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Assessment of morphological and molecular variation in local olive (*Olea europaea* L.) in the Northern part of Iran

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ABSTRACT

Iran is known as one of the origins of olive in the world with many different olive cultivars, mainly in the north. Eighty eight accessions belong to 4 main olive cultivars were investigated by 21 morphological characters and 11 ISSR markers. Analyses of morphological characters revealed the existence of high genetic variability among cultivars. Based on both morphological and ISSR cluster analyses, 88 accessions were grouped in five distinct clusters. The ISSR primers produced 77 polymorphic bands. AMOVA showed significant difference in both between and within olive cultivars. The highest and lowest coefficient of Nei's genetic distance was observed in 'Mari' and 'Shengeh' (0.105) and 'Zard' and 'Rowghani' (0.061), respectively. In both morphological and ISSR data analyses, 'Mari' showed the highest homogeneity. The olive cultivars were not clustered based on their geographical origin.

Key words: genetic diversity, ISSR, PIC, polymorphism

IZVLEČEK

OVREDNOTENJE MORFOLOŠKE IN MOLEKULARNE VARIABILNOSTI LOKALNE OLJKE (*Olea europaea* L.) V SEVERNEM DELU IRANA

Iran je prepoznan kot eden izmed svetovnih izvorov oljke z mnogimi sortami, predvsem v njegovem severnem delu. Raziskano je bilo 88 akcesij oljke, ki so pripadale 4 glavnim sortam na osnovi polimorfizma 21 morfoloških znakov in 11 ISSR molekulskih markerjev. Analiza morfoloških znakov je odkrila veliko genetsko variabilnost med sortami. Na osnovi morfoloških znakov in ISSR molekulskih markerjev se je 88 akcesij združilo v pet skupin. Z ISSR markerji so pomnožili 77 polimorfnih fragmentov. AMOVA je pokazala značilne razlike znotraj sort in med sortami. Največji vrednosti Neiovega koeficienta kot kazalnika genetske oddaljenosti sta bili ugotovljeni med sortami Mari in Shengeh (0.105) in Zard in Rowghani (0.061). Sorta Mari je pokazala največjo homogenost na osnovi analize morfoloIoških znakov in ISSR molekulskih markerjev. Sorte oljke se niso združevale na osnovi geografskega izvora.

Ključne besede: genetska raznolikost, ISSR, PIC, polimorfizem

1 INTRODUCTION

The olive (*Olea europaea* L.) is an economically important fruit crop of the Mediterranean basin (Rao et al., 2009). Archaelogical 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

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the north of Iran and more than 85 % of olive production belongs to these regions (Noormohammadi et al., 2007). A large number of olive accessions are growing in Iran; therefore, many workers have reported on morphological or molecular characteristics of these accessions (Samaee et al., 2003; Hosseini-Mazinani et al., 2004, Omrani-Sabbaghi et al., 2007; Sheidai et al., 2007; Noormohammadi et al., 2012; Dastkar et al., 2013). The genetic diversity could be an important resource for the development of modern olive culture towards typical olive oil and fresh products (Hegazi et al., 2012).

The ability to discriminate olive cultivars to estimate genetic variability is an important factor for a better management of genetic resources and successful breeding programs (Milotić et al., 2005). Morphological descriptors of International Olive Council (IOC, 1993) are usually applied for characterization and identification of olive cultivars. Although the morphological characters are strongly affected by environmental conditions, the age of trees, the training systems, and the phenological stage of plants, the morphological approach continues to be the initial main step for description and classification of olive germplasm (Rotondi and Magli, 1999). 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. In the last years, molecular markers, such as RAPD (Belaj et al., 2001; Besnard et al., 2001; Mekuria et al., 1999; Wiesman et al., 1998), AFLP (Angiolillo et al., 1999; Sanz-Cortes et al., 2003; Sensi et al., 2003; Owen et al., 2005) and SSR (Bandelj et al., 2002; Belaj et al., 2004; Cipriani et al., 2002; Diaz et al., 2006; Khadari et al., 2003; Rallo et al., 2000; Sefc et al., 2001), have been used to characterize olive germplasm. Also, ISSRs methods have been used (Hess et al., 2000; Pasqualone et al., 2001; Gemas et al., 2004; Terzopoulos et al., 2005). Little information is available on the genetic background of Iranian domestic olive genotypes (Dastkar et al., 2013). The cross-pollination between domesticated and wild varieties caused the significant proportion of genetic diversity. Sadeghi (1992) reported that some cultivars appeared by selection of superior trees and by selection of mutation in over the years. The goal of this study is characterizing main Iranian olive cultivar in two provinces of Gilan and Zanjan by the use of ISSR markers and morphological characteristics.

2 MATERIALS AND METHODS

2.1 Plant material

Eighty-eight trees belonging to the 4 endemic cultivars including ‘Mari’, ‘Zard’, ‘Shengeh’ and ‘Rowghani’ were used in the morphological and molecular study. Trees were sampled randomly from five different locations of two provinces of Gilan and Zanjan including Gilvan, Tarom, Aliabad, Manjil and Jamalabad. Observations were made on samples of 40 healthy adult leaves and fruits for each tree. Morphological characters were measured manually and recorded for all 21 characters including leaf characters (3 characters) and fruit characters (8 characters). Then, ten characters were recorded on free stone fruits. The morphological characters were coded as binary or multistate characters (Table 1).

2.2 DNA extraction

Total genomic DNA was extracted from fresh leaves using CTAB method with some modifications (Doyle and Doyle, 1990). Leaf tissue (0.5 g) is ground in liquid nitrogen and incubated at 65 °C for 30 min in 1ml extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl, 2 % CTAB, 1 % PVP). An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and 2 µl β-mercaptoethanol were added to the supernatant and the whole mixture was centrifuged at 12000 rpm for 10 min. The precipitation of the upper phase was obtained by adding 1 volume of 2-isopropanol at -20 °C for 20 min and then followed by centrifugation at 13000 rpm for 15 min. The DNA pellet was washed with 1 ml 75 % ethanol. The DNA pellet was resuspended in 50 µl TE (10 mM Tris-HCl, pH 8.0; 0.5 M EDTA, pH 8.0).

ISSR analysis was performed using 11 primers (Table 2). PCR reactions were performed in a 25 µl volume containing 1× PCR reaction buffer

(10 mM Tris- HCl; 50 mM KCl) 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 0.3 µM of a single primer; 20 ng genomic DNA and 1.0 U *Taq* DNA polymerase. The amplifications were performed in Applied Biosystems thermocycler under the following conditions: 94 °C, 5 min; 94 °C, 30 s; specific annealing temperature (Table 2), 45 s; 72 °C, 2 min; repeat to step 2, 45 times; 72 °C, 5 min.

2.3 Statistical analysis

Canonical discriminant, cluster analysis among cultivars by within group linkage analysis and correlation analysis were conducted using SPSS-V. 20. Molecular analysis of variance (AMOVA) was performed using GeneAlex 6.4 (Peakall and Smouse, 2006) to divide the total variation to between and within olive cultivars variation. Genetic distances between all pairwise combinations of the accessions were calculated using Nei's coefficients. Genetic diversity parameters including Ne (number of effective alleles), H (Nei's gene diversity) and I (Shannon's information index) were calculated by GeneAlex 6.4. PIC was estimated using the Excel software. ISSR polymorphism was scored for the presence (1) or absence (0) of amplified bands and was used to estimate the dissimilarity coefficients between cultivars using simple matching's coefficient method. The dissimilarity matrix was used to construct a dendrogram using the complete linkage method. These analyses were carried out using NTSYS pc ver. 2.01 (Rohlf., 1998). The agreement between dendrograms derived from morphological characters and ISSR markers, were compared using the Mantel (1967) matrix correspondence test.

Table 1: List of morphological characteristics and their codes and meaning (IOC)

	Variable	Intensity
1.	Leaf width (LW)	Narrow (1) Medium (2) Broad (3)
2.	Leaf length (LL)	Short (1) Medium (2) Long (3)
3.	Leaf shape (length/width) (LS)	Elliptic (1) Elliptic-Lanceolate (2) Lanceolate (3)
4.	Fruit shape (position A)	Spherical (1) Ovoid (2) Elongated (3)
5.	Fruit mass	Low (1) Medium (2) High (3) Very high (4)
6.	Fruit symmetry (position A)	Symmetry(1) Slightly asymmetry (2) Asymmetry (3)
7.	Position of maximum transvers diameter (position B)	Towards base (1) Central (2) Towards apex (3)
8.	Fruit apex (position A)	Acute (1) Obtuse (2) Rounded (3)
9.	Nipple	Absent (1) Tenuous (2) Obvious (3)
10.	Presence of lenticels	Few (1) Many (2)
11.	Size of lenticels	Small (1) Large (2)
12.	Stone shape (position A)	Spherical (1) Ovoid (2) Elliptic (3) Elongated (4)
13.	Stone mass	Low (1) Medium (2) High (3) Very high (4)
14.	Stone symmetry (position A)	Symmetry (1) Slightly asymmetry (2) Asymmetry (3)
15.	Stone symmetry (position B)	Symmetry (1) Slightly asymmetry (2)
16.	Position of maximum transvers diameter (position B)	Towards base (1) Central (2) Towards apex (3)
17.	Stone apex (position A)	Acute (1) Obtuse (2) Rounded (3)
18.	Stone base (position A)	Truncate (1) Pointed (2) Rounded (3)
19.	Stone surface (position B)	Smooth (1) Rugose(2) Scabrous (3)
20.	Number of grooves	Low (1) Medium (2) High (3)
21.	Distribution of the grooves	Regular (1) Grouped around the surface (2)

Table 2: Primers used for ISSR analysis: total number bands, polymorphic bands and % of polymorphism obtained

	Primers	Sequence 5′-3′	Annealing temperature	Total number of bands	Polymorphic bands	% polymorphism
1	UBC834	$(AC)_8C$	48.5	9	9	100
2	UBC807	$(AG)_8T$	43	7	7	100
3	UBC808	$(AG)_8C$	45	10	10	100
4	UBC809	$(AG)_8G$	44.7	7	6	85.71
5	UBC810	$(GA)_8T$	47.74	10	6	60
6	UBC822	$(TC)_8A$	43.14	10	9	90
7	HB12	$(CAC)_3GC$	34.92	8	6	75
8	UBC815	$(CT)_8G$	51.33	6	3	50
9	UBC816	$(CA)_8T$	55.37	7	7	100
10	UBC823	$(TC)_8C$	52.52	7	5	71.43
11	UBC825	$(AC)_8T$	56.56	10	9	90
Mean				8.27	7	70.84

3 RESULTS AND DISCUSSION

3.1 Morphological characterization

Correlation among morphological characteristics were worked out at phenotypic level and presented in Table 3. The significant correlation ($p<0.01$) were found between some characteristics, such as Fruit Shape and Fruit Apex (-0.592), Fruit Shape and Stone Shape (0.872), Fruit Symmetry (p A) and Fruit Shape (0.602), Fruit Symmetry (p A) and Fruit Apex (-.572), Fruit Symmetry (p A) and Stone Shape (0.587), Fruit Symmetry (p A) and Stone Symmetry (p A) (0.540), Fruit Symmetry (p A) and Stone Base (0.515), Fruit Apex and Stone Shape (-0.598), Fruit Apex and Stone Symmetry (p A) (-0.523), as well as Stone Shape and Stone Apex (-0.504).

The dendrogram obtained by within group linkage analysis grouped the 4 cultivars and 88 individuals into five clusters (Figure 1). The first cluster included the number of the individuals of ‘Rowghani’ and ‘Shengeh’. Individuals of ‘Mari’ with a limited number of ‘Rowghani’ and ‘Shengeh’ were grouped into cluster 2 and 3. All of the individuals of ‘Zard’ with a limited number of ‘Rowghani’ and ‘Shengeh’ (2 and 5 respectively) were placed into cluster 4 and 5. Among cultivars, ‘Zard’ and ‘Mari’ had the highest homogeneity. ‘Shengeh’ and ‘Rowghani’ showed high affinity. It could be due to synonymy in two cultivars. Grouping of such a mixture of accessions may be the result of the presence of synonymous/mislabeled accessions (Noormohammadi et al., 2007). These olive trees grow in the areas with close vicinity; therefore, the similarities observed among them may be due to the gene flow occurring among them.

The accuracy of the groups produced was reassessed using discriminant function analysis. The total success rate of discriminant function was 89.8 %, which indicates that it was successful in discriminating different groups. The canonical discriminant functions are described (Table 4). The first three functions had eigenvalues that are above 2 and jointly accounted for 98 % of total variance. The first two functions accounted for 82.1 % of the total variance within the individuals. Standardized discriminant function coefficient could be used to identify important characters causing variation cultivars (Table 5). In the first function, high coefficient was observed for stone shape. In the second function, stone base and leaf length had high coefficient values. In the third function, presence of lenticels and fruit mass had high coefficient values. These characters that loaded high in the three functions demonstrate their relevance in discriminating between the olive cultivars. This was further reaffirmed by the extraction of standardized canonical discriminant function coefficient.

Table 3: Correlation coefficients between 21 morphological characters

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1.00																				
2	.317**	1.00																			
3	.090	-.283**	1.00																		
4	.092	-.036	.056	1.00																	
5	.060	-.055	.174	-.184	1.00																
6	.305**	.072	.071	.602**	-.112	1.00															
7	.218*	.242*	.000	.298**	-.133	.329**	1.00														
8	-.253*	-.072	.000	-.592**	.082	-.572**	-.208	1.00													
9	.194	-.117	.113	.188	.007	.348**	.106	-.306**	1.00												
10	.280**	.039	.116	.183	.293**	.237*	.120	-.234*	.239*	1.00											
11	.242*	.046	.076	.205	-.093	.240*	.185	-.185	.277**	.051	1.00										
12	.073	-.081	-.013	.872**	-.096	.587**	.186	-.598**	.109	.229*	.079	1.00									
13	-.001	-.083	.000	-.054	.371**	-.214*	-.038	.081	-.330**	.043	-.208	.047	1.00								
14	.315**	.026	.068	.392**	-.124	.540**	.223*	-.523**	.092	.194	.031	.427**	.000	1.00							
15	.005	-.050	.106	.142	.059	.316**	.134	-.190	.121	.091	.088	.094	-.003	.332**	1.00						
16	.282**	.149	-.123	-.170	.137	-.020	.060	-.019	.015	.191	.088	-.115	-.041	.041	.104	1.00					
17	-.073	.165	-.198	-.455**	-.312**	-.325**	-.032	.430**	-.189	-.212*	.079	-.504**	-.070	-.369**	-.061	.041	1.00				
18	.365**	.183	.000	.455**	-.217*	.515**	.243*	-.495**	.179	.047	.479**	.376**	-.127	.437**	.355**	.180	-.271*	1.00			
19	-.065	.069	.000	.108	-.070	.123	.141	-.246*	.009	.212*	-.162	.131	-.002	.158	.072	-.078	-.163	-.007	1.00		
20	-.091	-.015	-.264*	.014	.032	-.049	-.194	.120	-.059	-.098	-.232*	.102	.058	-.038	-.151	-.072	-.052	-.289**	-.066	1.00	
21	.172	.010	.000	-.174	-.115	-.123	-.157	.084	.110	-.201	.243*	-.280**	-.052	-.053	.126	.082	.323**	.185	-.121	-.050	1.00

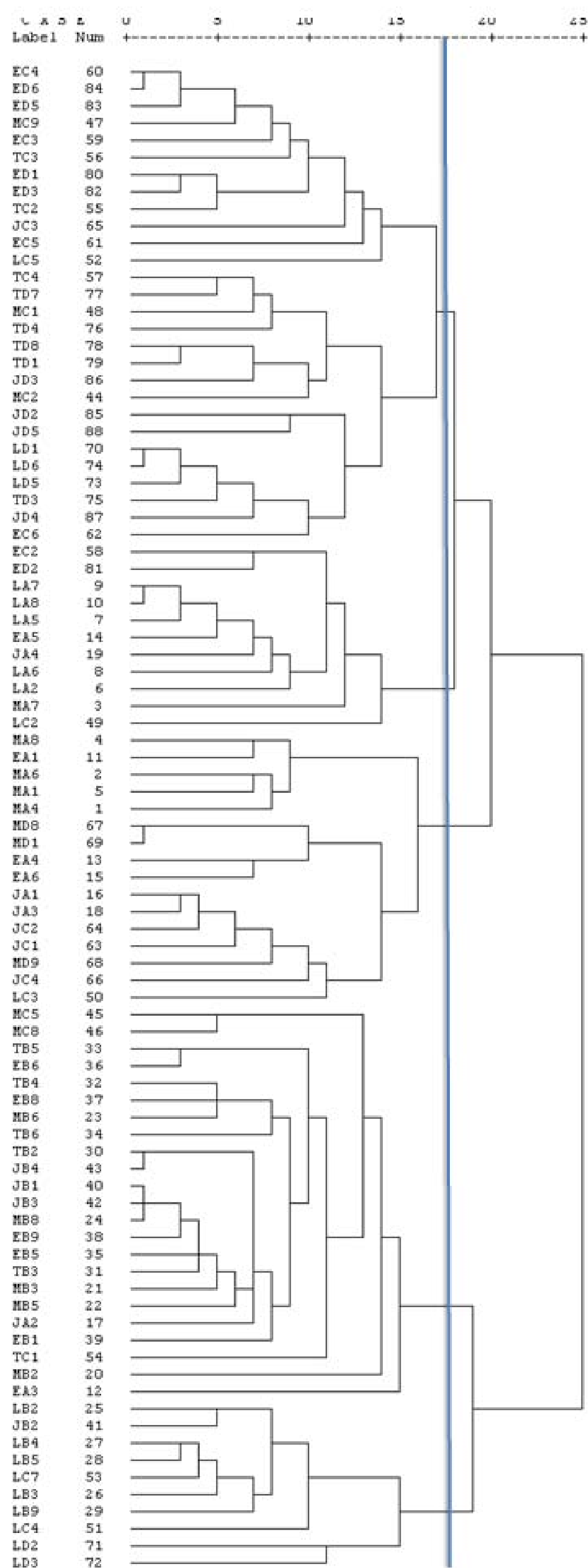

Figure 1: Dendrogram obtained from the 21 morphological characters, within group linkage method with squared Euclidean distance. Accessions are indicated with A, B, C and D for Mari, Zard, Shengeh and Rowghani cultivars respectively. Regions are indicated with L, M, E, T and J for Aliabad, Gilvan, Manjil, Tarom and Jamalabad respectively.

Table 4: Summary of canonical discriminant functions

Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation
1	7.245	55.2	55.2	0.937
2	3.536	26.9	82.1	0.883
3	2.077	15.8	98.0	0.822
4	0.266	2.0	100.0	0.460

Table 5: Standardized canonical discriminant function coefficients of characters in olive cultivars

	Function			
Characters	1	2	3	4
Leaf length	-0.05	<u>0.461</u>	0.363	-0.064
Fruit mass	-0.036	-0.152	<u>0.445</u>	0.351
Presence of lenticels	-0.058	0.006	<u>0.841</u>	0.151
Fruit apex	-0.305	0.321	-0.337	<u>0.827</u>
Stone shape	<u>0.670</u>	-0.655	-0.245	0.337
Stone base	0.614	<u>0.740</u>	-0.082	0.215

3.2 ISSR polymorphism

The 11 ISSR primers produced 77 polymorphic bands by each primer ranged from 4-10. The highest number of polymorphic bands was obtained by UBC 808 (10 bands), while UBC 815 produced the lowest number of polymorphic bands (3 bands). PIC values ranged from 0.31 9 (UBC 810) to 0.46 (HB 12) (Table 2). The mean values of EMR, MI, effective number of alleles (Ne), Nei's genetic diversity (H) and Shannon index (I) for all the primers were 6.14, 2.37, 1.73, 0.4 and 0.6 respectively (Table 6). Among the primers, UBC 822 and UBC 816 showed that the highest number of effective alleles (1.92)

The AMOVA showed that most of the genetic diversity was attributable to differences among individuals within cultivars (91 %) rather than among cultivars (9 %) (Table 7). The calculated PhiPT for all individuals (0.086) was significant ($P< 0.01$).

The highest amount of polymorphism, Shannon's index and heterozigosity was observed in 'Zard' cultivar and the lowest polymorphism and heterozigosity in 'Mari' (Table 8). The number of effective allele varied from 1.62 to 1.67. Percent of polymorphism varied from 92.39 to 97.83. The highest polymorphism may be due to high efficiency of markers. Based on the results, the highest and lowest coefficient of Nei's genetic distance between cultivars were belonged to 'Mari' with 'Shengeh' (0.105) and 'Zard' with 'Rowghani' (0.061), respectively (Table 9).

Table 6: Genetic diversity parameters estimated for the ISSR primers in 88 accessions. PIC = polymorphic information content; EMR = effective multiplex ratio; MI = marker index; Ne = number of effective alleles; H = Nei's index; I = Shannon's information index.

	Primer name	PIC	EMR	MI	Ne	H	I
1	UBC 834	0.42	9	3.78	1.63	0.36	0.55
2	UBC807	0.37	7	2.59	1.63	0.36	0.53
3	UBC 808	0.38	10	3.8	1.77	0.43	0.76
4	UBC 809	0.4	5.14	2.06	1.84	0.45	0.65
5	UBC 810	0.31	3.6	1.11	1.54	0.32	0.49
6	UBC 822	0.37	8.1	2.99	1.92	0.48	0.67
7	HB 12	0.46	4.5	2.07	1.87	0.46	0.65
8	UBC 812	0.33	1.5	0.49	1.72	0.41	0.6
9	UBC 816	0.42	7	2.94	1.92	0.47	0.67
10	UBC 823	0.38	3.57	1.36	1.49	0.31	0.48
11	UBC 825	0.35	8.1	2.83	1.67	0.37	0.55
	Mean	0.38	6.14	2.37	1.73	0.4	0.6

Table 7: Analysis of variance of olive cultivars based on molecular markers. Df = degree of freedom; SS = sum of squares; MS = mean squares; Est. Var = estimated variance; % = percent of diversity; PhiPT = AC/(WC+AC).

Source	df	SS	MS	Est. Var	%
Among Cultivar(AC)	3	128.739	42.913	1.319	9
Within Cultivar(WC)	84	11.72.465	13.958	13.958	91
Total	87	1301.205		15.277	100
PhiPT = 0.086			P Value = 0.001		

Table 8: Genetic diversity parameters among 4 cultivars based on ISSR loci. Na = number of different alleles; Ne = number of effective alleles; I = Shannon's information index; He = expected heterozygosity; SE = standard error

Cultivars	Number	Na±SE	Ne±SE	I±SE	He±SE	% polymorphism
Mari	19	1.88±0.049	1.65±0.036	0.533±0.023	0.365±0.018	92.21
Zard	24	1.96±0.029	1.67±0.033	0.554±0.020	0.379±0.016	97.4
Shengeh	23	1.95±0.025	1.67±0.035	0.547±0.021	0.375±0.016	94.81
Rowghani	22	1.96±0.022	1.66±0.036	0.544±0.021	0.371±0.016	96.1
Mean		1.93±0.017	1.66±0.018	0.544±0.011	0.373±0.008	95.13

Table 9: Nei's genetic distance between pairs of 4 olive cultivars

Cultivar	Mari	Zard	Shengeh	Rowghani
Mari	0			
Zard	0.082	0		
Shengeh	0.105	0.083	0	
Rowghani	0.073	0.061	0.081	0

3.3 Genetic diversity based on ISSR data

In the complete linkage dendrogram based on ISSR data, the 88 olive trees were separated in five clusters (Figure 2 and Table 10). The first cluster included all of the individuals of Manjil's 'Mari' and 'Zard' cultivars, four individuals of the Gilvan's 'Mari' and three individuals of the Aliabad's 'Mari'. The second cluster were placed all of the Aliabad's individuals of 'Shengeh' and 'Rowghani' cultivars and four individuals of the Gilvan's 'Shengeh'. Cluster 3 grouped all of the individuals of Jamalabad's 'Rowghani' and four individuals of the Aliabad's 'Zard'. All of the individuals of Tarom and Manjil's 'Shengeh' grouped in cluster 4. Claster 5 was formed by diverse individuals. Cultivars and origins couldn't form distinct cluster. Aliabad and then Manjil were able to place their individuals of each cultivar in similar cluster, in other words, these areas showed the most homogeneity. The Mantel analysis revealed a negative and significant correlation ($r = -0.164$ $p<0.001$, 1000 random permutations) between the morphological and ISSR marker-derived dissimilarity matrices.

Table 10: Grouping the accessions in 5 clusters. Accessions are indicated with A, B, C and D for Mari, Zard, Shengeh and Rowghani cultivars respectively. Regions are indicated with L, M, E, T and J for Aliabad, Gilvan, Manjil, Tarom and Jamalabad respectively.

Cluster	number	olive accessions
1	18	MA4, MA6, MA7, MA8, LA2, LA5, LA6, EA1, EA3, EA4, EA5, EA6, EB5, EB6, EB8, EB9, EB10, MC10
2	22	LA7, LA8, MB6, MB8, TB3, TB5, TB6, MC2, MC5, MC8, MC9, LC2, LC3, LC4, LC5, LC7, LD1, LD2, LD3, LD5, LD6, TD3
3	16	JA1, JA3, LB2, LB4, LB5, LB9, TB4, JB4, JC2, JC4, JC5, ED6, JD2, JD3, JD4, JD5
4	21	JA2, JA4, JB1, JB2, JB3, TC1, TC2, TC3, TC4, EC2, EC3, EC4, EC5, EC6, JC3, TD8, TD10, ED1, ED2, ED3, ED5
5	9	MA10, MB2, MB3, MB5, LB3, TB2, MD8, MD9, MD10

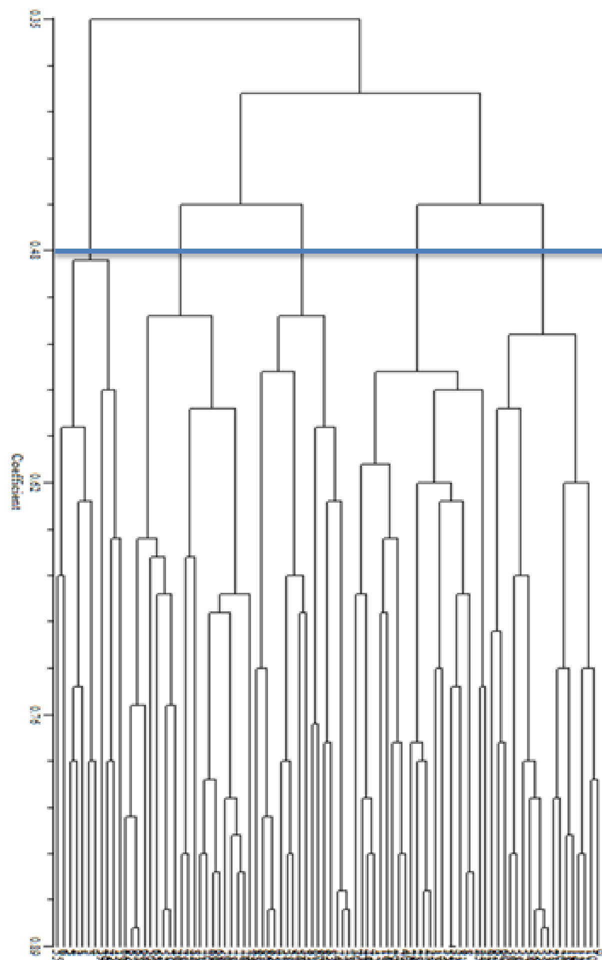

Figure 2: Complete linkage dendrogram based on simple matching coefficient illustrating the genetic similarities and distance among 88 olive accessions obtained by 11 ISSR primers data

4 DISCUSSION

Homogeneity in Aliabad trees was bigger than those of other regions. This orchard, in contrast to others, is vegetative collection. Among other cultivars, 'Zard' and 'Mary' had more homogeneity according morphological characters. Weakness of these classifications have been shown by the evidence that chemical and morphological changes in olive trees as well as other plants are influenced by domestication and agronomic selection (Sheidai et al., 2007). The high homogeneity in cultivars may reflect the selection pressure for fruit quality (Mekuria et al., 2002). Each of them was grouped in two clusters. Aliabad individuals were separated in cultivars. Cluster 2 of 'Mari' trees had acute fruit apex, rugose stone surface and few lenticels. Whereas, 'Mari' individuals in cluster 3 had obtuse fruit apex, smooth stone surface and many lenticels. 'Zard' individuals in cluster 4 had very high fruit mass, very high stone mass, spherical stone shape and many lenticels. While, 'Zard' individuals in cluster 5 had high fruit mass, high stone mass, ovoid stone shape and few lenticels.

The result showed high allelic variation in 11 ISSR markers, from which four ISSR primers had the highest amount (100 %). These four primers could be used to study genetic variation among olive genotypes. The highest level of polymorphism at ISSR loci indicates high genetic variability in olive cultivars which is in agreement with other studies (Gemas et al., 2004; Essadki et al., 2006; Terzopoulos et al., 2005; Martins-Lopez et al., 2009; Hess et al., 2000). Good discrimination

efficiency and high reproducibility of ISSRs make them particularly suitable to identify the closely related clones which are often the results of very local selection in fruit species (Essadki et al., 2006). The UBC 822 gave the highest number of effective alleles (1.92) and Nei's genetic diversity (0.48) among the ISSR primers, while the UBC 823 primer gave the lowest values for number of effective alleles (1.49), Nei's genetic diversity (0.31) and Shannon index (0.48).

The 'Mari' showed the highest homogeneity based on both data analyses and often their individuals were placed close to each other, while the highest genetic diversity compared to other cultivars was observed for the 'Shengeh'. Intra-cultivar variation has also been reported in 'Shengeh' by using morphological characters (Hosseini-Mazinani et al., 2004). 'Mari' cultivar showed the lowest mean of Shannon's index (0.533) and high genetic distance with the other cultivars, indicating little or low gene flow with other cultivars take place. 'Zard' and 'Rowghani' showed the lowest Nei's genetic distance. In the other study 'Zard', 'Rowghani' and 'Dezfool' were placed together and they showed low genetic distance, therefore, they reported probably those cultivar had the same origin (Koohi-dehkordi et al., 2006). A good correlation was not found between genetic distances estimated using ISSR markers and those based on morphological characteristics. This may be a consequence of the fact that molecular analysis probes a wider area of the genome than does morphological analysis (Rao et al., 2009). The lack of correlation between those two estimates is also influenced by the fact that a large proportion of the variation detected in trees by ISSR is, a priori, non-adaptive (Karhu et al., 1996), and hence not subject to selection, unlike phenotypic attributes (Rao et al., 2009).

Noormohammadi observed high intra-cultivar variation among North Iranian olive cultivars and without geographical separation (Noormohammadi et al., 2007; Noormohammadi et al., 2009; Noormohammadi et al., 2014), which was in agreement with the present study. Lack of a clear clustering of olive may be due to material exchanges by local gardeners and complicated in their denominations because of morphological similarity (Noormohammad et al., 2009). 'Mari' has narrow and elongated form of fruit, unlike other cultivars and identifying them is easier than others. The high intra-cultivar variation was obtained from most woody perennial outbreeding species, with most variation being within populations and existence of low gene flow (Hamrich and Godt, 1989).

5 MAIN CONCLUSION

In this paper, four olive cultivars have been investigated and characterized by combining morphological and molecular data. An important issue in identification of cultivars by morphological characters is the use of features receiving the least effects from environmental factors. Based on our analyses, characters of fruit and stone were more important than leaf characters. Both morphological and ISSR data analyses showed intra- and inter cultivar genetic diversity. These local cultivars must be exploited to identify individuals highly adaptive to extreme environmental conditions.

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