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Use of Microsatellite Markers for Genetic Diversity Analysis of Olive Germplasm in the North of Iran

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ABSTRACT: Olive (*Olea europaea* L.) is one of the subtropical fruit tree with remarkable economic importance. This study was conducted to evaluate the variation and inter-relationship of 31 olive genotypes including seven Iranian and 24 foreign genotypes collected in a germplasm using Simple Sequence Repeat (SSR) markers. In this research, 7 SSR primer pairs out of 17 SSRs were used for fingerprinting of the genotypes. Totally, 37 SSR polymorphic alleles were detected. Classification of genotypes based on UPGMA criterion located them in three major groups. In this study, Iranian cultivars and ecotypes of olive, clustered separately from Mediterranean cultivars. This is concluded that SSR markers could efficiently clarify the existent genetic variability in olive, and the identified genetic variability is somewhat in coincidence with the geographical distribution of olive genotypes.

Keywords: Olive, Simple sequence repeat, similarity, cluster analysis

INTRODUCTION

Olive (*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 is due to the long history of olive cultivation in this part of the world (Dastkar *et al*. 2013). Vavilov (1950) considered Iran one of the olive's main centers of origin and diversification in the world. 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).

The study of genetic diversity of olive is of interest for the conservation of genetic resources, broadening of the genetic base and practical applications in breeding programs. Several characteristics such as morphological (Zaher *et al*. 2011; Dastkar *et al*. 2013), biochemical characteristics (Gomez Coca *et al*. 2014) have already been used to study the genetic variation of olive germplasm. This is noticeable that morphological characteristics usually vary with environment (Lu, 1997). Now, with the emergence of molecular markers such as simple sequence repeat markers (SSR), this is possible to evaluate genetic divergence of plant germplasm in greater detail. Molecular markers are stable and detectable in all tissues, regardless of growth, differentiation and development or stage of the cell. They are not subject to environmental, pleiotropic or epistatic effects (Agarwal *et al*. 2008; Moose and Mumm, 2008).

In this sense, several studies were developed SSR markers (Dastkar *et al*. 2013; Muzzalupo *et al*. 2014; Mnasri Rahmani *et al*. 2014) to reveal genetic diversity of olive. Muzzalupo *et al*. (2014) reported that the use of molecular markers, like microsatellite, is imperative to build a database for cultivar analysis, for the traceability of processed food, and for the appropriate management of olive germplasm collections. In the studies conducted by Roubos *et al*. (2010) the microsatellites were used 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. Same results also cached by Sarri *et al*. (2006) by using SSR markers for distinguishing 118 cultivars from different Mediterranean countries. Also, Mnasri Rahmani *et al*. (2014) using SSR markers could classify thirty Tunisian olive genotypes in three groups. There are also some reports about fingerprinting of olive germplasm from Iran using SSRs (Sheidai *et al*. 2010; Hosseini-Mazinani *et al*. 2014). Noormohammadi *et al*. (2007) reported a number of olive species and subspecies, too many local cultivars, ecotypes and natural hybrids of olive occurring in Iran. Regarding robustness of SSR marker, this study was aimed to employed SSR technique to assess the genetic variation of 31 Iranian and foreign olive cultivars.

MATERIALS AND METHODS

A. Plant material and DNA extraction

From an olive germplasm established in the north of Iran (Ali-Abad region of Gilan province) (Bahmani *et al*., 2015), 31 cultivars (seven Iranian and 24 foreign cultivars) were used (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 genotypes 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 (Khavi)	Syria	T/O
6	Fishomi	Iran	T/O	22	Leccino	Italy-Toscana	O
7	Baladi	Palestine	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. SSR assay

Seventeen SSR primer pairs were initially tested; of these, seven pairs that produced informative, easily scored and clear products were selected for genotyping the olive cultivars (Table 2). The volume for all of the PCR reaction mixtures was 15 µl, containing 20 ng of genomic DNA, $MgCl_2$ (2 mM), 0.2 mM of each dNTP (Roche), 1 U of Taq DNA polymerase (Cinna Gen, Iran) and 0.1 mM of each 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 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 seconds, an annealing temperature of 53°C for 30 seconds and 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. PCR products were separated using 6% polyacrylamide gel electrophoresis and visualized using silver staining.

C. Data analysis

For each of the primer pairs, 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. Genetic distances were calculated based on Jaccard's similarity coefficient. The cultivars were grouped by cluster analysis using the neighbor-joining method. The computer program used was DARwin5 (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

Our results of SSR analysis rely on efficiency of SSR marker in evaluation of olive germplasm and existence of suitable genetic variability in the studied olive germplasm. These findings is accommodated with findings of Noormohammadi *et al.* (2007), Corrado *et al.* (2011), Mnasri Rahmani *et al*. (2014) and Muzzalupo *et al*. (2014). In this research, 7 SSR loci out of 17 SSRs were used for olive genotypes fingerprinting. Generally, 37 alleles were identified using 7 SSR loci in studied genotypes. The number of alleles per locus varied from 3 in loci ssrOeUA-DCA3/AJ279854 and ssrOeUA-DCA5/AJ279856 to 8 in locus UDO99-011F/R with an average of 5.3 alleles (Table 2). This finding to some extent is similar to findings of Dastkar *et al*. (2013) which reported mean of 4.3 alleles per SSR locus. Oppositely, Corrado *et al*. (2011) in the study of olive varieties from southern of Italy reported high genetic variability for SSR loci and calculated 11 to 12 allele per SSR locus. Also, Abdelhamid *et al*. (2013) manifested that number of alleles per SSR locus varied between 4 and 14 with the mean of 7.3 alleles per locus in Tunisian olive cultivars. Classification of olive genotypes based on SSR data by using UPGMA clustering method separated them into three major groups which each one subdivided into some subgroups (Fig. 2). Group I was included genotypes "Mission", "Fishomi", "Sevillana", "Manzanilla", "Cornicabra", "Frangivento", "Amigdalolia", "Zard", "Kavi", "Fuji", "Mari" and "Dan". Genotypes "Roghani", "Shenge", "Goluleh", "Dezful" and "Conservolea" were located in group III. In this research, other genotypes were located in group II (Fig. 2).

Table 2: Selected primers used for SSR analyses of 31 olive genotypes.

Primer	Sequence (5'-3')	Number of alleles	Reference
ssrOeUA-DCA1	CCTCTGAAAATCTACACTCACATCC	6	Sefe *et al.*, 2000
AJ279853	ATGAACAGAAAGAAGTGAACAATGC		
ssrOeUA-DCA3	CCCAAGCGGAGGTGTATATTGTTAC	3	
AJ279854	TGCTTTTGTCGTGTTTGAGATGTTG		
ssrOeUA-DCA5	AACAAATCCCATACGAACTGCC	3	
AJ279856	CGTGTTGCTGTGAAGAAAATCG		
UDO99-009F	TTGATTTCACATTGCTGACCA	6	Cipriani *et al.*, 2002
UDO99-009R	CATAGGGAAGAGCTGCAAGG		
UDO99-011F	TGACTCCCTTTAAACTCATCAGG	8	
UDO99-011R	TGCGCATGTAGATGTGAATATG		
UDO99-014F	TTCCCCTTATTCAATGTGAACC	6	
UDO99-014R	ACTGCAGTTTGGGAATCAAA		
UDO99-024F	GGATTTATTAAAAGCAAAACATACAAA	5	
UDO99-024R	CAATAACAAATGAGCATGATAAGACA		
Total		37	

The following primer pairs were also tested:
- ssrOeUA-DCA4/ AJ279855; ssrOeUA-DCA9/ AJ279859; ssrOeUADCA13/ AJ279862 (Sefe *et al.*, 2000).
- UDO99-008F/UDO99-008R; UDO99-031F/UDO99-031R; UDO99-039F/ UDO99-039R; UDO99-043F /UDO99-043R (Cipriani *et al.*, 2002).
- EMO2/ AJ416320; EMO3/ AJ416321; EMO13/ AJ416322 (De La Rosa *et al*., 2002).

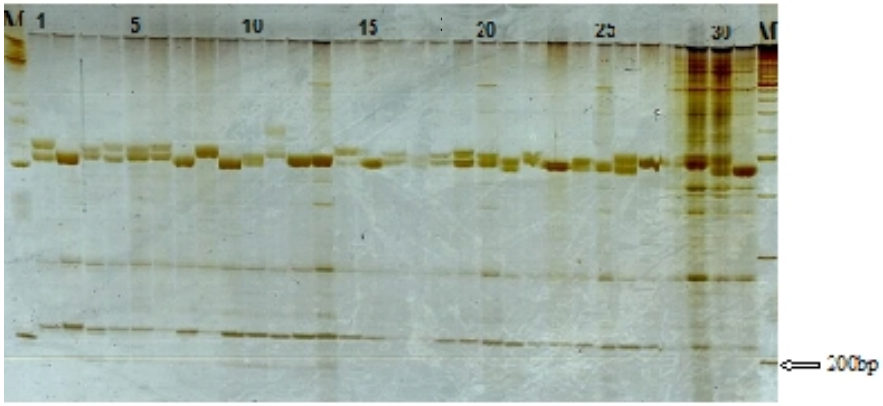

Fig. 1. Allelic variation of ssrOeUA-DCA3/AJ279854 locus detected in 31 olive cultivars. Numbers of the genotypes at the top are as same as those of Table 1. M: Size ladder.

Paralleled with the findings of Bahmani *et al*. (2015) SSR marker could distinguish Iranian olive genotypes from foreign genotypes. Regarding Fig. 2, genotypes "Zard" and "Mari" belonged to Guilan and Zanjan province of Iran were located in group I. In point of fruit morphology 'Mari' cultivar is unique by having an elongated fruit, well distinguishable from other Iranian olive cultivars, however; by SSR analysis here it showed affinity to "Zard" genotype. Results concerning to cluster analysis (Fig. 2) are in agreement with Hosseini-Mazinani *et al*. (2014) which suggested that Iranian cultivars and ecotypes of olive, clustered separately from Mediterranean cultivars using SSR marker. Concordance to previous report about this studied germplasm (Bahmani *et al*. 2015), SSR markers revealed the more affinity of Iranian olive cultivars to Syrian cultivars than to Mediterranean cultivars (cluster I of the dendrogram) (Fig. 2). This research depicted that classification of olive genotypes based on SSR markers is somewhat coincidence with the geographical distribution of genotypes. For instance, all of Italian olive genotypes were located in group II.

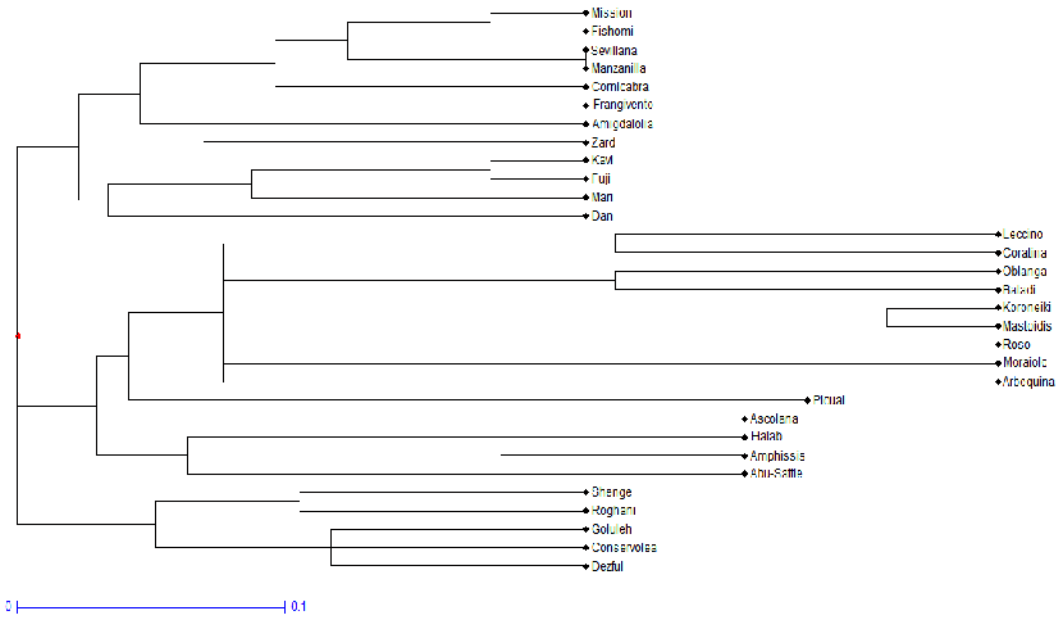

Fig. 2. Dendrogram of 31 olive genotypes analyzed based on genetic similarity. Distances were obtained using Jaccard coefficients and agglomerative analysis was performed with the UPGMA algorithm.

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