phenotype, a long juvenile phase and maintained vegetative growth until late autumn (Rugini et al. 2008). To enhance stress tolerance, Rugini et al. (2000) were able to transform somatic embryos of the same genotype with the osmotin gene. Obtained transgenic plants have shown an outstanding resistance to water stress as well as enhanced tolerance to peacock spot (*Spilocaea oleagina*) although they are particularly attractive to the cribate weevil, *Otiorhynchus cribricollis* (Rugini et al. 2016). In order to elucidate the role of flowering locus T (FT) gene in olive, somatic embryos derived from a mature embryo of cv. Picual were transformed with FT-homologue from *Medicago truncatula*, following the protocol of Torreblanca et al. (2010). Some of the obtained transgenic lines flowered either during the in vitro phase (Fig. 14.8g) or following acclimatization (Fig. 14.8h); inflorescences were formed all year around although they were more abundant in spring. These plants also showed profuse axillary branching and reduced size (Haberman et al. 2017).

14.5.6 In Vitro Storage

14.5.6.1 Low-Temperature Conservation

Initial attempts to preserve olives under low temperature regimes were carried out by Micheli et al. (1998) through encapsulation of apical and lateral buds of cv. Moraiolo in sodium alginate beads; buds could be stored up to 45 days at 4 °C, although those of apical origin responded better in terms of sprouting. A few years later, Micheli et al. (2007) showed that 3–4 mm long nodal sections with lateral buds could be kept for 30 days at room temperature, in alginate nutrient solution kept in plastic cuvettes, suggesting that this could be an useful methodology to exchange material between countries. The encapsulation method has also been used by Cabello Moreno et al. (2013) using nodal sections of cv. Arbequina; these authors encapsulated buds at 4 °C over a 4-week period, indicating that pretreatments with growth regulators such as abscissic acid are beneficial for the conservation process.

Shoot cultures of cvs. Leccino and Frantoio, were maintained at 4 °C under darkness in OM basal medium for 8 months with a regrowth capacity above 80% (Lambardi et al. 2002), while in the case of cv. Arbequina maintained in RP medium (Roussos and Pontikis 2002) at 8 °C under light, shoots could be kept viable for 12 months (Imbroda et al. 2014).

14.5.6.2 Cryopreservation

Attempts at long-term conservation of buds have been carried out using the apical dome with 1–2 pairs of leaf primordia as explants. Martinez et al. (1999) obtained a 30% bud recovery in cv. Arbequina while Lambardi et al. (2002) reported a 15% in cv. Frantoio; however, in both cases, regrowth and further development or cryopreserved material occurred at a very low rate. Subsequent attempts to improve regrowth after cryopreservation through the inclusion of hormones in post-thaw medium, allowed Lynch et al. (2007) to maintain growth up to 10 weeks. Histological examinations showed the occurrence of damage in subapical cells, which could explain the failure in shoot recovery.

Somatic embryos seem to be a more suitable material for cryopreservation than buds. Shibli and AI-Juboory (2000) were able to successfully cryopreserve somatic embryos derived from seedling roots, via the encapsulation-dehydration or the encapsulation-vitrification methods; in both cases, inclusion of adequate dehydration steps (drying beads up to 21.1% moisture in the first case or keeping them for 3 h in plant vitrification solution) was critical for the process. In addition, exposure of embryogenic callus to 30 °C for 1 day allowed achievement of 58% survival rate when using encapsulation-dehydration or 68% in the case of encapsulation-vitrification protocol. Moreover, plants derived from cryopreserved cultures showed no morphological differences with controls. The importance of a correct dehydration step has also been emphasized by Sanchez-Romero et al. (2009) when using the droplet vitrification method after a 60 min dehydration in PVS2 (Sakai et al. 2008). In addition, Bradai and Sánchez-Romero (2017) indicated that preculturing embryogenic structures in a medium with high sucrose concentration was beneficial for culture recovery. Following regeneration, no morphological differences were observed between control plants and those derived from cryopreserved embryos. A different approach, including a 3-day sucrose pretreatment followed by incubation in a cry protectant mixture, prior to freezing at controlled rate (0.5 °C/min until −35 °C) and plunging into LN, allowed Lynch et al. (2011) to get noticeable callus regrowth and subsequent embryo recovery. Biochemical analysis indicated that applied pretreatments could enhance glutathione reductase, proline and sugar levels in the tissue, preparing them to withstand freezing.

14.6 Conclusions and Prospects

The choice of plant material is the first decision of the olive grower at the time of establishing a new plantation. A high diversity of local cultivars is a common trait in traditional olive-producing countries. Most olive cultivars are old, and they are grown around areas in which they were likely selected. In most cases, cultivars are self-rooted. Grafted trees are found only in the case of difficult-to-root cultivars or due to top grafting onto wild olives or onto other obsolete cultivars. In new olive-producing countries, imported cultivars represent most of the olive orchards. The

intensification of olive plantations after World War II in producing countries is based on a few selected national or foreign cultivars. This change has heightened interest in the conservation of olive genetic resources and in breeding olives.

The exploration, cataloguing and conservation of true-to-type traditional olive cultivars are the first steps for the sustainable use of genetic resources in any olive-growing country. Currently, ex situ collections are common facilities for conserving cultivars in germplasm banks. In all banks, confusion between names of accessions and cultivars remain an unresolved issue. An International Network with 22 National Germplasm Banks coordinated by the International Olive Council (IOC) with more than 1300 accessions is attempting to identify and to authenticate them. Morphologic characterization and DNA markers, particularly SSRs, have been shown to be powerful and discriminant methods to authenticate accessions (true-to-type cultivars), duplications, homonyms, synonyms and incorrect denominations. The identification and authentication of the IOC Network accessions is on course. The publication of a World Catalogue of True-to-Type Olive Cultivars will aid in solving the confusion in olive varietal denominations and developing International Plant Certification Protocols that will guarantee true-to-type and pathogen- and pest-free nursery plants in a time of global exchange of plant material. Other strategies, such as agronomic evaluation in banks and comparative trials, are in their infancy. The exploration and evaluation of wild and ancient olives is also under development to expand the genetic diversity to confront new breeding challenges such as climate change and devastating pests and diseases, as well as for studies related to the origin and domestication of the olive, among others.

New plantations systems have increased the need for new specific cultivars that have not been previously considered by empirical local breeders, i.e. the traditional farmer and breeder. The new systems require a high investment and early return, adaptation to mechanical harvesting, high yield, high oil content, resistance or tolerance to biotic and abiotic stresses, and quality of the olive oil and table olives, among others. In the last sixty years, crossbreeding has progressively developed in various olive growing countries. The breeding programs have demonstrated the following issues:

(1) A consistent increase in trained qualified breeders and publications.
(2) Evaluation and selection of parents for crosses will improve breeding efficiency.
(3) Reduction of the juvenile period (JP) to 2–3 years after planting accelerates the breeding process.
(4) Implementation of early selection methods of evaluation.
(5) Genetic studies demonstrates transgressed segregation of any trait in any crossbred progeny, consistency among genotype values data during the different steps of evaluation for most evaluated traits, and gain in the evaluation of a smaller number of well-selected crossbred progenies rather than many crossbred progenies with a smaller size.
(6) Moderate and high values of heritability for relevant agronomic, olive oil, and table olives traits is observed

(7) Progressive and fast development of genomic tools, such as MAS and GWAS, in addition to a recent publication concerning the first sequenced genome will accelerate the process of crossbreeding.
(8) Cooperation with farmers or other stakeholders during the final step of evaluation is critical for a sound G × E evaluation and for visibility among farmers of the new material advancements.
(8) New materials from public breeding programs for olive oil and table olive cultivars and rootstock will be progressively released in the next decade, providing farmers with cultivars for new mechanized plantation systems and/or resistance or tolerance to verticillium wilt and other diseases.
(10) New joint public-private consortia will develop new breeding programs.

Finally, registration represents the birth of a new cultivar. Marketing strategies appear as a major factor for its diffusion. This last step will determine whether any bred cultivar is well adapted to different environments in which it is dispersing, thus providing information about the real value of this innovation. A further requirement is the need for certification of nursery plants to ensure that newly-bred cultivars are true to type and free from pests and pathogens to demonstrate the potential performance of the new cultivar.

Regarding the possibilities of using in vitro regeneration and transformation technologies in breeding programs, micropropagation protocols are currently available for different olive cultivars; hence, although the genotype has strong influence on in vitro behavior, the technology could be useful for rapid multiplication of new releases as well as for international exchange of material. Regeneration via somatic embryogenesis is well established for juvenile material and very promising results are being obtained for explants of adult origin. Optimizing this pathway would make it feasible the use of cryopreservation for long-term in vitro storage as well as to undertake transformation of selected genotypes with gene coding for important agronomical traits. In any case, it appears that the interest of private companies for genetic manipulation of commercial cultivars, will depend on changes of consumer's acceptance of transgenic products.

Acknowledgments We dedicated this review to the collective effort of all researchers working on olive genetic resources and breeding in different Andalusia institutions since the establishment of the Olive Work Germplasm Bank of Córdoba in 1970, a landmark on olive breeding. We also acknowledge Isabel Trujillo for critical reading of the Section on Genetic Resources and to the graduate students Diego Cabello, Hristofor Miho, Pablo Morello and Pedro Valverde for their photographs and support in preparing the manuscript. Authors also recognize the plant breeding group of IFAPA-Alameda del Obispo, the biotechnology groups at IHSM (UMA-CSIC), Plant Biology Department, Malaga University, and IFAPA-Churriana, for their contributions to part of the research reviewed. Finally, we acknowledge the Spanish funding research agencies for their economic support for more than 40 years. Project P11-AGR-7992 is currently supporting part of the research indicated in Sect. 14.5.

Appendix 1

Research institutes and online resources

Country	Institution	Specialization and research activities	Contact information and website
Albania[a]	Centre of Agricultural Technology Transfer. Centre of Agricultural Technology Transfer Shamogjin, Komuna Novosele, Vlorë. Phone.: 00355 33 404144/145 Fax: 00355 33 404144/145	Genetic resources	Ms. Aulona Veizi aulona10@gmail.com qttbvlore@yahoo.com
Algeria[a]	ITAF. Tessala El Merdja - Birtouta -Alger. Phone: +213 023 58 38 60/61/66 Fax: +213 023 58 38 64/65	Genetic resources	M. Mahmoud Mendil mbmendil@gmail.com Itafv.dg@gmail.com webmaster@itafv.dz
Argentina	Laboratory of Genetic and Health Quality/ Faculty of Agricultural Sciences/UNC. Faculty of Agricultural Sciences National University of Cuyo. Almirante Brown 500. Chacras de Coria - Luján de Cuyo. CPA M5528AHB - Mendoza - Argentina. Phone: (+54 261) 413-5010	Genetic resources Breeding Genomics	L.E. Torres itorres@agro.unc.edu.ar http://www.fca.uncu.edu.ar/
Argentina	IBAM/ CONICET/ INTA. Faculty of Agricultural Sciences National University of Cuyo. Almirante Brown 500. Chacras de Coria - Luján de Cuyo. CPA M5528AHB - Mendoza - Argentina. Phone: (+54 261) 413-5010	Genetic resources Breeding Abiotic stresses Biotic stresses. Oil quality Genomics	R. W. Masuelli masuelli@fca.uncu.edu.ar https://inta.gob.ar/mendoza
Argentina[a]	EEA/CONICET/ INTA. Agricultural Experiment Station San Juan, Calle 11 y Vidart (5427) Villa Aberastain San Juan. Phone: (0264) 492 1079, (0264) 492 1191	Genetic resources Abiotic stresses	Dra. Mariela Torres mtorres@sanjuan.inta.gov.ar https://inta.gob.ar

Country	Institution	Specialization and research activities	Contact information and website
Australia	WWAI/ NSW DPI. Wagga Wagga Agr Inst, EH Graham Ctr Agr Innovat, Wagga Wagga, NSW 2650, Australia. Phone: (02) 6938 1999 International: +61 2 6938 1999 Fax: (02) 6938 1809	Genetic resources	www.dpi.nsw.gov.au
Australia	UNE. Univ New England, Sch Environm & Rural Sci, Armidale, NSW, 2351 Phone: +61 2 6773 2323 Fax: +61 2 6773 2769	Genetic resources Breeding	msedgle2@une.edu.au ers@une.edu.au
Belgium	Ghent University. Univ Ghent, Dept Plant Biotechnol & Bioinformat, B-9052 Ghent, Belgium Phone. +32 9 331 38 00 Fax +32 9 331 38 09	Genomics	marc.vanmontagu@ugent.be
Croatia	Faculty of Agriculture/ University of Zagreb. University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia, Phone: +385 (0)1 2393 777 Fax: +385 (0)1 2315 300	Genetic resources Breeding Genomics	Z. Satovic zsatovic@agr.hr http://www.agr.unizg.hr/en
Croatia[a]	Institute for Adriatic Crops and Karst Reclamation. Inst Adriat Crops & Karst Reclamat, Put Duilova 11, Split 21000, Croatia. Phone: +385.21.43.44.44 Fax: +385.21.31.65.84	Genetic resources Biotic stresses	Mr. Slavko Perica Slavko.Perica@krs.hr info@krs.hr
Cyprus[a]	Agricultural Research Institute // Officer Olive Technology Laboratory. Agricultural Research Institute. P.O.Box 22016, 1516 Nicosia, Cyprus. Phone: ++357 22 403100 Fax: ++357 22 316770	Genetic resources	Ms. Dora Chimonidou dari@arinet.ari.gov.cy info@ari.gov.cy
France	AGAP/INRA/ Montpellier SupAgro. Montpellier SupAgro, 2 place Pierre Viala, 34060 MONTPELLIER Cedex 02. Phone: +33 (0)4 99 61 22 00 Fax: +33 (0)4 99 61 29 00	Genetic resources Breeding	L. Essalouh laila.essalouh@supagro.inra.fr https://www.supagro.fr

Country	Institution	Specialization and research activities	Contact information and website
France[a]	UMR-AGAP. Avenue Agropolis, 34398 Montpellier Cedex 5, France. Phone: +33 4 67 61 58 00	Genetic resources Breeding Oil Quality Genomics	B. Khadari khadari@supagro.fr https://umr-agap.cirad.fr
France	Université de Toulouse III/ EDB - UMR 5174. 118, route de Narbonne Bât. 4R131062 TOULOUSE cedex 9 Phone (+33) 05 61 55 73 84 Fax: (+33) 05 61 55 73 27	Evolution	G. Besnard guillaume.besnard@univ-tlse.fr http://www.edb.ups-tlse.fr/
Greece	Laboratory of Pomology/ Department of Crop Science/ Agricultural University of Athens. Agricultural University of Athens. Iera Odos 75, Athina 118 55, Greece. Phone.: +30 21 0529 4900 Fax. +30210-5294081.	Genetic resources Breeding	M. Hagidimitriou marianna@aua.gr http://www2.aua.gr
Greece	Institute of Viticulture, Floriculture and Vegetable Crops (I.V.F.V.H)/ NAGREF. Institute of Viticulture, Floriculture and Vegetable Corps of Herakleion // I.V.F.V.H // PO Box 2229 // 71003 Herakleion Phone: 2810 302 300// 245 851, 240 986. Fax 2810 245 873	Genetic resources Breeding Abiotic stresses Biotic Stresses Oil Quality	A.G. Doulis grandreas.doulis@nagref-her.gr http://www.nagref-her
Greece[a]	Olive Cultivation and Post Harvest Physiology Laboratory/ Institute for Olive Tree and Subtropical plants of Chania/NAGREF. Institute for Olive Tree and Subtropical plants of ChaniaLeof. Soudas 131, Chania 731 34, Greece. Phone:+30 2821 083472	Genetic resources Breeding Abiotic stresses Biotic Stresses Oil Quality	Dr. G.C. Koubouris koubouris@nagref-cha.gr Info@nagref-her.gr
Greece	Aristotle Univ. Thessaloniki. Aristotle Univ Thessaloniki, Dept Hort, Lab Biol Hort Plants, Thessaloniki 54124, Hellas, Greece	Biotic Stresses Breeding Oil Quality	Maria Tsimidou tsimidou@chem.auth.gr info@agro.auth.gr

Country	Institution	Specialization and research activities	Contact information and website
Iran	National Institute of Genetic Engineering and Biotechnology. Shahrak-e Pajoohesh, km 15, Tehran - Karaj Highway, Tehran, Iran P.O. Box: 14965/161. Phone: +98 21 44787301-9. Fax: +98 21 44787399	Genetic Resources Breeding Genomics	M. Hosseini-Mazinani hosseini@nigeb.ac.ir nigeb_manager@nigeb.ac.ir
Iran	Horticulture Department/Gorgan University of Agricultural Sciences. Gorgan University of Agricultural Sciences and Natural Resources. Gorgan, 49138-15739, Iran. Phone: +98-171-2220320. Fax: + 98-171-2220640	Genetic Resources Breeding Genomics	M. Sharifani mmsharif2@gmail.com International@gau.ac.ir
Iran	SPII/HD. SPII, Hort Dept, Mahdasht Rd,POB 31359-33181, Karaj, Iran	Breeding	A. Zeinanloo info@abrii.ac.ir
Israel	The Robert H. Smith Faculty of Agriculture, Food and Environment The Hebrew University of Jerusalem. Hebrew Univ Jerusalem, Fac Agr, Inst Plant Sci, IL-76100 Rehovot, Israel. P.O Box 12, Rehovot 76100	Genetic resources Breeding Abiotic stresses Biotic stresses. Oil Quality Genomics	A. Samach alon.samach@mail.huji.ac.il http://departments.agri.huji.ac.il
Israel[a]	ARO/ Volcani Center[a]. Agricultural Research Organization - the Volcani Center, 68 HaMaccabim Road, P.O.B 15159 Rishon LeZion 7505101, Israel Phone: +972-3-9683226 Fax: +972-3-9665327	Genetic Resources	Dr. Giora Ben Ari giora@agri.huji.ac.il http://www.agri.gov.il
Italy	IVALSA. National Research Council of Italy, Trees and Timber Institute Follonica (Grosseto) via Aurelia, 49 58022 - Follonica (GR) Phone. +39 056 652356	Genetic Resources Breeding Genomics	C. Cantini cantini@ivalsa.cnr.it http://www.ivalsa.cnr.it

Country	Institution	Specialization and research activities	Contact information and website
Italy	CNR/Institute of plant genetics. Institute of plant genetics. Via Madonna Alta, 130-06128 Perugia (PG) - Umbria Phone: +39 0755014862 Fax: 0755014869	Genetic resources Breeding Genomics	L. Baldoni luciana.baldoni@ibbr.cnr.it http://www.ibbr.cnr.it
Italy	DEMETRA/ University of Palermo. University of Palermo. Piazza Marina, 61// 90133 - PALERMO. Phone: +39 091 238 93011	Genetic Resources Abiotic stresses Biotic stresses	T. Caruso f tiziano.caruso@unipa.it http://www.unipa.it
Italy	Management Department of Agricultural and Forestry Systems/ University of the Mediterranean Studies of Reggio Calabria. Mediterranea University of Reggio Calabria. Salita Melissari 89124 Reggio Calabria. Tel.: +39 0965 169 1207 Fax: +39 0965 332201	Genetic Resources Breeding	R. Mafrica rocco.mafrica@unirc.it http://www.unirc.it
Italy	UNIFI/DISPAA. Universita degli studi fii Firenze. Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente Piazzale delle Cascine, 18 - 50144 Firenze Phone: +39 055275-5700	Genetic resources Breeding Abiotic stresses	http://dispaa.unifi.it
Italy	UNITU. Universita della Tuscia, DAFNE, Via San Camillo de Lellis Snc, I-01100 Phone +39 0761357581/554; Fax +39 0761357558/434	Biotechnology Genomics	Eddo Rugini rugini@unitus.it dafne@pec.unitus.it
Italy	CNR/ IVALSA. Sesto Fiorentino (Firenze) via Madonna del Piano, 10 50019 - Sesto Fiorentino (FI) Phone: +39 055 52251	Genetic Resources Genomics	M. Centrito centrito@ivalsa.cnr.it info@ivalsa.cnr.it

Country	Institution	Specialization and research activities	Contact information and website
Italy	UNIBA/DISAAT. Universita di Bari, Dipartimento di Scienze Agro Ambiental e Territoriali (Di.S.A.A.T.) Amendola 165-A, I-70126 Bari, Italy	Genetic resources Biotic stresses	Franco Nigro franco.nigro@uniba.it info@agr.uniba.it
Italy	ENTECRA OLI. CRA OLI, I-06049 Spoleto, PG, Italy. Centro di ricerca per l'olivicoltura e l'industria olearia (Rende) Via Nursina 2 06049 - *SPOLETO*. Phonel: +39 0743-49743 Fax: +39 0743-43634	Genetic resources Biotic stresses	Adolfo Rosati rosati@entecra.it info@entecra.it
Italy	UNIPG/DSAAA. Univ Perugia, Dipartimento Sci Agr Alimentari & Ambientali, Via Borgo 20 Giugno 74, I-06121 Perugia, Italy	Oil quality	Maurizio Servili maurizio.servili@unipg.iti nfo@unipg.it
Italy[a]	CRA-OLI Research center for olive growing and oil industry. Centro di ricerca per l'olivicoltura e l'industria olearia – Sede Scientifica di Città S.Angelo (OLI.PE) Viale Petruzzi 75 65013 - CITTA' SANT'ANGELO	Genetic resources Breeding Abiotic stresses Biotic stresses Oil quality	Enzo Perri enzo.perri@crea.gov.it http://sito.entecra.it
Jordan[a]	NCARE. National Center for Agricultural Research and Extension PO Box 639, Baq'a 19381, Jordania Phone: +962 (6) 4725071 Fax: +962 (6) 4726099	Genetic Resources Breeding Abiotic stresses Biotic stresses Oil quality	Dr. Salam Ayoub salamayoub@hotmail.com http://www.ncare.gov.jo/
Lebanon	Lebanese University/ Faculty of Agricultural Sciences. Faculty of Agricultural Sciences. PO Box 90775, Horst Tabet, Beirut - Lebanon Phone: 484130/01 484131/01 484132/01 Fax: 510870/01 510867/01	Genetic resources Breeding Genomics	L. Chalak lamis.chalak@gmail.com https://www.ul.edu.lb

Country	Institution	Specialization and research activities	Contact information and website
Lebanon[a]	LARI. Lebanese Agr Res Inst, Lab Olive Oil, Tal Amara, Bekaa, Lebanon	Genetic resources Oil quality	Milad El Riachy mraichy@lari.gov.lb info@lari.gov.lb
Montenegro[a]	Biotechnical Faculty/Centre For Subtropical Cultures. Biotechnical Faculty. Centre for Subtropical Cultures Bar-University of Montenegro. Ul. Bjelisi bb 85000 Bar// Montenegro Phone: (382) 69516165	Breeding Genomics	B. Lazovic biljanal@t-com.me http://www.ucg.ac.me
Morocco[a]	INRA/ URAP. Centre Régional de la Recherche Agronomique de Marrakech Unité de Recherche sur l'Amélioration des Plantes et de la qualité B.P. 533 Menara MARRAKECH Maroc Phone: +212 524447882/ +212 524435175/ +212 524432627 Fax: +212 524446380	Genetic Resources Breeding	Sikaoui Lhassane sikaouilhassane@yahoo.fr http://www.inra.org.ma
Portugal[a]	INIAV. UEI de Biotecnologia e Recursos Geneticos. Polo de Elvas Estrada de Gil Vaz, Apartado 67351-901 Elvas – Portugal Phone: (+ 351) 268 637 740	Genetic resources Breeding Oil quality Table olives quality Genomics	António M Cordeiro antonio.cordeiro@iniav.pt polo.elvas@iniav.pt
Slovenia[a]	Experimental center for olive growing. Agriculture and Forestry Institute Nova Gorica.Ulica 15. maja 17, 6000 Koper Tel: ++386 (0)5 631 32 32/ ++386 (0)41 815 302	Genetic resources	Ms. Vesel Viljanka viljanka.vesel@siol.net www.kmetijskizavod-ng.si
Spain[a]	IFAPA Centro Alameda del Obispo. Centro Alameda del Obispo Avda. Menéndez Pidal s/n 14004- Córdoba Phone. +34 957016000	Genetic resources Breeding Abiotic stresses Biotic stresses. Oil quality Genomics	Raul De la Rosa raul.rosa@juntadeandalucia.es cordoba.ifapa@juntadeandalucia.es

Country	Institution	Specialization and research activities	Contact information and website
Spain	IMIDRA. Finca El Encin, Autovía del Noreste A-2, Km. 38.200, 28805// Alcalá de Henares, Madrid Phone: +34 918 87 94 00	Genetic resources Breeding Oil quality Genomics	B. E. Sastre blanca.esther.sastre@madrid.org www.madrid.org/imidra/
Spain	Instituto de la Grasa/ CSIC. Instituto de la Grasa, CSIC. Ctra. de Utrera, km. 1. Campus Universitario Pablo de Olavide - Edificio 46. 41013 - SEVILLA (España) Phone:(+34) 95 461 1550 Fax:(+34) 95 461 6790	Genetic resources Breeding Oil quality Table olives quality Genomics	J. M. Martínez-Rivas mrivas@cica.es www.ig.csic.es
Spain	Plant Physiology/ Faculty of Science/ University of Extremadura. University of Extremadura. Avda. de Elvas, s/n. 06006 Badajoz Phone:+34 924 289 300 Fax.: +34 924 272 983	Genetic resources Abiotic stresses Biotic stresses. Oil quality Genomics	M. C. Gomez-Jimenez mcgomez@unex.es https://www.unex.es
Spain	IAS-CSIC. Instituto de Agricultura Sostenible Avenida Menéndez Pidal s/n Campus Alameda del Obispo 14004 Córdoba (España) Phone: +34 957 49 92 00 Fax:+34 957 49 92 52	Genetic resources Breeding Abiotic stresses Biotic stresses.	H. F. Rapoport hrapoport@ias.csic.es http://www.ias.csic.es/
Spain[a]	Department of Agronomy/ University of Córdoba[a]. Universidad de Córdoba Departamento Agronomía Campus Univ. de Rabanales Ctra. Madrid-Cádiz Km. 396 14071-Córdoba Phone +34 957218433/ 34/ 35 Fax +34 957218438	Genetic resources Breeding Abiotic stresses Biotic stresses. Oil quality Genomics	Diego Barranco ag1banad@uco.es infoetsiam@uco.es
Spain	IRTA Mas de Bover. Instituto de Investigación y Tecnología Agroalimentarias. Ctra. de Reus El Morell Km 4,5 Phone: 977 32 84 24 Fax: 977 34 40 55	Genetic resources Breeding Abiotic stresses Biotic stresses. Oil quality Genomics	Agusti Romero agusti.romero@irta.cat http://www.irta.cat

Country	Institution	Specialization and research activities	Contact information and website
Spain	CSIC/EEZ. Plant Reproductive Biology Laboratory, Estacion Experimental del Zaidin (CSIC), Profesor Albareda 1, 18008 Granada, Spain. Phone +34 958572757 Fax +34958572753	Abiotic stresses Genomics	Juan D. Alche juandedios.alche@eez.csic.es info @csic.eez.es
Spain	US/ETSIA. Departamento Agroforestal. ETSIA. Universidad de Sevilla. Ctra. Utrera km 1. 41013 Sevilla. Phone +34 954486455 Fax +34 954486436	Breeding Quality table olives	Pilar Rallo prallo@us.es agroforestal@us.es
Spain	CNAG CRG. Barcelona Inst Sci & Technol, Ctr Genom Regulat, CNAG CRG, Baldiri i Reixac 4, Barcelona 08028, Spain Phone +34 93 316 01 00 Fax +34 93 316 00 99	Genomics	Toni Gabaldon toni.gabaldon@crg.eu. info@crg.esi
Spain	UJA/DBE. Univ Jaen, Dept Biol Expt, Campus Lagunillas S-N, Edif B-3, Jaen 23071, Spain. Phone:+34 953 212527	Genomics Oil Quality	Francisco Luque fjluque@ujaen.es info@ujaen.es
Spain	CSIC/IRNAS. CSIC, IRNAS, Irrigat & Crop Ecophysiol Grp, Ave Reina Mercedes 10, Seville 41012, Spain. Phone: +34 95 462 47 11	Abiotic Stresses	Enrique Fernandez Luque jeferse@irnase.csic.es www.irnas.csic.es
Spain	IHSM/UMA-CSIC, Inst Hortofruticultura Subtrop & Mediterranea, Univ Malaga-CSIC, Dept. Biol Vegetal, Fac Ciencias, E-29071 Malaga, Spain. Phone: +34 952131947	Biotechnology	F. Pliego, ferpliego@uma.es
Tunisia	IRESA. Univ Sousse, IRESA, High Agron Inst, B.P. n° 47 -4042 Chott Meriem *Sousse*, *Tunisie Phone*: (+216) 73 327 544/ (+216) 73 327.592. Fax: (+216) 73 327	Genetic resources	Ibtisissem Laribi ibtissem.laaribi@yahoo.fr isa.chott@iresa.agrinet.tn

Country	Institution	Specialization and research activities	Contact information and website
Tunisia[a]	Institut de l'Olivier Sfax, Route de l'Aéroport, B.P. 1087 3000 Sfax Tél: (+216) 74 241 240/ 74 241 589 Fax: (+216) 74 241 033	Genetic Resources Breeding Biotic Stresses	Monji Msallem msallemonji@yahoo.fr http://www.iosfax.agrinet.tn/
Turkey	Department of Bioengineering/Ege University. Ege University Faculty of Engineering Department of Bioengineering. Erzene Quarter, Ege Unv. No:180, 35040 Bornova/İzmir Phine: 0 232 311 58 11 Fax: 0 232 311 58 80	Genetic Resources and Breeding Genomics	B. Tanyolaç bahattin.tanyolac@ege.edu.tr http://biyomuhendislik.ege.edu.tr info@ege.edu.tr
Turkey	Bornova Olive Research Station. Olive Culture Research Station Üniversite Cd. No: 43 35100 Bornova/ İzmir Phone: +90 (232) 462-7073	Genetic Resources	Dr. Unal Kaya unal.kaya@gthb.gov.tr posta@zae.gov.tr
Turkey[a]	GFAR. Olive Research Institute - İzmir Universite Cd. No:43 35100 BORNOVA IZMIR Phone: +90 232 462 70 73 Fax: +90 232 435 70 42	Genetic Resources	Melek Gurbuz melekgurbuz11@gmail.com izmirzae@tarim.gov.tr
Uruguay	INIA/ National Agricultural Research Institute. National Agricultural Research Institute. Andes 1365 - piso 12 CP 11100 Montevideo, Uruguay Phone: + 598 2902 0550 Fax: + 598 2902 3666	Genetic Resources and Breeding Abiotic stresses Biotic stresses	P. Conde pconde@inia.org.uy inia@inia.org.uy
USA	National Clonal Germplasm Repository/USDA. Nat'l Clonal Germplasm Rep - Tree Fruit & Nut Crops & Grapes. Davis, CA One Shields Ave, UC Davis Davis, CA 95616 Phone: (530)752-7009 Fax: 530-752-5974	Genetic Resources Breeding Genomics	J. E. Preece John.Preece@ars.usda.gov https://www.ars.usda.gov

[a]IOC Network Germplasm Bank

Appendix 2

Genetic resources: Main native cultivars

Country	Cultivar	Use	Fruit weight (g)	Oil content
Albania	Kalinjot	Oil, Table	3.5	High
Algeria	Azeradj	Oil, Table	4	Medium
	Chemlal de Kabylie	Oil	2	Low
	Sigoise	Table, Oil	3	Medium
Argentina	Arauco	Table, Oil	8	Medium
Chile	Azapa	Table, Oil	8	Medium
Croatia	Lastovka	Oil	3	High
	Oblica	Table	5	Medium
Cyprus	Ladoelia	Oil	2.5	High
Egypt	AggeiziShami	Table	8	Very low
	Touffahi	Table	12	Very low
France	Aglandau	Oil, Table	2.5	Medium
	Bouteillan	Oil	5	High
	Grosanne	Table, Oil	2.5	Low
	Lucques	Table	3	Low
	Picholine du Languedoc	Table, Oil	3	Medium
	Salonenque	Table, Oil	3	Medium
	Tanche	Oil, Table	2.5	High
Greece	Adramitini	Oil	2.5	High
	Amigdaloia	Table, Oil	8	Medium
	Chalkidikis	Table	8	Medium
	Kalamata	Table	3.5	Medium
	Konservolia	Table	4	Medium
	Koroneiki	Oil	1	Very high
	Mastoidis	Oil, Table	2.6	Very high
	Megaritiki	Oil	2	High
	Valanolia	Oil	2.5	Medium
Iran	Fishomi	Table		Medium
	Mari	Table, Oil	3.5	High
	Roghani	Oil	4	High
	Zard	Oil, Table	4.5	Medium

Country	Cultivar	Use	Fruit weight (g)	Oil content
Israel	Barnea	Oil, Table	3	Medium
	Nabali	Oil, Table	3	High
	Souri	Oil, Table	3	High
Italy	Ascolana	Table	7	Low
	Bosana	Oil	3	High
	Cellina di Nardo	Oil	2	Low
	Coratina	Oil	5	Medium
	Frantoio	Oil	3	High
	Leccino	Oil	2.5	Medium
	Moraiolo	Oil	2	High
	OgliarolaBarese	Oil	2	High
	Pendolino	Oil	2	Low
	Taggiasca	Oil	2	High
Jordan	Nabali Baladi	Table, Oil	3	High
	Rasei	Oil	3	Medium
Lebanon	Baladi	Oil, Table	3	High
	Souri	Oil, Table	3	High
Libya	Endory	Oil	1	High
	Hammudi	Oil	2.2	High
Malta	Bidni	Oil, Table	2	High
Montenegro	Zutica	Oil	3.2	High
Morocco	Picholine Marocaine	Oil, Table	3.5	Medium
Palestine	Nabali	Oil, Table	3.1	High
	Souri	Oil, Table	3	High
Perú	Criolla	Table	8	High
Portugal	Cobrançosa	Oil	3	Medium
	GalegaVulgar	Oil, Table	2.5	Medium
Slovenia	Buga	Oil		
Spain	Arbequina	Oil	1.9	High
	Arbosana	Oil	2	Medium
	Cornicabra	Oil	3	High
	Empeltre	Oil	2.7	Medium
	Farga	Oil	2.4	Medium

Country	Cultivar	Use	Fruit weight (g)	Oil content
	Gordal Sevillana	Table	12.5	Low
	Hojiblanca	Oil, Table	4.8	Medium
	Lechin de Sevilla	Oil	3	Medium
	Manzanilla de Sevilla	Table	4.6	Medium
	Picual	Oil	3.2	High
Syria	Doebli	Oil, Table	4.5	High
	Sorani	Oil, Table	3	High
	Zaity	Oil	2.5	Very high
	Kaissy	Table	5	Low
Tunisia	Chemlali Sfax	Oil	1	Very high
	Chetoui	Oil, Table	2.5	Medium
	Oueslati	Oil, Table	2	High
Turkey	Ayvalik	Oil	3.6	High
	Domat	Table	5.3	Medium
	Gemlik	Oil, Table	3.7	High
	Memecik	Oil, Table	4.8	Medium
USA	Mission	Oil, Table	3	Medium

References

Alagna F, D'Agostino N, Torchia L et al (2009) Comparative 454 pyrosequencing of transcripts from two olive genotypes during fruit development. BMC Genom 10:399. https://doi.org/10.1186/1471-2164-10-399

Alcántara E, Cordeiro AM, Barranco D (2003) Selection of olive varieties for tolerance to iron chlorosis. J Plant Phys 160(12):1467–1472

Arias-Calderón R, Rodríguez-Jurado D, Bejarano-Alcázar J et al (2015a) Evaluation of verticillium wilt resistance in selections from olive breeding crosses. Euphy 206(3):619–629. https://doi.org/10.1007/s10681-015-1463-7

Arias-Calderón R, Rodríguez-Jurado D, León L et al (2015b) Pre-breeding for resistance to verticillium wilt in olive: fishing in the wild relative gene pool. Crop Prot 75:25–33. https://doi.org/10.1016/j.cropro.2015.05.006

Arias-Calderon R, Rouiss H, Rodriguez-Jurado D et al (2014) Variability and heritability of fruit characters in olive progenies from open-pollination. Sci Hort 169:94–98

Atienza SG, De la Rosa R, Domínguez-García MC et al (2013) Use of DArT markers as a means of better management of the diversity of olive cultivars. Food Res Int 54:2045–2053

Atienza SG, de la Rosa R, León L et al (2014) Identification of QTL for agronomic traits of importance for olive breeding. Mol Breed 34:725–737. https://doi.org/10.1007/s11032-014-0070-y

Avidan B, Meni Y, Lavee S (2012) Inheritance potential of fruit quality traits within olive (*Olea europaea* L.) cultivars. Acta Hort 949:105–111

Azzarello E, Mugnai S, Pandolfi C et al (2009) Comparing image (fractal analysis) and electrochemical (impedance spectroscopy and electrolyte leakage) techniques for the assessment of the

freezing tolerance in olive. Trees-Struct Funct 23(1):159–167. https://doi.org/10.1007/s00468-008-0264-1
Baccouri B, Ben Temime S, Campeol E et al (2007) Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. Food Chem 102:850–856
Bacelar EA, Moutinho-Pereira JM, Gonçalves BC et al (2009) Physiological responses of different olive genotypes to drought conditions. Acta Phys Plant 31(3):611–621. https://doi.org/10.1007/s11738-009-0272-9
Badenes ML, Byrne DH (eds) (2012) Fruit breeding. Handbook of plant breeding, vol 8. Springer, Dordrecht
Bairu MW, Aremu AO, van Staden J (2011) Somaclonal variation in plants: causes and detection methods. Plant Growth Regul 63:147–173
Barranco D, Cimato A, Fiorino P et al (2000) World catalogue of olive varieties. International Olive Council, Madrid
Barranco D, Fernández-Escobar R, Rallo L (eds) (2010) Olive growing, 1st English edition of the 5th edition of El cultivo del olivo. Co-edition. Junta de Andalucia-Mundi-Prensa-RIRDC, Australia
Barranco D, Fernández-Escobar R, Rallo L (eds) (2017) El cultivo del olivo, 7th edn. Mundi- Prensa, Madrid
Barranco D, Rallo L (1984) Las variedades de olivo cultivadas en Andalucía. Ministerio de Agricultura-Junta de Andalucía, Madrid
Barranco D, Rallo L, Trujillo I (2005a) Elaiografía hispánica. In: Rallo L, Barranco D, Caballero JM et al (eds) Variedades de olivo en España. Junta de Andalucía, MAPA, Ediciones Mundi-Prensa, Madrid, pp 80–231
Barranco D, Ruiz N, Gómez-Del Campo M (2005b) Frost tolerance of eight olive cultivars. HortSci 40(3):558–560
Bartolini G (2008) Olive germplasm (*Olea europaea* L.): cultivars, synonyms, cultivation area, collections, descriptors. http://www.oleadb.it/
Bartolini G, Prevost G, Messeri C et al (1998) Olive germplasm: cultivars and world-wide collections. FAO, Rome
Bartolozzi F, Fontanazza G (1999) Assessment of frost tolerance in olive (*Olea europaea* L.). Sci Hort 81(3):309–319. https://doi.org/10.1016/S0304-4238(99)00019-9
Bazakos C, Manioudaki ME, Therios I et al (2012) Comparative transcriptome analysis of two olive cultivars in response to NaCl-stress. PLoS ONE 7:e42931. https://doi.org/10.1371/journal.pone.0042931
Bazakos C, Manioudaki ME, Sarropoulou E et al (2015) 454 pyrosequencing of olive (*Olea europaea* L.) transcriptome in response to salinity. PLoS ONE 10:e0143000. https://doi.org/10.1371/journal.pone.0143000
Belaj A, Trujillo I, de la Rosa R et al (2001) Polymorphism and discriminating capacity of randomly amplified polymorphic markers in an olive germplasm bank. J Am Soc Hort Sci 126:64–71
Belaj A, Satovic Z, Rallo L, Trujillo I (2002) Genetic diversity and relationships in olive (Olea europaea L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theor Appl Genet 105:638–644
Belaj A, Caballero JM, Barranco D et al (2003a) Genetic characterization and identification of new accessions from Syria in an olive germplasm bank by means of RAPD markers. Euphy 134:261–268
Belaj A, Satovic Z, Ismaili H et al (2003b) RAPD genetic diversity of Albanian olive germplasm and its relationships with other Mediterranean countries. Euphy 130:387–395
Belaj A, Rallo L, Trujillo I et al (2004a) Using RAPD and AFLP markers to distinguish individuals obtained by clonal selection of 'Arbequina' and 'Manzamila de Sevilla' olive. HortSci 39(7):1566–1570

Belaj A, Satovic Z, Cipriani G et al (2003c) Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. Theor Appl Genet 107:736–744
Belaj A, Satovic Z, Rallo L (2004b) Optimal use of RAPD markers for identifying varieties in olive (*Olea europaea* L.) germplasm collections. J Am Soc Hort Sci 129(2):266–270
Belaj A, Trujillo I, Barranco D et al (2004c) Characterization and identification of Spanish olive germplasm by means of RAPD markers. HortSci 39(2):346–350
Belaj A, Muñoz-Diez C, Baldoni L et al (2007) Genetic diversity and population structure of wild olives from the north-western Mediterranean assessed by SSR markers. Ann Bot 100:449–458
Belaj A, Muñoz-Diez C, Baldoni L et al (2010) Genetic diversity and relationships of wild and cultivated olives at regional level in Spain. Sci Hort 124:323–330
Belaj A, León L, Satovic Z et al (2011) Variability of wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*) analyzed by agromorphological traits and SSR markers. Sci Hort 129:561–569
Belaj A, Dominguez-Garcia MD, Atienza SG et al (2012) Developing a core collection of olive (*Olea europaea* L.) based on molecular markers (DArTs, SSRs, SNPs) and agronomic traits. Tree Genet Genom 8:365–378
Belaj A, Gurbuz VM, Sikaoui H et al (2016) Olive genetic resources. In: Rugini E, Baldoni L, Mulea R, Sabastini L (eds) The olive tree genome, compendium of plant genomes. Springer International Publishing AG, New York, pp 26–54. https://doi.org/10.1007/978-3-319-48887-5_3
Bellincontro A, Caruso G, Mencarelli F, Gucci R (2013) Oil accumulation in intact olive fruits measured by near infrared spectroscopy-acousto-optically tunable filter. J Sci Food Agr 93(6):1259–1265. https://doi.org/10.1002/jsfa.5899
Bellini E (1992) Behaviour of some genetic characters in olive seedlings obtained by cross-breeding. Acta Hort 317:197–208
Bellini E (1993) Variabilità genetica ed ereditarietà di alcuni caratteri in semenzali d'incrocio di olivo. Olivae 49:21–34
Bellini E, Giordani E, Parlati MV (2004) Arno, Tevere e Basento: nuove cultivar di olivo ottenute per incrocio. Olivae 102:42–46
Bellini E, Giordani E, Parlati MV et al (2002a) Olive genetic improvement: thirty years of research. Acta Hort 586:105–108
Bellini E, Giordani E, Parlati MV et al (2002b) Olive genetic improvement: variability within the progeny "Picholine x Grossane". Acta Hort 586:183–186
Bellini E, Giordani E, Ranalli A et al (2002c) Analytical characteristics of the virgin olive oils from three new genotypes obtained at Florence by cross-breeding. Acta Hort 586:125–128
Ben-Ari G, Biton I, Avidan B, Lavee S (2014) The diversity in performance of commercial olive clones selected from the autochthonous cv. Souri population for intensive irrigated cultivation. HortSci 49:425–429
Ben Sadok I, Celton JM, Essalouh L et al (2013) QTL mapping of flowering and fruiting traits in olive. PLoS ONE 8:e62831
Ben Sadok I, Martinez S, Moutier N et al (2015) Plasticity in vegetative growth over contrasted growing sites of an f1 olive tree progeny during its juvenile phase PLoS One 10(6). https://doi.org/10.1371/journal.pone.0127539
Benelli C, Fabri A, Grassi S et al (2001) Histology of somatic embryogenesis in mature tissues of olive (*Olea europaea* L.). J Hort Sci Biotech 76(1):112–119
Besnard G, Anthelme F, Baali-Cherif D (2012) The Laperrine's olive tree (Oleaceae): a wild genetic resource of the cultivated olive and a model-species for studying the biogeography of the Saharan Mountains. Acta Bot Gallica 159(3):319–328. https://doi.org/10.1080/12538078.2012.724281
Besnard G, Khadari B, Navascués M et al (2013) The complex history of the olive tree: from Late Quaternary diversification of Mediterranean lineages to primary domestication in the northern Levant. Proc R Soc B-Biol Sci 280:20122833. https://doi.org/10.1098/rspb.2012.2833
Bogani P, Cavalieri D, Petruccelli R et al (1994) Identification of olive tree cultivars by using random amplified polymorphic DNA. Acta Hort 356:98–101
Boulouha B (1986) Sélection clonale de la Picholine Marrocaine. Olea 17:67–70

Bourgin JP, Nitsch JP (1967) Production of haploid *Nicotiana* from excised stamens. Ann Phys Vég 9:377–382
Bradai F, Pliego-Alfaro F, Sanchez-Romero C (2016a) Long-term somatic embryogenesis in olive (*Olea europaea* L.): influence on regeneration capability and quality of regenerated plants. Sci Hort 199:23–31
Bradai F, Pliego-Alfaro F, Sanchez-Romero C (2016b) Somaclonal variation in olive (*Olea europaea* L.) plants regenerated via somatic embryogenesis: influence of genotype and culture age on phenotypic stability. Sci Hort 213:208–215
Bradai F, Sánchez-Romero C (2017) Efecto de la crioconsevación y de un precultivo con alta concentracion de sacarosa sobre la embriogénesis somática de olivo. Madrid, Libro de Resúmenes, XII Reunión de la SECIVTV, p 98
Brhadda N, Abousalim A, Walali LDE (2003a) Effets du milieu de culture et de la lumiére sur l'embriogenése somatique de l'olivier (*Olea europaea* L.) cv. Picholine Marocaine. Fruits 58:167–174
Brhadda N, Abousalim A, Walali LDE et al (2003b) Effets du milieu de culture sur le microbouturage de l'olivier (*Olea europaea* L.) cv. Picholine Marocaine. Biotechnol Agron Soc Envir 7(3–4):177–182
Briccoli-Bati C, Godino G, Monardo D et al (2006) Influence of propagation techniques on growth and yield of olive trees cultivars 'Carolea' and 'Nocellara Etnea'. Sci Hort 109:173–182
Caballero JM, Del Río C, Barranco D et al (2006) The olive world germplasm bank of Cordoba, Spain. Olea 25:14–19
Cabello Moreno B, Barceló Muñoz A, Padilla IMG (2013) Petratamientos y encapsulación en alginato como método de conservación in vitro de olivo. X Reunión de la SECIVTV, Zaragoza, Libro de Resúmenes, p 55
Caceres ME, Cecarelli M, Pupilli F et al (2015) Floral biology in *Olea europaea* subsp *cuspidata*: a comparative structural and functional characterization. Euphy 201(2):307–319
Cañas LA, Ávila J, Vicente M et al (1992) Micropropagation of olive (*Olea europaea* L.). In: Bajaj YPS (ed) Biotechnology in agriculture and forestry Vol 18: High-tech and micropropagation II. Springer, Berlin, pp 493–505
Cañas LA, Benbadis A (1988) In vitro plant regeneration from cotyledon fragments of the olive tree (*Olea europaea* L.). Plant Sci 54:65–74
Capelo AM, Silva S, Brito G et al (2010) Somatic embryogenesis induction in leaves and petioles of a mature wild olive. Plant Cell Tiss Organ Cult 103:237–242
Carmona R, Zafra A, Seoane P et al (2015) ReprOlive: a database with linked data for the olive tree (*Olea europaea* L.) reproductive transcriptome. Front Plant Sci 6:625. https://doi.org/10.3389/fpls.2015.00625
Caruso T, Marra FP, Costa F et al (2014) Genetic diversity and clonal variation within the main Sicilian olive cultivars based on morphological traits and microsatellite markers. Sci Hort 180:130–138
Casanova L, Suárez MP, Fernández-Cabanás VM et al (2014) From the juvenile to the adult vegetative phase in olive seedlings: the transition along the stem axis. Span J Agr Res 12(4):1149–1157. https://doi.org/10.5424/sjar/2014124-6363
Cerezo S, Mercado JA, Pliego-Alfaro F (2011) An efficient regeneration system via somatic embryogenesis in olive. Plant Cell Tiss Organ Cult 106:337–344
Chaari A, Chelly-Chaabouni A, Maalej M et al (2002) Meski olive variety propagated by tissue culture. Acta Hort 586:871–874
Charafi J, El Meziane A, Moukhli A et al (2008) Menara gardens: a Moroccan olive germplasm collection identified by a SSR locus-based genetic study. Genet Res Crop Evol 55:893–900
Chiappetta A, Muto A, Bruno L et al (2015) A dehydrin gene isolated from feral olive enhances drought tolerance in *Arabidopsis* transgenic plants. Front Plant Sci 6:392. https://doi.org/10.3389/fpls.2015.00392
Ciccarese F, Ambrico A, Longo O et al (2002) Search for resistance to verticillium-wilt and leaf spot in olive. Acta Hort 586:717–720

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

Colella C, Miacola C, Amenduni M et al (2008) Sources of verticillium wilt resistance in wild olive germplasm from the Mediterranean region. Plant Pathol 57(3):533–539

Cozza R, Turco D, Briccoli-Bati C et al (1997) Influence of growth medium on mineral composition and leaf histology in micropropagated plantlets of *Olea europaea*. Plant Cell Tiss Organ Cul 51:215–223

Cruz F, Julca I, Gómez-Garrido J et al (2016) Genome sequence of the olive tree, *Olea europaea*. Gigasci 5:29. https://doi.org/10.1186/s13742-016-0134-5

Daane KM, Johnson MW (2010) Olive fruit fly: managing an ancient pest in modern times. Ann Rev Entom 55. https://doi.org/10.1146/annurev.ento.54.110807.090553

Dabbou S, Chaieb I, Rjiba I et al (2012) Multivariate data analysis of fatty acid content in the classification of olive oils developed through controlled crossbreeding. J Am Oil Chem Soc 89:667–674

De la Guardia MD, Alcántara E (2002) A comparison of ferric-chelate reductase and chlorophyll and growth ratios as indices of selection of quince, pear and olive genotypes under iron deficiency stress. Plant Soil 241(1):49–56. https://doi.org/10.1023/A:1016083512158

De la Rosa R, Angiolillo A, Guerrero C et al (2003) A first linkage map of olive (*Olea europaea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. Theor Appl Genet 106:1273–1282. https://doi.org/10.1007/s00122-002-1189-5

De la Rosa R, Arias-Calderón R, Velasco L, León L (2016) Early selection for oil quality components in olive breeding progenies. Eur J Lipid Sci Tech 118:1160–1167. https://doi.org/10.1002/ejlt.201500425

De la Rosa R, James CM, Tobutt KR (2004) Using microsatellites for paternity testing in olive progenies. HortSci 39(2):351–354

De la Rosa R, Kiran AI, Barranco D, Leon L (2006) Seedling vigour as a preselection criterion for short juvenile period in olive breeding. Aust J Agr Res 57:477–481. https://doi.org/10.1071/ar05219

De la Rosa R, Klepo T, Arias-Calderon R et al (2014) Current status of conservation, evaluation and usefulness of wild olive germplasm. Acta Hort 1057:515–520

De la Rosa R, Leon L, Guerrero N et al (2007) Preliminary results of an olive cultivar trial at high density. Aust J Agric Res 58:392–395. https://doi.org/10.1071/AR06265

De la Rosa R, Talhaoui N, Rouis H et al (2013) Fruit characteristics and fatty acid composition in advanced olive breeding selections along the ripening period. Food Res Int 54(2):1890–1896

D'Hallewin G, Mulas M, Schirra M (1990) Characteristic of eleven table-olive clones selected from Nera cultivar. Acta Hort 286:48–52

Díaz A, Martín A, Rallo P et al (2007) Cross-compatibility of the parents as the main factor for successful olive (*Olea europaea* L.) breeding crosses. J Am Soc Hort Sci 132:1–6

Diez CM, Moral J, Cabello D et al (2016) Cultivar and tree density as key factors in the long-term performance of super high-density olive orchards Front. Plant Sci 7:1226. https://doi.org/10.3339/fpls.2016.01226

Diez CM, Trujillo I, Barrio E et al (2011) Centennial olive trees as a reservoir of genetic diversity. Ann Bot 108:797–807. https://doi.org/10.1093/aob/mcr194

Díez CM, Trujillo I, Martinez-Urdiroz N et al (2015) Olive domestication and diversification in the Mediterranean Basin. New Phytol 206:436–447. https://doi.org/10.1111/nph.13181

Dimassi-Theriou K (1994) In vitro propagation of cv. 'Kalamon' olives (*Olea europaea sativa* L.). Adv Hort Sci 8:185–189

Domínguez-García MC, Belaj A, De La Rosa R et al (2012) Development of DArT markers in olive (Olea europaea L.) and usefulness in variability studies and genome mapping. Sci Hort 136:50–60. https://doi.org/10.1016/j.scienta.2011.12.017

Driver JA, Kuniyuki A (1984) In vitro propagation of paradox walnut rootstock (*Juglans hindsii* x *Juglans regia*) by tissue culture. HortSci 19(4):507–509

El Aabidine AZ, Charafi J, Grout C et al (2010) Construction of a genetic linkage map for the olive based on AFLP and SSR markers. Crop Sci 50:2291. https://doi.org/10.2135/cropsci2009.10.0632

El Bakkali A, Haouan H, Moukhli A et al (2013) Construction of core collections suitable for association mapping to optimize use of Mediterranean olive (*Olea europaea* L.) genetic resources. PLoS ONE 8(5):e61265

El Riachy M, Priego-Capote F, Leon L, de Castro MDL (2012a) Virgin olive oil phenolic profile and variability in progenies from olive crosses. J Sci Food Agr 92(12):2524–2533

El Riachy M, Priego-Capote F, Rallo L et al (2012b) Phenolic profile of virgin olive oil from advanced breeding selections. Span J Agr Res 10(2):443–453

El Riachy M, Priego-Capote F, Rallo L et al (2012c) Phenolic composition of virgin olive oils from cross breeding segregating populations. Eur J Lipid Sci Tech 114(5):542–551

El Riachy M, Rallo L, de la Rosa R, Leon L (2011) May soil solarization reduce the juvenile period in olive? HortSci 46(9):1241–1244

Ersoy AH, Arsel F, Sefer U et al (2008) The first findings on the promising individuals from hybridization. Acta Hort 791:49–54

Fabbri A, Hormaza JI, Polito VS (1995) Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. J Am Soc Hort Sci 120(3):538–542

Farahani F, Yari R, Sheidai M (2011a) Molecular C-value and morphological analyses of somaclonal variation in three olive cultivars. Afr J Plant Sci 5(9):493–499

Farahani F, Yari R, Sheidai M (2011b) Somaclonal variation in Dezful cultivar of olive (*Olea europaea* subsp. *europaea*). Gene Cons 40:216–233

Faraloni C, Cutino I, Petruccelli R et al (2011) Chlorophyll fluorescence technique as a rapid tool for in vitro screening of olive cultivars (*Olea europaea* L.) tolerant to drought stress. Envir Exp Bot 73:49–56

Fendri M, Trujillo I, Trigui A et al (2010) Simple sequence repeat identification and endocarp characterization of olive tree accessions in a Tunisian germplasm collection. HortSci 45:1429–1436

Fernandes Serrano JM (1990) Clonal selection in Portuguese olive varieties. Acta Hort 286:53–56

Fernández JE, Moreno F (1999) Water use by the olive tree. J Crop Prod 2(2):101–162

Fernández-Ocaña A, García-López MC, Jiménez-Ruiz J et al (2010) Identification of a gene involved in the juvenile-to-adult transition (JAT) in cultivated olive trees. Tree Genet Genom 6:891–903. https://doi.org/10.1007/s11295-010-0299-5

Fiorino P (ed) (2003) Olea. Trattato di olivicoltura. Edagricole. Bologna

Fontanazza G, Baldoni L (1990) Proposed programme for the genetic improvement of the olive. Olivae 34:32–40

Fourati H, Cossentini M, Karray B, Khlif M (2002a) Classification of olive trees according to fruit and oil characterization. Acta Hort 586:687–690

Fourati H, Cossentini M, Khlif M (2002b) Study of the sterolic fraction of some free crossbreeding olive cultivars. Acta Hort 586:683–686

Frisullo S, Camele I, Agosteo GE et al (2014) Brief historical account of olive leaf scorch ("brusca") in the Salento peninsula of Italy and state-of-the-art of the olive quick decline syndrome. J Plant Path 96(3):441–449

Gabaldón-Leal C, Ruiz-Ramos M, de la Rosa R et al (2017) Impact of changes in mean and extreme temperatures caused by climate change on olive flowering in southern Spain. Int J Climat 37:940–957. https://doi.org/10.1002/joc.5048

Garantonakis N, Varikou K, Birouraki A (2017) Influence of olive variety on biological parameters of *Bactrocera oleae* (Diptera: Tephritidae). Appl Entom Zool 52(2):189–196. https://doi.org/10.1007/s13355-016-0467-7

Garcia Berenguer A (1988) Selección clonal en *Olea europaea* L. cultivar Picual. ITEA 75:9–13

Garcia JL, Avidan N, Troncoso A et al (2000) Possible juvenile-related proteins in olive tree tissues. Sci Hort 85:271–284

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

García-Férriz L, Ghorbel R, Ybarra M et al (2002) Micropropagation from adult olive trees. Acta Hort 586:879–882

Garcia-Gonzalez DL, Tena N, Aparicio R (2010) Quality characterization of the new virgin olive oil var. Sikitita by phenols and volatile compounds. J Agr Food Chem 58(14):8357–8364

Garcia-Lopez MC, Vidoy I, Jimenez-Ruiz J et al (2014) Genetic changes involved in the juvenile-to-adult transition in the shoot apex of *Olea europaea* L. occur years before the first flowering. Tree Genet Genom 10:585–603

Garcia-Mozo H, Orlandi F, Galan C et al (2009) Olive flowering phenology variation between different cultivars in Spain and Italy: modeling analysis. Theor Appl Climat 95(3–4):385–395. https://doi.org/10.1007/s00704-008-0016-6

Gaut BS, Díez CM, Morrell PL (2015) Genomics and the contrasting dynamics of annual and perennial domestication. Trends Genet 31:709–719. https://doi.org/10.1016/j.tig.2015.10.002

Gemas VJV, Almadanim MCC, Tenreiro R et al (2004) Genetic diversity in the olive tree (*Olea europaea* L. subsp *europaea*) cultivated in Portugal revealed by RAPD and ISSR markers. Genet Res Crop Evol 51:501–511. https://doi.org/10.1023/B:GRES.0000024152.16021.40

George EF (1993) Plant propagation by tissue culture, vol I: The technology. Exegetics, Edington, UK

Giovenzana V, Beghi R, Civelli R et al (2015) Postharvest characterization of olive oil fruits texture by NIR and Vis/NIR spectroscopy. In: Guidetti R, Bodria L, Best S (eds) Frutic Italy 2015: 9th Nut and Vegetable Production Engineering Symposium, vol 44, pp 61–66

Gomes S, Martins-Lopes P, Lima-Brito J et al (2008) Evidence for clonal variation in 'Verdeal-Trasmontana' olive using RAPD, ISSR and SSR markers. J Hort Sci Biotech 83:395–400

Gómez-del-Campo M, Barranco D (2005) Field evaluation of frost tolerance in 10 olive cultivars. Plant Genet Resour-C 3(3):385–390. https://doi.org/10.1079/PGR200592

Gonçalves MF, Malheiro R, Casal S et al (2012) Influence of fruit traits on oviposition preference of the olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), on three Portuguese olive varieties (Cobrançosa, Madural and Verdeal Transmontana). Sci Hort 145:127–135. https://doi.org/10.1016/j.scienta.2012.08.002

Grasso F, Coppola M, Carbone F et al (2017) The transcriptional response to the olive fruit fly (*Bactrocera oleae*) reveals extended differences between tolerant and susceptible olive (*Olea europaea* L.) varieties. PLoS One 12(8). https://doi.org/10.1371/journal.pone.0183050

Grigoriadou K, Vasilakakis M, Eleftheriou EP (2002) In vitro propagation of the Greek olive cultivar 'Chondrolia Chalkidikis'. Plant Cell Tiss Organ Cult 71:47–54

Guerfel M, Boujnah D, Baccouri B, Zarrouk M (2007) Evaluation of morphological and physiological traits for drought tolerance in 12 Tunisian olive varieties (*Olea europaea* L.). J Agron 6(2):356–361

Guerra D, Lamontanara A, Bagnaresi P et al (2015) Transcriptome changes associated with cold acclimation in leaves of olive tree (*Olea europaea* L.). Tree Genet Genom 11:113. https://doi.org/10.1007/s11295-015-0939-x

Haberman A, Bakhshian O, Cerezo-Medina S et al (2017) A possible role for flowering locus T-encoding genes in interpreting environmental and internal cues affecting olive (*Olea europaea* L.) flower induction. Plant, Cell Environ 40:1263–1280. https://doi.org/10.1111/pce.12922

Hackett WP (1985) Juvenility, maturation, and rejuvenation in woody plants. Hort Rev 7:109–155

Hammami SBM, De la Rosa R, Sghaier-Hammami B et al (2012) Reliable and relevant qualitative descriptors for evaluating complex architectural traits in olive progenies. Sci Hort 143:157–166. https://doi.org/10.1016/j.scienta.2012.06.009

Hammami SBM, Leon L, Rapoport HF, De la Rosa R (2011) Early growth habit and vigour parameters in olive seedlings. Sci Hort 129(4):761–768. https://doi.org/10.1016/j.scienta.2011.05.038

Hancock JF (2008) Temperate fruit crop breeding. Springer, New York

Haouane H, El Bakkali A, Moukhli A et al (2011) Genetic structure and core collection of the World Olive Germplasm Bank of Marrakech: towards the optimized management and use of Mediterranean olive genetic resources. Genet 139(9):1083–1094

Hartmann H, Schnathorst WC, Whisler J (1971) Oblonga—clonal olive rootstock resistant to verticillium wilt. Calif Agr 25(6):12–15

Hartmann HT, Kester DE, Davies FT Jr, Geneve RL (2002) Plant propagation principles and practices. Prentice Hall, Englewood Cliffs

Hartmann HT, Porlingis I (1957) Effect of different amounts of winter chilling on fruitfulness of several olive varieties. Bot Gaz 119(2):102–104

Hassani D, Tombesi A (2008) Vegetative growth of olive genotypes from a diallel cross. Acta Hort 791:137–142

Heinze B, Fussy B (2008) Somatic mutations as a useful tool for studying clonal dynamics in trees. Mol Ecol 17:4779–4781. https://doi.org/10.1111/j.1365-294X.2008.03964

Hernandez ML, Belaj A, Sicardo MD et al (2017) Mapping quantitative trait loci controlling fatty acid composition in olive. Euphy 213:7

Hosseini-Mazinani M, Torkzaban B, Arab J (2013) Iranian Olive Catalogue. National Institute of Genetic Engineering and Biotechnology Press, Tehran

Iannotta N, Noce ME, Ripa V et al (2007) Assessment of susceptibility of olive cultivars to the *Bactrocera oleae* (Gmelin, 1790) and *Camarosporium dalmaticum* (Thüm.) Zachos & Tzav.-Klon attacks in Calabria (southern Italy). J Envir Sci Heal B 42(7):789–793. https://doi.org/10.1080/03601230701551426

Imbroda I, Cabello Moreno B, Gallego J et al (2014) Frigoconservación in vitro de olivo (cultivar Arbequina). Acta Hort 69:139–140

IOC (2017). International Olive Council. http://www.internationaloliveoil.org. Accessed 13 Nov 2017

İpek A, İpek M, Ercişli S, Tangu NA (2017) Transcriptome-based SNP discovery by GBS and the construction of a genetic map for olive. Funct Integr Genom 17:493–501. https://doi.org/10.1007/s10142-017-0552-1

Jimenez R, Rallo P, Suarez MP et al (2011) Cultivar susceptibility and anatomical evaluation of table olive fruit bruising. Acta Hort 924:419–424

Jiménez-Díaz RM, Cirulli M, Bubici G et al (2012) Verticillium wilt, a major threat to olive production: status and future prospects for its management. Plant Dis 96(3):304–329. https://doi.org/10.1094/pdis-06-11-0496

Jiménez-Fernández D, Trapero-Casas JL, Landa BB et al (2016) Characterization of resistance against the olive-defoliating Verticillium dahliae pathotype in selected clones of wild olive. Plant Path 65(8):1279–1291. https://doi.org/10.1111/ppa.12516

Jiménez-Ruiz J, García-López MC, Vidoy I et al (2015) Transcriptional analysis of adult cutting and juvenile seedling olive roots. Tree Genet Genom 11:77. https://doi.org/10.1007/s11295-015-0898-2

Johnson R, Jellis GJ (1992) Breeding for disease resistance, vol 1. Kluwer Academic Publishers, Dordrecht

Kamoun NG, Khilf M, Ayadi M, Karray B (2002) Clonal selection of olive tree variety "Chemlali Sfax": preliminary results. Acta Hort 586:147–150

Kaya HB, Cetin O, Kaya HS et al (2016) Association mapping in Turkish olive cultivars revealed significant markers related to some important agronomic traits. Biochem Gen 54:506–533

Khlif M, Trigui A (1990) Olive cultivars investigations. Preliminary results. Acta Hort 286:65–68

Klepo T, Toumi A, de la Rosa R et al (2014) Agronomic evaluation of seedlings from crosses between the main Spanish olive cultivar 'Picual' and two wild olive trees. J Hort Sci Biotech 89(5):508–512

Koubouris GC, Breton CM, Metzidakis IT, Vasilakakis MD (2014) Self-incompatibility and pollination relationships for four Greek olive cultivars. Sci Hort 176:91–96. https://doi.org/10.1016/J.Scienta.2014.06.043

Lambardi M, Amorisi S, Caricato G et al (1999) Microprojectile-DNA delivery in somatic embryos of olive (*Olea europaea* L.). Acta Hort 474:505–509

Lambardi M, Benelli C, Carlo De et al (2002) Medium and long-term in vitro conservation of olive germplasm (*Olea europaea* L.). Acta Hort 586:109–112
Lambardi M, Ozudogru EA, Roncasaglia R (2013) In vitro propagation of olive (*Olea europaea* L.) by nodal segmentation of elongated shoots. In: Lambardi M, Ozudogru EA, Jain SM (eds) Protocols for micropropagation of selected economically-important horticultural plants. Springer, New York, pp 33–44
Larkin PJ, Scowcroft SC (1981) Somaclonal variation-a novel source of variability from cell culture for plant improvement. Theor App Genet 60:197–214
Lavee S (1990) Aims, methods, and advances in breeding of new olive (*Olea europaea* L.) cultivars. Acta Hort 286:23–36
Lavee S (2012) From the olive tree to olive oil—new trends and future challenges. Acta Hort 924:263–276
Lavee S (2013) Evaluation of the need and present potential of olive breeding indicating the nature of the available genetic resources involved. Sci Hort 161:333–339. https://doi.org/10.1016/j.scienta.2013.07.002
Lavee S, Avidan B (2011) Heredity diversity in populations of free-, self-, and specific cross-pollinated progenies of some olive (*Olea europaea* L.) cultivars. Isr J Plant Sci 59(1):29–37
Lavee S, Avidan N, Haskal A, Ogrodovich A (1996) Juvenility period reduction in olive seedlings-a tool for enhancement of breeding. Olivae 60:33–41
Lavee S, Harshemesh H, Haskal A et al (1999) 'Maalot' a new cultivar for oil extraction resistant to *Spilocaea oleagina* (Cast.). Acta Hort 474:125–128
Lavee S, Singer A, Haskal A et al (2008) Diversity in performance between trees within the traditional Souri olive cultivar (*Olea europaea* L.) in Israel under rain-fed conditions. Olivae 109:33–45
Leitao F, Serrano JF, Potes MF et al (1999) Preliminary results on clonal and sanitary selection of *Olea europaea* L Cv 'Santulhana' in north-east of Portugal. Acta Hort 474:163–166
Leon L (2012) Usefulness of portable near infrared spectroscopy in olive breeding programs. Span J Agr Res 10:141–148
Leon L, Arias-Calderon R, de la Rosa R et al (2016) Optimal spatial and temporal replications for reducing environmental variation for oil content components and fruit morphology traits in olive breeding. Euphy 207(3):675–684
Leon L, Beltran G, Aguilera MP et al (2011) Oil composition of advanced selections from an olive breeding program. Eur J Lipid Sci Tech 113(7):870–875
Leon L, De la Rosa R, Barranco D, Rallo L (2007) Breeding for early bearing in olive. HortSci 42(3):499–502
Leon L, De la Rosa R, Gracia A et al (2008) Fatty acid composition of advanced olive selections obtained by crossbreeding. J Sci Food Agr 88(11):1921–1926
Leon L, Downey G (2006) Preliminary studies by visible and near-infrared reflectance spectroscopy of juvenile and adult olive (*Olea europaea* L.) leaves. J Sci Food Agr 86:999–1004
Leon L, Martin LM, Rallo L (2004a) Repeatability and minimum selection time for fatty acid composition in olive progenies. HortSci 39:477–480
Leon L, Rallo L, Del Rio C, Martin M (2004b) Variability and early selection on the seedling stage for agronomic traits in progenies from olive crosses. Plant Breed 123:73–78
Leon L, Rallo L, Garrido A (2003) Near-infrared spectroscopy (NIRS) analysis of intact olive fruit: a useful tool in olive breeding programs. Grasas Aceites 54:41–47
Leon L, Velasco L, de la Rosa R (2015) Initial selection steps in olive breeding programs. Euphy 201(3):453–462
Leva A (2009) Morphological evaluation of olive plants micropropagated in vitro culture through axillary buds and somatic embryogenesis methods. Afric J Plant Sci 3(3):37–43
Leva AR, Muleo R, Petruccelli R (1995) Long-term embryogenesis from immature olive cotyledons. J Hort Sci 70(3):417–421
Leva AR, Petruccelli R (2012) Monitoring of cultivar identity in micropropagated olive plants using RAPD and ISSR markers. Biolog Plant 56(2):373–376

Leva AR, Sadeghi H, Petruccelli R (2013) Carbohydrates modulate the in vitro growth of olive microshoots. I. The analysis of shoot growth and branching patterns. J Plant Growth Regul 32:53–60

Leyva-Pérez MD, Jiménez-Ruiz J, Gómez-Lama Cabanás C et al (2017) Tolerance of olive (*Olea europaea*) cv Frantoio to *Verticillium dahliae* relies on both basal and pathogen-induced differential transcriptomic responses. New Phytol. https://doi.org/10.1111/nph.14833

Lloyd G, McCown B (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc Int Plant Prop Soc 30:421–427

López-Doncel LM, Trapero A, García-Berenguer A (1999) Resistance of olive tree cultivars to leaf spot caused by *Spilocaea oleagina*. Acta Hort 474:549–554

López-Escudero FJ, Mercado-Blanco J (2011) Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil 344(1):1–50

Luvisi A, Aprile A, Sabella E et al (2017) *Xylella fastidiosa* subsp. *pauca* (CoDiRO strain) infection in four olive (*Olea europaea* L.) cultivars: profile of phenolic compounds in leaves and progression of leaf scorch symptoms. Phytopath Medit 56(2):259–273. https://doi.org/10.14601/phytopathol_mediterr-20578

Lynch PT, Siddika A, Johnston JW et al (2011) Effects of osmotic pretreatments on oxidative stress, antioxidant profiles and cryopreservation of olive somatic embryos. Plant Sci 181:47–56

Lynch PT, Siddika A, Mehra A et al (2007) The challenge of successful cryopreservation of olive (*Olea europaea* L.) shoot tip. Adv Hort Sci 21:211–214

Mailer RJ (2004) Rapid evaluation of olive oil quality by NIR reflectance spectroscopy. J Am Oil Chem Soc 81:823–827

Malheiro R, Casal S, Baptista P, Pereira JA (2015) Physico-chemical characteristics of olive leaves and fruits and their relation with *Bactrocera oleae* (Rossi) cultivar oviposition preference. Sci Hort 194:208–214. https://doi.org/10.1016/j.scienta.2015.08.017

Mancuso S, Azzarello E (2002) Heat tolerance in olive. Adv Hort Sci 16(3–4):125–130

Marcelo A, Fernandes M, Fatima Potes M, Serrano JF (1999) Reactions of some cultivars of *Olea europaea* L. to experimental inoculation with *Pseudomonas syringae* pv. *savastanoi*. Acta Hort 474:581–584

Marchese A, Marra FP, Caruso T et al (2016) The first high-density sequence characterized SNP-based linkage map of olive (*Olea europaea* L. subsp. *europaea*) developed using genotyping by sequencing. Aust J Crop Sci 10:857–863

Marin L, Benlloch M, Fernández-Escobar R (1995) Screening of olive cultivars for salt tolerance. Sci Hort 64(1–2):113–116. https://doi.org/10.1016/0304-4238(95)00832-6

Martinez D, Arroyo-Garcia R, Revilla MA (1999) Cryopreservation of in vitro grown shoots-tips of *Olea europaea* cv Arbequina. CryoLett 20:29–36

Martins A, Santos L, Lopes J, Gouveia J (1998) Primeiros resultados da seleção da variedade de oliveira Cobrançosa. Rev Ciên Agr 21(1–4):35–46

Martins-Lopes P, Gomes S, Lima-Brito J et al (2009) Assessment of clonal genetic variability in *Olea europaea* L. "Cobrançosa" by molecular markers. Sci Hort 123:82–89. https://doi.org/10.1016/J.SCIENTA.2009.08.001

Mazri MA, Belkoura I, Pliego-Alfaro F et al (2013) Somatic embryogenesis from leaf and petiole explants of the Moroccan olive cultivar Dahbia. Sci Hort 159:88–95

Medina E, Morales-Sillero A, Ramirez EM et al (2012) New genotypes of table olives: profile of bioactive compounds. Int J Food Sci Tech 47(11):2334–2341

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

Mendil M, Sebai A (2006) L'olivier en Algérie. Institut Technique de l'Arboriculture Fruitiére et de la Vigne, pp 48–86

Micheli M, Hafiz IA, Standardi A (2007) Encapsulation of in vitro-derived explants of olive (*Olea europaea* L. cv. Moraiolo) II. Effects of storage on capsule and derived shoots performance. Sci Hort 113:286–292

Micheli M, Mencuccini M, Standardi A (1998) Encapsulation of in vitro proliferated buds of olive. Adv Hort Sci 12:63–168
Mitrakos K, Alexaki A, Papadimitriou P (1992) Dependence of olive morphogenesis on callus origin and age. J Plant Phys 139(3):269–273
Moore GA (2001) Oranges and lemons: clues to the taxonomy of citrus from molecular markers. Trends Genet 17:536–540. https://doi.org/10.1016/S0168-9525(01)02442-8
Moral J, Alsalimiya M, Roca LF et al (2015) Relative susceptibility of new olive cultivars to *Spilocaea oleagina*, *Colletotrichum acutatum*, and *Pseudocercospora cladosporioides*. Plant Dis 99(1):58–64. https://doi.org/10.1094/PDIS-04-14-0355-RE
Moral J, Diez CM, Leon L et al (2013) Female genitor effect on the juvenile period of olive seedlings. Sci Hort 156:99–105
Moral J, Trapero A (2009) Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. Plant Dis 93(10):1028–1036. https://doi.org/10.1094/PDIS-93-10-1028
Morales-Sillero A, Fernandez-Cabanas VM, Casanova L et al (2011) Feasibility of NIR spectroscopy for non-destructive characterization of table olive traits. J Food Eng 107(1):99–106. https://doi.org/10.1016/j.jfoodeng.2011.05.039
Moreno-Alías I, León L, De la Rosa R, Rapoport HF (2009) Morphological and anatomical evaluation of adult and juvenile leaves of olive plants. Trees 23:181–187
Moreno-Alías I, Rapoport HF, León L, de la Rosa R (2010a) Olive seedling first-flowering position and management. Sci Hort 124:74–77
Moreno-Alías I, Rapoport HF, López R et al (2010b) Optimizing early flowering and pre-selection for short juvenile period in olives seedlings. Hort Sci 45:519–522
Moutier N, Pinatel C, Martre A et al (2004) Identification et caractérisation des variétés d'olivier cultivées en France. Naturalia Publication
Muñoz-Diez C, Imperato A, Rallo L et al (2012) Worldwide Core Collection of olive cultivars based on simple sequence repeat and morphological markers. Crop Sci 52(1):211–221. https://doi.org/10.2135/cropsci2011.02.0110
Muñoz-Mérida A, González-Plaza JJ, Cañada A et al (2013) De novo assembly and functional annotation of the olive (*Olea europaea*) transcriptome. DNA Res 20:93–108. https://doi.org/10.1093/dnares/dss036
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 15:473–497
Muzzalupo I, Chiappetta A, Benincasa C, Perri E (2010) Intra-cultivar variability of three major olive cultivars grown in different areas of central-southern Italy and studied using microsatellite markers. Sci Hort 126:324–329
Nikoloudakis N, Banilas G, Gazis F et al (2003) Discrimination and genetic diversity among cultivated olives of Greece using RAPD markers. J Am Soc Hort Sci 128:741–746
Noormohammadi Z, Hosseini-Mazinani M, Trujillo I et al (2007) Identification and classification of main Iranian olive cultivars using microsatellite markers. HortSci 42(7):1545–1550
Orinos T, Mitrakos K (1991) Rhizogenesis and somatic embryogenesis in callus from wild olive [*Olea europaea* var. *sylvestris* (Miller) Lehr] mature zygotic embryos. Plant Cell Tiss Organ Cult 27:183–187
Ozdemir Y, Kurultay S (2015) Determination of physicochemical properties of some crossed olives and their convenience to black table olive fermentation by using *Lactobacillus plantarum* as a starter culture. J Int Sci Publ: Agric Food 3:416–424
Ozdemir Y, Ozturk A, Guven E et al (2016) Fruit and oil characteristics of olive candidate cultivars from Turkey. Not Bot Horti Agrobo 44(1):147–154
Padula G, Giordani E, Bellini E et al (2008) Field evaluation of new olive (*Olea europaea* L.) selections and effects of genotype and environment on productivity and fruit characteristics. Adv Hort Sci 22(2):87–94
Palliotti A, Bongi G (1996) Freezing injury in the olive leaf and effects of mefluidide treatment. J Hort Sci Biotech 71(1):57–63

Peixe A, Raposo A, Lourenço H et al (2007) Coconut water and BAP successfully replaced zeatin in olive (*Olea europaea* L.) micropropagation. Sci Hort 113:1–7

Pelsy F, Hocquigny S, Moncada X et al (2010) An extensive study of the genetic diversity within seven French wine grape variety collections. Theor Appl Genet 120:1219–1231. https://doi.org/10.1007/s00122-009-1250-8

Penyalver R, García A, Ferrer A et al (2006) Factors affecting *Pseudomonas savastanoi* pv. *savastanoi* plant inoculations and their use for evaluation of olive cultivar susceptibility. Phytopath 96(3):313–319. https://doi.org/10.1094/phyto-96-0313

Perez AG, de la Rosa L, Pascual M et al (2016) Assessment of volatile compound profiles and the deduced sensory significance of virgin olive oils from the progeny of Picual x Arbequina cultivars. J Chromatogr A 1428:305–315

Perez AG, Leon L, Pascual M et al (2014) Variability of virgin olive oil phenolic compounds in a segregating progeny from a single cross in *Olea europaea* l. and sensory and nutritional quality. PLoS ONE 9(3):e92898. https://doi.org/10.1371/journal.pone.0092898

Pérez-Barranco G, Torreblanca R, Padilla IMG et al (2009) Studies on genetic transformation of olive (*Olea europaea* L.) somatic embryos: I. Evaluation of different aminoglycoside antibiotics for nptII selection. II. Transient transformation via particle bombardment. Plant Cell Tiss Organ Cult 97:243–251

Peyvandi M, Farahani F, Noormohamadi Z et al (2009a) Mass production of *Olea europaea* L. (cv. Rowghani) through micropropagation. Gen Appl Plant Phys 35(1–2):35–43

Peyvandi M, Noormohamadi Z, Baninashemi O et al (2009b) Molecular analysis of genetic stability in long-term micropropagated shoots of *Olea europaea* L. (cv. Dezful). Asian J Plant Sci 8(2):146–152

Pontikis CA, Loukas M, Kousonis G (1980) The use of biochemical markers to distinguish olive cultivars. J Hort Sci Biotech 55:333–343

Priego JM (1935) Las variedades de olivo generalizadas en España. Instituto de Investigaciones Agronómicas, Madrid

Pritsa TS, Voyiatzis DG (2004) The in vitro morphogenetic capacity of olive embryo explants at different developmental stages, as affected by L-Glutamine, L-Arginine and 2,4-D. J Biol Res 1:55–61

Pritsa TS, Voyiatzis DG, Voyiatzis CJ, Sotiriou MS (2003) Evaluation of vegetative growth traits and their relation to time to first flowering of olive seedlings. Aust J Agr Res 54:371–376

Rallo L (1995) Selección y mejora genética del olivo en España. Olivae 59:46–53

Rallo L (2014a) Breeding oil and table olives for mechanical harvesting in Spain. Horttechn 24:295–300

Rallo L (2014b) Looking towards tomorrow in olive growing challenges in breeding. Acta Hort 1057:467–482

Rallo L, Barranco D, Caballero JM et al (eds) (2005) Variedades de olivo en España, Junta de Andalucía, MAPA. Ediciones Mundiprensa, Madrid, pp 80–231

Rallo L, Barranco D, Castro-Garcia S et al (2013) High-Density Olive Plantations. Hortic Rev 41:303–384. https://doi.org/10.1007/s00122-003-1301-5

Rallo L, Barranco D, De la Rosa R et al (2008a) 'Chiquitita' olive. HortSci 43(2):529–531

Rallo L, Barranco D, De la Rosa R, León L (2016a) New olive cultivars and selections in Spain: results after 25 years of breeding. Abstract, VIII International Symposium in Olive Growing. ISHS. Splitz. Croatia

Rallo L, Caruso T, Díez CM, Campisi G (2016b) Olive growing in a time of change: from empiricism to genomics. In: Rugini E, Baldoni L, Mulea R, Sabastini L (eds) The olive tree genome, compendium of plant genomes. Springer International Publishing AG, New York, pp 55–64. https://doi.org/10.1007/978-3-319-48887-5_4

Rallo L, Cuevas J, Rapoport HF (1990) Fruit-set pattern in self-pollinated and open-pollinated olive cultivars. Acta Hort 286:219–222

Rallo L, Díez CM, Morales-Sillero A et al (2017) Quality of olives: a focus on agricultural preharvest factors. Sci Hort in press

Rallo L, El Riachy M, Rallo P (2011) The time and place for fruit quality in olive breeding. In: Jenks MA, Bebeli JB (eds) Breeding for fruit quality. Wiley-Blackwell, West Sussex, pp 323–347
Rallo P (2000b) Desarrollo y aplicación de marcadores microsatélites en olivo (*Olea europaea* L.). PhD. diss. Universidad de Córdoba, Spain
Rallo P, Dorado G, Martin A (2000a) Development of simple sequence repeats (SSR) in olive tree (*Olea europaea* L.). Theor Appl Genet 101:984–989
Rallo P, Jiménez R, Morales-Sillero A et al (2012) Evaluation of table-olive quality parameters in progenies obtained by cross-breeding. Acta Hort 949:527–532
Rallo P, Jiménez R, Ordovás J, Suárez MP (2008b) Possible early selection of short juvenile period olive plants based on seedling traits. Aust J Agr Res 59:933. https://doi.org/10.1071/AR08013
Rallo P, Toledo E, Suárez MP et al (2008c) Variabilidad en los contenidos de azúcares y polifenoles en pulpa verde de aceituna: evaluación de progenies. Actas Hort 51:351–352
Rallo P, Morales-Sillero A, Brenes M et al (2018) Elaboration of table olives: assessment of new olive genotypes. Eur J Lipid Sci Tech 1800008. https://doi.org/10.1002/ejlt.201800008
Rama P, Pontikis CA (1990) In vitro propagation of olive (*Olea europaea sativa* L.) 'Kalamon'. J Hort Sci 65(3):347–353
Ramos A, Rapoport HF, Cabello D, Rallo L (2018) Chilling accumulation, dormancy release temperature, and the role of leaves in olive reproductive budburst: evaluation using shoot explants. Sci Hort 230 https://doi.org/10.1016/j.scienta.2017.11.003
Rapoport HF (2014) The reproductive biology of the olive tree and its relationship to extreme environmental conditions. Acta Hort 1057:41–50
Revilla MA, Pacheco J, Casares A et al (1996) In vitro reinvigoration of mature olive trees (*Olea europaea* L.) through micrografting. In Vitro Cell Dev Biol Plant 32:257–261
Rhouma A, Chettaoui M, Krid S et al (2013) Evaluation of susceptibility of an olive progeny (Picholine x Meski) to olive leaf spot disease caused by *Fusicladium oleagineum*. Eur J Plant Pathol 135(1):23–33. https://doi.org/10.1007/s10658-012-0062-x
Ripa V, De Rose F, Caravita MA et al (2008) Qualitative evaluation of olive oils from new olive selections and effects of genotype and environment on oil quality. Adv Hort Sci 22(2):95–103
Rjiba I, Dabbou S, Gazzah N, Hammami M (2010) Effect of crossbreeding on the chemical composition and biological characteristics of Tunisian new olive progenies. Chem Biodivers 7(3):649–655
Roca M, Leon L, de La Rosa R (2011) Pigment metabolism of 'Sikitita' olive (*Olea europaea* L.): a new cultivar obtained by cross-breeding. J Agr Food Chem 59(5):2049–2055
Rodriguez-Castillo E, Diaz A, Belaj A, De la Rosa R (2009) Inter-compatibility relationships in olive as revealed by paternity tests with SSR markers. Acta Hort 814:659–662
Roselli G and Donini B. (1982) 'Briscola' nuova cultivar di a sviluppo compatto. Rivista di ortoflorofrutticoltura italiana 66:103–114
Roussos PA, Pontikis CA (2002) In vitro propagation of olive (*Olea europaea* L.) cv. Koroneiki Plant Growth Rag 37:295–304
Rugini E (1984) In vitro propagation of some olive (*Olea europaea sativa* L.) cultivars with different root-ability, and medium development using analytical data from developing shoots and embryos. Sci Hort 24:123–134
Rugini E (1988) Somatic embryogenesis and plant regeneration in olive (*Olea europaea* L.). Plant Cell Tiss Organ Cult 14:207–214
Rugini E (1995) Somatic embryogenesis in olive. In: Jain SM, Gupta P, Newton R (eds) Somatic embryogenesis in woody plants, vol 2. Kluwer Academic Publishers, Dordrecht, pp 171–189
Rugini E, Baldoni L (2005) *Olea europaea* Olive. In: Litz RE (ed) Biotechnology of fruit and nut crops. CABI, Cambridge, pp 404–428
Rugini E, Biasi R, Muleo R (2000) Olive (*Olea europaea* var *sativa*) transformation. In: Jain SM, Minocha S (eds) Molecular biology of woody plants. Kluwer Academic Publishers, Dordrecht, pp 245–279
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, Cristofori V, Silvestri C (2016) Genetic improvement of olive (*Olea europaea* L.) by conventional and in vitro biotechnology methods. Biotech Adv 34:687–696. https://doi.org/10.1016/j.biotechadv.2016.03.004

Rugini E, De Pace C (2016) Olive breeding with classical and modern approaches. In: Rugini E, Baldoni L, Mulea R, Sabastini L (eds) The olive tree genome, compendium of plant genomes. Springer International Publishing AG, New York, pp 163–191. https://doi.org/10.1007/978-3-319-48887-5_10

Rugini E, Fontanazza G (1981) In vitro propagation of 'Dolce Agogia´ olive. HortSci 16(4):492–493

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

Rugini E, Jacoboni A, Luppino M (1993) Role of basal shoot darkening and exogenous putrescine treatments on in vitro rooting and on endogenous polyamine changes in difficult-to-root woody species. Sci Hort 53:63–72

Rugini E, Pezza A, Muganu M et al (1995) Somatic embryogenesis in olive (*Olea europaea* L.). In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry, vol 30, Somatic embryogenesis and synthetic seed I. Springer, Berlin, pp 404–414

Ruiz N, Barranco D, Rapoport HF (2006) Anatomical response of olive (*Olea europaea* L.) to freezing temperatures. J Hort Sci Biotech 81(5):783–790

Sakai A, Hirai D, Niino T (2008) Development of PVS-based vitrification and encapsulation-vitrification protocols. In: Reed B (ed) Plant cryopreservation: a practical guide. Springer, New York, pp 33–58

Sanchez de Medina V, Calderon-Santiago M, El Riachy M et al (2015a) Influence of genotype on the fatty acids composition of virgin olive oils from advanced selections obtained by crosses between Arbequina, Picual, and Frantoio cultivars along the ripening process. Eur J Lipid Sci Tech 117(8):1261–1270

Sanchez de Medina V, El Riachy M, Priego-Capote F, Luque de Castro MDL (2015b) Composition of fatty acids in virgin olive oils from cross breeding segregating populations by gas chromatography separation with flame ionization detection. J Sci Food Agr 95(14):2892–2900

Sanchez de Medina VM, Priego-Capote F, Luque de Castro MDL (2015c) The effect of genotype and ripening index on the phenolic profile and fatty acids composition of virgin olive oils from olive breeding programs. Eur J Lipid Sci Tech 117(7):954–966

Santos-Antunes F, León L, de la Rosa R et al (2005) The length of the juvenile period in olive as influenced by vigor of the seedlings and the precocity of the parents. HortSci 40:1213–1215

Santos-Antunes F, Mohedo A, Trujillo I, Rallo L (1999) Influence of the genitors on the flowering of olive seedlings under forced growth. Acta Hort 474:103–105

Sanchez-Romero C, Swennen R, Panis B (2009) Cryopreservation of olive embryogenic cultures. CryoLett 30(5):359–372

Saponari M, Boscia D, Nigro F, Martelli GP (2013) Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). J Plant Path 95(3):668. https://doi.org/10.4454/JPP.V95I3.035

Sarkar N, Schmid-Siegert E, Iseli C et al (2017) Low rate of somatic mutations in a long-lived oak tree. bioRxiv 149203. https://doi.org/10.1101/149203

Seifi E, Guerin J, Kaiser B, Sedgley M (2011) Sexual compatibility and floral biology of some olive cultivars. New Zeal J Crop Hort 39:141–151. https://doi.org/10.1080/01140671.2011.560165

Selak GV, Cuevas J, Ban SG, Perica S (2014) Pollen tube performance in assessment of compatibility in olive (*Olea europaea* L.) cultivars. Sci Hort 165:36–43. https://doi.org/10.1016/j.scienta.2013.10.041

Sgamma T, Jackson A, Muleo R et al (2014) TEMPRANILLO is a regulator of juvenility in plants. Sci Rep-UK 4:3704. https://doi.org/10.1038/srep03704

Sghir S, Chatelet P, Ouazzani N et al (2005) Micropropagation of eight Moroccan and French olive cultivars. HortSci 40(1):193–196

Shibli RA, AI-Juboory KH (2000) Cryopreservation of 'Nabali' olive (*Olea europaea* L.) somatic embryos by encapsulation-dehydration and encapsulation-vitrification. CryoLett 21(6):357–366
Shibli RA, Shatnawi M, Abu E et al (2001) Somatic embryogenesis and plant recovery from callus of 'Nabali' olive (*Olea europaea* L.). Sci Hort 88:243–256
Sofo A, Dichio B, Xiloyannis C, Masia A (2004) Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. Phys Plant 121(1):58–65. https://doi.org/10.1111/j.0031-9317.2004.00294.x
Sorrentino G, Muzzalupo I, Muccilli S et al (2016) New accessions of Italian table olives (*Olea europaea*): characterization of genotypes and quality of brined products. Sci Hort 213:34–41
Stokstad E (2015) Italy's olives under siege: blight alarms officials across Europe. Science 348(35):620. https://doi.org/10.1126/science.348.6235.620
Suárez MP, Casanova L, Jiménez R et al (2011) Variability of first flower to ground distance in olive seedlings and its relationship with the length of the juvenile period and the parent genotype. Sci Hort 129(4):747–751
Suarez MP, Lopez-Rivares EP, Cantero ML, Ordovas J (1990) Clonal selection on 'Manzanilla de Sevilla'. Acta Hort 286:117–119
This P, Lacombe T, Thomas MR (2006) Historical origins and genetic diversity of wine grapes. Trends Genet 22:511–519. https://doi.org/10.1016/j.tig.2006.07.008
Tous J, Romero A, Plana J (1999) IRTA-i 18 clon de la variedad de olivo Arbequina. Olivae 77:50–52
Tous J, Romero A, Plana J et al (2005) Selección clonal en variedades. In: Rallo L, Barranco D, Caballero JM et al (eds) Variedades de olivo en España (Libro II: Variabilidad y selección). Junta de Andalucía, MAPA y Ediciones Mundi-Prensa, Madrid
Torreblanca R, Cerezo S, Palomo-Ríos E et al (2010) Development of a high throughput system for genetic transformation of olive (*Olea europaea* L.) plants. Plant Cell Tiss Organ Cult 103:61–69
Trapero C, Díez CM, Rallo L et al (2013a) Effective inoculation methods to screen for resistance to verticillium wilt in olive. Sci Hortic 162:252–259. https://doi.org/10.1016/j.scienta.2013.08.036
Trapero C, Rallo L, López-Escudero FJ et al (2015) Variability and selection of verticillium wilt resistant genotypes in cultivated olive and in the *Olea* genus. Plant Pathol 64(4):890–900. https://doi.org/10.1111/ppa.12330
Trapero C, Serrano N, Arquero O et al (2013b) Field resistance to Verticillium wilt in selected olive cultivars grown in two naturally infested soils. Plant Dis 97(5):668–674. https://doi.org/10.1094/PDIS-07-12-0654-RE
Trabelsi EB, Bouzid S, Bouzid M et al (2003) In vitro regeneration of olive tree by somatic embryogenesis. J Plant Biol 46(3):173–180
Trigui A, Msallem M, Yengui A et al (2002) Oliviers de Tunisie (1). Ministère de l'Agriculture IRESA, Institute l'Olivier, République Tunisienne
Trigui A, Yengui A, Belguith H (2006) Olive germplasm in Tunisia. Olea 25:19–23
Trujillo I, Arus P, Rallo L (1995) Identification of olive cultivars by isozyme analysis. J Amer Soc Hort Sci 120(2):318–324
Trujillo I, Ojeda MA, Urdiroz NM et al (2014) Identification of the Worldwide Olive Germplasm Bank of Córdoba (Spain) using SSR and morphological markers. Tree Genet Genom 10:141–155. https://doi.org/10.1007/s11295-013-0671-3
Tugendhaft Y, Eppel A, Kerem Z et al (2016) Drought tolerance of three olive cultivars alternatively selected for rain fed or intensive cultivation. Sci Hort 199:158–162. https://doi.org/10.1016/j.scienta.2015.12.043
Unver T, Wu Z, Sterck L et al (2017) Genome of wild olive and the evolution of oil biosynthesis. Proc Nat Acad Sci USA 114:E9413–E9422. https://doi.org/10.1073/pnas.1708621114
Van Nocker S, Gardiner SE (2014) Breeding better cultivars, faster: applications of new technologies for the rapid deployment of superior horticultural tree crops. Hort Res 1:14022. https://doi.org/10.1038/hortres.2014.22
Velasco L, Fernandez-Cuesta A, De la Rosa R et al (2014) Selection for some olive oil quality components through the analysis of fruit flesh. J Am Oil Chem Soc 91(10):1731–1736

Velázquez K, Agüero J, Vives MC et al (2016) Precocious flowering of juvenile citrus induced by a viral vector based on citrus leaf blotch virus: a new tool for genetics and breeding. Plant Biotech J. https://doi.org/10.1111/pbi.12555

Vidoy-Mercado I, Imbroda-Solano I, Barceló-Muñoz A et al (2012) Differential in vitro behavior of the Spanish olive (*Olea europaea* L.) cultivars 'Arbequina' and 'Picual'. Acta Hort 949:27–30

Viruega JR, Roca LF, Moral J, Trapero A (2011) Factors affecting infection and disease development on olive leaves inoculated with *Fusicladium oleagineum*. Plant Dis 95(9):1099–1108. https://doi.org/10.1094/PDIS-11-10-0795

Wilhelm S, Taylor JB (1965) Control of verticillium wilt of olive through natural recovery and resistance. Phytopath 55(3):310–316

Wu S, Collins G, Sedgley M (2004) A molecular linkage map of olive (*Olea europaea* L.) based on RAPD, microsatellite, and SCAR markers. Genome 47(1):26–35. https://doi.org/doi.org/10.1139/g03-091

Wu SB, Collins G, Sedgley M (2002) Sexual compatibility within and between olive cultivars. J Hort Sci Biotech 77(6):665–673. https://doi.org/10.1080/14620316.2002.11511554

Xavier CJ (2015) Resistencia y control químico en la Antracnosis del olivo causada por *Colletotrichum* spp. PhD Thesis, ETSIAM, Universidad de Córdoba

Young JM, Wilkie JP, Fletcher MJ et al (2004) Relative tolerance of nine olive cultivars to Pseudomonas savastanoi causing bacterial knot disease. Phytopathol Mediterr 43(3):395–402

Zeinanloo A, Shahsavari A, Mohammadi A, Naghavi MR (2009) Variance component and heritability of some fruit characters in olive (*Olea europaea* L.). Sci Hort 123(1):68–72

Zouari I, Mezghani A, Labidi F (2017) Flowering and heat requirements of four olive cultivars grown in the south of Tunisia. Acta Hort 1160:231–236. https://doi.org/10.17660/ActaHortic.2017.1160.34