# Genetic and morphological variation in Iranian olive (Olea europaea L.) germplasm

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## ABSTRACT

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Olive cultivars with specific characteristics have been developed thanks to Iran's particular climatic conditions and long-term olive cultivation. The genetic variation and relationships among 40 olive cultivars, as well as 17 unknown genotypes from the national olive collection orchard at Tarom Research Station, Zanjan, Iran, were evaluated using SSR markers. Using 10 microsatellite primer pairs, 43 polymorphic bands were obtained on 57 olive genotypes. In addition to molecular markers, 14 morphological traits were measured in all olive genotypes. Based on discriminant and cluster analysis, the group of Iranian genotypes showed the greatest genetic distance from Spanish, Greek, Syrian, Italian and French groups. Based on cluster analysis using molecular and morphological data, most of the unknown genotypes showed high genetic similarity with genotypes from Spain and Syria. Despite the high genetic variation among cultivars in each group, geographical origin had significant impact on observed variability using Shannon's information index and polymorphism information of olive accessions. Climatic conditions in Iran, which are very different from conditions in Mediterranean countries, probably played a significant role, although this needs to be investigated further. The lowest mean of Shannon's index (0.76) for Iranian accessions indicated limited gene exchange with other genetic resources worldwide. The different genetic background of Iranian olive cultivars may help breeders access new and valuable genetic resources for national olive breeding programs.

Keywords: climatic conditions, germplasm, markers, olive, SSR, Shannon's index

## **INTRODUCTION**

live (Olea europaea L.) is one of the subtropical fruit tree species with remarkable cultural and economic importance. The main olive producing area is located in Mediterranean basin, however some other countries out of this region also grow and produce olive. Growing of very old trees in different parts of Iran due to the long history of olive cultivation in this part of the world. Vavilov (1950) considered Iran one of the olive's main centers of origin and diversification in the world. The olive's wide distribution in Iran, which is geographically and climatically different from the Mediterranean Basin, may lead to the development of cultivars that are genetically very different from those of Mediterranean origin. A considerable number of domestic and exotic olive cultivars and genotypes has been collected and grown since 1997 at Tarom Olive Research Station located in Tarom. Zanjan, Iran.

Currently, more than 200 olive genotypes and about 70 olive cultivars as well as some native olive genotypes are grown at Tarom Research Station. Little information is available on the genetic background of Iranian domestic olive genotypes. It is not clear, for example, how closely related Iranian olive cultivars and genotypes are to those from the main olive producing regions of Spain and Italy. Since there is very little germplasm exchange

between Iran and other olive growing countries, one may wonder how this is reflected in the genetic variation within Iranian cultivars. Various types of genetic markers have been widely used over the last decade to study the variability and genetic structure of olive cultivars (Belaj *et al.*, 2004). Due to recent advances in DNA technology, most of these studies have been performed by means of molecular markers (Belaj *et al.*, 2011). Lamantia *et al.* (2006) compared the fingerprints of 100 olive genotypes using SSR and RAPD markers. They showed that SSR markers can discriminate among olive genotypes better than RAPD markers.

Bracci *et al.* (2009) addressed the advantages of SSRs or microsatellites in a comparative olive study using RAPDs and AFLPs, and stressed the high efficiency of this marker technology for genotyping olive cultivars. Among available molecular markers, SSRs are among the most efficient markers for identifying olive genotypes because of their co-dominant inheritance and repeatability (Lamantia *et al.*, 2006). Noormohammadi *et al.* (2009) used 13 SSR markers to study the inter-clonal variation of Iranian olive cv. Zard, which showed great polymorphism as well as synonyms.

Roubos *et al.* (2010) used microsatellites to fingerprint olive genotypes and study the genetic structure of the most important Greek olive cultivars. Although they did not find a significant correlation between the genetic distance and geographical origin of these genotypes, they successfully discriminated different olive cultivars using microsatellite markers. Sarri *et al.* (2006) also used SSR markers for distinguishing 118 cultivars from different Mediterranean countries.

Since morphological and pomological variations originate from genetic structure, morphological traits are as important as molecular markers for studying the genetic structure and phylogenetic relationships among different olive genotypes. Morphological traits such as structure and shape of fruit and stone, leaf morphology and leaf size have been commonly used to discriminate olive cultivars (Hannachi *et al.*, 2008). However, grouping Turkish olive genotypes based on morphological and RAPD markers gave different results (Ozkaya *et al.*, 2006).

In this study, the genetic structure and variation of Iranian olive genotypes were compared with those of exotic olive cultivars mainly from Spain, Greece and Syria using both molecular (microsatellites) and morphological markers.

# MATERIALS AND METHODS

# Plant materials and molecular characterization

This study was carried out on 9-year-old olive trees in two consecutive years (2010-2011) at Tarom Olive Research Station (49° 05'E, 36°47'N). The test materials included 57 olive cultivars and genotypes, having different geographic origins, as well as 17 genotypes of unknown origin. They were placed in six different groups: genotypes from Iran (group 1), Italy and France (group 2), Spain (group 3), Syria (group 4), Greece (group 5) and unknown genotypes (group 6).

Because there were few genotypes from Italy and France, we put them together in one group in order

to avoid errors in the statistical analysis (Table 1). For the same reason, a single cultivar from Egypt (cv. Toffahi) and one from Lebanon (cv. Beladi) were combined with those of Syria. Total DNA was extracted using the protocol described by Vroh Bi *et al.* (1996) from young and mature leaves collected from the upper part of olive trees.

Fifty SSR primers were initially tested for genotyping the olive cultivars; of these, 10 primer pairs that produced informative, easily scored and clear products were selected for genotyping olive trees (Table 2), according to the method described by Cipriani *et al.* (2002) and Carriero *et al.* (2002).

The reaction mixture for all primer pairs was 20 ml volumes, containing 100 ng of genomic DNA, MgCl<sub>2</sub> 1.5 mM, 0.2 mM of each dNTP (Roche), 1 U of Taq DNA polymerase (Cinna Gen, Iran) and 0.5 mM of forward and reverse primers (Biolegio, The Netherlands). The PCRs were carried out in a thermal cycler (Bio Rad, USA) PCR system with initial denaturation at 95 °C for 5 minutes, followed by 35 cycles at 94 °C for 45 seconds, an annealing temperature of 57°C for 45 seconds and 72 °C for 45 seconds, and the final extension at 72 °C for 8 minutes. PCR products were separated using 8% *POLYACRYLAMIDE GEL* electrophoresis and visualized using silver staining.

# Morphological characterization

In this study, we measured and recorded for all genotypes 14 morphological and pomological traits including leaf characters (length, width, length/width fruit characters (length, ratio), diameter. length/diameter ratio, fruit fresh weight and pulp fresh weight), stone characters (length, width, length/width ratio and stone weight), pulp/stone ratio and oil content (%) (Barranco Navero et al., 2000). Data were collected from three trees and considered representative of morphological characters. Olive oil was extracted using the combined Soxhlet method. After oven-drying the fruit flesh (for 72 hours at 70 °C), three samples (weighing 2 g each) from all cultivars were measured and olive oil was calculated using the following formula: weight of samples Soxhlet-weight samples before of after Soxhlet/weight of samples before Soxhlet  $\times$  100.

# Statistical analysis

Canonical discriminant and cluster analysis among groups were performed using SPSS-16 and PopGene-32 software, respectively. Molecular analysis of variance (AMOVA) was performed using GenAlex 4.1 software to assess the total diversity among and within olive groups. Cluster analyses were performed using SPSS-16 to Dastkar et al.: Genetic and morphological ...

No.	Cultivar	Region	No.	Cultivar	Region
1	Zard	Iran	30	Mavi	Syria
2	Roghani	Iran	31	Doebli	Syria
3	Zard-e-gloleh	Iran	32	Beladi	Lebanon
4	Shengeh	Iran	33	Toffahi	Egypt
5	Mari	Iran	34	Voliotiki	Greece
6	Picholine	France	35	Koroneiki	Greece
7	Grossane	France	36	Kalamata	Greece
8	Cailletier	France	37	Karidolia	Greece
9	Frantoio	Italy	38	Mastoidis	Greece
10	Leccino	Italy	39	Konservolia	Greece
11	Lechin de Granada	Spain	40	Valanolia	Greece
12	Manzanilla de Sevilla	Spain	No.	Genotype	Region
13	Corfolia	Spain	41	N1	Unknown
14	Oblonga	Spain	42	N2	Unknown
15	Arbequina	Spain	43	N3	Unknown
16	Picual	Spain	44	N4	Unknown
17	Manzanilla	Spain	45	N5	Unknown
18	Cronicabra	Spain	46	N6	Unknown
19	ManzanilloCacereña	Spain	47	N7	Unknown
20	Verdal de Jaen	Spain	48	N8	Unknown
21	Picudo	Spain	49	N9	Unknown
22	Khodeiri	Syria	50	N11	Unknown
23	Don	Syria	51	N12	Unknown
24	Moussa,abi	Syria	52	N13	Unknown
25	Abu-Satl	Syria	53	N14	Unknown
26	Souri	Syria	54	N15	Unknown
27	Jlott	Syria	55	N16	Unknown
28	Kaissy	Syria	56	N17	Unknown
29	Sourani	Syria	57	N18	Unknown

Table 1. List of olive cultivars and genotypes and their geographical origins.

#### Table 2. List of primer pairs used in this study.

No.	Locus	Reference	(5´→3´) F-Primer (3´→5´) R-Primer	Allele size (bp)	Annealing temp. (°C)
1	UDO99-006	Cipriani <i>et al.</i> , 2002	TCAGTTTGTTGCCCTTTAGTGGA TTGTAATATGCCATGTAACTCGAT	172	57
2	UDO99-007	Cipriani <i>et al</i> ., 2002	TGTGTTCTTTATTTGAAGGAATCTT TCGCTTTTGTGTTACATATTCG	120	57
3	UDO99-009	Cipriani <i>et al</i> ., 2002	TTGATTTCACATTGCTGACCA CATAGGGAAGAGCTGCAAGG	119	55
4	UDO99-020	Cipriani <i>et al</i> ., 2002	TGAGGAGCCTTTGAACACTTT CGGACCCTGAAGTGATGATT	197	57
5	UDO99-025	Cipriani <i>et al</i> ., 2002	AACATGCCGTTGCATTTTTA GGCATCAATCTACTTCCCACA	158	57
6	UDO99-027	Cipriani <i>et al</i> ., 2002	TCCGTGCAAACCATGAAATA TTGATGACTAGCACACATGTAGGA	122	57
7	UDO99-035	Cipriani <i>et al</i> ., 2002	AATTTAATGGTCACACACAC ATTGCGAAATAGATCTACGA	145	55
8	GAPU 103A	Cipriani <i>et al</i> ., 2002	TGAATTTAACTTTAAACCCACACA	245	57
9	GAPU 108	Cipriani <i>et al.</i> , 2002	GATCCTTAGAGGATTCAATGAGAA	274	57
10	GAPU 59	Cipriani <i>et al.</i> , 2002	CCCTGCTTTGGTCTTGCTCAA CAAAGGTGCACTTTCTCTCG	227	57

determine the position of unknown genotypes and their relationship to the other groups. Genetic diversity parameters including N (total number of alleles), Na (number of different alleles), Ne (number of effective alleles), Ho (observed heterozygosity), He (expected heterozygosity) and I (Shannon's information index) were calculated by GenAlex 4.1 software.

## RESULTS

A total of 43 polymorphic markers were scored using 10 SSR primer pairs. With some primer pairs, in addition to expected alleles, a few other polymorphic bands of different sizes were also observed. Discriminant analysis showed that the first and second functions (P < 0.01) together explained 66.2% of the total variation among groups (Table 3). The AMOVA results revealed that 84.4% of olive cultivars were classified according to their geographical origin based on three initial functions. Unknown cultivars were classified close to the Syrian and Spanish groups, and the Iranian group showed a greater genetic distance from the other groups (Fig. 1). Cluster analysis of the groups produced two main clusters: 3 and 4 (Fig. 2). The Iranian, Italian, French and Greek groups were together, while the Spanish, Syrian and unknown groups were located close to each other.

		variance				
Function	Eigen value	(%)	Cumulative variance	<b>Canonical correlation</b>	Wilks' Lambda	Chi-square
1	16.816	38.4	38.4	0.972	0.000**	330.257
2	12.188	27.8	66.2	0.961	0.000**	239.533
3	7.937	18.1	84.4	0.942	0.007**	158.285
4	5.019	11.5	95.8	0.913	0.059	89.293
5	1.829	4.2	100.0	0.804	0.354	32.753

Table 3. Canonical functions and Wilks' Lambda coefficient for 43 alleles in 57 individual olive plants.

\*\* Significant at the 1% probability level.



Fig 1. Distribution of olive groups based on the first and second canonical functions using molecular markers.



Fig. 2. Cluster analysis using the UPGMA method based on Nei genetic distance coefficients and molecular characterization.

Clustering among individuals formed 4 groups 15 (Fig. 3). Each group consisted of several sub-groups. Clustered together in group 3 were 8 of the 17 unknown genotypes, along with 10 cultivars from Syria. In addition, 5 unknown genotypes and 6 cultivars from Spain were clustered in group 4. These findings are in agreement with the results of a scatter plot of the groups based on canonical functions (Fig. 1) and cluster analysis (Fig. 2).

The AMOVA showed that most of the genetic diversity was attributable to differences among individuals within groups (96%) rather than among groups (4%) (Table 4). The calculated PhiPT (analogue of  $F_{ST}$  fixation index) for all individuals (0.042) was significant (P< 0.05). Based on the results, the highest coefficient of genetic distance between groups belonged to Iranian and Spanish cultivars (0.128) (Table 5).

Using 10 microsatellite primer pairs, 43 polymorphic bands were obtained on 57 olive



Fig. 3. Cluster analysis of olive genotypes using Ward's method based on squared Euclidean distance similarity coefficients using molecular markers.

Table 4. Analysis of variance of olive groups based on molecular markers

morecular markers.								
Source	df	SS	MS	Est. Var.	%			
Among groups	5	63.940	12.788	.400	4			
Within groups	51	466.446	9.146	9.146	96			
Total	56	530.386		9.546	100			
A			*					

 \*PhiPT = 0.042
 P Value = 0.020\*

 Significant at the 5% probability level.

\*PhiPT = AP / (WP + AP); AP = Est. Var. Among groups; WP = Est. Var. Within groups.

genotypes. The maximum (8 bands) and minimum (2 bands) number of bands per primer pair occurred on locus UDO99-007 and GAPU59, respectively. Shannon's information index (I) varied from 1.9 (UDO99-007) to 0.69 (GAPU59) with an average of 1.22 (Table 6). All microsatellite loci showed high PIC values (from 0.250 to 0.620) and allowed the clustering of all individuals (Table 6). The maximum and minimum PIC values were observed in UDO99-007 (0.624) and UDO99-027 (0.148), respectively, in the Syrian group (Table 7).

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Table 5. Coefficient of genetic distance between groups based on molecular characterization

Group	Iran	Italy & France	Spain	Syria	Greece	Unknown
Iran	0.0					
Italy & France	0.026	0.0				
Spain	0.128	0.066	0.0			
Syria	0.055	0.009	0.053	0.0		
Greece	0.000	0.000	0.052	0.004	0.0	
Unknown	0.100	0.021	0.035	0.032	0.050	0.0

## Table 6. Descriptive analysis of 10 microsatellite loci in 57 olive genotypes.

Locus	Ν	Na	Ne	Не	Ho	I	PIC	
GAPU 108	49	3	2.687	0.628	0.367	1.034	0.352	
GAPU 59	50	2	1.999	0.500	0.220	0.693	0.250	
GAPU 103A	55	6	5.189	0.807	0.545	1.711	0.567	
UDO99-006	54	7	5.746	0.826	0.648	1.835	0.601	
UDO99-007	53	8	5.989	0.833	0.585	1.921	0.620	
UDO99-009	51	3	1.993	0.498	0.549	0.862	0.322	
UDO99-020	50	4	2.701	0.630	0.460	1.182	0.410	
UDO99-025	56	3	3.000	0.667	0.554	1.099	0.370	
UDO99-027	53	3	1.844	0.458	0.528	0.768	0.288	
UDO99-035	54	4	2.598	0.615	0.519	1.153	0.401	
Mean	52.500	4.3	3.375	0.646	0.498	1.226	0.418	

N= total number of alleles; Na = number of different alleles; Ne = number of

effective alleles; He = expected heterozygosity; Ho = observed heterozygosity;

I = Shannon's information index; PIC= polymorphic information content.

Table 7. Descriptive analysis of 10 microsatellite loci in 6 groups of olive genotypes.

											<u> </u>	<u> </u>		
		Iran			Italy&Fra	nce		Spain			Syria			
Locus	Ho	PIC	I	Ho	PIC	Ι	Ho	PIC	Ι	Ho	PIC	Ι	Ho	
GAPU 108	0.400	0.250	0.673	0.500	0.359	1.040	0.714	0.307	0.898	0.364	0.349	0.995	0.000	(
GAPU 59	0.250	0.171	0.377	0.200	0.244	0.611	0.125	0.245	0.621	0.364	0.250	0.689	0.400	(
GAPU 103A	0.200	0.381	1.089	0.800	0.500	1.505	0.909	0.566	1.709	0.500	0.465	1.418	0.333	(
UDO99-006	0.000	0.250	0.673	1.000	0.413	1.213	0.636	0.576	1.775	0.600	0.437	1.331	0.714	(
UDO99-007	0.600	0.455	1.359	0.250	0.444	1.321	0.818	0.513	1.534	0.500	0.624	1.891	0.571	(
UDO99-009	0.200	0.244	0.611	0.400	0.256	0.639	0.556	0.358	1.037	0.700	0.289	0.791	0.500	(
UDO99-020	0.333	0.349	1.011	0.250	0.327	0.900	0.556	0.385	1.120	0.455	0.407	1.212	0.500	(
UDO99-025	0.400	0.320	0.898	0.800	0.342	0.950	0.636	0.367	1.084	0.273	0.340	0.996	0.429	(
UDO99-027	0.600	0.244	0.611	0.400	0.218	0.500	0.700	0.250	0.688	0.182	0.148	0.368	0.667	(
UDO99-035	0.200	0.149	0.325	0.400	0.429	1.280	0.818	0.358	1.083	0.200	0.334	0.914	0.857	(
Mean	0.318	0.281	0.763	0.500	0.353	0.996	0.647	0.393	1.155	0.414	0.364	1.060	0.497	(

Ho = observed heterozygosity; I = Shannon's information index; PIC = polymorphism information content.

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Table 8 Mean and standar	d deviation	of morphological	traits for siv	olive ground
Table 0. Mican and standar	u ucviation	or morphological	traits for six	onve groups

						Gro	oups		
		1		2		3		4	
Traits	Mean	Std. Deviation <sup>*</sup>	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean
Leaf Length (mm)	66.71	3.35	58.38	5.81	54.06	3.63	56.68	5.98	61.73
Leaf Diameter (mm)	13.30	0.98	9.95	1.67	10.93	1.48	11.84	1.72	11.38
Leaf L:D <sup>**</sup> ratio	5.04	0.39	6.63	2.35	5.03	0.68	4.90	0.79	5.45
Fruit Length (mm)	24.37	1.41	20.70	3.46	21.82	3.85	25.50	3.64	24.54
Fruit Diameter (mm)	18.42	1.69	16.36	2.50	16.54	3.71	19.29	2.98	17.77
Fruit L : D <sup>**</sup> ratio	1.33	0.16	1.27	0.15	1.35	0.21	1.34	0.15	1.41
Stone Length (mm)	17.41	1.89	14.08	2.48	15.74	3.05	17.25	2.52	16.52
Stone Diameter (mm)	8.96	1.00	8.13	0.72	8.59	2.19	9.07	1.39	8.33
Stone L : D <sup>**</sup> ratio	1.99	0.41	1.74	0.30	1.89	0.37	1.95	0.42	2.03
Fruit Fresh Weight (g)	4.59	0.70	3.38	1.41	4.26	2.11	5.83	2.27	5.00
Stone Fresh Weight (g)	0.70	0.07	0.57	0.13	0.62	0.18	0.83	0.27	0.63
Pulp Fresh Weight (g)	3.89	0.64	2.81	1.31	3.64	2.01	4.99	2.08	4.37
Pulp : Stone Ratio	5.56	0.47	4.86	1.47	5.84	2.38	6.02	1.69	6.68
Oil content (%)	55.99	4.44	58.97	3.57	55.67	5.25	58.83	4.72	60.01
* 64 1 11 * 4*									-

\* = Standard deviation. \*\*L : D = Length : Diameter.

Table 9. Canonical functions and Wilks' Lambda coefficient for 14 morphological traits in 57 individual

		Variance			
Function	Eigen value	(%)	Cumulative variance	<b>Canonical correlation</b>	Wilks' Lambda
1	1.209	36.3	36.3	.740	.000**
2	.857	25.8	62.1	.679	.005**
3	.630	18.9	81.0	.622	.044*
4	.404	12.1	93.2	.536	.191
5	.227	6.8	100.0	.430	.392

\* Significant at the 5% probability level. \*\* Significant at the 1% probability level.

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Mean and standard deviation of morphological traits for each group are presented in Table 8. Canonical analyses based on morphological traits showed that the first and second canonical functions of morphological traits together explained 62.1% of the variation (P < 0.01) (Table 9). These results confirmed the initial grouping of olive genotypes based on geographical origin. Distribution of olive groups based on the first and second canonical components of morphological traits is shown in Fig. 4.



Fig. 4. Distribution of olive groups based on the first and second canonical functions of morphological traits.

# DISCUSSIONS

Results of discriminant analysis showed that the initial grouping based on geographical origin was likely correct. Given the high intra-group variation (94%) among individual olive genotypes, it can be concluded that indigenous cultivars from each geographical region evolved by genetic exchange through cross-pollination between individuals of the same region. Based on AMOVA analysis, Belaj *et al.* (2011) showed that most genetic diversity occurred among individuals within populations (97.55%).

Unknown genotypes collected at Tarom Olive Research Station belong to various research centers all over the country. Cluster analysis of individual olive genotypes indicated that most of the unknown genotypes were genetically very similar to those from Spain and Syria. It is likely that the unknown genotypes were introduced into Iran from those countries or are the result of cross-pollination between Spanish and Syrian cultivars. Bracci *et al.* (2009) showed the influence of geographical distribution on olive genetic structures and found a clear distinction between olive cultivars from Italy and those from other Mediterranean countries.

Descriptive analysis showed high polymorphism

in all SSR loci among 57 olive genotypes. The high amount of PIC, number of total alleles (N) and Shannon's information index (I) of locus UDO99-007 indicate its efficiency to determine and explain the genetic distance among individual olive genotypes. PIC values were different among groups as well as among different SSR loci. Several factors such as number of alleles per locus, number of guanine and thymine nucleotides in repeated regions, length of repeated sequences, number of individuals in each group and number of SSR markers may influence pic values (Roder *et al.*, 1998).

In the current study, the highest PIC value was observed in Spanish and unknown genotypes which had the highest number of cultivars and genotypes in their respective groups. Effective allele number is the number of alleles in a population that is expected for a locus and *its heterozygosity*. The Spanish cultivars and unknown genotypes showed the highest average number of alleles and Shannon's index. This may be due in part to the high genetic diversity present in these two groups and, in the case of Spanish cultivars, to the fact that most of them are the result of targeted crosses by different breeding programs rather than randomized or clonal selection from a few cultivars (Leon et al., 2004). Another reason may be the high number of individuals in both the Spanish and unknown groups.

The Iranian group had the lowest mean of Shannon's index (0.76), indicating the limited exchange of genetic resources with other gene pools worldwide. Morphological traits of different olive genotypes could easily classify different genotypes in several groups. Although these genotypes have different geographical origins, they were grown similar conditions. under It seems that morphological traits such as fruit, leaf and stone morphology were less adapted than others to environmental conditions, and were therefore able to discriminate genotypes based on their geographical origins. This may indicate the higher heritability of these traits. Results of morphological traits are in accord with those of molecular data on grouping of olive genotypes.

It is noteworthy that, in this study, both morphological traits and molecular markers placed Iranian genotypes in a distinct group, far from foreign cultivars. This finding is in agreement with earlier work where they used microsatellite markers for grouping domestic and exotic olive cultivars in Iran (Omrani Sabbaghi *et al.*, 2007). Omrani Sabbaghi *et al.* (2007) showed that most Iranian olive cultivars tended to cluster in a distinct group and were clearly different and genetically distant from Syrian cultivars. However, our results showed that molecular markers are more distinctive (84.4%) in grouping different olive genotypes than morphological traits (82.5%).

## CONCLUSION

Despite the high genetic interference among cultivars, geographical origin has had significant influence on the genetic structure of olive trees. The high genetic diversity identified among individuals within a group from a geographical area may be useful for establishing olive breeding programs using hybridization and mass selection. The group of Iranian cultivars showed the greatest genetic distance from other groups based on discriminant and cluster analysis. Although experimental climatic conditions were the same for all test cultivars/genotypes, the climatic conditions in Iran, which are different from those in the Mediterranean Basin in the long term, probably influenced the genetic structure of Iranian genotypes. The Iranian cultivars that grow at Tarom Station are representative of the old trees that were widely planted in Iran. Although this needs to be studied further, the different genetic background of Iranian cultivars may lead to improved agronomic behavior such as biotic and abiotic stress tolerance; therefore, they could serve as potentially important genetic resources for future breeding programs.

### REFERENCES

- Barranco Navero, D., A. Cimato, P. Fiorion, L. Rallo Romero, A. Touzani, C. Castaneda, F. Serafini, and I. T. Navas. 2000. World Catalogue of Olive Varieties. Intl. Olive Oil Council (IOOC). 360 pp.
- Belaj, A., L. Leon, S. Satovic, and R. de la Rosa. 2011. Variability of wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*) analyzed by agromorphological traits and SSR markers. Sci. Hort. 129: 561-569.
- Belaj, A., I. Trujillo, D. Barranco, and L. Rallo. 2004. Characterization and identification of Spanish olive germplasm by means of RAPD markers. Hort Sci. 39 (2): 346-350.
- Bracci, T., L. Sebastian, M. Busconi, C. Fogher, A. Belaj, and I. Trujillo, 2009. SSR markers reveal the uniqueness of olive cultivars from the Italian region of Liguria. Sci. Hort. 122: 209-215.
- Carriero, F., G. Fontanazza, F. Celliniand, and G. Giorio. 2002. Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theo. Appl. Genet. 104: 301-307.

- Cipriani, G., M. T. Marrazzo, R. Marconi, A. Cimato, and R. Testolin. 2002. Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. Theo. Appl. Genet. 104: 223-228.
- Hannachi, H., B. Breton, M. Msallem, S. Ben El Hadj, M. El Gazzah, and A. Berville. 2008. Differences between native and introduced olive cultivars as revealed by morphology of drupes, oil composition and SSR polymorphisms: A case study in Tunisia. Sci. Hort. 116: 280–290.
- Lamantia, M., J. Guerin, M. Sedgle, and E. Barone. 2006. Identification of olive (*Olea europaea* L.) genotypes using SSR and RAPD markers. Pp. 9-14. In: Actes Editions. Rabat.
- Leon, L., R., de la Rosa, D. Barranco, and L. Rallo. 2004. Ten years of olive breeding in Cordoba (Spain). Acta Hort. 663: 747-750.
- Noormohammadi, Z., M. Hosseini-Mazinani, I. Trujillo, and A. Belaj. 2009. Study of intra-cultivar variation among main Iranian olive cultivars using SSR markers. Acta Biol. Szegediensis 53 (1): 27-32.
- Omrani-Sabbaghi, A., M. Shahriari, M. Falahati-Anbaran, S. A. Mohammadi, A. Nankali, M. Mardi, and B. Ghareyazie. 2007. Microsatellite marker-based assessment of genetic diversity in Iranian olive (*Olea europaea* L.) collections. Sci. Hort. 112: 439–447.
- Ozkaya, M. T., E. Cakir, Z. Gokbayrak, H. Ercan, and N. Taskin. 2006. Morphological and molecular characterization of Derik Halhali olive (*Olea europaea* L.) accessions grown in Derik–Mardin Province of Turkey. Sci. Hort. 108: 205-209.
- Roder, M. S., V. Korzun, K. Wendehake, J. Plaschke, M. H. Tixier, P. Leroy, and M. W. Ganal. 1998. A Microsatellite Map of Wheat. Genet. 149: 2007-2023.
- Roubos, K., M. Moustakas, and F. A. Aravanopoulos.
  2010. Molecular identification of Greek olive (*Olea europaea*) cultivars based on microsatellite loci.
  Genet. Mol. Res. 9(3): 1865-1876.
- Sarri, V., L. Baldoni, A. Porceddu, N. G. Cultrera, A. Contento, M. Frediani, A. Belaj, I. Trujillo, and P. G. Cionini. 2006. Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. Genome 49: 1606-1615.
- Vavilov, N. 1950. The origin, variation, immunity and breeding of cultivated plants. Chron. Bot. 13(1/6): 36-37.
- Vroh Bi, I., L. Hraventg, A. Chandelier, G. Mergiai, and P. DuJardin. 1996. Improved RAPD amplification of recalcitrant plant DNA by use of activated charcoal during DNA extraction. Plant Breed. 115: 205-206.