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Variation Assessment of Olive Cultivars in a Germplasm Established in the North of Iran Using ISSR Markers

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ABSTRACT: In this study, variation and inter-relationship of 31 olive cultivars (seven Iranian and 24 foreign cultivars), collected in a germplasm in the north of Iran, were analyzed using Inter Simple Sequence Repeat (ISSR) markers. Nine primers were used for allele scoring. In total, 44 ISSR polymorphic alleles were detected. Cluster analysis of the ISSR data showed inter-cultivar variation for Mediterranean cultivars of Italy, Spain and Greece and they were clearly distinct from the Iranian and Syrian cultivars. The principal coordinate analysis (PCoA) explained 22.55% and 20.78% of the total variation along the first (PC1) and second (PC2) component, respectively. In general, the PCoA plot revealed same affinities for the cultivars as the phylogenetic tree. The outcome of this study could be useful for construction of a molecular database of the cultivars which can be used in breeding and cultivar improvement.

Key words: Cluster analysis, Genetic diversity, ISSR, Olive

INTRODUCTION

Olive (*Olea europaea* L. subspeuropaea var. europaea) is native to the Mediterranean Basin. Referring to the Food and Agriculture Organization of the United Nations (FAO); Spain, Italy, Greece, Turkey and Morocco are the top five olive producing countries in the world. Besides the traditionally popular Mediterranean basin, the cultivation of olive tree is spread to other countries like the United States, India, Australia, Japan, Chile, Pakistan, Afghanistan and other Asian, African and Middle Eastern countries.

Olive cultivation and industry has a long history in Iran. According to FAO statistics, the cultivated areas of olive in Iran were 110,000 hectares of which 48,000 hectares were bearing. Also the total olive production of the world was 18 million tons per year and Iran by producing 102000 tons was ranked the sixteenth (FAO, 2010). Olive is mainly being produced in Fars, Kerman, Hormuzgan, Sistan-Baluchestan, Qazvin, Gilan, Golestan and Zanjan provinces of Iran. With respect to high economic value of olive industry in Gilan province (north of Iran), a rich germplasm of both Iranian and foreign cultivars was established in Ali-Abad town, near to Rudbar city of Gilan province (Fig. 1).

For efficient utilization of this germplasm in breeding, commercial selection and propagation; different morphological and molecular aspects of the cultivars should be characterized in detail. Many molecular markers such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat (ISSR) have been extensively used in olive for cultivar identification, phylogenetics and germplasm characterization (Belaj *et al.* 2003; Owen *et al.* 2005; Taamalli *et al.* 2008; Gomes *et al.* 2009; Koehmsted *et al.* 2010; Haouane *et al.* 2011; Hegazi *et al.* 2012; Delgado-Martinez *et al.* 2012).

A part from the controversy that exists for the number of olive species and subspecies occurring in Iran, too many local cultivars, ecotypes and natural hybrids of olive have been reported in the country (Sheidai *et al.* 2010; Hosseini-Mazinani *et al.* 2013). These variations have been extensively studied based on morphological traits and molecular markers, however in case of molecular analysis; mostly RAPD, SSR and AFLP have been employed (Samaee *et al.* 2003; Noormohammadi *et al.* 2007; Sheidai *et al.* 2010; Hosseini-Mazinani *et al.* 2014). Moreover, using these molecular markers relationships between Iranian and some Mediterranean olive cultivars have been studied in a limited number of researches (Omrani-Sabbaghi *et al.* 2007; Trujillo *et al.* 2012), and ISSR has not been used for these purposes. In the present study, ISSR markers were used to evaluate molecular variation and revealing inter-relationship between 31 olive cultivars (both Iranian and foreign cultivars). Having a clear knowledge of this issue will be useful for their conservation, selection and breeding program.

MATERIALS ANDMETHODS

A. Plant material and DNA isolation

Thirty-one cultivars (seven Iranian and 24 foreign cultivars) of olive from a germplasm in the north of Iran (Ali-Abad) were used (Fig. 1 and Table 1).

Total genomic DNA was isolated from fresh leaves following the procedure described by Murray and Thompson (1980) with some modification. After quantity determination of the DNA using a spectrophotometer (Nano Drop 2000, Thermo Scientific), the templates were diluted to 25 ng/µl for using in PCR reactions.

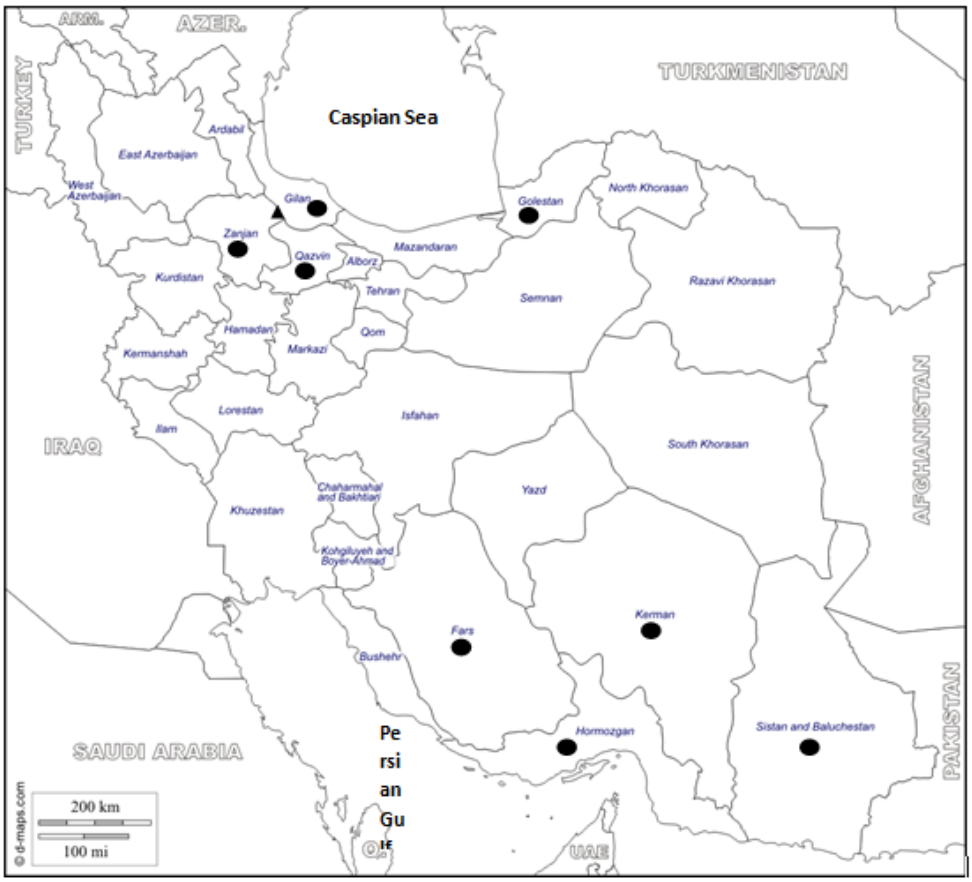

Table 1: List of olive cultivars used in this study.

Number	Cultivar name	Country of Origin	Use	Number	Cultivar name	Country of Origin	Use
1	Roghani (Rowghani)	Iran	T/O*	17	Fuji	Syria	T
2	Zard	Iran	T/O	18	Sevillana	Spain	T
3	Amigdalolia	Greece	T	19	Mission	USA	T
4	Mari	Iran	T/O	20	Cornicabra	Spain	O
5	Manzanilla	Spain	T	21	Kavi(Khav)	Syria	T/O
6	Fishomi	Iran	T/O	22	Leccino	Italy/ Toscana	O
7	Baladi	Palestie	T/O	23	Koroneiki	Greece	O
8	Amphissis	Greece	T/O	24	Dan	Syria	T
9	Shenge (Shengeh)	Iran	T/O	25	Oblanga	Italy	T
10	Arbequina	Spain	T/O	26	Abo-Sattle	Syria	T
11	Dezful	Iran	T/O	27	Halab	Syria	T
12	Coratina	Italy	O	28	Moraiolo	Italy	O
13	Frangivento	Italy	O	29	Roso	Italy	O
14	Conservolea	Greece	T	30	Ascolana	Italy	T
15	Mastoidis	Greece	O	31	Golule(Goluleh)	Iran	O
16	Picual	Spain	T				

T*= Table olive, O = Olive oil, T/O = dual use.

B. Marker analyses

ISSR assays were performed using 13 primers of the Biotechnology Laboratory, The University of British Columbia, Canada. The PCR amplifications were conducted in a total volume of 15 µl [PCR buffer (1×), 50 ng of genomic DNA, 0.1 mM of each dNTP, 0.1 mM of the primer, 1 unit of DNA Taq polymerase (Fermentas) and 1.5 mM of $MgCl_2$]. The touch down PCR condition was: 94°C for 2min, 10 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 2 min, 25 cycles of 94°C for 30 s, 46°C for 45 s, 72°C for 2 min ending with 72°C for 2 min. The PCR products were separated on 2% agarose gel in TBE buffer (1×) (45 mM Tris-Boric, 1mM EDTA pH 8.0). The gels were stained with ethidium bromide (100 mg/ml).

C. Data analysis

For each of the primers, the presence of an amplified product (fragment) was identified as "1" and the absence was designated as "0". Unclear and instable fragments were not scored. Based on the Jaccard coefficient of similarity genetic distances were calculated. The cultivars were grouped by cluster analysis using the neighbor-joining method. The computer program used was DARwin5 (Perrier and Jacquemoud-Collet, 2006). PIC (polymorphic information content) values were calculated as described in detail by Kumar *et al.* 2014. PIC provides an estimate of the discriminatory power of a locus by taking into account not only the number of alleles but also the relative frequencies of those alleles. A principle coordinate analysis was carried out based on a pairwise individual-by-individual genetic distance matrix calculated using the GenAlEX 6 software (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

After screening of 13 ISSR primers, nine primers were selected for final PCR amplification and data scoring because of producing unambiguous PCR fragments and polymorphism (Table 2). The primers, UBC813 [(CT)8T], UBC855 [(AC)8YT], UBC862 [(AGC)6], UBC873 [(GACA)4] were excluded due to producing either monomorphic or ambiguous, fragments. Fig. 2 is a typical gel profile of polymorphic ISSR markers obtained by UBC825 primer. According to Table 2, in total 44polymorphic alleles were obtained.

Table 2: Selected primers used for ISSR analyses in 31 olive cultivars of this study.

Primer	Sequence	Polymorphic bands	Monomorphic bands	Not-scored bands	Total number of bands	PIC
UBC807	$(AG)_8T$	1	10	-	11	0.45
UBC808	$(AG)_8C$	6	2	1	9	0.42
UBC809	$(AG)_8G$	8	1	5	14	0.27
UBC810	$(GA)_8T$	5	1	5	11	0.32
UBC811	$(GA)_8C$	1	5	-	6	0.13
UBC812	$(GA)_8A$	6	2	4	12	0.28
UBC822	$(TC)_8A$	6	1	6	13	0.30
UBC825	$(AC)_8T$	9	0	1	10	0.34
UBC834	$(AG)_8Y^*T$	2	7	1	10	0.14
Total		44	29	23	96	-
Mean						0.29

*Y: T or C.

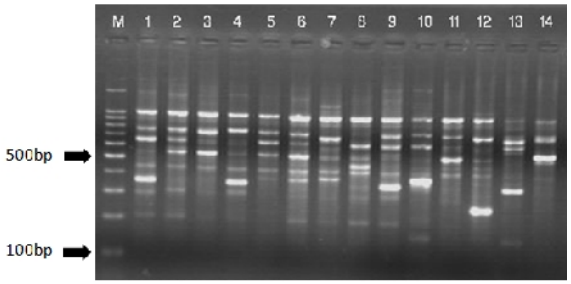

Fig. 2. Polymorphism of ISSR obtained by UBC825 primer in the olive cultivars of this study. M: DNA size marker. The numbers at the top are related to the cultivars of Table 1.

The highest (9) and the lowest (1) number of polymorphic alleles were obtained by UBC825 and UBC807 primers, respectively. PIC values ranged from 0.45 for UBC807 to 0.14 for UBC834. PIC for dominant markers like ISSR is a maximum of 0.5 (Kumar *et al.* 2014). The mean value of PIC (0.29) was relatively high which confirmed the high polymorphism among the cultivars. According to Fig. 3, the dendrogram shows that the 31 olive cultivars analyzed in this study could be distinguished from one another. Among Mediterranean olive cultivars of each country, i.e., Italy, Spain and Greece, inter-cultivar variation was easily detected and they were clearly distinct from Iranian and Syrian cultivars, excepting for 'Ascolana', 'Amphissis' and 'Moraiollo'. Four of five cultivars of Spain were grouped in a cluster (cluster III) in which no Italian cultivars were placed. Four of seven cultivars of Italy were grouped in cluster IV. 'Moraiolo' and 'Ascolana' were placed in cluster I, whereas 'Coratina' was placed in cluster II. This result supports the distance of Italian olive cultivars from Spain olives that have been already reported using different molecular markers (Besnard and Berville, 2000; Owen *et al.* 2005; Bracci *et al*. 2009).

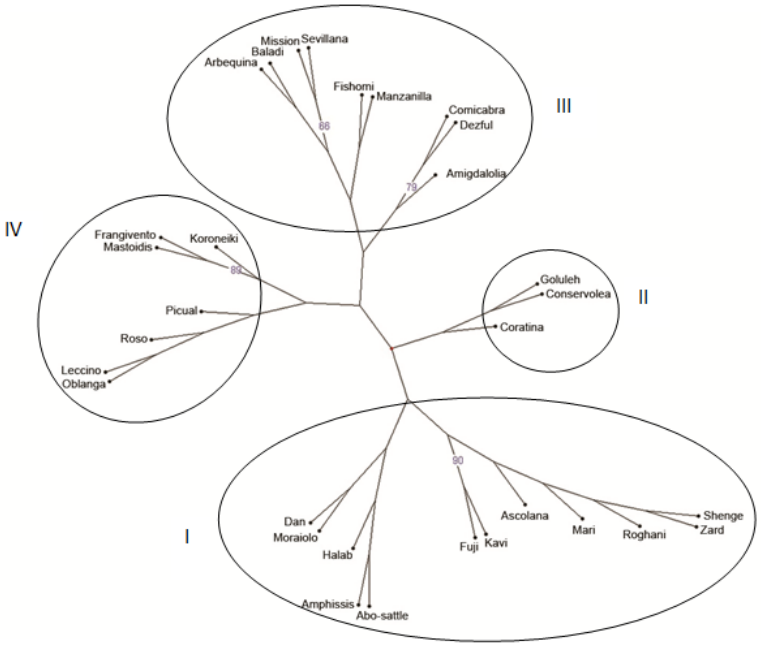

Fig.3. Phylogenetic tree for 31 olive cultivars used in this study based on Jaccard coefficient and obtained from ISSR data. Bootstrap values more than 50 are indicated.

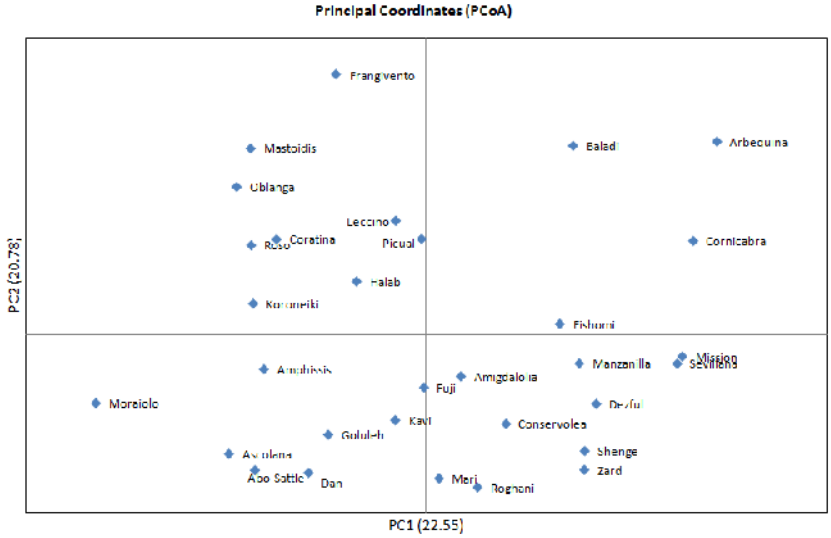

Fig. 4. Two-dimensional plot of the Principal Coordinates analysis (PCoA) of ISSR data includes 31 olive cultivars.

Of thirty-one olive cultivars analyzed in this study, Greece cultivars ('Amigdalolia', 'Amphissis', 'Conservolea', 'Mastoidis' and 'Koroneiki') were the most variable as they scattered on all four clusters of the dendrogram. This finding is congruent with the results of previous studies in which based on SSR, RAPD and ISSR no particular clustering was found for Greece olive cultivars due to high genetic variation among them (Belaj *et al.* 2002; Essadki *et al*. 2006; Omrani-Sabbaghi *et al.* 2007).

Four main Iranian olive cultivars, 'Mari', 'Roghani', 'Zard' and 'Shenge', belonging to the northern provinces of the country (Gilan, Zanjan, Ghazvin and Golestan) were clustered together, whereas each of other three Iranian cultivars 'Goluleh', 'Dezful' and 'Fishomi', were placed in different clusters of the dendrogram. The cultivar 'Dezful' which is belonged to the south of Iran was also remote from the cultivars of north provinces of the country based on SSR markers (Noormohammadi *et al.* 2007). Iranian olive cultivars revealed more affinity to Syrian cultivars than to Mediterranean cultivars (cluster I of the dendrogram).

The principal coordinate analysis (PCoA) explained 22.55% and 20.78% of the total variation along the first (PC1) and second (PC2) component, respectively. In general, the PCoA plot revealed same affinities for the cultivars as the phylogenetic tree (Fig. 4). In this plot the cultivar 'Dezful' placed closer to four main cultivars of Iran, 'Mari', 'Roghani', 'Zard' and 'Shenge'.

The divergence of 'Golule' cultivar from other Iranian olive cultivars, found by ISSR analysis here, is in agreement with its distinct and unique fruit morphology. Comparing to other Iranian olive cultivars which have mostly ovoid fruit shape, 'Goluleh' has mostly spherical or round fruit shape (Hosseini-Mazinani *et al.* 2013). This congruity between fruit morphology and ISSR data of this study was not found for 'Mari' cultivar. In point of fruit morphology 'Mari' cultivar is also unique by having an elongated fruit, well distinguishable from other Iranian olive cultivars, however; by ISSR analysis here it showed affinity to 'Roghani', 'Zard' and 'Shenge' cultivars.

Hosseini-Mazinani *et al.* 2014; suggested that Iranian cultivars and ecotypes of olive, clustered separately from Mediterranean cultivars using SSR markers, and showed a high number of private alleles.In another study on genetic diversity of Iranian olive collections, most of the Iranian olive accessions were clustered to a main distinct group separated from Syrian and other introduced cultivars (Omrani-Sabbaghi *et al.* 2007).The results of these researches along with the result of the present study could show that a Mediterranean-Middle East divergence of olive cultivars occurred.

Relatively few ISSR primers were used in our study, nevertheless; high genetic variation could be distinguished among the olive cultivars preserved in Ali-Abad germplasm in the north of Iran. The outcome of this study could be useful for construction of a molecular database of the germplasm.

Collecting of different olive cultivars having high genetic variation in a germplasm, provides an opportunity not only to develop breeding programs but also to select tolerant genotypes to biotic and abiotic restricts. For instance the cultivars of this germplasm at the same time can be evaluated to dryness occurring frequently in the summers of the region. Being genetically different, it is anticipated that the cultivars will react differently to this environmental restrict. As Iran is a dry country, selecting olive cultivars more tolerant to dryness is highly valuable.

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