Molecular identification of Greek olive (*Olea europaea*) cultivars based on microsatellite loci

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ABSTRACT. *Olea europaea* is one of the oldest species of domesticated trees. We used microsatellite markers for fingerprinting and for evaluation of genetic similarity and structure of 26 Greek olive cultivars, which cover most of the olive cultivation regions of Greece, including previously undescribed denominations from northern Greece. Eighty-one alleles were revealed with six SSR loci that were selected as most informative of 10 SSR primers that were initially investigated. The number of alleles per locus varied from 7 to 20 (mean, 13.5). Heterozygosity ranged from 0.240 at locus DCA-3 to 0.826 at locus UDO99-9, with a mean value of 0.600. Analysis of 104 trees representing 26 denominations (four trees per denomination) revealed 26 distinct SSR profiles, indicating 26 olive cultivars; no intracultivar variability was observed. Genetic and geographic distances were not significantly correlated, based on the Mantel test. These SSR loci

allowed unequivocal identification of all the cultivars and will be useful for future breeding and olive germplasm management efforts.

Key words: Olea europaea; Molecular markers; SSR; DNA fingerprinting

INTRODUCTION

An important part of everyday life in ancient Mediterranean civilizations, the olive (*Olea europaea*) remains one of the most abundantly cultivated tree species throughout the Mediterranean basin. Olive trees are cultivated in a variety of climates, from the coastline of the Mediterranean basin to interior regions with a cooler climate (Bracci et al., 2009).

Olive production throughout the world uses more than 1275 cultivars; most cultivars have been identified in southern European countries, including 538 in Italy, 183 in Spain, 88 in France, and 52 in Greece (Sarri et al., 2006; Ipec et al., 2009). Greece holds third place in world olive production, with more than 117 million olive trees; 80% of Greek orchard land is devoted to olive culture.

Olive trees are predominantly allogamous (Díaz et al., 2006a), which leads to high levels of heterozygosity and DNA polymorphisms among cultivars (Rallo et al., 2000). The wealth of genetic variability, coupled with considerable confusion in olive cultivar nomenclature, makes evaluation and characterization of olive genetic resources necessary. DNA fingerprinting is recognized as very important, since both olive productivity and oil quality are traits inherent to varieties. It is presumed that crosses between wild local olive trees and introduced selected cultivars have led to new cultivars (Besnard et al., 2001b). *Olea europaea* is a highly variable species, with hundreds of denominations, cultivated mainly in the Mediterranean basin (Banilas et al., 2003).

Until recent years, cultivar identification has been based on morphological and agronomic traits. However, the recognition of olive cultivars based on phenotypic characters is often problematic, especially at the early stages of tree development (Banilas et al., 2003). This has led to great confusion and uncertainty about the current status of olive germplasm in many countries, including Greece. The ability to discriminate olive cultivars and estimate genetic variability is important for successful breeding programs and improved management of genetic resources (Owen et al., 2005). The use of molecular markers to manage olive germplasm is particularly advantageous, due to the fact that the olive has an exceptionally long juvenile period (Montemurro et al., 2005). Many of the main Greek olive cultivars are identified using RAPD (Nikoloudakis et al., 2003; Hagidimitriou et al., 2005), AFLP (Hagidimitriou et al., 2005; Owen et al., 2005) and ISSR markers (Terzopoulos et al., 2005).

Microsatellite markers (simple sequence repeats, SSRs) are a powerful tool for olive cultivar identification because they are transferable, co-dominant, highly polymorphic, widely distributed along the genome, and easily reproducible (Rallo et al., 2000). SSRs have proven to be efficient for cultivar characterization, and several loci have been developed for *O. europaea* (Rallo et al., 2000; Sefc et al., 2000; Carriero et al., 2002; Cipriani et al., 2002; De la Rosa et al., 2002; Díaz et al., 2006b; Baldoni et al., 2009). The use of SSRs to characterize olive cultivars could become an efficient tool for germplasm conservation and management strategies.

The main areas of olive cultivation in Greece are the south and central provinces

of the country, the coastline regions and the islands. Currently, olive cultivation is expanding rapidly in the northern part of Greece. A number of olive varieties have been used in inland areas of northern Greece during the past 600 years, but they remain largely unexploited. The recent rapid expansion of olive cultivation in this area makes these varieties particularly important. Cultivated areas in Northern Greece use both traditional cultivars (Koroneiki, Megaritiki, Kalamon) and local denominations (Maronias, Chondrolia Chalkidikis, Galatistas, Petrolia Serron, Lefkolia Serron, Arvanitolia Serron, Pierias). Study of genetic resources and discrimination of Greek olive denominations are particularly important for efforts to preserve biodiversity and maintain the advantages of local varieties. Furthermore, genotype information can assist breeding programs and help clarify cases of homonymy and synonymy in Greek olive cultivars.

We utilized SSRs to fingerprint and assess the genetic diversity of olive cultivars that represent almost all *O. europaea* cultivation range in Greece. Previously undescribed local denominations of Northern Greece were included in addition to traditional Greek olive cultivars. The hypothesis that northern Greek denominations represent new cultivars and form a distinctive genetic group within Greek olive cultivars was tested.

MATERIAL AND METHODS

Plant material

Samples were collected before the flowering period in May 2007 from the olive cultivar collection of the Alexander Technological Educational Institute of Thessaloniki. One hundred and four individuals were sampled, representing 26 Greek olive denominations (four individuals per denomination) that included seven denominations from north Greece and 19 traditional Greek olive cultivars (Table 1, Figure 1; Kostelenos, 2006). All trees displayed the typical morphological characteristics (i.e., leaf size and shape, fruit shape) of the cultivars they represented.

DNA isolation

DNA was extracted from fresh leaves according to Sefc et al. (2000) with minor modifications. After phenol:chloroform:isoamyl alcohol extraction, the supernatant was subjected to two ether extractions to remove residual phenol.

PCR amplification

Six SSR loci (Table 2) were selected of 10 that were originally investigated, from three groups of SSR primers: DCA (Sefc et al., 2000), UDO99 (Cipriani et al., 2002) and EMO (De La Rosa et al., 2002). Selection was made based on degree of polymorphism, as well as clear, stable and repeatable amplified DNA fragments. Polymerase chain reactions (PCRs) were performed in a final volume of 20 μL, containing 1X PCR buffer (Invitrogen), 0.2 mM of each dNTP (Invitrogen), 1.5 mM $MgCl_2$, 0.2 mM of each primer (Invitrogen), 20 ng genomic DNA and 1 U Taq Polymerase enzyme (Invitrogen). Amplification was performed in a Mastercycler® thermal cycler (Eppendorf). The temperature profile was the following: initial denaturation for 5 min at 95°C; 35 cycles of three repetitive steps consisting of a denaturation step for 45 s at 94°C, a

Table 1. Greek olive cultivars studied, with code number, main biomorphological characteristics, use of fruits, and geographical origin (Kostelenos, 2006).

Code	Cultivar name	Fruit weight[a]	Productivity[b]	Oil content[c]	Cold resistance[d]	Use[e]	Origin
1	Adramitini	2	3	A	2	T/O	Lesvos
2	Agouromanakolia	2	3	H	3	O	Arkadia
3	Amfissis	4	3.5	A	2	T	Amfissa
4	Amigdalolia	5	3	A	1	T	Attiki
5	Arvanitolia Serron	4	2.5	L	3	T	Serres
6	Chondrolia Chalkidikis	4	4	L	3	T	Chalkidiki
7	Dafnelia	3	2	L	2	T/O	Samos
8	Gaidourelia	5	3	L	1	T	Arkadia
9	Galatistas	3	3.5	A	4	T/O	Chalkidiki
10	Kalamon	3	2	L	1	T	Messinia
11	Kolimbada	4	2	L	1	T	Messinia
12	Koroneiki	1	5	H	2	O	Messinia
13	Kothreiki	3	3	A	2.5	T/O	Argolida
14	Koutsourelia	1	3	H	2	O	Ahaia
15	Lefkolia Serron	3	3.5	A	3.5	O	Serres
16	Lianolia Kerkiras	1	3	A	2.5	O	Kerkira
17	Maronias	3	3	H	3	T/O	Komotini
18	Mastoidis	2	3	A	3	O	Kriti
19	Mavrelia Messinias	1	3.5	A	2.5	O	Messinia
20	Megaritiki	3	4	A	2.5	T/O	Attiki
21	Petrolia Serron	3	3	H	2.5	T/O	Serres
22	Pierias	3	3	H	3	O	Pieria
23	Throumbolia	2	3	H	1	T/O	Rodos
24	Tragolia	1	3	H	2	O	Messinia
25	Valanolia	3	3	H	2.5	T/O	Lesvos
26	Vasilikada	4	2	L	3	T	Evia

[a]1 = 0-2.5 g; 2 = 2.6-4.0 g; 3 = 4.1-6.0 g; 4 = 6.1-7.5 g; 5 = >7.6 g. [b]2 = low; 3 = moderate; 4 = high; 5 = very high. [c]L = low; A = average; H = high. [d]1 = sensitive; 2 = moderately sensitive; 3 = resistant; 4 = highly resistant. [e]T = table olive; O = olive oil; T/O = dual use.
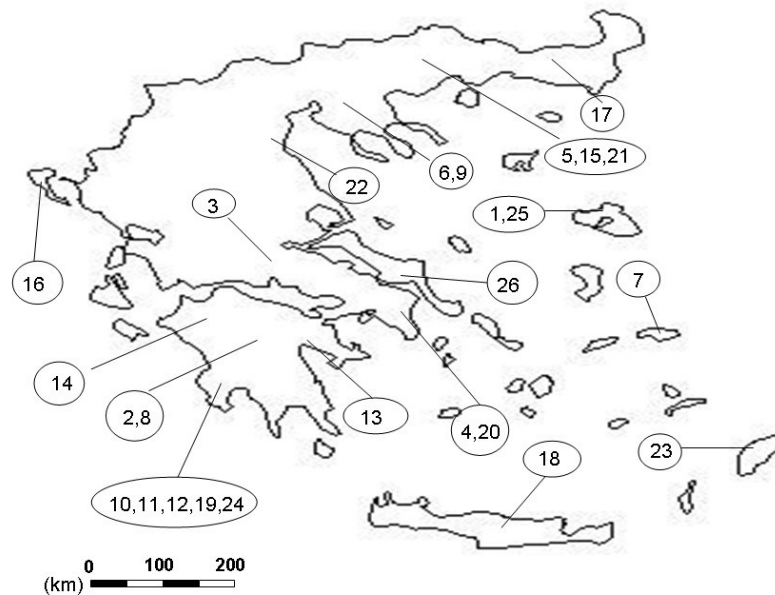
Figure 1. Map of Greece indicating the main area of cultivation of the olive cultivars in this study. The numbers refer to the codes of cultivars shown in Table 1.

primer annealing step for 45 s at 50°C for DCA-3, -16, -17, and -18, for 45 s at 55°C for DCA-9 and -10, for 45 s at 57°C for UDO99 (-9, -11 and -35), for 45 s at 60°C for EMO-3, followed by a primer extension step at 72°C for 45 s, and a final extension step at 72°C for 8 min.

Table 2. List of the SSR used to analyze the olive cultivars, repeat motif, suggested annealing temperature (Ta), annealing temperature used in this study (in parentheses) and primer sequences.

Locus	Repeat motif	Ta (°C)	Forward primer sequence	Reverse primer sequence
DCA-3	(GA)19	50 (55)	cccaagcggaggtgtatattgttac	tgcttttgtcgtgtttgagatgttg
DCA-9	(GA)23	55 (55)	aatcaaagtcttccttctcatttcg	gatccttccaaaagtataacctctc
DCA-18	(CA)4CT(CA)3(GA)19	50 (55)	aagaaagaaaaaggcagaattaagc	gttttcgtctctctacataagtgac
UDO99-9	(AG)16	57 (57)	ttgatttcacattgctgacca	catagggaagagctgcaagg
UDO99-35	(CA)15	57 (57)	aatttaatggtcacacacac	attgcgaaatagatctacga
EMO-3	(CA)7	60 (60)	ggtgtagcccaagcccttat	tgcatgaccgtggtgtaagt

Electrophoresis

PCR products were checked by agarose gel electrophoresis and then separated on a gel containing 4.5% high-resolution agarose, UltraPure™ Agarose-1000 (Invitrogen). In order to avoid band diffusion and to increase resolution, we used a refrigerated, re-circulating bath (Fisher Scientific, No. 3006) and a horizontal gel electrophoresis unit (Fisher Scientific, No. CHU25), with a built-in cooling block that ensures uniform cooling of the gel. Before applying the electrical current, the bath temperature was adjusted to -4°C and running buffer temperature to 13°C. Samples were run at 200 V for 4 h. A 10-bp ladder (Invitrogen) was used as a size standard. Gels were visualized and photographed under UV light with the Molecular Imager® Gel Doc™ XR system (Biorad). Images were analyzed with the Quantity 1.0 software (Biorad).

Data analysis

Polymorphic information content values and heterozygosity (H_o) were computed using CERVUS 3.0 (Kalinowski et al., 2007). Probability of identity was calculated by the IDENTITY 1.0 program (Wagner and Sefc, 1999). Amplified alleles were scored for presence (1) or absence (0). Genetic distances were calculated using the Jaccard coefficient of similarity. A dendrogram was constructed via the unweighted pair-group method with arithmetic averages (UPGMA), using the NTSYS-pc program version 2.11e (Rohlf, 2002). A principal coordinate analysis was carried out based on a pairwise, individual-by-individual (N x N) genetic distance matrix calculated for codominant data, using the GenAlEx 6 software (Peakall and Smouse, 2006). In addition, the SSR data were analyzed by factorial correspondence analysis using the GENETIX 4.0 software. Mantel tests were also performed using the NTSYS program in order to investigate the relationship between the genetic and geographic distances of the cultivars.

RESULTS

Six SSR loci of the 10 loci originally scored were selected for detailed analysis based on clear amplification products, non-ambiguous scoring data, and efficient detection of polymorphism in the olive germplasm (Khadari et al., 2008). The loci that were excluded were UDO99-11, DCA-16, DCA-17, and DCA-10. The selected SSR loci revealed 81 alleles, ranging from seven at

the DCA-3 locus (Figure 2) to 20 at the DCA-9 locus (Figure 3), with a mean of 13.5 alleles per locus. Size ranges were as expected (Table 3). Observed heterozygosity ranged from 0.240 at locus DCA-3 to 0.826 at locus UDO99-9, with a mean of 0.600 (Table 3). Polymorphic information content values (Table 3) varied from 0.756 (DCA-3) to 0.922 (DCA-9). The probability of sampling identical genotypes differed among loci from 0.019 (UDO99-9) to 0.116 (DCA-18; Table 3). The highest frequencies were observed for alleles 243 (36%), 221 (31%) and 177 (23%) at loci DCA-3, EMO-3 and DCA-18, respectively (Table 4). Allele frequencies based on the estimated null allele frequency were also calculated (Table 4). Analysis of 104 individuals representing 26 cultivars and denominations identified 26 distinct SSR profiles. No intracultivar variability was observed.
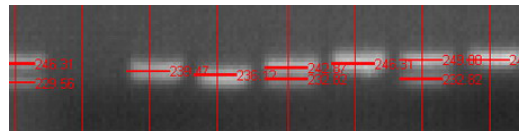

Figure 2. Primer DCA-3 alleles from eight cultivars after scoring with Quantity 1.0.

**Figure 3.** Primer DCA-9 alleles from eight cultivars after scoring with Quantity 1.0.

Table 3. Size range (SR), expected size range (ESR), number of alleles (n), observed heterozygosity (H_o), polymorphic information content (PIC), and probability of identity (PI) of microsatellite data for 26 Greek olive cultivars.

Locus	SR	ESR	n	H_o	PIC	PI
DCA-3	(226-253)	228-250	7	0.240	0.756	0.114
DCA-9	(156-238)	161-205	20	0.773	0.922	0.020
DCA-18	(142-183)	168-184	15	0.615	0.889	0.116
UDO99-9	(83-128)	83-119	19	0.826	0.919	0.019
UDO99-35	(131-170)	136-168	11	0.750	0.880	0.045
EMO-3	(218-249)	205-215	9	0.375	0.791	0.082
Average			13.5	0.597	0.860	1.65×10^{-5}

Table 4. Allele size (in bp), allele frequencies (in parentheses) and allele frequencies based on the estimated null allele frequency (in brackets).

DCA-3	DCA-9	DCA-18	UDO99-9	UDO99-35	EMO-3
230(0.02)[0.014]	156(0.14)[0.095]	142(0.04)[0.038]	83(0.04)[0.044]	131(0.05)[0.025]	218(0.08)[0.037]
232(0.06)[0.029]	159(0.02)[0.023]	148(0.08)[0.077]	85(0.04)[0.044]	133(0.05)[0.025]	221(0.31)[0.200]
237(0.10)[0.058]	162(0.02)[0.023]	151(0.04)[0.038]	87(0.02)[0.022]	140(0.10)[0.105]	224(0.21)[0.115]
239(0.18)[0.089]	166(0.05)[0.046]	153(0.08)[0.077]	89(0.07)[0.044]	142(0.18)[0.192]	227(0.10)[0.056]
243(0.36)[0.154]	170(0.05)[0.046]	156(0.04)[0.038]	90(0.02)[0.022]	144(0.10)[0.105]	230(0.06)[0.037]
245(0.12)[0.044]	172(0.05)[0.046]	159(0.08)[0.077]	91(0.02)[0.022]	147(0.13)[0.133]	233(0.15)[0.115]
249(0.16)[0.074]	174(0.02)[0.023]	163(0.04)[0.038]	93(0.02)[0.022]	149(0.10)[0.105]	236(0.02)[0.018]
-	181(0.02)[0.023]	165(0.04)[0.038]	97(0.13)[0.140]	152(0.05)[0.051]	240(0.04)[0.037]
-	182(0.07)[0.070]	167(0.04)[0.038]	99(0.02)[0.022]	165(0.10)[0.078]	249(0.02)[0.018]
-	187(0.05)[0.046]	169(0.04)[0.038]	102(0.02)[0.022]	168(0.13)[0.078]	-
-	189(0.07)[0.070]	171(0.04)[0.038]	103(0.04)[0.044]	170(0.03)[0.025]	-
-	192(0.05)[0.046]	173(0.08)[0.038]	105(0.07)[0.044]	-	-
-	194(0.09)[0.070]	175(0.04)[0.038]	107(0.07)[0.067]	-	-
-	200(0.05)[0.046]	177(0.23)[0.118]	109(0.11)[0.091]	-	-
-	202(0.14)[0.095]	183(0.12)[0.077]	111(0.13)[0.140]	-	-
-	208(0.02)[0.023]	-	113(0.04)[0.044]	-	-
-	210(0.05)[0.046]	-	115(0.04)[0.022]	-	-
-	217(0.02)[0.023]	-	126(0.07)[0.067]	-	-
-	220(0.02)[0.023]	-	128(0.02)[0.022]	-	-
-	238(0.02)[0.023]	-	-	-	-

Clustering according to the UPGMA method revealed five major groups (Figure 4). The first group contained five cultivars (Amfissis, Vasilikada, Galatistas, Lefkolia Serron, Agouromanakolia) two of which (Amfissis, Vasilikada) have large fruits and are used as table olives. Lefkolia Serron and Agouromanakolia are used for oil production, while Galatistas is typically a dual-use cultivar. The second group was the largest, including 12 cultivars (Chondrolia Chalkidikis, Kolimbada, Amigdalolia, Megaritiki, Maronias, Adramitini, Throumbolia, Mastoidis, Kothreiki, Valanolia, Gaidourelia, Arvanitolia Serron). Five were table cultivars (Chondrolia Chalkidikis, Kolimbada, Amigdalolia, Gaidourelia, Arvanitolia Serron) six were dual-use cultivars (Megaritiki, Maronias, Adramitini, Throumbolia, Kothreiki, Valanolia) and one was an oil-producing cultivar (Mastoidis). The third group consisted of the cultivars Koutsourelia and Mavrelia Messinias, which have small fruit that are used for oil production. The fourth group contained cultivars Tragolia, Dafnelia, Lianolia Kerkiras, and Koroneiki. These cultivars are used for oil production, except Dafnelia, which is a dual-use cultivar. The fifth group (Pierias, Petrolia Serron, Kalamon) contained three cultivars, all of which have medium-sized fruits. Pierias is cultivated for oil production, while Petrolia Serron is a dual-use cultivar and Kalamon a table cultivar (Table 1).
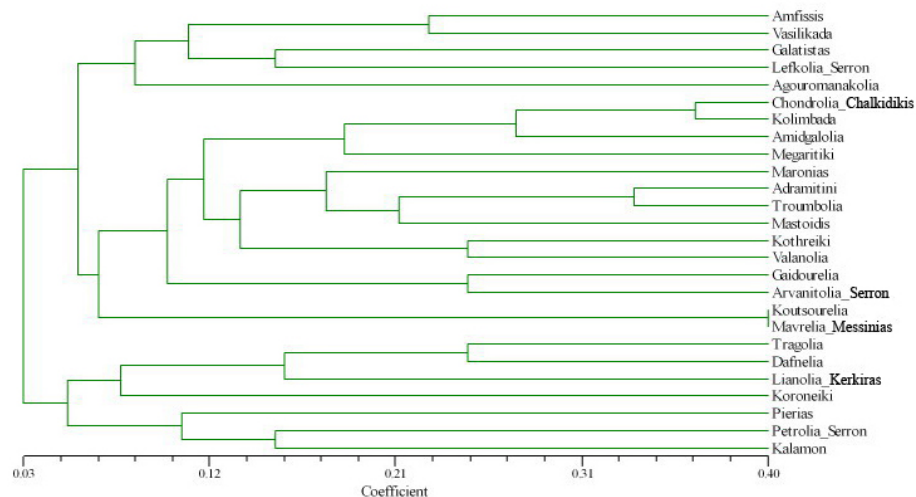
Figure 4. UPGMA dendrogram obtained from microsatellite data for Greek olive cultivars.

The principal coordinate analysis results revealed that a considerable amount of variation (61%) was explained by the first three axes. Axes 1, 2 and 3 explained 24, 20 and 17% of the variation, respectively. Five loosely defined groups were also identified and grouping results based on principal coordinate analysis (Figure 5) were similar to dendrogram clustering using UPGMA. A single difference in grouping between principal coordinate analysis and UPGMA was observed regarding cultivars Koutsourelia and Mavrelia Messinias. The above cultivars grouped together in the third group of UPGMA, but did not cluster in principal coordinate analysis.

Figure 5. Principal coordinate analysis derived from 81 microsatellite alleles.

The first three axes of the factorial correspondence analysis explained 23.08% of the total variability. The multivariate analysis gave more abstract grouping than UPGMA and principal coordinate analysis depicted two main groups (Figure 6), a large one (20 cultivars) and a small one (four cultivars). Three cultivars (Throumbolia-code 23, Tragolia-code 24, Vasilikada-code 26) were placed separately.

Figure 6. Factorial correspondence analysis based on SSR data. Last digits correspond to the cultivar code in Table 1. The first or first two digits correspond to a random cultivar number.

Genetic and geographic distance differences among cultivars were not concordant, based on the Mantel test (r = 0.03, P = 0.29). Additional Mantel tests that considered the southern and northern Greek cultivars separately also indicated a lack of significant correlation (r ≤ 0.01, P ≥ 0.50).

DISCUSSION

A set of 26 Greek olive denominations were genotyped at six SSR loci in order to examine genetic variation at the molecular level. In general, our results were in agreement with those of Baldoni et al. (2009), regarding locus stability and usage. Allele numbers and sizes were also within the range in previous studies (Baldoni et al., 2009). Relatively few SSR loci were used; nevertheless, most were highly polymorphic and therefore allowed unequivocal identification of all the plant material. Previous studies (Besnard et al., 2008; Gomes et al., 2009) also discriminated cultivar collections using six SSR loci. The selected loci, due to the easily interpretable alleles on high-resolution agarose gels, can be used by laboratories that do not have access to automated sequencers or silver-staining polyacrylamide gels. Markers DCA-9, DCA-18 and UDO99-9 in particular were highly polymorphic and sufficient to discriminate all cultivars. These markers would be suitable for DNA fingerprinting of Greek olive cultivars. On the other hand, four SSR loci were excluded from our study because of unfavorable characteristics, such as absence of stability and repeatability, non-interpretable fingerprinting patterns, low degree of polymorphism, and few alleles. A non-interpretable fingerprinting pattern associated with multiple locus amplification was observed at locus DCA-10. This pattern could be due to duplication of a particular genomic region (Rallo et al., 2000). Markers UDO99-11, DCA-16 and DCA-17 were deemed unsuitable because they were not highly polymorphic, presenting few alleles in these Greek genotypes (two alleles for UDO99-11 and three for DCA-16 and DCA-17).

Microsatellite loci DCA-9, UDO99-9, DCA-18, UDO99-35, EMO-3, and DCA-3 revealed 20, 19, 15, 11, 9, and 7 alleles, respectively (Table 3). This large number of alleles per locus may have been influenced by the sampling strategy, as most cultivars and denominations were selected from diverse areas of cultivation. The mean number of alleles per locus observed in our study (A = 13.50) is similar to the value obtained from a Mediterranean variety collection (A = 14.50; Breton et al., 2008), using 12 DCA primers. Mean heterozygosity was high, indicating a wealth of genetic variation in the cultivar collection. Similar heterozygosity values for the same SSR primers in *O. europaea* have been reported (Khadari et al., 2008). Polymorphic information content classified all SSR loci as both informative and suitable for mapping (polymorphic information content >0.70; Poljuha et al., 2008). The total exclusion probability (P > 0.99) and the total probability of identity (P > 1.65 x 10^{-5}) indicate that the chance of finding two individuals with the same genotype in the population is almost zero.

Allele frequencies were generally low, particularly at loci with a high allelic number, as expected. Differences between observed allele frequencies and allele frequencies based on the estimated null allele frequency were not statistically significant (Table 4). Null alleles arise when mutations occur in the annealing site, preventing one or both of the primers from binding (Pemberton et al., 1995). In our study, null alleles did not alter the overall outcome of assignment testing. Therefore, the six SSR loci could be deemed as appropriate for this type of study based on this property as well. Unequivocal identification of all entries and absence of any intra-denomination variability support the notion that all northern Greek denominations represent distinct olive cultivars.

No particular clustering was observed among cultivars from northern Greece, based both on principal coordinate analysis and the UPGMA dendrogram. For example, two cultivars (Galatistas, Lefkolia Serron) of the seven from northern Greece were included in the first

group. Chondrolia Chalkidikis, Arvanitolia Serron and Maronias were placed in the second group, while Pierias grouped with Petrolia Serron in the fifth group (Figure 4). The graphical representation of the first two principal coordinates, which explained 44% of the total variability, also showed that northern Greek cultivars are scattered in the different groups (Figure 5). Factorial correspondence analysis defined two major groups in three-dimensional space, where 23.08% of the total variability was explained. This value was not particularly high, but is within the range of similar studies (Breton et al., 2008; Zitoun et al., 2008). In the northern Greek cultivars, factorial correspondence analysis results agreed with the outcome of principal coordinate analysis and UPGMA, as these were not associated with a particular group. Therefore, the hypothesis that northern Greek cultivars form a distinctive group is not supported by either univariate or multivariate analyses. Mantel tests did not show correlation between geographic and genetic distances. The present distribution of olive cultivars in Greece clearly appears to be the result of strong anthropogenic influences. This finding is in agreement with previous studies on Greek olive cultivars (Nikoloudakis et al., 2003; Hagidimitriou et al., 2005).

Several molecular studies have discriminated various Greek olive cultivars (Fabbri et al., 1995; Besnard et al., 2000, 2001a,b; Belaj et al., 2001, 2002; Nikoloudakis et al., 2003; Rallo et al., 2003; Owen et al., 2005; Terzopoulos et al., 2005; Hagidimitriou et al., 2005; Montemurro et al., 2005; Díaz et al. 2006b; Essadki et al., 2006; Omrani-Sabbaghi et al., 2007; Bracci et al., 2009). Greek genotypes seem to carry the same chloroplast DNA polymorphisms (Besnard et al., 2000) and the same mitotypes (ME1), except Amigdalolia, which carries the ME2 mitotype (Besnard et al., 2001b). However, mitotypes and chlorotypes are less efficient in discriminating cultivars compared to other markers (Besnard et al., 2001a). Four previous studies (Belaj et al., 2001, 2002; Besnard et al., 2001a,b) have analyzed Greek cultivars along with other Mediterranean cultivars using RAPD markers; none of them found a particular clustering among the Greek cultivars. Most of the genetic diversity was found to be attributable to differences among genotypes within a country. Belaj et al. (2002), Terzopoulos et al. (2005) and Essadki et al. (2006) observed various groupings of Greek cultivars based on ISSR markers. Omrani-Sabbaghi et al. (2007) did not find any particular clustering between seven Greek genotypes using SSR markers. Fabbri et al. (1995), using RAPD markers, Rallo et al. (2003) using SSR markers, Montemurro et al. (2005) using SSR and AFLP markers, and Bracci et al. (2009) using SSR markers, included only two Greek genotypes in their studies from which no relevant information could be deduced. Discrimination of Greek olive cultivars according to SSR marker analysis does not produce clusters similar to those resulting from ISSR (Terzopoulos et al., 2005) and RAPD (Nikoloudakis et al., 2003; Hagidimitriou et al., 2005) analyses. On the contrary, our dendrogram presents similarities with the dendrograms reported in previous AFLP marker-based studies (Hagidimitriou et al., 2005; Owen et al., 2005). For example, Megaritiki, Kolimbada, Adramitini, Kothreiki, and Amigdalolia are grouped together in our study, as in the Hagidimitriou et al. (2005) AFLP study. Moreover, Koroneiki, Tragolia, Dafnelia grouped together in our study, as in the AFLP study conducted by Owen et al. (2005).

Greek olive cultivars have been traditionally classified according to their fruit size and consequently their usage, since size and fruit use are in most cases interdependent. Cultivars that produce large fruits are used mainly as table olives, while small-fruited cultivars are used mainly for olive oil production. Previous molecular studies reported genetic distance clustering of olive cultivars according to their fruit size and usage (Loukas and Krimbas, 1983; Fabbri et al., 1995; Besnard et al., 2001b; Nikoloudakis et al., 2003; Hagidimitriou et al., 2005). It

is possible that this diversity resulted from selection pressure for desirable traits (fruit size, oil content). However, in our study, as in Belaj et al. (2001), only a weak concordance of cultivar classification to fruit size and usage was observed.

In conclusion, after testing our hypotheses, we have shown that the northern Greek cultivar denominations that we investigated represent distinctive olive cultivars. However, these northern Greek cultivars do not seem to form a distinct genetic group, when compared to a collection of typical Greek olive cultivars. This is the first study on DNA fingerprinting of Greek olive cultivars using microsatellite markers. Although the major cultivated genotypes that cover most of the *Olea* cultivation range in Greece have been fingerprinted, further study is needed in order to elucidate the variation and genetic structure of the Greek olive tree genome in order to conserve olive germplasm cultivated in restricted areas. This research should be useful for breeding programs, for nurseries dealing with olive cultivar propagation and dissemination and subsequently for commercialization of olives and related products.

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