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Identification and Classification of Main Iranian Olive Cultivars Using Microsatellite Markers

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Abstract. **Numerous olive cultivars are cultivated in Iran, mainly in the north. Ninety-two accessions belonging to 10 main olive cultivars were screened by 13 microsatellite markers revealing high genetic variability both within and between cultivars. In total, 72 alleles were detected with a mean number of 5.5 alleles per locus. Twenty-four unique allelic patterns were observed, whereas six genotypes showed 15 unique alleles. Heterozygosity ranged from 0.00 to 0.98, whereas the mean number of discrimination power and polymorphic information content were 0.55 and 0.54, respectively. The combination of 5 simple sequence repeat markers made discrimination of 84% of all accessions included in the study possible. The existence of homonyms, synonyms, or mislabeling as well as intracultivar polymorphism was revealed by allele differences between accessions of the same denomination. The phenogram showed variability among as well as between some cultivars, but most accessions with the same generic names were grouped together.**

Olive (*Olea europaea* L.) is as an important oil-producing crop in the Mediterranean region whose domestication occurred during the Chololithic period (5700–5500 years BC) in the Near-East (Zohary and Hopf, 1994). It has been suggested that the olive was introduced into the Mediterranean Basin from the Middle East or Eastern Africa (Chevalier, 1948; Green and Wickens, 1989; Turrill, 1951).

Archaeological findings revealed that olive cultivation in Iran dates back 2000 years ago (Sadeghi, 1992). At present, the old commercial olive orchards are located mainly in the north of Iran and more than 85% of olive production belongs to these regions. In the last 10 years, olive plantation has grown in several provinces and currently, olive cultivation covers more than 100,000 ha in Iran. The total olive production was ≈60,000 tons in 2005 (Zeinanloo, 2006). Although a large number of olive accessions are growing in Iran, there have been few limited reports on morphological, cytogenetic, and molecular characteristics of these accessions (Hosseini-Mazinani et al., 2004; Omrani-Sabbaghi et al., 2007; Samaee et al., 2003; Sheidai et al., 2007).

Initial exploration surveys throughout the country found 10 main traditional olive cultivars (Sadeghi, 1992). More recently, investigations based on International Olive Council morphological descriptors have found a considerable variation within Iranian olive cultivars (Hosseini-Mazinani et al., 2004). The use of morphological markers has been useful for olive germplasm characterization and identification in many olive-growing countries (Cantini et al., 1999; Rallo et al., 2005). However, discrimination of varieties based on morphology evaluation is limited by the effect of environmental conditions, the need for extensive observations of mature plants, and the requirement of very well-trained staff (Belaj et al., 2001). Therefore, more comprehensive studies using reliable markers are needed to gain a better understanding of the level and distribution of genetic diversity in olive cultivars, which may be of use in cultivar identification.

In recent years, molecular markers such as isozymes (Trujillo et al., 1995) randomly amplified polymorphic DNA (RAPD) (Belaj et al., 2001; Besnard et al., 2001; Mekuria et al., 1999; Wiesman et al., 1998), and amplified fragment length polymorphism (Angiolillo et al., 1999) have been increasingly used to characterize and distinguish the olive cultivars.

As a result of their multiallelic hypervariability, codominant nature, high information content, and amenability to automation, microsatellites are becoming one of the most popular and reliable marker systems for genetic characterization of varieties, genetic diversity, plant breeding programs, genetic map development, and linkage analysis (Morgante and Olivieri, 1993; Powell et al., 1996; Rafalski et al., 1996). Microsatellites have also been proven to be very suitable markers for fingerprinting and revealing the genetic diversity in olive cultivars (Bandelj et al., 2002; Belaj et al., 2004; Cipriani et al., 2002; De la Rosa et al., 2002; Diaz et al., 2006; Khadari et al., 2003; Rallo et al., 2000; Sefc et al., 2000).

The present study aims at characterizing, by the use of microsatellite markers, main Iranian olive cultivars in three north provinces of Gilan, Zanjan, and Ghazvin, which are considered the most important regions of olive cultivation in Iran. We also try to verify homonymy (one denomination for several genotypes), synonymy (one genotype with several denominations), intracultivar polymorphism, and mislabeling. To our knowledge, this is the preliminary report on the identification and classification of the main Iranian olive cultivars in detail by means of microsatellite markers.

Materials and Methods

Plant materials and DNA extraction. A panel of 92 accessions belonging to the 10 major Iranian olive cultivars was used in the molecular study (Table 1). Trees were sampled randomly from eight different locations selected in 3 provinces of Gilan, Zanjan, and Ghazvin so as to be representative of the majority of Iranian olive varieties (Fig. 1). Total genomic DNA was extracted from fresh leaves using the CTAB method of Murry and Tompson (1980) with modification described by De la Rosa et al. (2002).

Microsatellite assay. To reduce the number of replication of each genotype, screening was performed on 92 olive accessions by using five high polymorphic simple sequence repeat (SSR) markers (ssrOeUA-DCA3,ssrOeUA-DCA9, ssrOeUA-DCA16, ssrOeUA-DCA18, and UDO99-043), which have been shown to be efficient for cultivar

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Table 1. Cultivar accession included in the study, English translation, the number of studied trees per accession or source of material, geographical diffusion and use of fruit, and ordering number (Nr).

Nr	Cultivar accession	English translation	No. of trees	Source of materials	Geographical diffusion	Use of fruits
1	Dakal	Olive of Dakal	4	Research Garden	Gilan	—
2	Dezful	Olive of Dezful	3	Research Garden	Gilan	O[z]&T[z]
3	Fishomi	Olive of Fishom	6	Research Garden	Gilan	O&T
4	Geloleh	Round olive	4	Harzebil	Gilan	O
5	Geloleh	Round olive	7	Ettka Garden	Gilan	O
6	Geloleh	Round olive	1	BahramAbad	Ghazvin	O
7	Geloleh	Round olive	3	Manjil	Gilan	O
8	Geloleh	Round olive	3	Motahari Garden	Zanjan	O
9	Khara	No translation	2	Ettka Garden	Gilan	O
10	Khorma zeitoon	Date-shaped olive	7	Harzebil	Gilan	O&T
11	Khorma zeitoon	Date-shaped olive	1	Motahari Garden	Zanjan	O&T
12	Khorma zeitoon	Date-shaped olive	1	Anjilak	Gilan	O&T
13	Mari	Sneaky olive	2	Motahari Garden	Zanjan	O&T
14	Mari	Sneaky olive	2	Research Garden	Gilan	O&T
15	Mari	Sneaky olive	2	Ettka Garden	Gilan	O&T
16	Rowghani	Oily olive	3	Research Garden	Gilan	O&T
17	Rowghani	Oily olive	2	BahramAbad	Ghazvin	O&T
18	Rowghani	Oily olive	4	Vakhman	Ghazvin	O&T
19	Rowghani	Oily olive	3	Motahari Garden	Zanjan	O&T
20	Rowghani	Oily olive	2	Ettka Garden	Gilan	O&T
21	Shengeh	No translation	1	Ettka Garden	Gilan	O&T
22	Shengeh	No translation	16	Research Garden	Gilan	O&T
23	Shengeh	No translation	2	Manjil	Gilan	O&T
24	Zard	Yellow	4	Research Garden	Gilan	O&T
25	Zard	Yellow	4	Ettka Garden	Gilan	O&T
26	Zard	Yellow	3	Motahari Garden	Zanjan	O&T

[z]O = oil; T = table olive; O&T = oil and table olive.

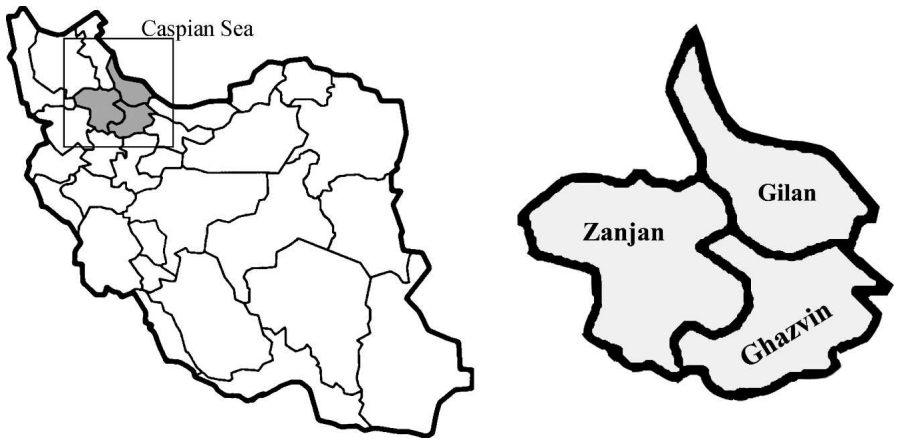

Fig. 1. Iran map showing three provinces of Gilan, Zanjan, and Ghazvin in the north of the country.

identification in the World Germplasm Bank of Córdoba (I. Trujillo, unpublished data), although the remaining accessions were analyzed by 13 microsatellite markers of ssrOeUA-DCA3, ssrOeUA-DCA9, ssrOeUA-DCA11, ssrOeUA-DCA15, ssrOeUA-DCA16, ssrOeUA-DCA18 (Sefc et al., 2000), UDO99-011, UDO99-019, UDO99-043, UDO99-024 (Cipriani et al., 2002), GAPU59, GAPU71B, and GAPU101 (Carriero et al., 2002). Amplification of microsatellites was performed in polymerase chain reactions (PCRs) in a total volume 20 μL containing 2 ng genomic DNA, 1× supplied PCR buffer (Biotools, Madrid, Spain), 200 μM of each dNTP (Roche, Switzerland), 0.25 unit of Taq DNA polymerase (Biotools), and 0.2 μM of forward (fluorescently labeled) and reverse primers. The PCR reaction was carried out on a thermal cycler (Perkin-Elmer-9600, GMI, Ramsey, MN) programmed with a denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 20 s, annealing temperature 50 °C for 30 s and 72 °C for 30 s, and final extension at 72 °C for 7 min. Finally, the analysis was carried out on an automatic capillary sequencer, ABI 3130 Genetic Analyzer (Applied Biosystems/HITACHI, Tokyo) using fluorescent dyes, and fragment sizes were determined using internal standards.

Data analysis. The detection of amplification products was carried out with an automated sequencer (ABI PRISM 3130 DNA sequencer; Applied Biosystems, Foster City, CA). Sizing was performed using the programs GENESCAN 3.7 and GENOTYPER 3.7 from Applied Biosystems. Three reference samples were used in all runs. Observed heterozygosity (H_0) was obtained as the ratio of the heterozygous individuals to the total number of genotypes per locus expected heterozygosity (H_E) (Nei, 1987). Polymorphic information content (PIC) (Botstein et al., 1980), null alleles frequency (r) (Brookfield, 1996), H_0, and H_E were calculated by Cervus version 2.0 (Marshall et al., 1998) software.

The discriminating power (*Dj*), confusion probability (*Cj*) of each SSR locus (Tessier et al., 1999) as well as the probability of identity (PI) (Paetkan et al., 1995) were computed. Genetic distances between all pairwise combinations of the accessions were calculated using Dice coefficients. Grouping of the genotypes was determined by using the unweighted paired group using average (UPGMA) method as well as ordination based on principal coordinate analysis (PCO) (Chatfield and Collins, 1995; Ingrouille, 1986). Cophonetic correlation was also determined for different clustering methods. NTSYS-pc version 2.02 (Rohlf, 1998) software was used for these statistical analyses.

Results and Discussion

Simple sequence repeat diversity in Iranian olive cultivars. The initial screening with five primers (ssrOeUA-DCA3, ssrOeUA-DCA9, ssrOeUA-DCA16, ssrOeUA-DCA18, and UDO99-043) revealed the presence of the same allelic profiles among different trees (replications) of the same cultivars. So, among 92 accessions, 40 trees could be considered duplications of the plant materials and they were excluded from further analysis. The identification of duplications within and between olive orchards is very important for better management of olive genetic resources in the collections. Table 2 indicates the genetic diversity found by means of 13 SSR on 52 olive accessions belonging to 10 Iranian cultivars. All 13 microsatellite markers except UDO99-019 were polymorph, revealing the presence of 72 alleles in all cultivars analyzed. The number of alleles in each locus varied from one (UDO99-19) to 10 (DCA9) with an average number of 5.6 alleles per locus (Table 2). The allele 174 base pair at DCA18 locus showed the highest frequency (0.62) (data not shown), whereas the lowest frequency (0.01) was observed as unique alleles at eight loci, which is given in Table 3.

High variability in average number of alleles per locus in olive cultivars has been also reported by other workers (Belaj et al., 2004; Carriero et al., 2002; De la Rosa et al., 2002; Khadari et al., 2003) in studies from other geographical locations. They have reported average values of 7.5, 6.4, 5.7, 7.4, and 5.2 alleles per locus related to different numbers of loci in their studies. Variation reported in the number of alleles in olive cultivars by different workers may be related to variation in the loci studied as well as the number of genotypes and their localities (Lopes et al., 2004).

Under the "Hardy Weinberg" equilibrium, the observed heterozygosity varied from 0.000 in monomorphic locus of UDO99-019 to 0.976 in DCA11 locus with an average value of 0.660. The observed heterozygosity at loci DCA3, DCA9, DCA11, UDO99-43,

Table 2. Allele size, number of alleles, unique alleles, and heterozygosity indices for studied cultivars in 13 simple sequence repeat (SSR) primers.

Locus	Size range	No. of alleles	No. of unique alleles	No. of unique allele patterns	No. of allele patterns	H_O	H_E	PIC	Probability of null alleles	*Dj*	*Cj*	*PI*
ssrOeUA-DCA3	229/253	7	2	3	13	0.929	0.819	0.781	–0.0724	0.825	0.175	0.064
ssrOeUA-DCA9	159/207	10	3	3	14	0.810	0.792	0.753	–0.0119	0.795	0.205	0.078
ssrOeUA-DCA16	122/178	6	1	3	13	0.634	0.667	0.607	0.0142	0.674	0.325	0.166
ssrOeUA-DCA18	162/180	6	2	3	8	0.524	0.548	0.492	+0.0110	0.544	0.456	0.266
UDO99-043	170/216	9	4	6	11	0.762	0.697	0.651	–0.0639	0.700	0.300	0.136
ssrOeUA-DCA11	140/178	6	1	1	9	0.976	0.759	0.710	–0.1395	0.767	0.233	0.102
ssrOeUA-DCA15	243/263	3	0	0	3	0.073	0.319	0.293	+0.6180	0.322	0.678	0.491
UDO99-011	112/130	6	1	2	7	0.860	0.701	0.640	–0.01223	0.590	0.410	0.310
UDO99-019	129	1	0	0	1	0.000	0.000	0.000	0.000	0.000	1.000	—
UDO99-024	166/189	5	0	1	8	0.488	0.667	0.597	+0.1552	0.672	0.328	0.178
GAPU59	206/216	3	0	0	3	0.326	0.350	0.310	+0.0935	0.353	0.647	0.463
GAPU71B	118/135	4	0	1	6	0.721	0.651	0.574	–0.0444	0.656	0.344	0.196
GAPU101	189/217	6	1	1	11	0.814	0.767	0.721	–0.0348	0.771	0.229	0.096
Mean[z]	—	72	15	24	107	0.66	0.594	0.547	—	0.543	0.457	0.195

[z]Numbers of alleles, unique alleles, unique allele patterns, and allele patterns are total.
H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content; *Dj* = discriminating power; *Cj* = confusion probability; *PI* = probability of identity.

UDO99-11, GAPU71B, and GAPU101 was higher than expected. The heterozygosity deficiency found in DCA15, DCA16, DCA18, GAPU59, and UDO99-24 loci, which was nonsignificant based on χ^2 test ($P < 0.01$), might be attributable to the presence of null alleles at these loci (Table 2; Ishibashi et al., 1996). The presence of null alleles is a consequence of sequence polymorphisms in the flanking regions of the locus resulting from point mutations or insertion/deletions (Jones and Ardren, 2003).

Discrimination and identification of Iranian olive cultivars. The study of allelic polymorphism obtained in the present work allows discrimination of 84% of the olive accessions analyzed by producing unique genotype profiles (Table 3). Meanwhile the microsatellite locus UDO99-43 revealed the highest number of unique alleles (4), whereas some loci did not show any unique alleles (Table 2). Six genotypes possessed unique alleles with 'Dezful' possessing the highest number (8) in DCA3, DCA9, DCA11, GAPU101, UDO99-11, and UDO99-43 loci (Table 3).

Calculated PIC values were in a range from 0.000 to 0.781 in 13 loci. It has been suggested that PIC values greater than 0.5 (for example, ssrOeUA-DCA16, UDO99-011, and GAPU71B) are informative markers, whereas loci with PIC values greater than 0.7 (ssrOeUA-DCA3, ssrOeUA-DCA9, ssrOeUA-DCA11, and GAPU101) are suitable for genetic mapping (Bandelj et al., 2004). Therefore, in the present study, nine loci may be considered informative, whereas the other three loci may be used in genetic mapping. The PIC values obtained here are close to the values observed by Bandelj et al. (2004) and Omrani-Sabbaghi et al. (2007).

The power of discrimination of microsatellites varied between 0.000 (UDO99-19) to 0.825 (DCA3) with an average of 0.543. On the other hand, the lowest confusion probability belonged to DCA3 (0.175) and highest value was obtained for UDO99-19 (1.00) (Table 2). The high discriminatory power of the microsatellite loci examined in this study is comparable with the other studies performed on different olive cultivars (Belaj et al., 2004; Khadari et al., 2003).

The low probability of identical genotypes (PI) observed ranging from 0.064 (DCA3) to 0.491 (DCA15) is considered to be useful in genotype discrimination (Table 2). The total cumulative PI value for this set of markers was 1.2×10^{-9}, which shows the high discrimination power of the selected primers.

The microsatellite locus UDO99-043 revealed the highest number of polymorphic alleles allowing the identification of six different genotypes (Table 2), whereas the combination of five microsatellites of DCA3, DCA9, DCA16, DCA18, and UDO99-043 identified 84% of the accessions studied (Table 3).

Therefore, the olive cultivars studied can be well discriminated by using the previously mentioned indices as a result of the presence of a high amount of genetic variability among these cultivars.

Synonymous and homonymous cultivars. Analysis of 13 microsatellites used revealed the presence of both synonyms and homonyms in the genotypes studied. Some cultivars such as 'Khara' and two accessions of 'Rowghani' (1050 and 209) produced identical allelic profiles. Moreover, the accession of ''Rowghani-122′ showed the same allelic profiles of two accessions of 'Zard-1107' and 'Zard-1109' (Table 3). Therefore, they may be considered as synonyms or mislabeled accessions as a result of morphological difficulties. The previous studies on Iranian olive cultivars have also revealed such misnaming (Hosseini-Mazinani et al., 2004; Samaee et al., 2003).

On the other hand, several accessions with identical names showed a low level of genetic similarity and were placed in different clusters far from each other in the phenogram obtained in which differences in their microsatellite loci is the reason for the separation of these accessions. Like other Mediterranean countries, homonymy is one of the problems in Iranian olive germplasm. Naming of cultivars have been based on common morphological traits, particularly of the fruit ('Geloleh' = round fruit; 'Mari' = sneaky fruit; 'Khorma zeitoon' = date-shaped fruit), toponymys ('Fishomi', 'Dezful', and 'Dakal'), or practical use of cultivars like 'Rowghani' = oily cultivar. Our results demonstrate that generic names of Iranian olive cultivars include different genotypes. For example, few accessions of 'Rowghani' such as 'Rowghani-1048' and 'Rowghani-367' differed in 10 loci (Table 3). Four accessions of 'Geloleh' (389, 1127, 316, and 497) differed from 10 to 11 loci, whereas the accessions of 'Shengeh-1090' and 'Shengeh-263' differed in 12 loci (Table 3). Therefore, these cultivars may be considered homonyms or mislabeling. Discrimination of homonymous cases in olive germplasm has also been reported by using SSRs and other molecular markers by other workers (Belaj et al., 2001, 2002; Khadari et al., 2003; Mekuria et al., 1999; Rallo et al., 2005; Wiesman et al., 1998). On the other hand, studies on characterization of olive cultivars in Eastern Mediterranean countries also have shown homonymy cases (Belaj et al., 2003a, 2003b; Lopes et al., 2004).

Two accessions of 'Geloleh', i.e., 'Geloleh-1117' and 'Geloleh-1122', differed in six loci, whereas accessions of the cultivars 'Dakal', 'Fishomi', 'Zard', and 'Khorma zeitoon' differed in one to two loci. These levels of differences are considered intracultivar polymorphism or somatic mutation occurring in the process of vegetative propagation. The existence of intracultivar variation in Iranian olive has been also reported by Omrani-Sabbaghi et al. (2007). Future comparison using detailed morphological and agronomical data will shed light if these individuals are different cultivars. According to our results, a new way of naming of Iranian cultivars is needed in which both molecular and morphological information should be included.

Genetic relationships. Different similarity coefficients determined among the cultivars studied showed the highest value of similarity (r = 1.0) among eight pairs of accessions (for example, among 'Khara', 'Rowghani-1050', and 'Rowghani-209' also

Table 3. Allelic composition of studied olive accessions for thirteen microsatellite markers.

Cultivar accession	Genotype profiles												
	DCA3	DCA9	DCA16	DCA18	UDO43	DCA11	DCA15	UDO11	UDO19	UDO24	GAPU59	GAPU71B	GAPU101
Dakal-284	243/251	184/206	155/178	162/162	174/210	144/178	243/243	112/127	129/129	185/189	206/210	121/124	193/199
Dakal-285	243/251	184/206	122/156	162/162	174/210	144/178	243/243	112/127	129/129	185/189	206/210	121/124	193/199
Dakal-281	243/251	184/202	122/156	162/162	174/210	144/178	243/243	112/127	129/129	185/189	206/210	121/124	193/199
Dezful-292	**247[y]/247**	**160[y]/199**	**122/124[y]**	162/166	**185[y]/208[y]**	**140[y]/144**	243/243	119/127	129/129	**183/185**	206/210	**118/121**	**189[y]/217**
Fishomi-1052	232/253	170/202	122/122	**174/179[y]**	170/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	191/217
Fishomi-253	232/253	170/202	122/122	174/174	170/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	191/217
Fishomi-255	232/253	170/199	122/122	174/174	170/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	191/217
Fishomi-293	232/253	170/202	122/122	174/174	170/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	191/217
Geloleh-1136	229/232	186/199	122/122	174/174	174/174	142/146	—[z]	116/127	129/129	181/183	206/216	121/135	191/191
Geloleh-1147	229/232	186/199	122/122	174/174	**176/176**	142/146	243/243	116/127	129/129	181/183	206/216	121/135	191/191
Geloleh-1158	229/232	**190[y]/199**	122/122	174/174	**178[y]/178**	142/146	243/243	119/127	129/129	181/183	206/216	121/135	191/191
Geloleh-1127	229/243	170/170	**155/155**	174/174	170/174	142/178	243/245	116/119	129/129	166/181	216/216	124/124	191/191
Geloleh-389	251/253	170/186	122/176	**162/174**	172/174	168/178	263/263	119/127	129/129	181/181	206/216	124/124	191/199
Geloleh-501	229/232	184/186	122/176	174/174	174/174	142/178	243/245	114/127	129/129	166/183	206/216	121/135	191/217
Geloleh-502	229/232	186/199	122/122	174/174	174/174	142/178	243/243	116/127	129/129	181/183	206/216	121/135	191/191
Geloleh-497	229/243	170/170	176/176	166/174	170/174	142/168	243/243	116/119	129/129	181/181	216/216	124/135	193/197
Geloleh-1117	229/232	184/186	122/122	174/174	174/174	142/178	243/245	114/127	129/129	166/183	206/216	121/135	191/217
Geloleh-1122	229/232	186/199	122/122	174/174	174/174	142/146	243/243	119/127	129/129	181/183	206/216	121/135	191/191
Geloleh-316	232/251	**186/196[y]**	—	174/174	**174/216[y]**	142/178	243/243	**114/130[y]**	129/129	166/181	206/216	121/124	191/217
Khara-351	229/253	186/202	122/176	166/174	172/174	146/168	243/243	119/127	129/129	181/183	206/216	121/124	191/193
Khorma Zeitoon-1134	243/253	170/202	155/178	174/174	170/172	142/168	—	116/127	129/129	181/181	216/216	124/124	191/197
Khorma Zeitoon-1135	243/253	170/202	155/176	**174/181[y]**	170/172	142/168	243/243	116/127	129/129	181/181	216/216	124/124	191/197
Khorma Zeitoon-1145	243/253	170/202	122/156	174/174	170/172	142/168	243/243	116/127	129/129	181/181	216/216	124/124	191/197
Khorma Zeitoon-299	229/253	184/202	122/176	166/174	174/174	142/168	243/243	114/127	129/129	166/181	216/216	121/124	191/217
Khorma Zeitoon-402	**232/243**	170/170	122/176	166/174	170/174	**142/142**	243/243	116/119	129/129	181/183	216/216	124/135	191/193
Mari-340	232/251	186/186	122/176	166/174	172/174	142/168	243/243	119/127	129/129	166/166	216/216	124/135	193/193
Mari-368	232/251	186/186	176	166/174	172/174	142/168	243/243	119/127	129/129	166/166	216/216	124/135	193/193
Rowghani-1048	229/253	186/202	122/176	166/174	**172/176**	146/168	243/243	119/127	129/129	181/183	206/216	121/124	191/193
Rowghani-1050	229/253	186/202	122/176	166/174	172/174	146/168	243/243	119/127	129/129	181/183	206/216	121/124	191/193
Rowghani-367	**243/245[y]**	170/202	155/178	174/174	170/172	142/168	243/243	116/127	129/129	181/181	216/216	124/124	191/197
Rowghani-376	243/253	170/202	155/176	174/174	170/172	142/168	243/243	116/127	129/129	181/181	216/216	124/124	191/197
Rowghani-209	229/253	186/202	122/176	166/174	172/174	146/168	243/243	118/127	129/129	181/183	206/216	121/124	191/193
Rowghani-112	229/251	184/186	122/176	162/166	172/174	146/178	245/245	114/127	129/129	181/181	216/216	124/124	191/193
Rowghani-119	229/251	184/186	155/176	162/166	170/174	146/178	245/245	114/127	129/129	181/181	216/216	124/124	191/193
Rowghani-122	229/232	170/186	176/176	166/174	174/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	193/217
Rowghani-332	251/253	184/186	122/176	166/174	172/174	142/146	243/243	114/127	129/129	166/181	216/216	**121/121**	191/193
Shengeh-1094	229/253	170/202	122/176	166/174	172/174	146/168	243/243	119/127	129/129	181/183	216/216	124/135	193/193
Shengeh-1104	243/251	170/184	122/176	166/174	170/174	168/178	263/263	116/127	129/129	181/181	216/216	124/124	197/217
Shengeh-363	243/251	170/184	122/176	168/174	170/174	168/178	263/263	116/127	129/129	181/181	216/216	124/124	197/217
Shengeh-1082	232/253	170/170	122/155	174/174	172/174	142/146	243/243	116/127	129/129	181/183	216/216	121/124	197/217
Shengeh-1090	229/251	184/206	122/122	174/174	**174/176**	142/146	243/243	119/127	129/129	183/183	206/216	121/135	191/191
Shengeh-263	243/251	170/184	122/176	168/174	170/174	168/178	263/263	116/127	129/129	181/181	216/216	124/124	197/217
Shengeh-1115	243/253	170/202	155/176	174/174	170/172	142/168	—	116/127	129/129	181/181	216/216	124/124	191/197
Shengeh-1089	251/251	170/170	122/122	166/174	172/174	146/178	243/243	119/127	129/129	183/183	216/216	121/124	197/217
Shengeh-1098	251/251	170/170	122/176	166/174	172/174	146/178	243/243	119/127	129/129	181/181	216/216	121/124	197/217
Shengeh-1083	229/232	170/202	122/122	174/174	174/174	142/146	263/263	116/127	129/129	181/183	216/216	124/135	191/199
Shengeh-1086	229/232	170/202	122/176	174/174	174/174	142/146	—	119/127	129/129	181/183	216/216	124/135	191/199
Shengeh-1102	232/253	170/170	122/155	174/174	172/174	142/146	243/243	119/127	129/129	181/183	216/216	121/124	197/217
Zard-1156	229/232	170/186	**178/178**	166/174	174/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	193/217
Zard-322	229/232	170/186	122/176	166/174	172/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	193/217
Zard-1107	229/232	170/186	176/176	166/174	174/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	193/217
Zard-1109	229/232	170/186	176/176	166/174	174/174	142/146	243/243	119/119	129/129	183/183	216/216	121/135	193/217

[z]Missing data.
[y]Unique alleles.
Unique allelic patterns are shown in bold. More than one tree with the same genotype profile is shown in italics.

between 'Zard-1107' and 'Rowghani-122'). The lowest value of similarity occurred between 'Dezful' and 'Rowghani-376' and also between 'Dezful' and 'Khorma zeitoon-1135' (r = 0.136).

Data obtained from 13 SSR markers were used for elucidating the genetic relationships among olive cultivars by constructing the UPGMA clustering method (Fig. 2). The cophonetic coefficients determined for different clustering methods revealed the highest value for UPGMA (r = 0.86) indicating good fit of the original data to the clustering. Therefore, results of UPGMA are discussed subsequently.

In general, seven major groups/clusters were identified in cluster analysis. The first major cluster is comprised of 'Dezful' and 'Dakal' accessions in which 'Dakal' accessions showed higher similarity and were placed close to each other. This result was also observed by Omrani-Sabbaghi et al. (2007). The same is true for two cultivars, Zard and Fishomi, because they were shown to be similar to each other in our study. These two cultivars formed the second major cluster. This cluster also included a pair of synonymous/mislabeled accessions (Zard-1107, 1109, and Rowghani-122) (Fig. 2).

Accessions of 'Geloleh' along with specimens from 'Shengeh' formed the third major cluster.

The accessions of 'Geloleh', 'Rowghani', and 'Shengeh' are distributed in different

clusters possibly as a result of their genetic variability or misnaming (Table 3). Intracultivar variations have also been reported in 'Shengeh' by using morphological characters (Hosseini-Mazinani et al., 2004). Accessions of 'Mari' showed similarity to each other and have been placed in cluster 4, whereas accessions of 'Khorma zeitoon', 'Rowghani', and 'Shengeh' have been also included. Accession of 'Khara' along with two accessions of 'Rowghani' (1050 and 209) have been also grouped in this cluster. Grouping of such a mixture of accessions may be the result of the presence of synonymous/mislabeled accessions. One accession of 'Geloleh-316' formed cluster 5, whereas cluster 6 consisted of two accessions of 'Rowghani-112' and 'Rowghani-119'. Accessions of 'Khorma zeitoon' mainly have been placed in cluster 7 along with two accessions of 'Geloleh' and 'Rowghani' and four accessions of 'Shengeh' also included in this cluster.

In the present work, all of the clusters included accessions (except cluster 1) are from three northern provinces of Iran (Gilan, Zanjan, and Ghazvin). Cluster 1 included 'Dakal' and 'Dezful' cultivars, which are mainly cultivated in the south of Iran and have been introduced in the north of the country. Therefore, it may be suggested that separation of these two cultivars from the others may be the result of their adaptation to the environmental and cultivation practices available in the south of the country. A similar result has also been revealed by RAPD analysis of the same genotypes reported earlier (Samaee et al., 2003).

The stability of the groups was also confirmed by partitioning the variants of data sets using PCO. The first and second principal components accounted for 21.5% and 14.2% of total variation, respectively. Generally, the PCO plot supported the clustering results obtained (Fig. 3).

In conclusion, the results of present study are the following: 1) 84% of olive accessions analyzed were discriminated; 2) genetic variation among and between cultivars was revealed by using 13 SSR markers; 3) synonymy and homonymy cases were identified among the cultivars studied; and 4) the genetic relationship among these olive cultivars was studied. In general, the present study showed the use of SSR markers in identification and discrimination of the olive cultivars under study.

Attempts have been started to improve and develop olive germplasm collection and olive orchards in Iran. Programs such as identification of germplasm, conservation, improvement, development, and finally management of olive orchards will be greatly augmented through accurate identification of the local cultivars. Presently, a large-scale program is underway to examine the degree of diversity of Iranian olive varieties at intra- and intercultivar levels using phenotypic characteristics and molecular markers. In the framework of this research program, the studied cultivars have been propagated for planting in three major Iranian olive collections, Zanjan (north), Gorgan (northeast), and Fars (south), for further assessments.

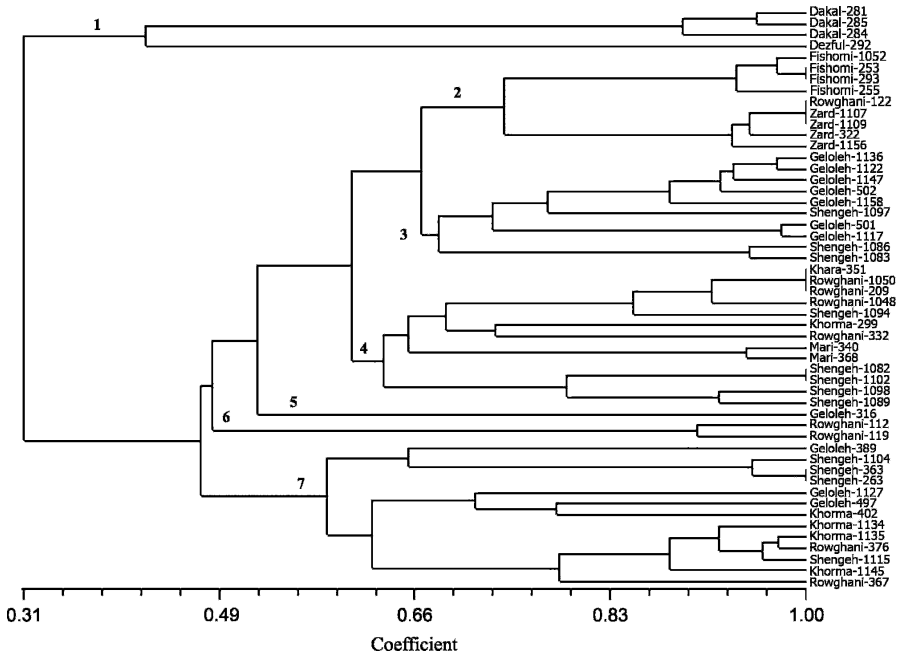

Fig. 2. Unweighted paired group using average dendrogram of Iranian olive cultivars based on Dice's coefficient.

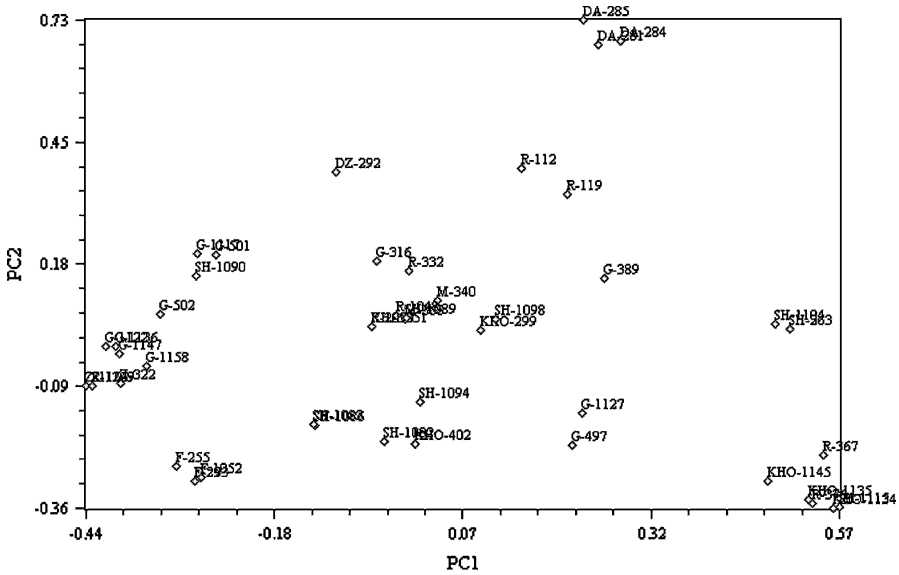

Fig. 3. Principal coordinate analysis of the studied olive cultivars based on simple sequence repeat markers. DA = Dakal; DZ = Dezful; F = Fishomi; Z = Zard; G = Geloleh; SH = Shengeh; KHA = Khara; R = Rowghani; M = Mari; KHO = Khorma zeitoon.

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