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REVIEW

Recent developments in olive (*Olea europaea* L.) genetics and genomics: applications in taxonomy, varietal identification, traceability and breeding

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Abstract

Key message **The latest results in DNA markers application and genomic studies in olive.**

Abstract Olive (*Olea europaea* L.) is among the most ancient tree crops worldwide and the source of oil beneficial for human health. Despite this, few data on olive genetics are available in comparison with other cultivated plant species. Molecular information is mainly linked to molecular markers and their application to the study of DNA variation in the *Olea europaea* complex. In terms of genomic research, efforts have been made in sequencing, heralding the era of olive genomic. The present paper represents an update of a previous review work published in this journal in 2011. The review is again mainly focused on DNA markers, whose application still constitutes a relevant percentage of the most recently published researches. Since the olive genomic era has recently started, the latest results in this field are also being discussed.

Keywords Functional Genomics · Germplasm · Molecular Markers · Olive Genome · Olive Oil

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Why olive?

Olive (*Olea europaea* L.) cultivation began thousand years ago in the Mediterranean. Recent archaeobotanical studies suggest that olive domestication started about five to six thousand years ago in the Near East (reviewed in Kaniewski et al. 2012). It is highly likely that this plant was one of the first domesticated tree species, becoming a symbol that for centuries has been shaping the landscape and marking the history of the rural population. Nowadays, olive is, after oil palm, among the most valuable oil fruit tree, covering more than 10 million hectares of land worldwide (FAOSTAT 2014—faostat3.fao.org). Olive cultivation is concentrated (>98% of the devoted surface) in the Mediterranean basin, where the plant is used for table olive and oil production and where a large proportion of the produced olive oil is also used (Baldoni and Belaj 2009). Moreover, olive cultivation is currently expanding in non-traditional producing territories such as the United States, South America, Australia, Japan and China. In Australia and Argentina, olive is now a well-established production reality, with irrigated and highly mechanised orchards.

Olive oil and table olives are perceived as healthy foods, and in fact, olive oil, mainly Extra Virgin Olive Oil (EVOO), has unique organoleptic and nutraceutical properties that have been highlighted by several studies. Olive oil represents the most important lipid fraction of the Mediterranean cuisine and is characterised by a peculiar fatty acid composition (55–83% of oleic acid, 3.5–21% of linoleic acid and 1% of linolenic acid—Cunnane 2003); in addition, it is high in antioxidant substances. The consumption of EVOO is related to reduced risks of cardiovascular disease and lower death rates in persons at high cardiovascular risk (Guasch-Ferré et al. 2014), in addition

to a variety of other health-related aspects (Keys 1995; Pérez-Jiménez et al. 2007; Rigacci and Stefani 2016).

Regarding the beneficial molecules in EVOO, Oleaceae fruits are rich in compounds such as simple phenolics, carotenoids, tocopherols and anthocyanins, which are frequent in several plant species, but they also possess an entirety of phenolic compounds, oleosides or secoiridoids, specific of the Oleaceae and few other families (Ryan et al. 2002). The protective activity of EVOO against degenerative and chronic diseases as well as tumours could be mainly ascribed to this complex set of secondary metabolites, which are also important for many of the sensory characteristics of EVOO (Servili et al. 2004, 2014).

Regarding the species, olive is a member of the Oleaceae family, which contains approximately 25 genera and at least 600 different species distributed in temperate and tropical areas (Besnard et al. 2009). Differing opinions exist on the nomenclature and hierarchies concerning the olive genus and species. Green (2002) classified 33 species and nine subspecies in the genus *Olea*. The *Olea europaea* complex comprises six subspecies: *O. europaea* subsp. *europaea*, *O. europaea* subsp. *cuspidata*, *O. europaea* subsp. *laperrinei*, *O. europaea* subsp. *maroccana*, *O. europaea* subsp. *cerasiformis* and O. *europaea* subsp. *guanchica*.

These subspecies have spread across the Mediterranean basin (subsp. *europaea*), the Macaronesia (subsp. *cerasiformis*, *guanchica* and *maroccana*), the Saharan mountains (subsp. *laperrinei*), and from South Africa to South Asia (subsp. *cuspidata*) (Médail et al. 2001; Green 2002). Cultivated olive and its wild Mediterranean relatives were further recognised as different varieties and, respectively, defined as *O. e.* subsp. *europaea* var. *europaea* and *O. e.* subsp. *europaea* var. *sylvestris* (Green 2002). This distinction is currently still debated as it was largely based on morphological traits, such as the small fruit and pit size characteristics of the wild variety, an approach that is considered to be highly imprecise and a potential source of inaccuracies (Ganino et al. 2006). Finally, another interesting and still open debate in olive studies, concerning the origin of cultivated olive, is whether domestication should be considered a single (Besnard and Rubio de Casas 2016) or multiple (Diez et al. 2015) event.

Olive trees are evergreen, outcrossing and can be vegetatively propagated. The species has an extensive genetic patrimony that resulted from plant lifetime and scarcity of genotype turnover. The large germplasm includes more than 1500 cultivars that are predominantly present in southern European countries such as Italy, Spain, France and Greece (Bartolini 2008). Therefore, olive represents an interesting case among agricultural crops and its wide genetic patrimony could represent an invaluable source of variability for breeding purposes (Baldoni and Belaj 2009).

Cytogenetic and ploidy analyses in *Olea europaea*

Olea europaea has 46 chromosomes and is a diploid species ($2n = 2x = 46$) (Breviglieri and Battaglia 1954). However, in olive, karyological studies are difficult since the chromosomes are small, similar in morphology and numerous. As previously revised (see Bracci et al. 2011 for more detailed references), cytogenetic studies have been carried out to distinguish the olive chromosome pairs and to determine the genome size content of Mediterranean olive genotypes from different countries. Values ranging between 1.45 and, circa, 2.3 pg of DNA per haploid genome, with 1C = 1400–1500 Mbp (Loureiro et al. 2007), have been reported, evidencing high variability between genotypes. Further, it has recently been estimated that nuclear DNA content can diminish following radiation, as a consequence of the irradiation dose, from 2.972 to 2.963 and 2.935 pg/2C nucleus of the control, for 10 and 30 Gy (grey) treated plants, respectively (Oražem et al. 2013).

Ploidy level was also investigated in the Olive complex. The presence of tetraploids and hexaploids was detected in subsp. *cerasiformis* and *maroccana*, respectively (Besnard et al. 2008; Brito et al. 2008; García-Verdugo et al. 2009). In addition, the coexistence of diploid and triploid individuals within the same population was also detected in a South Algerian population of the Laperrine's olive using nuclear microsatellite profiles and flow cytometry (Besnard and Baali-Cherif 2009). The frequency of triploids remain relatively low (ca. 3%), but until now, such a pattern has not been detected in any other olive subspecies.

The effect of tetraploidy on olive floral and fruit biology has been studied in Leccino Compact (LC), a mixoploid olive mutant of the cultivar Leccino (L) (Caporali et al. 2014). The mutant has both diploid and tetraploid cells. The authors concluded that tetraploidy induced larger floral structures as normally occurring in tetraploid plants, but with little influence on fruit size despite the much larger cell size. The genetic stability of two micropropagated wild olive species (*Olea europaea subsp. cerasiformis* and *O. europaea* ssp. *europaea* var. *sylvestris*) using flow cytometry and microsatellite markers was compared (Brito et al. 2010). No changes in ploidy level by flow cytometry or mutations were found among micropropagated plants, suggesting for the tested markers, genetic uniformity throughout the process.

Ribosomal and cytoplasmic DNA variation

Polymorphisms in ribosomal and cytoplasmic DNA, such as intergenic spacer (IGS), and internal transcribed spacer (ITS), are frequently employed for phylogenetic analysis. Since these sequences do not have an active and strict mechanism of conservation, they can show high nucleotide variability that can be detected and evidenced in various ways.

Ribosomal DNA polymorphism

The origin of the *Olea europaea* complex has been studied analysing the variability in the sequence of the ITS of the nuclear ribosomal genes. Nuclear ribosomal genes are repeated in tandem in the genome but the analysis of their variation in the olive complex has been a challenge. Early works with restriction fragment length polymorphisms (RFLP) revealed a high complexity of internal spacers that was difficult to use for investigating phylogenetic relationships between olive taxa (e.g. Besnard et al. 2001). Several pseudogenic ribosomal regions were then identified in the Olive complex (Besnard et al. 2007b). The first ITS (ITS1) of a pseudogene type was successfully isolated by Hess et al. (2000) and Besnard et al. (2007a), and used to investigate phylogenetic relationships between Macaronesian olives or to identify subspecies (and hybrids) in the invasive range (Australia and Hawaii). A phylogenetic study of the whole olive complex based on this pseudogene was also proposed by Besnard et al. (2007b). Finally, Besnard et al. (2009) isolated ITS1 of functional ribosomal genes with specific primers and investigated phylogenetic relationships in Olea and related genera. These data (coupled to plastid DNA polymorphisms) supported a polyphyletic origin of Olea and the distinction of four main lineages. Among Olea species, the split between subgenera Olea and Paniculatae was estimated in the Early Miocene or Late Oligocene. Within subgenus Olea, section Ligustroides and the Olive complex (section Olea) diverged in the Early Miocene. The diversification of these two sections in Africa was likely contemporary to the Saharan desertification.

Cytoplasmic DNA polymorphism

Mitochondrial (mtDNA) and plastidial DNA (cpDNA) markers have been widely used in olive with different aims, such as to study the genetic structure of the olive trees in the Mediterranean (Besnard et al. 2002) and to evaluate the biogeographical history of the Oleaceae family (Besnard et al. 2009). Besnard et al. (2007c) used plastid DNA to obtain a phylogenetic reconstruction of olive *laperrinei* (*O. europaea* subsp. *laperrinei*). This analysis supported a maternal origin of *laperrinei* populations in South Algeria, where allelic richness was observed, and proved that the barrier represented by the desert limits long-distance gene flow. After the sequencing of the olive chloroplast genome reported by Mariotti et al. (2010), cytoplasmic DNA, focusing on plastid DNA, has been extensively used. Besnard et al. (2011) carried out a sequencing of the whole olive plastid genome of trees with different cpDNA lineage with the goal to develop markers useful for profiling olive cpDNA haplotypes. A low variability of cpDNA was confirmed, evidencing, at the same time, a nucleotide divergence between the different chloroplast genomes not higher than 0.07%. Besnard et al. (2014), using 64 cpDNA loci (maternally inherited) and 11 nuclear microsatellites, examined the origins and spread of invasive Australian olive populations by analysing a large sample of native and invasive accessions. Similarly, Besnard et al. (2013) used both nuclear and plastid markers to analyse population genetics of Mediterranean and Saharan olives. They reported the presence of a mixture between Mediterranean and Saharan olives, confirming that Laperrine's olive has been involved in the diversification of cultivated olives. Further studies carried out using plastid DNA markers on Laperrine's and cultivated olives have been recently reviewed (Besnard et al. 2012). Finally, Chalak et al. (2015) used cpDNA to study the extent of the genetic diversity in Lebanese olive (*Olea europaea* L.), evidencing a mixture of an ancient germplasm and recently introduced varieties.

The low discriminating power of plastid DNA polymorphisms (ca. 80% of cultivars share the same cytoplasm; Besnard et al. 2011) is a limitation for traceability analyses, and consequently nuclear DNA polymorphisms are more suitable (see below). However, rare plastid haplotypes are potentially quite informative, and due to the high number of plastids in a cell, PCR methods are easily amenable to detect such polymorphisms even from degraded DNA (Pérez-Jiménez et al. 2013).

Applications of DNA-based molecular markers in *Olea europaea*

Studies on genetic variability of the *Olea europaea* complex

The detection and study of genetic resources are key factors in crop breeding. In olive, this information is of paramount importance since a high number of different genotypes are currently cultivated. For these reasons, molecular markers have been extensively used for germplasm characterisation, enhancing and refining the classical morphological description, which is strongly limited by environmental influences. *Olea europaea* classification has also resulted in improving our understanding of *Olea* taxa inter-compatibility with the Mediterranean olive. Historically, several classes of markers have been used for this purpose, such as RAPDs (random amplified polymorphic DNAs), AFLPs (amplified fragment length polymorphisms), ISSRs (inter simple sequence repeats), SSRs (simple sequence repeats), and more recently, mainly because of the introduction of next generation sequencing, SNPs (single nucleotide polymorphisms). See Bracci et al. (2011) for a deeper description of all these marker classes.

Microsatellites (SSRs) are usually considered as the marker of choice for germplasm analysis, which is evident

by the high number of papers on that can be retrieved by a database (SCOPUS) survey by searching for analysis of olive genetic diversity by means of microsatellites. Despite of this, a surprisingly high number of papers, mainly based on RAPDs, AFLPs and ISSRs, have been published recently. In previous years, when sequence information about olive was scarce, these markers had the advantage to enable genetic analysis without any a priori knowledge of DNA sequencing. Nowadays, irrespective of the high number of microsatellites already available for olive, these markers are still frequently being used in several studies.

RAPDs, ISSRs and AFLPs

The use of RAPD markers has been extremely common, alone or in combination with other markers (AFLPs, ISSRs, SSRs), to analyse the extent of genetic variability present in the germplasm of varieties of countries characterised by an old history of olive cultivation and oil production: Malta (Mazzitelli et al. 2014), Jordan (Brake et al. 2014), Greece (Linos et al. 2014), Egypt (Elsheikh et al. 2014), Iran (Sheidai et al. 2014), Pakistan (Awan et al. 2011), and Turkey (Coskun and Parlak 2013). In other papers, RAPD markers have been considered to study the potential presence of intra-cultivar variability (Çelikkol Akçay et al. 2014; Figueiredo et al. 2013; Leva and Petruccelli 2012), the genetic relationships between cultivars (Gomes et al. 2012), the genetic characterisation of minor cultivars in local regions (Parra-Lobato et al. 2012), the results of in vitro mutagenesis, and the detection of possible mutants in olive calli caused by the use of sodium azide (Alborzian Deh Sheikh and Moradnejad 2014).

Generally, ISSR markers have been used in combination with other markers, and the number of manuscripts based on the use of ISSR alone to analyse the genetic diversity of olive cultivars is relatively low (Kaya 2015). In addition to other markers, ISSR have been used to study and characterise the genetic variability in local olive germplasm (Zhan et al. 2015; Brake et al. 2014; Elsheikh et al. 2014; Linos et al. 2014; Leva and Petruccelli 2012; Noormohammadi et al. 2012) Further, Kaya and Yilmaz-Gokdogan (2016) have used ISSRs in addition to two retrotransposon-based marker systems (IRAP—inter-retrotransposon amplified polymorphism and REMAP—retrotransposon-microsatellite amplified polymorphism) for the molecular characterisation of olive cultivars.

The AFLP markers have been considered for a wide range of applications, such as association mapping to discover interesting traits (Kaya et al. 2016; Ipek et al. 2015b), analysis of the true hybrid nature of crosses between different cultivars (Cáceres et al. 2015), analysis of the mutagenic effect of X-ray irradiation on olive shoot cultures (Oražem et al. 2013) and the characterisation, in association with other marker types, of the olive germplasm of particular regions (Kaya et al. 2013; Strikić et al. 2011; Albertini et al. 2011).

SSRs

In the last five years, SSRs have been used to survey the diversity in national olive germplasms of both Mediterranean and non-Mediterranean countries, such as China (Qin et al. 2016; Zhan et al. 2015), Argentina (Torres et al. 2014), Colombia (Beghè et al. 2015), Iran (Mardi et al. 2016; Sorkheh and Khaleghi 2016; Noormohammadi et al. 2014; Mousavi et al. 2014), Palestine (Obaid et al. 2014), Turkey (Sakar et al. 2016a, b; Ipek et al. 2012; Ercisli et al. 2012; Işik et al. 2011), Algeria (Abdessemed et al. 2015; Dominguez-Garcia et al. 2012), Spain (Fernández i Martí et al. 2015; Delgado-Martinez et al. 2012), Italy (Caruso et al. 2014; Las Casas et al. 2014; Marra et al. 2013; Colao et al. 2011; Rotondi et al. 2011b; Corrado et al. 2011), Greece (Linos et al. 2014), Tunisia (Ben-Ayed et al. 2014a, b; Abdelhamid et al. 2013), Morocco (El Bakkali et al. 2013a, b), Australia (Rehman et al. 2012) and Croatia (Ercisli et al. 2012). Independent research groups (Ben Ayed et al. 2016; Trujillo et al. 2014; Xanthopoulou et al. 2014; Haouane et al. 2011) have carried out analysis of the genetic relationships between the different accessions maintained in ex situ conservation germplasms, collecting large amounts of samples from all over the world. Olive trees have a long lifespan, because of this and of thousands of years of olive cultivation numerous ancient trees are still present. These plants are extremely important from a scientific point of view, because apart from their historical and cultural significance, they are of high interest because of their genetic potential and their relationships with other olive varieties currently cultivated (Lazović et al. 2016). Ancient trees have been recently investigated using SSR markers (Lazović et al. 2016; Sakar et al. 2016b; Barazani et al. 2014; Petruccelli et al. 2014; Salimonti et al. 2013). Chalak et al. (2015) analysed 73 olive trees, including six monumentals from four main Lebanese areas, with 12 nuclear SSRs and 39 plastid DNA markers. The six monumental trees evidenced three different molecular profiles, one of which corresponding with profile of "Baladi", a widespread traditional cultivar. The findings of the study suggest that Lebanese olives were locally selected in ancient times, in the preliminary stages of olive cultivation, and they served as starting material for deriving, both by vegetative and sexual propagation, the present traditional varieties. Diez et al. (2011) analysed centennial olive trees by nuclear SSRs. The results evidenced a high percentage of unidentified genotypes among the oldest olives supporting their incredible value as a reservoir of genetic diversity. Barazzani et al. (2016) analysed the genetic structure of naturally growing olive trees in Israel.

The microsatellite analysis evidenced the genetic diversity of these trees from old cultivated olive trees supporting they might represent wild var. *sylvestris*. Further, it was showed a similarity in genetic structure of these naturally growing trees with the suckers of old cultivated trees suggesting that wild trees were used as rootstocks.

The evaluation of genetic diversity is of high interest for the adequate management of germplasm collections and for efficient breeding programs. In this respect, a topic of interest is the evaluation of the genetic diversity existing between and within cultivars. However, the complexity of the olive germplasm, the lack of references and the errors or inaccuracies in the denominations of the cultivars impedes the classification process. A high number of studies dedicated at cultivar genotyping have been carried out recently, with the specific aim to solve these problems. When SSRs have been used for such purpose, they evidenced that some cultivar names refer to a polyclonal population (Bracci et al. 2009; Ben-Ari et al. 2014; Caruso et al. 2014; Ipek et al. 2012).

Various analyses have recently been carried out to characterise the genetic variability in Mediterranean wild olive, and along with the analysis of cultivated olive, to reconstruct the domestication origin of this species (Diez et al. 2015; Hosseini-Mazinani et al. 2014; Noormohammadi et al. 2012; Belaj et al. 2011). Overall, these studies allowed identification of genepools both in cultivated olives (Haouane et al. 2011; Belaj et al. 2012; Besnard et al. 2013; Diez et al. 2015), and in oleasters (Belaj et al. 2011; Besnard et al. 2013; Diez et al. 2015), as well as the detection of recent admixture events between Mediterranean olive and non-Mediterranean relatives (Besnard et al. 2013, 2014; Cáceres et al. 2015).

SNPs

Single nucleotide polymorphisms (SNPs) are small variations in the DNA sequence and the most abundant and ubiquitous markers in any living organisms. For this reason, SNPs, coupled with next generation high-throughput genomic sequencing technologies, will be the marker system of choice in the future. Since a high number of SNPs starts to be present also in olive, they have been used more frequently for studying genetic variability in olive. Biton et al. (2015) developed a large set of 145,974 SNPs using next generation sequencing technology and subsequently used a subset of 138 SNPs to analyse 119 cultivars maintained in the Israeli germplasm collection. Interestingly, they found that cultivars were grouped more in terms of function (oil, table or double purpose) than in terms of their geographic origin. Kaya et al. (2013) developed a set of 2987 SNPs by transcriptome sequencing, focusing solely on transcribed genes, and successfully used these markers, together with AFLPs and SSRs, in characterising 96 olive genotypes from different areas of Turkey. Dominguez-Garcia et al. (2012) compared SNPs and SSRs to study a panel of cultivars from Algeria. While being less polymorphic than microsatellites, they showed an interesting level of polymorphism. At the same time, the authors recognised the necessity of developing more SNPs to make them as discriminative as SSRs. Colao et al. (2011) reported on the development of SNP markers in genes of interest and on their use to characterise olive cultivars grown in the Latium region (central Italy), in combination with microsatellites and morphological features. Belaj et al. (2012) joined molecular marker (SSR, SNP and DArT) data and agronomical traits to analyse 361 olive accessions of the IFAPA germplasm in Cordoba (Spain), defining their genetic diversity and structure. These data were further considered to construct a set of core collections of the olive accessions maintained in the germplasm. The SNPs were further used for: (1) the analysis of genetic identity among five putative clones of the olive cultivar Souri (Ben-Ari et al. 2014); (2) the traceability of olive oil (Kalogianni et al. 2015) also by developing peptide nucleic acid (PNA) and modified-PNA microarrays (Rossi et al. 2012); (3) genetic mapping (Marchese et al. 2016a; Ipek et al. 2016; Kaya et al. 2016).

Olive oil traceability

Olive oil, a premium product for its nutritional value and health benefits, is frequently being reported as the most adulterated plant-derived product in the world, and several cases of adulterations and frauds have been reported in the last years, as retrievable from the food fraud database available online (http://www.foodfraud.org). To prevent deliberate frauds and sophistications and to distinguish between dishonest practices and unwanted contamination during food production processes, the implementation of currently available traceability procedures is necessary. Since the mid 1990s, as extensively reviewed by us (Bracci et al. 2011) and others (Costa et al. 2012; Agrimonti et al. 2011), scientists from the largest olive oil-producing countries have developed a number of methods for recovering PCR grade DNA of the production cultivars from high-quality PDO productions. The rationale for this was that since DNA is not influenced by the environment and less influenced by processing with respect to other molecules (i.e. metabolites), the molecular tools could represent a promising and valid implementation of the traceability of food in general and olive oil in particular (Busconi et al. 2003; Ramos-Gómez et al. 2014, 2016; Bracci et al. 2011; Pafundo et al. 2010). Research groups have considered and tested several approaches, and specific protocols were adapted to existing commercial kits, but the results were,

and still are, uncertain and have different drawbacks. Among such drawbacks are the small quantity and quality of the recovered DNA, the low reproducibility of both extractions and PCR amplifications with the documented absence of correspondence between the profiles of olive oil and fruit DNA of the same cultivars, and the possibility to obtain the marker profile of the main cultivar while no signal was usually obtained for the secondary varieties eventually present (see Bracci et al. 2011 for individual references). Despite of this, the development of methods to trace olive oil production by DNA analysis is still a topic of great interest, as stated by the high number of respective papers published in the last four years, as retrieved by main databases (SCOPUS, Web of knowledge, Pubmed, all accessed in October 2016).

In the following, we are discussing the results of studies using standard and innovative approaches.

Innovative methods to analyse DNA from olive oil

A first aspect needs to be discussed is that—after at least 20 years of efforts—there is still an uncertainty about the methods to be used to recover PCR-compatible DNA. This step is still the limiting process influencing the overall results of any traceability purposes and despite the availability of established methods (see Pasqualone et al. 2016; Bracci et al. 2011 for further references), new papers dealing with the development of protocols (Ramos-Gómez et al. 2014; Raieta et al. 2015) or the comparison of different methods to find the adequate approach (Scollo et al. 2016; Ramos-Gómez et al. 2014) are published, denoting the necessity of a continuous and active improvement of DNA extraction for oil traceability.

Recently, Scollo et al. (2016) applied droplet digital PCR (ddPCR) to quantify the DNA recovered from oil by applying different methods. Again, the authors evidenced that the methods can modify the quantity/quality of recovered DNA and overall reproducibility. In this paper, the use of ddPCR is to quantify precisely the extracted DNA suggested, considering that spectrophotometric and electrophoretic DNA quantification methods, but also quantitative real-time PCR (qRT-PCR), because of the possible presence of polymerase inhibitors, do not permit reliable assessments. The ddPCR is an endpoint PCR (see Scollo et al. 2016; Pinheiro et al. 2012; Hindson et al. 2011 for further details of the technique), enabling the quantification of DNA irrespective of the efficiency of the PCR. This parameter can be influenced by the presence of inhibitors whose presence can result in the frequently observed lack of reproducibility, or in the non-complete concordance between genetic profiles of DNA from olive oils and fruits, reported by Doveri et al. (2006). Further, using adequate molecular markers, ddPCR could be used to detect the presence of small amounts of DNA from different species or varieties in the same species (Scollo et al. 2016).

The use of qRT-PCR alone or associated with High Resolution Melting (HRM) analysis was applied to oil traceability. For example, Ramos-Gómez et al. (2014), in developing a new method to recover PCR grade DNA from different vegetable oils, applied qRT-PCR to evaluate the performance of the extraction protocols. Similarly, Ramos-Gómez et al. (2016) compared eight different methods based on their efficiency in the qRT-PCR assay, sensitivity to DNA detection and DNA mixtures, sensitivity and specificity to recover olive DNA from the oils and from commercial oil-derived foodstuffs. The use of HRM allows the discrimination of DNA fragments with only a single different base. Montemurro et al. (2015) developed an SSR-based method coupled with HRM analysis to distinguish Terra di Bari olive oil from non-Terra di Bari olive oil, using different mixtures suggesting the possible distinction and identification of the PDO mixtures. The authors of other papers (Ganopoulos et al. 2013; Vietina et al. 2013) used appropriate molecular markers to develop conserved nuclear and plastidial DNA plant sequences (fatty acid desaturase, oleosin and chloroplast barcoding rbcL regions) and applied HRM analysis to trace adulteration of olive oils from different plant species at the level of 1% (w/w).

Molecular markers for DNA-based traceability of oil productions

Concerning the use of DNA markers for traceability purposes, there is an increasing interest in using SNPs (Single Nucleotide Polymorphism) for food analysis. While SSRs are usually the most frequently used DNA markers in these studies, the use of SNPs has been suggested based on the following criteria: (1) as the DNA recovered from processed food is usually highly degraded, SNPs provide the advantage of enabling genotyping with shorter DNA fragments (Uncu et al. 2015); (2) SNPs are abundant in both coding and non-coding regions of a genome, allowing an easy discrimination of related genotypes. Bazakos et al. (2012, 2016), using SNPs markers residing in restriction sites, associated with PCR–RFLP capillary electrophoresis, could discriminate both olive cultivars and the corresponding monovarietal oil from Greece and from different Mediterranean countries (Tunisia and Lebanon), eventually evidencing admixtures at the limit of 10%. Kalogianni et al. (2015) reported on the first multiplex SNP genotyping assay for olive oil cultivar identification. The assay was carried out on a suspension of fluorescence-encoded microspheres and allele discrimination was obtained by primer extension reaction. Using three selected SNPs, the authors could identify five common Greek olive cultivars

(Adramytini, Chondrolia Chalkidikis, Kalamon, Koroneiki and Valanolia). Ganopoulos et al. (2013) associated SNPs in plastidial regions and HRM to find contamination with oils of different plant species. In addition, Ben-Ayed et al. (2014a, b) used some of the SNPs identified in the olive genome to authenticate Tunisian oil productions. Other than SNPs, SSRs continue to be highly considered and used in traceability studies: Montemurro et al. (2015), Ben-Ayed et al. (2012), Rotondi et al. (2011a), Vietina et al. (2011) and Corrado et al. (2011). Ben Ayed et al. (2016) presented the Olive Genetic Diversity Database (OGDD) (http://www.bioinfo-cbs.org/ogdd/), which contains genetic, morphologic and chemical information about different olive cultivars. In this database, the genetic information about the cultivars is related to their SSR allele size.

An important outcome of *Olea europaea* genomic studies has been the DNA sequencing of the entire plastome of the Italian cultivar 'Frantoio' (Mariotti et al. 2010). At the same time, papers reporting on plastidial DNA markers for traceability were published. Spaniolas et al. (2010) used SNPs identified in the *trnL* (UAA) intron to identify the botanical origin of plant oils, with a special reference to olive and sesame oils. Pérez-Jiménez et al. (2013) tested the performance of plastidial markers of different types to fingerprint 17 Spanish cultivars, evidencing a rare haplotype in high-value genotypes used to produce regional commercial oil. This supports the utility of chloroplast DNA for oil traceability. As already reported, Ganopoulos et al. (2013) used plastidial SNPs on chloroplast *rbcL* barcoding regions for traceability purposes of vegetable oils. Ramos-Gómez et al. (2014) used chloroplast markers based on *rbcL* and on the intron of the *trnL* (UAA) gene to optimise an extraction method of PCR grade DNA from vegetable oils. The same authors (Ramos-Gómez et al. 2016), with the aim to develop a new QRT-PCR method to be applied for oil traceability, designed markers on different regions of chloroplast DNA genes (*trnE*, *trnQ*, *ycf1*, *clpP*) and a region between *PetN* and *PsbM* genes.

Finally, a complete review has been published recently (Pasqualone et al. 2016), reporting on the evolution and perspectives of traceability from tree to oil and table olives by means of DNA markers.

Genetic improvement of olive: paternity analysis and molecular linkage maps

Like in many other woody plants, the olive juvenile phase is relatively long, ranging between 10 and 15 years. This represents an obstacle to breeding by making it difficult and expensive. Seedling-forcing protocols have been developed to decrease the time of the juvenile phase. However, the assessment of the agronomic performance of mature plants still requires years of testing (Santos-Antunes et al. 2005). Therefore, molecular markers can be useful both to confirm the parental origins of the progeny and to select early agronomical characteristics associated with the markers, thereby reducing the duration and cost of olive breeding.

Paternity analysis

Regarding the paternity analysis, SSRs are appropriate to evidence the genetic contribution from the parents to the offspring since they are codominant and highly polymorphic (Mookerjee et al. 2005). In olive breeding programs, the effectiveness of SSRs in the identification of paternity contribution to the progeny has been proven by a number of authors (De la Rosa et al. 2004; Diaz et al. 2007; Klepo et al. 2013; Shemer et al. 2014; Cáceres et al. 2015). The results have shown that SSRs are convenient to assess routinely the crosses and to check self-incompatibility in olive cultivars (Diaz et al. 2006). Rehman et al. (2012) used nuclear SSR to distinguish Australian olive genotypes; the results supported the use of SSRs as a tool for cultivar differentiation and identification as well as reliable identification of mother plants for commercial propagation.

Biton et al. (2012), using microsatellite data of different cultivars and phenotypic data of the corresponding hybrids, evidenced the presence of significant correlation in terms of genetic distance between cultivars and F_1 performance for three traits: percentage of dry fruit weight, oil content and commercial oil production.

De la Rosa et al. (2013) reported on the development of hexa-nucleotide expressed sequence tags derived from simple sequence repeats (EST-SSRs). These markers had high levels of polymorphism, and the long-core repeat motif permitted an accurate genotyping. The markers were tested successfully on a set of cultivars used as genitors in the olive breeding program of Córdoba (Spain).

The self-(in)compatibility of two cultivars, the Spanish "Arbequina" and the Greek "Koroneiki", recently introduced in cultivation in Sicily, has been investigated (Marchese et al. 2016b). Self-pollination and open-pollination tests, observation of fruit set and DNA analyses with SSR markers were carried out to ascertain whether these cultivars were self-fertile and/or inter-compatible. The results evidenced that none of the "Arbequina" seeds originated from self-fertilisation and none of them had "Koroneiki" as the pollen parent. In contrast, "Koroneiki" was predominantly self-compatible, with 70% of the seeds originating from self-fertilisation.

The clarification of the genetic determination of self-incompatibility in olive has been carried out by Saumitou-Laprade et al. (2017). The study evidenced that self-

incompatibility in olive is under sporophytic genetic control and that olive is characterised by a homomorphic diallelic self-incompatibility system as other plant species (Fraxinus ormus and Phillyrea angustifolia). The clarification of the self-incompatibility in *O. europaea* will be extremely useful to optimise fruit production.

Recently, Beghè et al. (2017) used SSRs and paternity analysis to investigate the pollen flow, measuring pollen immigration rate and dispersal distance, and the spatial genetic structure in an endemic wild olive forest in Andalusia (southern Spain). The study was carried out to understand the mechanisms shaping the spatial distribution of genetic variation in *Olea europaea* subsp. *europaea* var. *sylvestris*.

Genetic mapping

Traditionally, genetic mapping has been carried out by linkage analysis on segregating populations, estimating the frequency of the recombination between the markers used to genotype the population and between the markers and genomic loci influencing traits of interest.

The first olive linkage map by means of 279 RAPDs, 304 AFLPs, few RFLPs and SSRs was obtained using 95 seedlings and the pseudo testcross strategy, derived from Leccino (female parent) × Dolce Agogia (male parent) cultivars in 2003 (De la Rosa et al. 2003). Two genetic maps were obtained, covering, respectively, 2765 cM and 22 major linkage groups in the cv Leccino and 2445 cM and 27 major linkage groups in the cv Dolce Agogia. Subsequently, Wu et al. (2004) used 104 seedlings from the crossing between the cultivars Frantoio (female parent) × Kalamata (male parent) to obtain two parental and an integrate linkage map using the pseudo-testcross strategy. Recently, new and interesting results were obtained in this sector; in 2010, two papers reported on the development of linkage maps: Khadari et al. (2010), using 147 seedlings of a cross between the cultivars Olivière (female parents) × Arbequina (male parents), and El Aabidine et al. (2010), using 140 cross progenies from the cultivars Picholine Marocaine (female parent) × Picholine du Languedoc (male parent). The first population (Khadari et al. 2010) was analysed using more than 400 markers (AFLPs, ISSRs and SSRs) while the second population (El Aabidine et al. 2010) was characterised using nearly 600 markers (SSRs, AFLPs, ISSRs, RADs, SCARs). With respect to the first maps, these two maps were obtained using a higher number of markers, making them a more suitable tool for applications such as QTLs detection.

In 2012, Dominguez-Garcia et al. (2012), using the progeny derived from Picual (female) × Arbequina (male), generated a map using 1630 DArT (diversity arrays technology) and 38 SSR markers. Ben Sadok et al. (2013) increased the number of markers mapped on the existing Olivière × Arbequina map (Khadari et al. 2010); the resulting female map consisted of 25 linkage groups covering 1745 cM, while the paternal map consisted of 21 linkage groups covering 1597 cM and the integrated map of 26 linkage groups covering 2148 cM. This map is currently the most saturated map obtained for olive with classic molecular markers. In 2014, Essalouh et al. (2014) added new markers, EST (Expressed Sequence Tags) derived SSRs, to further increase the saturation of the map obtained by Khadari et al. (2010).

With respect to the maps so far developed, usually based on small numbers of molecular markers and characterised by a medium–low saturation, the recent development of Next Generation Sequencing technologies has enabled the generation of new maps with a high saturation of markers using high-throughput markers such as SNPs. Ipek et al. (2016) and Marchese et al. (2016a) used GBS (Genotyping by Sequencing) to generate a high number of SNPs to obtain highly saturated genetic maps with a higher resolution capacity than those of the existing genetic maps. Ipek et al. (2016) identified 10,941 new SNPs by applying GBS. The resulting high-density genetic linkage map was developed using 121 cross-pollinated full-sib F1 progenies, derived from the crossing between cultivars Gemlik and Edincik-Su, and 5643 markers (21 SSRs, 203 AFLPs, and 5736 SNPs). The map was constituted of 25 linkage groups, spanning 3049 cM of the genome. Marchese et al. (2016a) developed a saturated map with 1597-tagged SNPs on the segregating progeny from the selfing of the cultivar Koroneiki. Twenty-three linkage groups composed the map, covering 11,897 cM. A further 6658 SNPs were associated with the 23 linkage groups, albeit their order was not determined. These articles showed that GBS is a valuable tool to identify thousands of SNPs to generate highly saturated olive maps.

Genetic maps are a useful tool to map QTLs (Quantitative Trait Loci). Usually, the high number of characteristics useful from an agronomic or economic point of view is quantitative traits and controlled by a high number of loci (QTLs) spread in the genome. Ben Sadok et al. (2013) identified 12 QTLs on the genetic map based on Olivière × Arbequina (Khadari et al. 2010), with small effects for interesting traits such as flowering; fruiting and production at whole tree scale. The highest part of QTLs was linked to alleles from the paternal cultivar Arbequina.

Atienza et al. (2014) analysed the same mapping population of Dominguez-Garcia et al. (2012). Twenty-two putative QTLs were identified in the map of Arbequina; more specifically, they detected QTLs for: (1) oil traits on linkage groups 1, 10 and 17; the QTLs on linkage groups 1 and 10 explained circa the 20–30% of phenotypic variance; (2) moisture-related traits on linkage groups 1, 10 and 17;

(3) pulp/stone ratio in linkage groups 10 and 17 explaining 15–20% of phenotypic variation. Five additional QTLs were evidenced in the map of Picual: four QTLs for fruit weight on linkage group 17 and an additional QTL for trunk diameter on linkage group 14, explaining 16% of the phenotypic variance.

The classic approach to map loci of interest is based on the analysis of biparental populations obtained by crossing contrasting parental material for the phenotype of interest. Recently, association mapping has been proposed as a valid and complementary strategy to genetically map agronomic traits. For association mapping studies, also termed Genome-wide Association Study (GWAS), the following factors are necessary: availability of a high number of molecular markers (as SNPs) for genotyping a germplasm and precise phenotypic characterisation of the same germplasm. The aim of this analysis is to find linkage disequilibrium (LD) between markers and traits. In classic linkage mapping, the association between a marker and the trait of interest is a consequence of the recombination events taking place in the segregating population of a biparental cross. Contrarily to this, in a GWAS experiment, the association is a consequence of the numerous recombination events that occurred over the years during the evolution of the germplasm. This can lead to a more precise characterisation of loci of interest with respect to classic approaches. Phenotyping a large germplasm can be expensive and time-consuming, especially for perennial plant species. To avoid this, the definition of a core collection of an individual representative of the whole germplasm can be an alternative. With this aim, El Bakkali et al. (2013a, b) and Khadari et al. (2014), by analysing the global olive germplasm bank (OWGB Marrakech, Morocco), proposed two core collections useful for such studies, consisting of 50 and 94 individuals. These collections grouped some of the cultivars considered as the most important ones in Mediterranean countries and displayed a limited genetic structure between east and west/centre gene pools. Consequently, they could efficiently be used in association studies. Belaj et al. (2012), using different kinds of markers, analysed the IFAPA germplasm to define a core collection. Different subsets of 18, 27, 36, 45 and 68 olive accessions, corresponding to 5, 7.5, 10, 12.5 and 19%, respectively, of the whole germplasm collection were selected. Based on the obtained results, the core collection of 68 accessions, because of a high efficiency at capturing the alleles/traits states found in the whole collection, could be of special interest for studies such as GWAS.

Kaya et al. (2016) reported the first GWAS study in olive to find an association between molecular markers and traits of interest. The authors analysed 96 olive genotypes using SNP, AFLP, and SSR markers and five traits related to yield, detecting eleven significant associations. The highest number of significant associations was found for fruit and stone weight. These results evidenced the utility of GWAS in detecting loci of interest without the development of a mapping population. Ipek et al. (2015a) characterised an olive core collection using SSRs to detect markers associated with the content of fatty acids in the oil to be used in marker-assisted breeding of olive. The authors found strong correlations between SSR DCA14 and stearic acid content and between SSR GAPU71B and oleic acid content.

Genomics studies

Genome sequencing

Knowledge of genome sequences is crucial for our understanding of plant biology; therefore, the genome sequences of a large number of organisms are expected to be completed in the near future. In olive, three sequencing projects started in the last years, namely the Italian OLEA project (Muleo et al. 2012), the international IOGC International Consortium, sequencing the genome of a wild olive (*O. europaea*, var. *sylvestris*; Unver et al. 2016), and the sequencing of an over 1000-year-old tree of the Spanish variety Farga (Cruz et al. 2016). The latter project was the first one to provide a draft of the olive genome, spanning a length of 1.31 Gb out of the estimated 1.38 Gb genome size, with a median C-value of 1.59 pg. The predicted number of protein gene-coding sequences, supported also by RNA sequencing from the different plant tissues, was estimated to be higher than 56,000. Recently, Barghini et al. (2014, 2015) carried out an analysis of the repeated fraction of the olive genome and reported a peculiar structure of this genome, as compared to that of other plants, with a large percentage of satellite DNA related to a few satellite tandem repeat families Oe80, Oe86, Oe178, Oe179 and Oe218, representing approximately 97% of this class of repeated elements. Among the repeated sequences, retrotransposons represent 40.265%; DNA transposons 5.514% tandem repeats 31.161% of the olive genome. In terms of the two-main superfamilies of LTR-retrotransposons, *Gypsy* and *Copia*-like LTR are present in a ratio of 1.17:1, indicating that the first ones are more abundant, even if the number of *Gypsy* families is smaller than the number of *Copia*, and that the *Gypsy* retro-transcription event started earlier in the genetic flow of the olive tree genome development (Barghini et al. 2014). The characterisation of olive short interspersed nuclear elements (SINEs) nonautonomous retrotransposons was recently carried by Barghini et al. (2016) providing one of the first sets of these elements in dicotyledonous species and adding new information on olive genome evolution. Generally,

SINEs have been detected in single copy or just lowly repeated and often associated with genic sequences. The comparison of olive SINEs and other LTR families suggested that SINE expansion in the genome occurred in very ancient times, preceding LTR expansion, and likely before the separation of the rosids from the asterids.

Functional genomics

Functional genomics is the study of the gene function as well as of other parts of the genome. In olive, the identification and annotation of genes prior to genomic sequencing were based on expressed sequence tag (EST) identification, initially focused on pollen allergens and fruit traits. Olive pollen causes respiratory allergies worldwide (cases have been reported in Mediterranean countries, North and South America and Australia). In the Mediterranean area, a high percentage (above 30%) of the residing people is affected by this problem (Bousquet et al. 1984), and allergens (Ole e 1 to Ole e 10) have been detected in olive pollen (Rodríguez et al. 2002; Hamman-Khalifa et al. 2008). The nucleotide sequence of Ole e 1 gene is characterised by high heterogeneities and polymorphisms. Hamman-Khalifa et al. (2008) evidenced that the origin of the cultivar is basically the main factor for Ole e 1 variance among pollens. Polymorphisms in the nucleotide sequence influence the amino acid sequence and the folding of the protein, leading to high variability in Ole e 1 allergenicity. Data agree with observations carried out both in vivo and in vitro about the capacity of olive cultivar allergens in binding IgE antibodies. Interestingly, polymorphisms in gene Ole e 1 are that high that even strictly related varieties can be recognised as dissimilar, which is the case in cultivars 'Picholine marocaine' and 'Menara', usually considered a clonal selection of the first.

A number of studies have focused on genes important in determining fruits and oil traits, such as the genes involved in fatty acid biosynthesis, namely enoyl-ACP reductase, plastidial stearoyl-ACP desaturase (*SAD*), omega 6 microsomal (*FAD2-1, FAD2-2*) and plastidial (*FAD6*) desaturase, omega 3 microsomal (*FAD3-A, FAD3B*) and plastidial desaturase (*FAD7-1 and FAD7-2*), acyl-CoA diacylglycerol acyltransferase (*DAGAT*) and the respective enzymes for electron donor cytochrome b5 reductase for microsomal desaturases and ferredoxin-NAD(P) reductase for plastidial desaturases (Hernández et al. 2011). Oleosin enzymes have been identified (Hatzopoulos et al. 2002). Some studies have been carried out to clone, characterise and analyse the spatial/temporal activation of genes involved in these pathways (Banilas et al. 2005; Poghosyan et al. 2005; Hernández et al. 2005, 2009, 2016). In olive, Cultrera et al. (2014) isolated six acyl carrier protein *ACP* cDNAs and characterised their genomic sequences coding for plastidial ACP isoforms. Three *OeACP* loci, with one pair of alleles each, were recognised and transcript abundance of *OeACP1* was found extremely low in comparison to the other *OeACP* genes. The *OeACP2* was abundant in fruit and leaf tissues, while *OeACP3* was highly expressed in flowers. The expression pattern of *ACP* genes during fruit development was correlated with the amount of protein, suggesting that *OeACP2* and *OeACP3* proteins are involved in the synthesis of fatty acids and triacylglycerol accumulation. Recently, Parvini et al. (2016) studied the specific contribution of different stearoyl-ACP desaturase (*SAD*) genes to the oleic acid content in olive fruit and isolated three distinct cDNA clones encoding three *SAD* isoforms. Measuring lipid content and gene expression analyses, they showed that *OeSAD2* seems to be the main gene contributing to the oleic acid content of the olive fruit. Furthermore, data show that the microsomal oleate desaturase gene *OeFAD2-2* is responsible for the linoleic acid content in virgin olive oil.

Beside fatty acid composition, minor components play a crucial role in human health and protect DNA, proteins and lipids from oxidative damage. Regarding this aspect, phenolic compounds have been extensively analysed, and as previously reviewed by Hatzopoulos et al. (2002), high-quality olive oils resistant to oxidation processes can be obtained by increasing these antioxidant molecules in olive fruits. Studies related to the biosynthetic pathways of antioxidants have been performed (Shibuya et al. 1999; Hatzopoulos et al. 2002; Saimaru et al. 2007), resulting in the cloning of a monosaccharide transporter (*OeMST2*) (Conde et al. 2007). In 2009, the identification of genes differentially expressed during fruit development was achieved by Alagna et al. (2009), using high-throughput sequences (454-sequencing). The authors sequenced four different cDNA libraries obtained from transcripts isolated in different periods of fruit development (at the beginning and at the end) from varieties Coratina and Tendellone (characterised, respectively, by high and low-phenolic contents). Galla et al. (2009) identified a large set of differentially expressed genes at three different olive phenological stages (i.e. initial fruit set, completed pit hardening and veraison) in the Leccino cultivar. In this case, four subtractive hybridization libraries were constructed and the sequenced clones (1132 in total) showed 60% of similarities to known proteins. Alagna et al. (2012) studied several phenolics (oleuropein, demethyloleuropein, 3–4 DHPEA-EDA, ligstroside, tyrosol, hydroxytyrosol, verbascoside and lignans) in fruits of 12 olive cultivars, identifying transcripts homologous to genes involved in the pathways of these secondary metabolites. The mRNA levels of transcripts putatively involved in phenolic biosynthesis measured in fruits of high- (Coratina) and low-phenolic (Dolce d'Andria) varieties at three different developmental

stages of fruits significantly correlated with the concentration of phenolic compounds. 45 days after flowering, *OeDXS*, *OeGES*, *OeGE10H* and *OeADH*, encoding putative 1-deoxy-D-xylulose-5-P synthase, geraniol synthase, geraniol 10-hydroxylase and arogenate dehydrogenase, respectively, were highly represented, suggesting that they play a key role in secoiridoid accumulation during fruit development. Recently, Alagna et al. (2016) could identify candidate genes belonging to the secoiridoid pathway in olive. The functional characterisation of the olive homologue of iridoid synthase (*OeISY*) proved that it is an unusual terpene cyclase that couples an NAD (P)H-dependent 1,4-reduction step with a subsequent cyclisation. These results are evident that *OeISY* likely generates the monoterpene scaffold of oleuropein.

A number of other functional genomics investigations concern the fitness of olive in stressful environmental conditions. The expression of aquaporin genes was studied by Secchi et al. (2007) to evaluate the impacts of drought on olive. The authors found a strong down-regulation in these genes following drought, most likely resulting in reduced membrane permeability to water, preventing water loss. Bruno et al. (2009) isolated a geranylgeranyl reductase (*OeCHLP*) gene and hypothesised its role in organ development and responses to abiotic and biotic stresses in relation to tocopherol activity. Recently, Perez-Martin et al. (2014) carried out an experiment of short-term water-stress and recovery in olive, measuring the changes in leaf gas exchange, chlorophyll fluorescence and plant water status and evaluating correlations with the expression levels of three genes: two aquaporins (*OePIP1.1* and *OePIP2.1*) and one stromal carbonic anhydrase. Based on their results, both aquaporins and carbonic anhydrase are involved in the regulation of stomatal (g_s) and mesophyll conductance (g_m). Using structural equation modelling, the authors proved that both *OePIP1.1* and *OePIP2.1* could explain most of the variations observed for g_s and g_m, while the carbonic anhydrase had a small effect on g_m.

Olive is moderately resistant to salinity and shows mechanisms of exclusion/retention of salt at the root level, thereby avoiding Na^+ and Cl^- accumulation in the aerial organs. Up to now, specific molecular and genomic studies on this topic in olive are scarce. Bazakos et al. (2016) investigated the molecular responses of olive leaves and roots to salinity using next generation sequencing technology. In this study, many differentially expressed genes related to salt tolerance were found, and in leaf transcripts corresponding to glutathione reductase, superoxide dismutase and proline dehydrogenase were identified. Rossi et al. (2016) showed that phenolic compounds remain stable or are strongly depleted under long-time treatment with sodium in the salt-sensitive cultivar Leccino, determining a strong up-regulation of key genes of the phenylpropanoid pathway. In the salt-resistant cultivar Frantoio, the content of phenolic compounds was always high and the up-regulation of the phenylpropanoid genes was less intense.

Beside abiotic stresses, low winter temperatures can cause extensive damage to olive plants. Transcriptome analysis of olive leaves during cold acclimation resulted in the identification of 6309 differentially expressed transcripts in cv Picual (de la Leyva-Perez et al. 2015). Similarly, Guerra et al. (2015) performed an RNA-Seq analysis of short- and long-term transcriptional changes during cold acclimation, identifying specific cold response genes of olive.

Finally, olive must cope with several biotic stressors (such as insects, fungi, bacteria and viruses), and the understanding of the molecular basis of the olive response to these stressors is of fundamental importance for breeding and innovative crop management solution. Since an exhaustive description of all these genomic studies is not possible in this review, a comprehensive and detailed analysis can be found in a book recently edited by Rugini et al. (2016).

Conclusions and future perspectives

In this review, an update of the research work done in olive genetics and genomic has been given. Considering the state of the art reported in Bracci et al. (2011) the progress in these areas have been relevant as proved by the number of publications released. Very likely this result has been determined by the increasing relevance of olive oil and fruits for human nutrition that represent a driving factors for researcher's activities and curiosity. The recent release of the olive genomic sequence and the expected resequencing of several olive varieties will speed up further the application of molecular tools in taxonomy, varietal identification, traceability and breeding of this species. In the next decade, functional genomics studies assisted by agronomical and physiological phenotyping tools and by a tighter integration of the different omics sciences (genomics, epigenomics, transcriptomics, proteomics and metabolomics) will be helpful in explaining olive fitness and yield performance in hostile environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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