Volume 56(2):155-163, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Inter-population genetic diversity in *Olea cuspidata* subsp. *cuspidata* revealed by SSR and ISSR markers

Zahra Noormohammadi*[1], Masoud Sheidai[2], Alireza Dehghani[2], Farshid Parvini[2], Seyed-Mehdi Hosseini-Mazinani[3]

[1]Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University (IAU), Tehran, Iran, [2]Shahid Beheshti University, GC, Faculty of Biological Sciences, Tehran, Iran, [3]Genetic Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

ABSTRACT The olive tree (*O. europaea* subsp. *europaea* var. *europaea*) is an ancient economic plant species with both cultivated and wild forms. In the present study, simple sequence repeat; SSR and inter simple sequence repeat; ISSR markers were used to study the genetic variation present in a few wild olive (*O. europaea* subsp. *cuspidata*) populations growing in the southern and western parts of Iran. Five SSR loci were analyzed which showed the presence of 12.6 alleles in average. The observed heterozygosity obtained for SSR markers ranged from 0.444 to 0.795 (mean value = 0.625), while Shannon index and polymorphic information content ranged from 1.46 (UDO99-043) to 2.47 (DCA3) and 0.620 (UDO99-43) to 0.871 (DCA3) respectively. Similarly ISSR analysis produced a total of 41 reproducible bands ranging from 240 bp (UBC-807) to 1750 bp (UBC823). The highest Nei's genetic diversity as well as Shannon index was obtained for UBC823 locus (0.37 and 0.54 respectively). Kerman population had the highest mean number of alleles while Jareh population showed the lowest number of alleles. Analysis of molecular variation (AMOVA) showed significant difference ($p < 0.05$) both among and between olive populations for SSR supported by *F*st pair-wise test. The highest value of within population ISSR genetic diversity occurred in Pahtak population, while Jareh population showed the highest value of among populations genetic diversity. UPGMA and NJ dendrograms obtained based on SSR and ISSR markers grouped the individuals of each population together in a distinct cluster, separated from the other populations due to their genetic distinctness.

Acta Biol Szeged 56(2):155-163 (2012)

KEY WORDS

ISSR
SSR
wild olive population

The olive tree is an ancient plant species with grate economic value to mankind (Zohary and Hopf 2000). It belongs to the genus *Olea* (*O. europaea* subsp. *europaea* var. *europaea*) with both cultivated and wild forms growing in Africa, Asia, Europe, and Oceania. The Mediterranean wild olive called oleaster (*O. europaea* subsp. *europaea* var. *sylvestris* Miller) shows close affinity to the cultivated olive and possibly is its progenitor. The non-Mediterranean wild olives have been geographically isolated from the oleaster and are adapted to new environmental conditions showing different morphological characters. Although recently, Green (2002) grouped all morphological forms of wild olive in a single aggregate *i.e. Olea europaea* subsp. *cuspidata*, other studies indicate the presence of several intra-specific forms (possibly ecotypes) both in Africa and Iran (Besnard et al. 2002; 2008; Sheidai et al. 2010).

Phylogeographic studies indicate that *O. europaea* subsp. *cuspidata* has diverged from the North African and Mediterranean olive trees and due to geographic isolation and fragmentation of habitat, in the north-west Africa, four subspecies have been formed namely subsp. *europaea*, subsp. *maroccana*, subsp. *guanchica* and subsp. *cerasiformis* (Rubio de Casas et al. 2006; Besnard et al. 2008). Furthermore, recent phylogenetic study of different subspecies of *Olea* by using both nuclear and plastid DNA sequences (Besnard et al. 2009) differentiate the subsp. *cuspidata* and the other subspecies of the section *Olea*.

Occurrence of natural hybridization between *europaea* subspecies; *O. cuspidate* and *O. africana* has been suggested by Besnard and Bervillé (2000), as well as Costa (1998). Omrani-Sabbaghi et al. (2007) suggested hybridization of subsp. *cuspidata* and the cultivated olive in South Africa and Iran and Sheidai et al. (2010) identified a hybrid population by using morphological and RAPD markers.

Different molecular markers have been used to study genetic relationship of cultivated and wild olives including RAPD (Belaj et al. 2004; Breton et al. 2006; Martins-Lopes et al. 2007; Besnard et al. 2008; Sheidai et al. 2010), microsatellite (simple sequence repeat; SSR) (Mookerjee et al. 2005; Gomes et al. 2009) and inter simple sequence repeat; ISSR

Accepted Oct 27, 2012
*Corresponding author. E-mail: marjannm@yahoo.com , z-nouri@srbiau.ac.ir

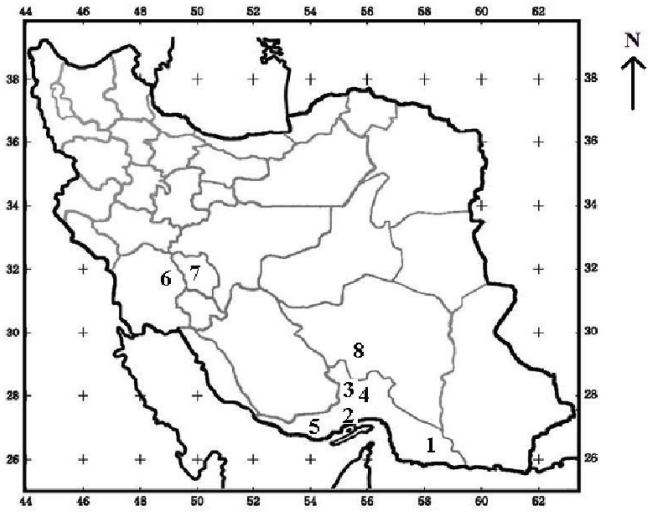

Figure 1. Distribution map and localities of wild olive (*O. cuspidata* subsp. *cuspidata*) populations studied. Population codes: 1-5: Pahtak, Geno, Homag, Bokhoon, and Anveh populations of Hormozgan province; 6: Jareh population of Khoozestan province; 7: Khersan population of Charmahal-Bakhteyari province; 8: Kerman population of Kerman province.

markers (Hess et al. 2000; Essadki et al. 2006; Martins-Lopes et al. 2007; Gomes et al. 2009; Terzopoulos et al. 2005).

In the present work, microsatellite and ISSR markers have been used to study the genetic variation of some wild olive populations collected from south and west of Iran for the first time, comparing the results obtained with previous study made on these populations by using morphological and RAPD analyses (Sheidai et al. 2010).

Materials and Methods

Plant materials

The olive trees of *Olea europaea* subsp. *cuspidate* were collected from 8 provinces in Southern and Western parts of Iran (Fig. 1), namely Hormozgan (Pahtak (4), Geno (5), Homag (2), Bokhoon (4) and Anveh (4) populations), Kerman (8) (Kerman population), Khoozestan (Jareh (2) population), and Charmahal-Bakhteyari (Khersan (4) population). Different number of olive trees was growing in these regions, for example in Jareh population only two trees were available. In order to establish the relationships between these wild olive populations and cultivated olive, 8 accessions of two Iranian olive cultivars (Dakal and Dezful) which are cultivated throughout the country in the olive farms were also included in the study.

Microsatellite and ISSR assay

Total genomic DNA was extracted from fresh leaves using the CTAB method by Murray & Thompson (1980) with the modification described by De la Rosa et al. (2002). Five olive microsatellite markers including ssrOeUA-DCA3, ssrOeUA-DCA9, ssrOeUA-DCA11 (Sefc & al. 2000) UDO99-011 and UDO99-043 (Cipriani et al. 2002) were selected as being the most polymorphic ones available.

PCR reactions were carried out in Techne thermocycler (Germany). Each 20 µl PCR reaction contained 20 ng genomic DNA, 1X supplied PCR buffer (Kowsar, Tehran, Iran) 200µM of each dNTP (Roche, Switzerland), 0.25 unit of Taq DNA polymerase (Kowsar, Tehran, Iran) and 0.2 µM of forward and reverse primers. One of each set of two primers was labeled with a fluorescent dye (ABI dyes: 6-FAM or HEX).

Amplification was performed following the program: initiation denaturation at 94ºC for 5 min, 35 cycles of 94ºC for 20 s, the annealing temperature 50ºC for 30 s and 72ºC for 30 s and final extension at 72ºC for 7 min. Capillary electrophoresis was performed on an automated sequencer (ABI Prism 3130 DNA sequencer, Applied Biosystems, Foster City, CA, USA) and the GeneScan internal size standard labelled with LIZ-500 (Applied Biosystems) was used.

The ISSR primers used in the present study were selected in a set of four primers; UBC807, UBC811, UBC823 and UBC849, commercialized by UBC (the University of British Columbia). PCR reactions were performed in a 25 µL volume containing 10 mM Tris–HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP; 0.2 µM of a single primer; 20 ng genomic DNA and 3 unit of Taq DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techne thermocycler (Germany) with following program: 5 min initial denaturation step 94°C, 30 s at 94°C; 1 min at 50°C, 1 min at 72°C. The reaction was completed by final extension step of 7 min at 72°C. Amplification products were visualized by running on 2% agarose gel, following ethidium bromide staining. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder (Fermentas, Germany). ISSR bands more than 1700 bp were not scored for more accuracy of scoring (Vargas and Kadereit 2001).

Data analysis

Microsatellite allelic size was determined by using the program GENEMAPPER 3.1 from Applied Biosystems. The number of SSR alleles, the number of unique alleles, observed (H_o) and expected (H_e) heterozygosity (Nei 1987), polymorphic information content (PIC) (Botstein et al. 1980) were determined by using Cervus version 2.0 (Marshall et al. 1998). In addition, the effective number of alleles (N_e) and Shannon's index were determined for both SSR and ISSR loci by POPGENE program (Yeh and Boyle 1999).

Analysis of Molecular Variance (AMOVA) and the *Fst* pair-wise test (with 10100 permutations) were performed to reveal significant genetic difference between populations as well as among individuals in each population by using Arlequin 3.0 (Excoffier 2005) for AMOVA analysis.

The level and distribution of genetic diversity was determined by Nei's gene diversity (H). H was calculated at the population level (Hpop) and species level (Hsp). The proportion of variation found within population was determined from Hpop/Hsp, whereas the proportion of variation distributed among populations was determined by (Hsp-Hpop)/Hsp (Nei 1973).

Cluster analysis of the olive trees were done by using UPGMA (Unweighted Paired Group using Arithmetic Average), Neighbor Joining (NJ) and Bayesian trees as well as ordination plots based on Principal Components Analysis (PCA) and Principal Coordinate Analysis (PCO) (Podani 2000). Bayesian clustering by using Markov chain Monte Carlo (MCMC) was performed and the results were compared with NJ and UPGMA dendrograms. Cophenetic correlation and bootstrapping (10000 replications) was performed to check the fit of dendrograms obtained (Podani 2000). NTSYS-pc version 2.02 (Rohlf, 1998), POPGENE ver. 1.31 (1997) and Mr. Bayes ver. 3.1 (2005) were used for molecular analyses.

Results

Microsatellite and ISSR diversity

Amplification products were obtained in all the genotypes studied at five SSR loci analyzed. We used olive specific SSR primers and obtained only two alleles in each locus. Amplification of the SSR markers produced 106 reproducible alleles that ranged from 97 bp (UDO-11 locus) to 286 bp (DCA-3 locus) in size (Table 1). The number of alleles per locus ranged from 8 (UDO99-043) to 17 (DCA03) with the average No. of 12.6 alleles (Table 1).

The value of observed heterozygosity ranged from 0.444 to 0.795 (mean value = 0.625). The mean value of expected heterozygosity (0.839) was higher than the observed heterozygosity (0.625) in SSR loci studied (Table 1). Shannon index as well as polymorphic information content (PIC) as the measure of genetic polymorphism, ranged from 1.46 (UDO99-043) to 2.47 (DCA3) and 0.620 (UDO99-43) to 0.871 (DCA3) respectively showing high genetic variation among the genotypes studied.

ISSR analysis showed a total of 41 reproducible bands ranging from 240 bp (UBC-807) to 1750 bp (UBC823).

Table 1. SSR alleles studied, their size range, number of alleles (N_a), number of effective alleles (N_e), Shannon Index (*I*), observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphic information content (PIC).

Locus	Size range	N_a	*Ne*	*I*	H_o	H_e	*PIC*
ssrOeUA-DCA03	226-286	17	8.42	2.47	0.795	0.892	0.871
ssrOeUA-DCA09	160-204	12	8.32	2.27	0.486	0.891	0.868
ssrOeUA-DCA11	113-200	14	8.15	2.3	0.692	0.888	0.866
UDO99-011	97-144	13	7.3	2.2	0.707	0.873	0.844
UDO99-043	115-221	8	2.79	1.46	0.444	0.642	0.62
Mean	--	12.6	7	2.14	0.625	0.839	0.814

UBC-849 primer showed 100% polymorphism while, ISSR primer UBC-807 showed the lowest value of polymorphism (86.67%).

The only specific ISSR band obtained was UBC-845-1400, while the band UBC-807-375 occurred in all genotypes studied except one. The highest values for Nei's genetic diversity and Shannon index were obtained for ISSR primer UBC823 (0.37 and 0.54 respectively, Table 2).

In SSR analysis, the mean value of observed heterozygosity varied from 0.521 (Kerman population) to 0.950 (Khersan population). The mean PIC value varied from 0.272 (Jareh population) to 0.627 (Anveh population). The highest mean number of effective alleles (N_e) and Shannon's index occurred in Bokhoon and Anveh populations (3.52 and 1.27 respectively, Table 3).

In ISSR markers, Pahtak population showed the highest mean number of effective alleles, the highest values of Shannon index and Nei's genetic diversity index (1.26, 0.22 and 0.15 respectively, Table 4). Homag population showed the lowest values for these genetic parameters because a single tree was available in this locality.

Population diversity and genetic relationships

Analysis of molecular variation (AMOVA) showed significant difference ($p < 0.05$) for SSR markers both among and between populations studied (Table 5). The *F*st pair-wise test supported the significant difference among most of the populations studied (Table 5). For example, Kerman population

Table 2. ISSR alleles studied, their size range, number of alleles (N), percentage of polymorphism (P%), number of effective alleles (N_e), Shannon Index (*I*), Nei's genetic diversity (*h*) and Standard Error (SE).

Locus	Size range	*N*	*P%*	N_e	*I*	*h*
UBC807 (AG)8T	240-1600	15	86.67	1.50 (0.34)	0.45 (0.24)	0.30 (0.18)
UBC811 (GA)8C	370-1400	7	100	1.55 (0.31)	0.50 (0.19)	0.33 (0.15)
UBC823 (TC)8C	300-1750	11	99	1.66 (0.35)	0.54 (0.21)	0.37 (0.16)
UBC849 (GT)8YA	350-1400	8	100	1.57 (0.35)	0.50 (0.21)	0.33 (0.16)
Mean (SE)	---	41	92.6	1.57(0.33)	0.49(0.21)	0.33(0.16)

Table 3. Genetic parameters in the wild olive. (H_o observed Hetrozygosity, H_e expected Hetrozygosity, PIC Polymorphic Information Content, N_e Number of effective allele).

Populations	SSR loci / Indices	DCA3	DCA9	DCA11	UDO11	UDO43	Mean
Bokhoon	No. Alleles	6	4	6	2	2	4
	No. Unique alleles	4	1	4	0	0	1.8
	H_o	1.00	0.500	0.750	0.500	0.667	0.683
	H_e	0.929	0.821	0.929	0.429	0.533	0.728
	PIC	0.786	0.667	0.786	0.305	0.346	0.578
	N_e	5.33	3.55	5.33	1.60	1.80	3.52
	Shannon's Index	1.73	1.32	1.73	0.56	0.63	1.19
Anveh	No. Alleles	5	4	5	4	3	4.2
	No. Unique alleles	3	2	2	2	1	2
	H_o	1.00	0.750	0.750	0.250	0.500	0.650
	H_e	0.857	0.750	0.893	0.750	0.607	0.771
	PIC	0.712	0.605	0.746	0.605	0.468	0.627
	N_e	4.00	2.90	4.57	2.90	2.13	3.30
	Shannon's Index	1.49	1.21	1.50	1.21	0.90	1.27
Khersan	No. Alleles	2	2	3	2	3	2.4
	No. Unique alleles	0	0	1	0	1	2
	Ho	1.00	1.00	0.750	1.00	1.00	0.950
	He	0.600	0.667	0.607	0.571	0.733	0.636
	PIC	0.375	0.375	0.468	0.375	0.535	0.426
	Ne	2.00	2.00	2.13	2.00	2.57	2.14
	Shannon's index	0.69	0.69	0.90	0.69	1.01	0.79
Homag	No. Alleles	3	2	2	3	1	2.2
	No. Unique alleles	2	1	2	2	0	5
	Ho	0.50	0.50	1.00	1.00	0.00	0.600
	He	0.833	0.50	1.00	0.833	0.00	0.633
	PIC	0.555	0.301	0.375	0.555	0.00	0.358
	Ne	2.66	1.60	2.00	2.66	1.00	1.98
	Shannon's index	1.03	0.56	0.69	1.03	0.00	0.66
Jareh	No. Alleles	2	2	2	1	2	1.8
	No. Unique alleles	0	2	1	0	1	4
	Ho	1.00	1.00	0.500	0. 00	0.500	0.80
	He	0.667	1.00	0.500	0. 00	0.500	0.533
	PIC	0.375	0.375	0.305	0. 00	0.305	0.272
	Ne	2.00	2.00	1.60	1.00	1.60	1.64
	Shannon's index	0.69	0.69	0.56	0.00	0.56	0.50
Pahtak	No. Alleles	4	4	4	4	1	3.4
	No. Unique alleles	1	0	3	2	0	6
	Ho	1.00	0.750	0.750	0.750	0.00	0.650
	He	0.821	0.857	0.643	0.750	0.00	0.614
	PIC	0.667	0.703	0.524	0.605	0.00	0.500
	Ne	3.55	4.00	2.28	1.00	2.90	2.75
	Shannon's index	1.32	1.38	1.07	0.00	1.21	0.99
Kerman	No. Alleles	9	4	3	1	6	4.6
	No. Unique alleles	6	0	1	0	3	10
	Ho	0.750	0.375	0.857	0.00	0.625	0.521
	He	0.858	0.725	0.604	0.00	0.800	0.598
	PIC	0.787	0.624	0.465	0.00	0.712	0.518
	Ne	5.12	3.12	2.28	1.00	4.0	3.10
	Shannon's index	1.92	1.24	0.89	0.00	1.54	1.12

differed significantly ($p < 0.05$) from the other populations except from Homag population.

Bokhoon and Geno populations showed the highest value of Nei's genetic identity (0.84) while, Bokhoon and cultivated olive populations revealed the lowest genetic identity (0.01) (Table 6). The highest value of within population genetic diversity (H_{pop}/H_{sp}) for ISSR markers was observed in Pahtak population (0.45), while Jareh population had the highest value of among populations genetic diversity ($H_{sp,}$ Table 4).

NJ, UPGMA and Bayesian trees obtained based on SSR markers produced similar results, separating cultivated olive (out-group) from wild olive populations studied. All of these

Table 3. Continued.

Geno	No. Alleles	7	5	3	5	1	4.2
	No. Unique alleles	5	2	0	3	0	10
	Ho	1.00	0.400	0.600	1.00	0.00	0.600
	He	0.911	0.822	0.689	0.800	0.00	0.644
	PIC	0.798	0.701	0.548	0.676	0.00	0.545
	Ne	1.55	1.55	2.16	2.50	4.57	2.47
	Shannon's index	0.65	0.65	0.92	1.06	1.63	0.98
Cultivars	No. Alleles	3	3	3	4	6	3.8
	No. Unique alleles	1	1	0	2	2	6
	Ho	0.286	0.143	0.500	0.875	1.00	0.560
	He	0.385	0.385	0.575	0.642	0.833	0.564
	PIC	0.325	0.325	0.482	0.525	0.748	0.481
	Ne	5.55	3.84	2.63	1.00	3.57	3.32
	Shannon's index	1.83	1.47	1.02	0.00	1.41	1.15

analyses showed bootstrap values. Therefore NJ tree of SSR markers are discussed below (Fig. 2).

The individuals (trees) of each population were almost grouped together forming a distinct cluster, separated from the other populations. Three populations of Anveh, Bokhoon and Jareh showed close affinity in SSR tree. Similarly, the Populations of Kerman, Pahtak and Geno were placed close to each other, while the Khersan population was placed in between cultivated forms and wild olive populations (Fig. 2).

Trees obtained based on ISSR data produced similar results, separating the wild olive populations studied. UPGMA tree (Fig. 3) with the highest cophenetic correlation (r = 0.98) showed 3 main clusters. The Jaccard's coefficient ranged from 0.40 to 0.97 among these populations. The highest similarity coefficients were obtained between individuals of Anveh population (0.97), while the lowest were observed between Pahtak and Khersan populations (0.40).

First major cluster contains two sub-clusters with trees collected from Anveh and Homag populations were placed in the first sub-group and trees collected from Jareh and Khersan populations were placed in the second sub-group. The second major cluster contains trees of Bokhoon and Kerman populations and the third cluster is comprised of trees of Geno and Pahtak populations.

The Mantel test performed between SSR and ISSR similarity matrices showed low correlation (r = 0.099; t = 1.6280; p = 0.9482) which was not significant.

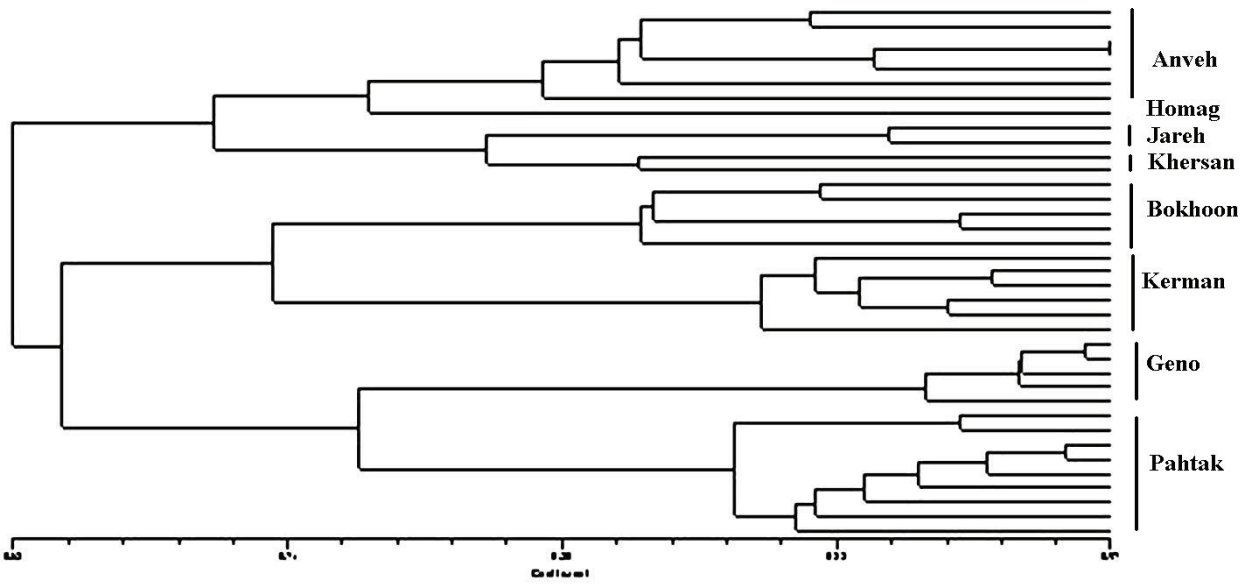

Figure 2. NJ dendrogram of SSR data. Populations abbreviations: A = Anveh, B = Bokhoon, D = Dezful cultivar, DK = Dakal cultivar, G = Geno, H = Homag, J = Jareh, K= Kerman, KH = Khersan and P = Pahtak.

Figure 2.

Table 4. Genetic parameters in wild olive population. (N_e = Number of effective allele, I = Shannon Index, H_{pop} = Heterozygosity at population level and H_{sp} = Heterozygosity at species level).

Population	Polymorphism%	N_e	I	No. Unique allele	Nei's genetic diversity	H_{pop}/H_{sp}	$H_{sp} - H_{pop}/ H_{sp}$
Bokhoon	29.27	1.14 (0.24)	0.14(0.23)	3	0.09 (0.15)	0.28	0.72
Anveh	34.15	1.19 (0.31)	0.17(0.26)	2	0.11(0.17)	0.33	0.67
Khersan	17.00	1.12 (0.27)	0.10(0.23)	0	0.07(0.16)	0.21	0.79
Homag	0.00	0.00	0.00	0	0.00	0.00	0.00
Jareh	9.76	1.07 (0.21)	0.06(0.18)	0	0.04 (0.12)	0.12	0.88
Pahtak	41.46	1.26 (0.37)	0.22(0.28)	3	0.15 (0.20)	0.45	0.54
Kerman	26.83	1.17 (0.32)	0.14(0.25)	2	0.10 (0.17)	0.29	0.71
Geno	19.51	1.14 (0.32)	0.11(0.24)	2	0.08 (0.17)	0.23	0.77

Table 5. Analysis of molecular variance (AMOVA) in 9 populations. (P- values are estimated using 10100 random permutation).

Source of variation	Degree of freedom	Sum of squares	Variance component	Percent of variation	P-value
Among population	8	28.397	0.28835	22.56	<0.05
Within population	73	72.237	0.98955	77.44	<0.05
Total	81	100.634	1.27791		
Fixation Index FST: 0.22565					

Table 6. Nei's standard genetic identity (upper diagonal) and pair-wise F*st* values (lower diagonal) among wild olive populations studied.

population	Bokhoon	Anveh	Khersan	Homag	Jared	Pataki	Kerman	Cultivar	Geno
Bokhoon		0.633	0.094	0.466	0.102	0.566	0.509	0.010	0.841
Anveh	0.099		0.178	0.553	0.216	0.678	0.558	0.118	0.741
Khersan	0.278	0.209*		0.109	0.210	0.058	0.114	0.258	0.098
Homag	0.069	0.029	0.194		0.139	0.557	0.685	0.092	0.751
Jareh	0.380	0.282	0.487	0.351		0.050	0.034	0.398	0.136
Pahtak	0.152*	0.117*	0.324*	0.111	0.369		0.636	0.089	0.680
Kerman	0.160*	0.153*	0.231*	0.013	0.351*	0.205*		0.090	0.725
Cultivar	0.363*	0.286*	0.321*	0.265	0.412*	0.332*	0.296*		0.179
Geno	0.018	0.062	0.230*	-0.005	0.274*	0.148*	0.081*	0.240*	

*Significant at 0.05 (P-value<0.05). P-values are estimated 10100 permutation.

Discussion

We obtained only two alleles in each SSR locus indicating diploid nature of *O. europaea* subsp. *cuspidate* trees studied. Combination of SSR alleles and flow cytometery have been used to indicate polyploidy nature of olive subspecies, for example, Besnard et al. (2008) reported that individuals of *O. europaea* subsp. *maroccana* and *cerasiformis* display three or more alleles at most of their SSR loci and along with DNA-ploidy levels measured with flow cytometry, they suggest that subsp. *maroccana* is hexaploid while, subsp. *cerasiformis* is tetraploid.

Rallo et al. (2003) reported that Iranian populations of subsp. *cuspidata* are polyploid while, Besnard et al. (2008) reported diploid nature of these trees by SSR and flow cytometry analyses. Our previous cytological study (Sheidai et al. 2009) also revealed diploid level of subsp. *cuspidate* populations in Iran which is supported by present SSR analysis showing presence of only two alleles at each locus.

Although SSR primers used in this study were originally designed for cultivated olive populations, a high genetic variation was observed among wild olive populations studied, for instance, high values of PIC, heterozygosity and Shannon indices, indicate the presence of high genetic variability among the olive genotypes studied. Such level of genetic diversity agrees with the previous reports of SSR analysis in both olive cultivars and wild olive genotypes (Carriero et al. 2002; De la Rosa et al. 2002; Khadari et al. 2003; Belaj et al. 2004; Poljuha et al. 2008).

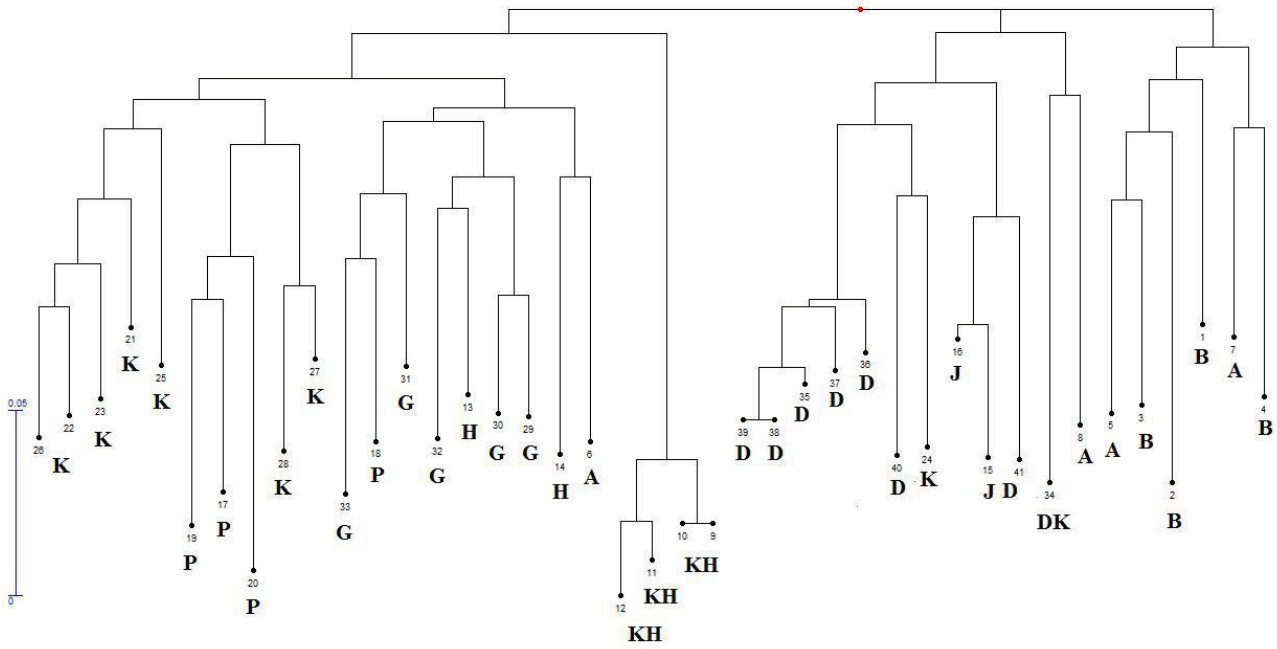

Figure 3. UPGMA dendrogram of ISSR markers in wild olive populations studied.

The reason for the higher expected heterozygosity versus the observed heterozygosity in SSR loci might be due to the presence of null alleles at these loci (Ishibashi et al. 1996). The presence of null alleles is a consequence of sequence polymorphisms in the flanking regions of the locus due to point mutations or insertion/deletions (Jones et al. 2003).

The high level of polymorphism in ISSR loci studied also indicates the presence of high genetic variability in wild olive trees studied which is in agreement with other studies made on cultivated olive trees (Terzopoulos et al. 2005; Martins-Lopes et al. 2007; 2009; Gomes et al. 2009), confirming that the olive is a highly variable species.

Hess et al. (2000) and Gomes et al. (2009) used ISSR markers to study genetic diversity in wild olive species as well as cultivated olive genotypes reporting lower level of polymorphism in ISSR loci with (CA) dinucleotide, while Terzopoulos et al. (2005) reported higher polymorphism in ISSR locus (UBC-818) based on a (CA) dinucleotide against other dinucleotide motifs in olive cultivars.

The present study shows the usefulness of ISSR (dominant) and SSR (codominant) molecular markers in revealing the genetic diversity within and among wild olive populations, however it seems that these molecular markers differ somewhat in results they produce due to variation in the DNA sequence they amplify. For example, specimens (olive trees) collected from Anveh population show the highest genetic variation in SSR markers compared to that of other populations while, Pahtak population shows the highest genetic variability in ISSR markers. Incongruence between these results is also demonstrated by Mantel test performed producing a non-significant correlation between SSR and ISSR markers. In our previous RAPD analysis (Sheidai et al. 2010), the highest genetic diversity was observed in Anveh and Khersan populations, partly supporting the present SSR result.

However, irrespective of these differences, both dominant molecular markers (RAPD and ISSR) as well as codominant markers (SSR) used can differentiate wild olive populations studied. This indicates that irrespective of molecular marker inheritance, strong genetic signal exists in different populations.

Dendrograms obtained from both SSR and ISSR markers are almost similar in separating individuals of each population from the other populations, further illustrating the genetic distinctness of the populations studied. The close affinity observed among Anveh, Bokhoon, Homag and Jareh populations in both ISSR and SSR trees, agrees with the morphological and RAPD trees reported earlier (Sheidai et al. 2010). Kerman population stands little far from the other populations in both ISSR and SSR trees which is also in agreement with morphological and RAPD trees.

Garcia-Verdugo et al. (2009), while studying genetic diversity of *Olea* subsp. *maroccana*, *serasiformis* and *guanchica* found a good agreement in both dominant (AFLPs) and codominant (SSRs) markers in showing genetic diversity and subspecies differentiation.

Some of the olive trees collected from Anveh, Bokhoon, Pahtak and Geno populations are placed among the trees of the other populations in dendrogram obtained. These olive

trees grow in the areas with close vicinity, therefore, the similarities observed between them may be due to the gene flow occurring among them. Such a consideration is also supported by the high heterozygosity values (>0.44) observed in these populations. These values are even much higher than the heterozygosity range (0.20-.0.25) given for dominant markers in out crossing long lived endemic species (Nybom 2004; Garcia-Verdugo et al. 2009).

The olive trees collected from Khersan population were placed in a single cluster separated from the other olive populations studied in both SSR and ISSR markers. In our previous study based on morphological ad RAPD markers (Sheidai et al. 2010), it was suggested that Khersan population is of hybrid nature formed by hybridization between cultivated and wild olive populations in the nearby vicinity. This assumption is supported by the dendrograms obtained here and also by the highest value of SSR observed heterozygosity (0.99) present in this population.

References

Belaj A, Cipriani G, Testolin R, Rallo L, Trujillo I (2004) Characterization and identification of the main Spanish and Italian olive cultivars by simple-sequence-repeat markers. Hortscience 39:1557-1561.

Besnard G, Bervillé A (2000) Multiple origins for Mediterranean olive (*Olea europaea* L. subsp. *europaea*) based upon mitochondrial DNA polymorphisms. C.R. Acad. Sci., Paris, Sér III, 323, pp. 178-181.

Besnard G, Khadari B, Villemur P, Bervillé A (2000) Cytoplasmic male sterility in the olive (*Olea europaea* L.). Theor Appl Genet 100:1018-1024.

Besnard G, Khadari B, Baradat P, Berville´ A (2002) *Olea europaea* (Oleaceae) phylogeography based on chloroplast DNA polymorphism. Theor Appl Genet 104:1353-1361.

Besnard G, Garcia-Verdugo C, Rubio De Casas R, Treier U, Galland N, Vargas P (2008) Polyploidy in the Olive Complex (*Olea europaea*): Evidence from Flow Cytometry and Nuclear Microsatellite Analyses. Ann Bot 10:25-30.

Besnard G, Rubio De Casas R, Pascal-Anoine C, Vargas P (2009) Phylogenetics of *Olea* (Oleaceae) based on plastid and nuclear ribosomal DNA sequences: Tertiary limatic shifts and lineage differentiation times. Ann Bot 104:143-160.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of genetic linkage map in man using restriction fragment length polymorphism. Am J Hum Genet 32:314-331.

Breton C, Tersac M, Bervillé A (2006) Genetic diversity and gene flow between the wild olive (oleaster, *Olea europaea* L.) and the olive: several Plio-Pleistocene refuge zones in the Mediterranean basin suggested by simple sequence repeats analysis. J Biogeography 33:1916-1928.

Carriero F, Fontanazza G, Cellini F, Glorio G (2002) Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). Theor Appl Genet 104:301-307.

Cipriani G, Marruzzo MT, Marconi R, Cimato A, Testolin R (2002) Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal Polymorphism within ancient cultivars. Theor Appl Genet 104:223-228.

Costa C (1998) Olive production in South Africa. A handbook for olive growers. Agricultural Research Council, Pretoria, p. 124.

De la Rosa R, James C, Tobutt KR (2002) Isolation and characterization of polymorphic microsatellite in olive (*Olea europaea* L.) and their transferability to other genera in the *Oleaceae.* Primer Note. Mol Ecol Notes 2:265-267.

Essadki M, Ouazzani N, Lumaret R, Moumni M (2006) ISSR variation in olive-tree cultivars from Morocco and other western countries of the Mediterranean Basin Genetic Res. Crop Evol 53:475-482.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinformatics Online 1:47-50.

Garcia-Verdugo C, Fay MF, Granado-Yela C, Rubio De Casas R, Balaguer L, Besnard G, Vargas P (2009) Genetic diversity and differentiation processes in the ploidy series of *Olea europea* L.: a multiscale approach from subspecies to insular populations. Mol Ecol 18:454-467.

Gomes S, Martins-Lopes P, Lopes J, Guedes-Pinto H (2009) Assessing genetic diversity in *Olea europaea* L. Using ISSR and SSR markers. Plant Mol Biol Report 27:365-373.

Green PS (2002) A revision of *Olea* L. (Oleaceae). Kew Bull 57: 91-140.

Hess J, Kadereit W, Vargas P (2000) The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and inter-simple sequence repeats (ISSR). Mol Ecol 9:857-868.

Ishibashi Y, Saitoh T, Abe S, Yoshida C (1996) Null microsatellite alleles due to nucleotide sequence variation in the grey-sided vole *Clethrionomys rufocanus*. Mol Ecol 5:589-590.

Jones AG and Ardren WR (2003) Methods of parentage analysis in natural populations. Mol Ecol 12:2511-2523.

Khadari B, Breton C, Moutier N, Roger JP, Besnard G, Berville A, Dosba F (2003) The use of molecular markers for germplasm management in a French olive collection. Theor Appl Genet 106:521-529.

Marshall TC, Slate J, Kruk L, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639-655.

Martins-Lopes P, Lima-Brito J, Gomes S, Meirinhos J, Santos L, Guedes-Pinto H (2007) RAPD and ISSR molecular markers in Olea europaea L.: Genetic variability and molecular cultivar identification. Genetic Res Crop Evol 54:117-128.

Martins-Lopes P, Gomes S, Lima-Brito J, Lopes J, Guedes-Pinto H (2009) Assessment of clonal genetic variability in *Olea europaea* L. 'Cobrançosa' by molecular markers. Sci Hort 123:82-89.

Murray MG and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 8:4321-4325.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321-3.

Nei M (1978) Molecular Evolutionary Genetics. Columbia University Press, New York.

Nybom H (2004) Comparision of different nuclear molecular DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143-1155.

Omrani-Sabbaghi A, Shahriari M, Falahati-Anbaran M, Nankali A, Gharehyazei B (2007) Microsatellite markers based assessment of genetic diversity in Iranian olives (*Olea europaea* L.) collections. Sci Hort 112:439-447.

Podani J (2000) Introduction to the Exploration of Multivariate Data. English translation. Backhuyes Publishers, Leide, pp. 407.

Poljuha D, Sladonja B, Setić E, Milotić A, Bandelj D, Jakse J, Javornik B (2008) DNA fingerprinting of olive varieties in Istria (Croatia) by microsatellite markers. Sci Hort 115:223-230.

Rallo P, Tenzer I, Gessler C, Baldoni L, Dorado G, Martín A (2003) Transferability of olive microsatellite loci across the genus *Olea*. Theor Appl Genet 107:940-946.

Rohlf FJ (1998) NTSYS-pc. Numerical taxonomy and multivariate analysis system. Version 2.00. Exeter Software, Setauket, N.Y.

Rubio de Casas R, Besnard G, Schönswetter P, Balaguer L, Vargas P (2006) Extensive gene flow blurs phylogeographic but not phylogenetic signal in *Olea europaea* L. Theor Appl Genet 113(4):575-583.

Sefc KM, Lopes MS, Mendoca D, Rodrigues Dos Santos M, Laimer Da Camara Machado M, Da Camara Machado A (2000) Identification of microsatellite loci in olive (*Olea europaea* L.) and their characterization in Italian and Iberian olive trees. Mol Ecol 9:1171-1173.

Sheidai M, Noormohammadi Z, Hoshiar-Parsian H, Chegini F (2009) Cyto-Morphology and molecular study of wild olives in Iran. Cytologia 74:369-377.

Sheidai M, Noormohammadi Z, Dehghani AR, Parvini F, Hoshiar-Parsian H, Hosseini-Mazinani M (2010) Intra-specific morphological and molecular diversity in brown olive (*Olea cuspidata*) of Iran. Sci Asia 36: 187-193.

Terzopoulos PJ, Kolano B, Bebeli PJ, Kaltsikes PJ, Metzidakis I (2005) Identification of *Olea europaea* L. cultivars using inter-simple sequence repeat markers. Sci Hort 105:45-51.

Vargas P and Kadereit JW (2001) Molecular Wngerprinting evidence (ISSR, Inter-simple sequence repeats) for a wild status of *Olea europaea* L. (*Oleaceae*) in the Eurosiberian North of the Iberian Peninsula. Flora 196:142-152.

Vargas P, Muñoz Garmendia F, Hess J, Kadereit J (2009) *Olea europaea* subsp. *guanchica* and subsp. *maroccana* (Oleaceae), two new names for olive tree relatives. Ann Jard Bot Madrid 58:360-361.

Yeh FC, Yang RC, Boyle T (1999) Population Genetic analysis (POPGENE 1.31). A joint project of University of Alberta and Center for International Forestry Research.

Zohary D and Hopf M (2000) Domestication of plants in the Old World, third edn. Oxford, Clarendon Press.