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Olive Tree Genetic Resources Characterization Through Molecular Markers

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1. Introduction

The *Olea europaea* L. is considered one of the most widely grown fruit crop in the countries of the Mediterranean basin. The olive products, such as olive oil, table olives, and olive pastes are the basic constitutes of the Mediterranean diet due to their benefits for human health, besides other applications such as in cosmetics field.

The olive is one of the most ancient cultivated fruit trees. Olive cultivation has a very long history which started from the Third Millennium BC (Loukas & Krimbas, 1983) in the Eastern region of the Mediterranean sea and spread later around the basin following land and maritime routes to Italy, Spain, North Africa and France. Nowadays there are about 805 million of olive trees, 98% of which are grown in the Mediterranean countries (Tsitsipis et al., 2009). The foremost consuming countries are also the main olive oil producers. According to Food and Agriculture Organization of the United Nations, Mediterranean countries produce more than 90% of world olives, and the biggest olive producers are Spain, Italy, Greece, Turkey, Tunisia, Morocco, Syria, and Portugal (FAO, 2008). Other consuming countries are the United States, Canada, Australia and Japan (Hatzopoulos et al., 2002; Pinelli et al., 2003). Though the olive oil consumption has been mainly constricted to Mediterranean countries, actually it has been extended to other areas due to its health beneficial proprieties (Bracci et al., 2011). Over the centuries, olive trees were propagated mainly vegetatively and were selected based on olive quantitative and qualitative traits. However, this procedure did not exclude the problematic of natural crossing between the newly introduced cultivars and the local germplasm and somatic mutation events, and genetic variability among the olive tree collections has been reported by several authors (Angiolillo et al., 1999; Bautista et al., 2003; Belaj et al., 2002, 2003, 2004, 2006; Cordeiro et al., 2008; Gemas et al., 2000; Gomes et al., 2008, 2009; Martins-Lopes et al., 2007, 2009; Sefc et al., 2000). In addition, the olive tree is allogamous, easily generating crosses between cultivars which give rise to high genetic variability between and within cultivars (Mekuria et al., 1999; Ouazzani et al., 1996; Zohary, 1994).

More than 2600 cultivars have been described for *Olea europaea* L. using morphologic analyzes (Rugini & Lavee, 1992), although many of them might be synonyms, homonyms, ecotypes or the result of crosses between neighbouring olive cultivars (Barranco et al., 2000). Bartolini et al. (1998) reported that there are 79 olive collections located in 24 countries

which contain about 1200 cultivars with more than 3000 different names. The high number of olive cultivars causes a huge problem in the germplasm collections management and traceability and authenticity of olive oils produced, once there is an uncertainty about its olive cultivar correct denomination (Cipriani et al., 2002).

Until recent years, cultivars' identification was based only on morphological and agronomic traits. However, recognition of olive cultivars based on phenotypic characters revealed to be problematic, especially in early stages of tree development. Traditionally diversity within and between olive tree cultivars was determined by assessing differences in olive tree, namely leaf shape and color, and olive fruits morphology. These measures have the advantage of being readily available, do not require sophisticated equipment and are the most direct measure of phenotype, thus they are accessible for immediate use, an important attribute. However, these morphological and phenological markers have the disadvantage of the small number of polymorphism detected and of being environmentally dependent (Mohan et al., 1997; Tanksley & Orton, 1983). Besides that, some of the phenological characteristics are only accessible for a limited (e.g., olive fruits) or when the olive tree achieves a mature stage, which may delay the correct identification. Due to the high genetic diversity level observed in olive germplasm and the presence of homonyms and synonyms cases, efficient and rapid discriminatory methods are urgently.

In recent years, molecular markers have been applied in olive germplasm to identify cultivars and to determine the relationships between cultivars. Molecular markers are, according to Kahl (2004), any specific DNA segment whose base sequence is polymorphic in different organisms. Such markers can be visualised by hybridization-based techniques such as restriction fragment length polymorphism (RFLP) or by polymerase chain reaction (PCR)-based methods.

Molecular markers present numerous advantages over conventional phenotype based alternatives. The choice and selection of an adequate marker system depends upon the type of study to be undertaken and whether it will fulfil at least a few of the mentioned criteria: (a) highly polymorphic between two organisms, inherited codominantly, (b) evenly distributed throughout the genome and easily visualized, (c) occurs frequently in the genomes, (d) stable over generations, (e) simple, quick and inexpensive, (f) small amounts of DNA samples required, and (g) no prior information about the sample's genome (Agarwal et al., 2008; Hatzopoulos et al., 2002).

Because of their high polymorphism level and discerning power, molecular markers have been used as a powerful tool for olive gene pools' characterization. Molecular markers have played a crucial role to distinguish, characterize, and to elucidate olive germplasm origin and diversity. Different molecular markers have been applied for olive genetic diversity assessment, such as the dominant random amplified polymorphic DNA (RAPD) (Belaj et al., 2003; Cordeiro et al., 2008; Gemas et al., 2004; Gomes et al., 2009; Martins-Lopes et al., 2007, 2009; Trujillo et al., 1995) and inter simple sequence repeat (ISSR) markers (Essadki et al., 2006; Gemas et al., 2004; Gomes et al., 2009; Martins-Lopes et al., 2007, 2009). The codominant microsatellite (SSR) (Belaj et al., 2003; Bracci et al., 2009; Gomes et al., 2009; Sabino et al., 2006; Sarri et al., 2006; Sefc et al., 2000), and amplified fragment length polymorphism (AFLP) (Ercisli et al., 2009; Grati-Kamoun et al., 2006; Montemurro et al., 2005) have been used for olive germplasm characterization.

However, the disadvantageous associated with some type of markers, like the less sensibility, and reproducibility of RAPD or the complexity of the AFLP assay, makes it necessary to convert interesting markers (bands) into sequence-characterized amplified regions (SCAR) or sequence-tagged site (STS) markers (Olson et al., 1989; Paran & Michelmore, 1993). During the olive genome exploration different molecular markers have emerged. The single nucleotide polymorphisms (SNP) has been used to discriminate 49 olive cultivars, selected among the most widely cultivated, for olive oil production, in the Mediterranean area (Consolandi et al., 2007). The presence of retrotransposon-like elements in the olive genome was reported during SCAR development for olive cultivar identification (Hernández et al., 2001b). It is generally accepted that retrotransposons have played an important role in olive genetic instability and genome evolution. The use of retrotransposon sequences to generate molecular markers (e.g., REMAP: retrotransposon microsatellite amplification polymorphism) has been used in olive tree (Natali et al., 2007).

The increasing openness of genetic markers in olive tree allows the detailed studies and evaluation of genetic diversity. Within this context, a review of the state of the art of molecular marker techniques applied for olive cultivars characterization and their applicability in olive germplasm conservation will be presented. This will give a prospect of what has been attained and what still needs to be done in order to better understand this crop that has lived for centuries and still remains to be discovered and understood.

2. Olive tree origins

Mythologically olive tree was a gift of Athens goddess to the Greeks. However, olive tree geographical origin still remains unclear. According to botanists, the olive tree and oleaster correspond to *Olea europaea* subsp. *europaea* L. var. *europaea* and var. *sylvestris*, respectively. Oleaster is the wild form, while the olive is the cultivated form (Breton et al., 2006). The olive tree is self-incompatible. Out-crossing is mediated by the wind that transports pollen over long distances, with cytoplasmic male-sterile cultivars being pollinated efficiently by surrounding cultivars or even by oleasters (Besnard et al., 2000). It is assumed that cultivars have originated from the wild Mediterranean olive (oleasters), and have been disseminated all around the Mediterranean countries following human displacement. It is also presumed that crosses between wild and cultivated forms could have led to new cultivars around Mediterranean countries (Besnard et al., 2001).

In order to understand olive domestication, random amplified polymorphic DNA (RAPD) profiles of 121 olive cultivars were compared to those of 20 natural oleaster populations from Eastern and Western parts of the Mediterranean Basin. The differences observed between groups of cultivars were clear (Besnard et al., 2001). Cultivars from Israel, Turkey, Syria, Greece and Sicily were close to the Eastern oleasters group; on the other hand, clones from Italy, France, Corsica, Spain and the Maghreb were closer to the Western group. Multiple origins for Mediterranean olive (*Olea europaea* L. ssp. *europaea*) based upon mitochondrial DNA variations have been reported (Besnard & Bervillé, 2000). The phylogeographic study revealed the presence of three mitotypes (ME1, MOM and MCK) in both cultivated olive and oleaster; while a fourth mitotype, ME2, was unique to a few cultivars from East to West. This information led to the conclusion that a great majority of the cultivars were originated by maternal descent from the Eastern populations once they carry the mitotypes ME1 or ME2. The cultivars with the Western mitotypes, MOM or MCK,

generally kept a nuclear RAPD profile close to the profile of Western natural populations. Consequently, they could result from exclusively local material (as for Corsica), while ME1 and ME2 are characteristic of the East Mediterranean populations. The presence of these different mitotypes reflects the complexity of olive domestication: the Western Mediterranean is probably a zone where olive trees from the East, once introduced, have been hybridized and back-crossed with the indigenous olives (Besnard et al., 2001).

Bronzini de Caraffa et al. (2002) have performed a study of nuclear and mitochondrial DNAs of cultivated and wild olives, from two Corsican and Sardinian Mediterranean islands, using both random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. The results have indicated that the combination of mitotype and RAPD markers can be used as a powerful tool for differentiating two groups in the wild forms: the Western true oleasters and the feral forms. A recently study has investigated the genetic diversity between Eastern and Western oleasters and between cultivars (Breton et al., 2006). The oleaster genetic diversity, obtained by chloroplast and SSR markers, is divided into seven *reconstructed panmictic oleaster populations* regions (RPOP) in both Eastern and Western populations that could overlay glacial refuges. The authors argue that the gene flow has occurred in oleasters mediated by cultivars spread by human migration or through trade. However, a complex origin for this species, higher than expected initially, was reported (Breton et al., 2006).

3. Non PCR-based markers

3.1 Restriction Fragment Length Polymorphism (RFLP)

The restriction fragment length polymorphism (RFLP) markers are based on the analysis of patterns derived from cleaved DNA sequences with known specific restriction enzymes and hybridization with specific probes (Mohan et al., 1997). Based on this method genetic variation and relationship between 89 very old olive trees and 101 oleasters, cultivated around the Mediterranean basin, have been evaluated by cytoplasmic DNA markers (Amane et al., 1999). A similar approach was used to study chloroplast DNA variation in wild and cultivated Morocco olives (Amane et al., 2000). The analysis revealed the presence of four distinct chlorotypes. Nowadays, restriction fragment length polymorphism (RFLP) markers are not very widely used due to several constrains of the method: (a) time consuming, (b) radioactive and/or toxic reagents, (c) large quantity of high quality genomic DNA, and (d) prior sequence information for probe generation; increasing overall the complexity of the methodology (Agarwal et al., 2008). With the development of PCR based methodologies, this marker has been limited for diversity studies, once PCR methods are more expedite. However, the use of RFLP combined with other molecular techniques has been used for olive tree diversity studies. Besnard et al. (2002) combined the RFLP technique with PCR to analyze the chloroplast DNA diversity in the olive complex.

4. PCR-based markers

Since the PCR has been introduced by Mullis et al. (1986) the molecular studies have profoundly changed the way in which they are conducted. The subsequent development of methods for DNA fingerprinting has introduced the possibility to univocally identify cultivars or clones from a specific area. The different PCR based methods used for olive

diversity evaluation and germplasm characterization will be described in this review, considering always the purpose of the work performed in olive around Mediterranean countries.

4.1 Random Amplified Polymorphic DNA (RAPD)

A new DNA polymorphism assay was first described in 1990 by Williams et al. (1990) and Welsh & McClelland (1990). The random amplified polymorphic DNA (RAPD) marker is based on the amplification by PCR of random DNA segments, using single primers of arbitrary nucleotide sequence. The amplified DNA fragments, referred to as RAPD markers, were shown to be highly useful in the construction of genetic maps. With RAPD method the resulted polymorphisms are detected by electrophoresis as different DNA fragments. The different DNA fragments are generated once the primers used usually anneal with multiple sites in different regions of the genome, producing multiple amplified products that often contain repetitive DNA sequences (Paran & Michelmore, 1993).

The first study using RAPD markers, to evaluate olive germplasm polymorphism was reported by Fabbri et al. (1995). All RAPD data suggest a high degree of genetic diversity in the olive germplasm (Belaj et al., 2006). Several reports detected also a high degree of genetic variability within cultivars of different countries: Iran (Shahriari et al., 2008), Spain (Belaj et al., 2002), and Portugal (Cordeiro et al., 2008; Gemas et al., 2004; Martins-Lopes et al., 2007). Most of the olive cultivars in these studies were clustered according to their fruit's end-use and ecological adaptation. Belaj et al. (2004) found that a combination of three highly polymorphic RAPD primers (OPK16, OPA19 and OPX09) was optimal to discriminate among 103 cultivars. Inter- and intravarietal variation of three olive cultivars, 'Galega Vulgar', 'Cordovil de Serpa' and 'Verdeal Alentejana', were also observed with RAPD markers (Gemas et al., 2000). The clonal diversity has been accessed using RAPD markers in combination with inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) in two important Portuguese olive cultivars, 'Verdeal-Transmontana' (Gomes et al., 2008) and 'Cobrançosa' (Martins-Lopes et al., 2009). The highest proportion of polymorphic products, observed in 'Verdeal-Transmontana' clones was generated using primer OPO10 (88%), and the mean level of polymorphism was 28%. In the 'Cobrançosa' the authors reported a considerable polymorphism among the DNA fingerprints of the clones. The RAPD primers amplified 150 reproducible fragments, of which 75% were polymorphic. The high level of polymorphism reported demonstrates that the Portuguese 'Verdeal-Transmontana' and 'Cobrançosa' cultivars were genetically heterogeneous, confirming that olive is a highly variable species. Recently, genetic similarities and distances among Turkish wild olive trees were studied in order to improve genetic resources and knowledge of cultivars evolutionary background (Sesli & Yegenoglu (2009).

However, RAPD methodologies have its criticisms due to the low data reproducibility between laboratories, although it may be quite reliable at the same laboratory. The fact of low cost, low time usage, low DNA amount even if not of good quality and no previous DNA sequence knowledge made this molecular marker technique one of the first to be used to access genetic variability. A variation of the RAPD technique, such as arbitrarily primed PCR (AP-PCR) (Welsh & McClelland, 1990), that involves the increase of annealing temperature during the PCR cycles, has been used, in Turkey, to characterize and select six important olive clones used for olive oil production Kockar & Ilıkcı (2003). Claros et al.

(2000) used the same methodology for olive geographic location and confirmed the hypothesis of autochthonic origin of most olive-tree cultivars. Nowadays, obtaining specific markers, such as sequence characterized amplified region (SCAR) and sequence-tagged-site (STS), from RAPD markers could be a way to overcome the lack of reproducibility, proper of RAPD markers.

4.2 Amplified Fragment Length Polymorphism (AFLP)

The principle of amplified fragment length polymorphism (AFLP) (Vos et al., 1995) technique is basically simple and its procedure consists of three main steps: (a) template preparation, (b) fragment amplification, and (c) gel analysis. The fingerprinting patterns are obtained by detection of genomic restriction fragments by PCR amplification. This technique has been widely employed. Because of its effectiveness, reliability and efficiency in genetic diversity studies (Ercisli et al., 2009), the AFLP technique has been widely used in olive Spanish cultivars considering intra-varietal diversity (Sanz-Cortés et al., 2003), and to assess genetic inter-relationships among cultivated cultivars in the Eastern Mediterranean Basin (Owen et al., 2005). The results showed significant genetic distance between Greek and Turkish cultivars, and a clear separation of most of the Spanish and Italian clones, suggesting that an East-West divergence of olive cultivars occurred. Using the AFLP markers Angiolillo et al. (1999) have shown that wild olives from the Western Mediterranean and cultivated cultivars did not cluster together, and were relatively distant. However, a few oleasters clustered with the cultivars suggesting a common origin.

The first linkage map of the olive genome was constructed using a combination of molecular markers (e.g., RAPD, AFLP, RFLP and SSR) (De la Rosa et al., 2003). Maps can be used to select important traits and to study genes that control expression of polygenic traits. Molecular marker linkage maps are widely recognized as essential tools for genetic research and breeding in many species.

4.3 Microsatellites (SSR)

The simple sequence repeat (SSR) (Tautz et al., 1986; Litt & Luty, 1989) consists of short (1-6 base pair long) stretches of DNA tandem repeated several times, occurring in the genomes of many higher organisms (Rafalski & Tingey, 1993; Wu & Tanksley, 1993). The simple sequence repeat or microsatellites, as one of the most popular marker system, are widely used in plant genetic research for diversity studies, namely in olive tree and to test the breeding success as they are transferable, highly polymorphic, ideal for genetic map development, linkage analysis, marker-assisted selection and fingerprinting studies (Bracci et al., 2009; Cipriani et al., 2002; De la Rosa el al., 2004; Gomes et al., 2009; Karp et al., 1996; Muzzalupo et al., 2009; Rallo et al., 2002; Sefc et al., 2000). When compared with RAPD or AFLP markers, the SSR have the advantage of their codominant nature, as two alleles may be identified at each locus. The main constrain of SSR markers is the development requires previous DNA sequencing for primer designing.

The microsatellites loci have been isolated from olive tree (Carriero et al., 2002; Cipriani et al., 2002; De la Rosa et al., 2002; Rallo et al., 2000; Sefc et al., 2000) and are used either alone or in combination with other molecular markers to characterize olive cultivars (Belaj et al., 2004; Gomes et al., 2009; Khadari et al., 2003; Wu & Sedgley, 2004). This methodology has

been used to analyze the genetic variability of the somatic embryogenesis induction process in *Olea europaea* L. and *Olea europaea* var. *maderensis*. The authors reported the maintenance of the genomic integrities between species suggesting the absence of somaclonal variation (Lopes et al., 2009). New insights about genetic diversity and gene flow between the wild (oleaster) and the cultivated form, using SSR marker was reported (Breton et al., 2006). A database containing a consensus list of SSR profiles for true-to-type olive genotyping has been constructed. This platform will allow results' comparison among laboratories, in order to establish a common olive database (Baldoni et al., 2009).

During many years the agarose gel electrophoresis has been used as the common detection method for SSR analysis. The agarose gel is efficient when the alleles are long enough, that is, more than 200-300 base pair and the differences among alleles are also significant to be visualized (i.e., more than 10-20 base pair). The high resolution polyacrylamide gels have been used when small differences between alleles, less than 1-10 base pair, must be identified. Nowadays, the separation of SSR markers using sequencing apparatus revealed to be very suitable, since the detection of alleles is performed automatically. The major advantages of automated detection are: (a) faster in obtaining results, (b) automated data analysis, (c) multiplex analysis, (d) high reproducibility, and (e) exclusion of silver-staining procedure. However, between different apparatus there may be found a shift among allele size, which has to be undertaken when comparing results among laboratories.

4.4 Inter Simple Sequence Repeats (ISSR)

In order to resolve some of the inconveniences associated with RAPD (low reproducibility), the high AFLP cost, and the need to know the flanking sequences in order to developed primers for SSR polymorphism, ISSR were developed (Terzopoulos et al., 2005; Zietkiewicz et al., 1994). ISSR markers are based on the amplification of regions (200-2000 base pair) between inversely oriented closely spaced microsatellites. The ISSR show the specificity of microsatellite markers, but need no sequence information for primer synthesis. The ISSR alone or in combination with other marker systems, have been widely used to analyze clonal variation and genetic variability in olive cultivars (Gemas et al., 2004; Gomes et al., 2008; Martins-Lopes et al., 2007, 2009; Terzopoulos et al., 2005).

Previous studies have concluded that ISSR markers are efficient in assessing phylogenetic relationships in the *O. europaea* complex (Gemas et al., 2004; Hess et al., 2000) and for olive fruits and leaves identification (Pasqualone et al., 2001). The simultaneous use of ISSR with other markers such as RAPD has made possible the discrimination between 30 Portuguese and 8 foreign olive cultivars (Martins-Lopes et al., 2007).

4.5 Sequence Characterized Amplified Region (SCAR)

Since PCR-based molecular markers have been developed, several PCR-based markers modifications have emerged. Due to the certification process of orchards and regions, crucial for protected denomination of origin (PDO), there is an urgent need for early and efficient methods able to discriminate and indentify olive cultivars. The development of cultivar-specific DNA markers can also be useful in olive industry in order to avoid olive oil adulteration that affects the oil quality (Marieschi et al., 2011; Pafundo et al., 2007).

The sequence characterized amplified region (SCAR) have been widely developed for plant breeding studies in several species such as wheat (Hernández et al., 1999), grapevine (Vidal et al., 2000), tomato (Zhang & Stommel, 2001), and pear (Lee et al., 2004; Marieschi et al., 2011). In olive, this type of marker has also been applied for olive germplasm evaluation and mapping (Bautista et al., 2003; Busconi et al., 2006; Hernández et al., 2001a), and for analysis of complex agro-food matrixes (olive oil traceability) (Pafundo et al., 2007).

The development of sequence characterized amplified region (SCAR) involves cloning of the amplified product, and then sequencing the two ends of the cloned product that appeared to be specific. The SCAR has the advantage of being inherited in a codominant fashion in contrast to RAPD which are inherited in a dominant manner (Mohan et al., 1997). Bautista et al. (2003) used this technology to develop specific markers useful for olive cultivar identification and mapping. They demonstrated that the use of SCAR markers is enough to provide a simple, cheap, and reliable procedure to identify geographically related olive cultivars. The development of SCAR markers by directly sequencing olive RAPD bands was reported by Hernández et al. (2001a) and they demonstrated that the generated markers were useful for the marker assisted selection of the high flesh/stone ratio. This type of marker has also been applied for olive germplasm evaluation and mapping (Bautista et al., 2003; Busconi et al., 2006). Wu et al. (2004) combined RAPD, SCAR and SSR markers to construct a linkage map from a cross-pollinated F_1 population of 'Frantoio' × 'Kalamata' olive cultivars.

4.6 Single Nucleotide Polymorphisms (SNP)

The single nucleotide polymorphisms are a marker system that can differentiate individuals based on variations detected at the level of a single nucleotide base in the genome. Such variations are present in large abundance in the genomes of higher organisms including plants (Agarwal et al., 2008). The SNP-based markers have been used in many plant species.

In olive, due to olive unknown genome, this technique has not been widely applied. Reale et al. (2006) used SNP markers to genotype 65 olive samples obtained from Europe and Australia, and observed that 77% of the cultivars were clearly discriminate. However, the authors developed SNP markers from olive gene sequences available in the GenBank database and from arbitrary sampling using the sequence-related amplification polymorphism (SRAP) method.

5. Conclusions

Nowadays, the olive industry requires certified olive cultivars with elite agronomic characteristics and adapted to modern intensive mechanized orchards (Hatzopoulos et al., 2002). Very few cultivars are grown commercially in more than one region or country, while most of them have a local diffusion. The cases of cultivars homonyms and synonyms associated with high genetic diversity makes the olive tree germplasm very difficult to characterize. The PCR-based markers opened the possibility to develop, over the last two decades, new molecular techniques for cultivar identification and further certification purposes in order to certify the propagated material. It is essential to study the genetic base of olive germplasm in order to characterize and compare with other genetic, phenotypic and agronomic data. Different molecular markers have been used in genetic diversity studies which give us information about the relationships between cultivars and the olive domestication process.

The choice on which molecular technique would be the most suitable for olive genetic resource characterization depend on a number of factors as the level of variability of the species, and the resources available (Belaj et al., 2006). Technological advancement has contributed to the development, in every aspect, of molecular genetic markers, making them technically simpler, efficient, cost-effective, and faster than the classic methods.

However, molecular approaches (nuclear and cytoplasmic) should not be considered alone or as substitutes of morphological characterization but as complementary tools, more complete and effective, for olive genetic resources studies. The several molecular markers used for germplasm variability studies may play a major role in olive tree breeding programs when using marker assisted selection for biotic and abiotic stress tolerance, olive fruits and oil quality traits.

However, there are still aspects of cultivars synonymous that still needed to be addressed in order to develop a complete database, in order to have an overview of the genetic variability available. As soon as the recent olive genome sequence is released new strategies may be taken in order olive germplasm management, breeding strategies and certification issues.

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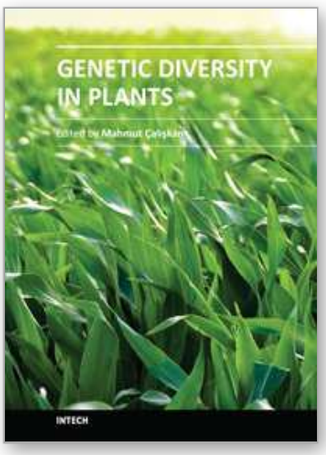

Genetic Diversity in Plants

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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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