American-Eurasian J. Agric. & Environ. Sci., 14 (5): 471-475, 2014
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2014.14.05.12333

Genetic Polymorphism among Picual Olive Samples Collected from Different Locations of Egypt

[1]El Said S. Hegazi, [1]Ayman A. Hegazi and [2]Hossam A. Sayed

[1]Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]National Gene Bank and Genetic Resources, Giza, Egypt

Abstract: Molecular fingerprints of Picual olive cultivar collected from different locations of Egypt using 16 primers (inter simple sequence repeats ISSRs) were tested to explore their genetic diversity. The highest number of polymorphic bands was obtained by H15 (14 bands), while Primer 5 produced the lowest number of polymorphic bands (5 bands). The percentage of polymorphism revealed by the different primers ranged from Zero to 75 % with an average 29.18%. Among all tested ISSR primers, only 4 primers (1789-A, HB10, 5 and 4) failed to produce any unique markers, while 12 primers generated positive and /or negative unique markers that could be used to identify Picual genotypes.

Key words: Differentiation · Genetic diversity · ISSRs · *Olea europaea* · Polymorphic information content

INTRODUCTION

Identification of olive cultivars is mainly performed through the analysis of biomorphological traits. In some cases, the descriptions and measurements varied considerably due to environmental variations and human judgment. In addition to this, there were also problems related to the great number of varieties described in the species. The numerous synonyms and homonyms and the presence of cultivar groups with biomorphological traits that are highly similar but not identical, constitute the so-called "varietal populations" [1]. The introduction of molecular markers (RAPD, RFLP, AFLP and ISSR) that can ensure rapid and certain characterization has been proposed recently as an alternative to the traditionally used methods [2, 3].

Used 12 microsatellite markers to evaluate 60 olive accessions including several cultivars that were developed in Brazil the largest allelic diversity and polymorphic information contents were also found at the two markers [4]. Microsatellite markers are also known as simple sequence repeats, are co-dominantly inherited. Therefore, they are considered to be ideal genetic markers and are widely used to study the germplasm [5].

The main objective of this study is to identify polymorphism and to assess the genetic variability of Picual olive cultivar growing in different olive producing environments of Egypt.

MATERIALS AND METHODS

Plant Material: Four Picual samples were collected from different locations (Siwa Oasis, Ismailia, Alexandria - desert Road and the farm of Agriculture Research station at Faculty of Agriculture, Cairo University). DNA was extracted from very young, healthy leaf tissues collected from three trees per each cultivar. About 2-3 g of tissue was lyophilized and kept at -80°C.

DNA Isolation: DNA Isolation was performed according to Junghans and Metzlatt [6]. Method extraction buffer (pH > 8.0)

- 50 mM Tris –HCl, 100 mM NaCl, 50 mM EDTA, 0.5 % SDS, 100 ul for 100 ml Mercator ethanol were added under hood and 100 ml distilled water.
- 10 Mm TE Tris buffer (pH 8.0), 1 Mm EDTA (pH 8.0).

DNA analysis was tested using 1% agarose gel electrophoresis while its concentration was determined spectrophotometrically.

Corresponding Author: El Said S. Hegazi, Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt.

Table 1: ISSR primers names and their sequences.

Primer name	Sequence	Ta (°C)	Primer name	Sequence	Ta (°C)
17898-A	$(CA)_6$ AC	40°C	HB-13	$(GAG)_3GC$	45°C
17898-B	$(CA)_6$ GT	40°C	H15	$(GTG)_3GC$	40°C
17899-A	$(CA)_6$ AG	40°C	ISSR-1	$CAC(TCC)_5$	50°C
HB-9	$(gt)_6$ gg	40°C	ISSR-2	$AGA(TCC)_5$	50°C
HB-10	$(ga)_6$cc	40°C	842	$(GA)_8$ TG	53°C
HB-8	$(GA)_6$GG	48°C	5	$(ca)_8$ gt	49°C
HB-11	$(GT)_6$CC	48°C	4	$(ca)_8$ ac	49°C
HB-12	$(CAC)_3GC$	45°C	17899-B	$(CA)_6$ GG	42°C

Polymerase Chain Reaction: The PCR reaction was carried out in a 20-ml volume of a mixture containing 10 ng of genomic DNA, 2.5µl dNTPs (8mM mix), 3.0 µl 15mM $MgCl_2$, , 5 unit Taq polymerase (Gibco-BRL, New York, USA), 1Taq buffer (20 mMTris–HCl, pH 8.4, 50 mM KCl) and 1 mM primer. 16 ISSR primers (Table 1) were used amplification was carried out in Start gene PCR which was programmed for 30 cycles as follows: Denaturation (one cycle) 94°C for 2 minutes, followed by 30 cycles: as follows 94°C for 30 seconds, 44°C for 45 Sec, 72°C for 1 minute and 30 Sec and finally one cycle at 72°C for 20 minutes and 4°C for infinitive.

Primers products were first tested by electrophoresis of amplification products on 1.2 % agarose gels, containing ethidium bromide in 0.5 Tris–borate buffer, in order to identify those primers producing clear amplified products. The gels were examined under UV light and photographed.

Statistical Analysis: ISSR gels were scored as 0/1 for absence/presence of the bands, respectively and the resulting data were analyzed using the both SPSS and NTSYS-pc2.0 software [7]. Phylogenetic dendrogram was constructed using the UPGMA method (Unweighted Pair-Group Method with arithmetical algorithms averages [8].

RESULTS AND DISCUSSION

Polymorphisms as Detected by 5` and 3` Anchored Primers: In the present study, molecular fingerprinting of "Picual" olive cultivar using 16 inter simple sequence repeats (ISSRs) was tested to explore the genetic diversity among four Picual genotypes based on the clear scorable band patterns. Total number of amplified bands was 136 and the number of amplified DNA fragments by each primer ranged from 5-14 bands. The highest number of polymorphic bands was obtained by H15 (14 bands), while p58 produced the lowest number of polymorphic bands (5 bands). The average number of bands/ primer was 8.5 bands/ template and the approximate size of amplification product ranged from 64-1769 pb. (Fig. 1). All primers produced polymorphic bands ranging in number from 1 to 8 fragments with an average polymorphism/ primer of 2.75 (Table 2). The percentage of polymorphism revealed by the different primers ranged from zero to 75% with an average 29.18%.

Genetic Relationships as Revealed by ISSRs Markers: The genetic relationship among the four Picual genotypes were determined. The 16 used primers were tested to compute the similarity matrices according to Dice [8]. The genetic similarity matrices based on the Dice coefficient were used in the cluster analysis to generate a dendrogram using the UPGMA analysis. The genetic similarity ranged from 0.92 to 0.98 (Table 3) in the Picual genotypes. The highest genetic similarity revealed by the ISSRs analysis (0.98) was found between Picual genotype collected from field Germplasm in Faculty of Agriculture, Cairo University and Picual genotype collected from Alexandria - desert Road.

This was followed by 0.97 and 0.96 between Picual genotype [collected from Alexandria - desert Road] and Picual genotype [collected from Ismailia Gov.], Picual genotype [collected from field Germplasm Cairo Uni.] and Picual genotype [collected from Alexandria - desert Road], respectively (Table 3). On the other hand, the low percentage of similarity was found between Picual genotype [collected from field Germplasm in Cairo Univ.] and Picual genotype [collected from Siwa Oasis]. This might be due to their origin [9, 10].

Genotype Identification by ISSR Unique Markers: As shown in (Table 4), the ISSR assay permitted the identification of four Picual genotypes by unique positive and/ or negative markers. Four out of one hundred thirty six ISSRs (about 2.94%) were found to be useful as cultivar specific markers which could be distinguished as 7 unique bands for Picual genotype coming from Cairo University Faculty of Agriculture through the FAO research network, 2 unique bands for Picual genotype collecting from Alexandria- desert Road, 2 unique bands

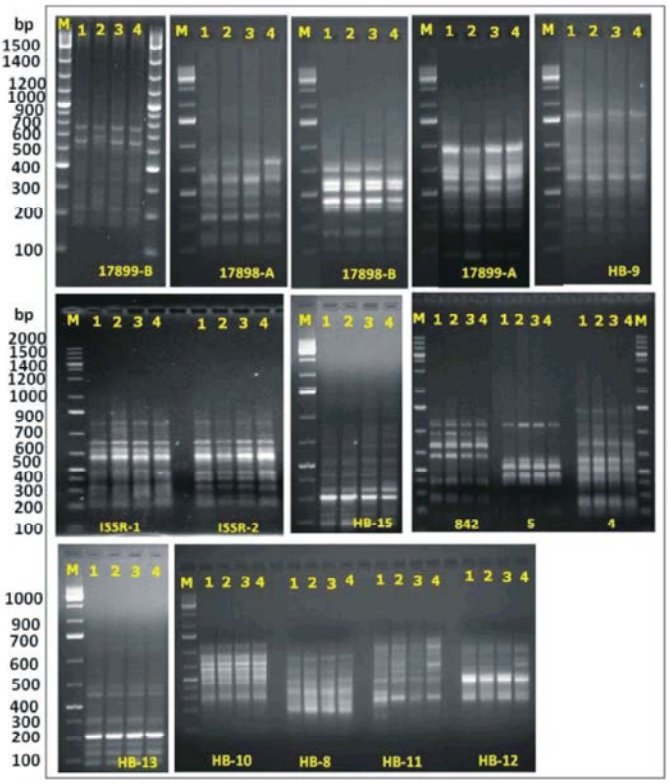

Fig. 1: ISSR banding patterns obtained from the four Picual genotypes analyzed by using 16 primers

Table 2: Total number of amplicons, size of amplified fragments, monomorphic amplicons, polymorphic amplicons and the percentage of polymorphism as revealed by ISSR markers among Picual genotypes

Primer	Total number of amplicons	Size of amplified fragments (bp)	Monomorphic amplicons	Polymorphic amplicons	Polymorphism (%)
17898-A	7	1769-237	7	-	0
17898-B	10	1143-213	7	3	30
17899-A	8	943-180	6	2	25
HB-9	7	1614-283	6	1	14.3
HB-10	7	1160-310	7	-	0
HB-8	8	919-220	2	6	75
HB-11	11	1380-166	3	8	72.7
HB-12	10	1098-288	6	4	40
HB-13	9	787-64	7	2	22.2
H15	14	841-195	6	8	57.1
ISSR-1	9	1228-206	5	4	44.4
ISSR-2	9	1228-223	8	1	11.1
842	8	1191-264	7	1	12.5
5	5	1123-400	5	-	0
4	8	1414-187	7	1	12.5
17899-B	6	773-270	3	3	50
Total	136	-	92	44	-
Average	8.5	-	5.75	2.75	29.18

Table 3: Similarity matrix among the four Picual genotypes collected from different location base on ISSR-PCR analysis

Cultivar	Picual 1	Picual 2	Picual 3	Picual 4
Picual 1	1	-	-	-
Picual 2	0.98	1	-	-
Picual 3	0.96	0.97	1	-
Picual 4	0.92	0.93	0.96	1

Table 4: Cultivars characterised by unique positive and/or negative ISSR markers, marker size and total number of markers identified each olive cultivar (based on 16 primers)

	Unique Positive			Unique Negative			
Cultivars	Primer	Marker size	Total No. of markers	Primer	Marker size	Total No. of markers	Grand Total
Picual (1)	HB-11	595 bp	3	HB-11	1380 bp	1	4
		333 bp					
		166 bp					
	ISSR-1	456 bp	1	ISSR-1	491 bp	1	2
	17899-B	656 bp	1	-	-	-	1
Picual (2)	17899-B	683 bp	1	17899-B	609 bp	1	2
Picual (3)	HB-11	815 bp	1	HB-11	744 bp	1	2
Picual (4)	17898-B	1086 bp	2	17898-B	1143 bp	1	3
		323 bp					
	17899-A	811 bp	1	17899-A	617 bp	1	2
	-	-	-	HB-9	283 bp	1	1
	HB-8	513 bp	2	HB-8	638 bp	2	4
		260 bp			367 bp		
	HB-12	808 bp	2	HB-12	751 bp	2	4
		700 bp			642 bp		
	HB-13	283 bp	1	HB-13	302 bp	1	2
	H15	261 bp	1	H15	276 bp	1	2
	ISSR-1	323 bp	1	ISSR-1	206 bp	1	2
	-	-	-	ISSR-2	611 bp	1	1
	842	872 bp	1	-	-	-	1
Total			18			15	33

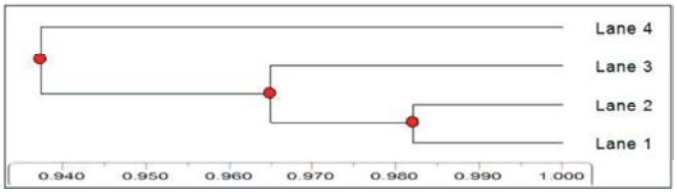

Fig. 2: UPGMA dendrogram based on the proportion of shared ISSR fragments obtained by using 16 primers in the total DNA of 4 Picual cultivars

for Picual genotype collecting from Ismailia Governate and 22 unique bands for Picual genotype collecting from Siwa Oasis; some of them were present in one cultivar and absent in the other cultivars or vice versa. The number of ISSR- PCR fragments generated by using the 16 primers could be used as cultivar specific markers. The ISSR markers generating primers and the positive and/ or negative markers approximate size are shown in (Table 4). Among all tested ISSR primers, only 4 primers (17898-A, HB10, 5 and 4) failed to produce any unique markers, while 12 primers, generated positive and/ or negative unique markers, that could be used to identify Picual genotypes with the percent of 75%. The number of generated unique markers ranged from 2 to 22 markers. The maximum number of unique markers was identified with the Picual genotype (4) [collecting from Siwa Oasis]. On the other hand, Picual genotype (2) and Picual genotype (3) were only characterized by unique positive bands and a negative one.

Cluster Analysis as Revealed by ISSR Markers: The UPGMA cluster analysis of genetic distance among different Picual genotypes is shown in Fig. (2). Phylogenetic analysis showed high degree of genetic variation among genotypes of different locations. However, interrelated genotypes of the same genotype Picual (1) and Picual (2) were grouped in the same clusters. The dendrogram ranked different genotypes into two major clusters at 93% level of similarity, the first included Picual 4 [collecting from Siwa Oasis] while, the second comprised Picual 3, Picual 2 and Picual 1 genotypes. Picual 3 and both Picual 2 and Picual 1 were further clustered into two separated lineages at 97% level of similarity. The highest genetic similarity (98%) was detected between the two genotypes of Picual 1 and Picual 2. On the other hand, the genetic variation between Picual 1 and Picual 4 genotypes was higher than Picual 1 and Picual 3 genotypes by 92% and 96%. The results obtained demonstrate that varietal groups with the same

name are not actually the same cultivars, even though, based on an analysis of similarity index and the level of difference among the genetic similarity values are low. The grouping obtained by Vergari *et al.* [10] doesn't reveal any relationship among 15 "Manzanillo" genotypes analyzed, either in terms of morphology or geographic origin. Moreover, Lavee *et al.* [11] reported that a cluster analysis of eight "Nabali" types using the results of 17 OPN primers showed that the eight types selected were genetically different through a relatively high similarity found for some. The genetic variability found in the population of the "Nabali" trees in the West Bank justifies an extensive "clonal" selection.

CONCLUSION

ISSR is a powerful tool for the identification of olive cultivars. The primers H15, HB11, HB12 and 17898-B generated the ISSR bands with high degrees of polymorphism. Using these bands, all of the Picual genotypes of olive studies were differentiated. Primer H15 was better than HB11, HB12 and 17898-B since it yielded more bands which were polymorphic. Our work shows that it is possible to obtain highly discriminating results with only 4 primers (H15, HB11, HB12 and 17898-B), which we will utilize for future analyses of new olive accessions.

REFERENCES

1. Fontanazza, G., 1993. Olivicoltura intensiva Meccanizzata. Edagricole, Bologna, Italy, pp: 312.
2. Angiolillo, A., L. Baldoni and A.G. Abbot, 1996. Relazione filogenetiche tra Varieta Coltivate di Olivo (*Olea europaea* L.) ed Altre Specie Affini Medianate Useo di Marcatori AFLP Comparazione Della Sequenza SAD. Atti Congresso Siga.
3. Vergari, G., M. Patumi and G. Fontanazza, 1996. Utilizzo Dei Marcatori RAPDs Nella Caratterizzazione Del Germoplasm Di Olivo. Olivae, 60: 19-22.
4. Val, A.D.B., J.L. Ferreira, J. Vieira Neto, M. Pasqual, A.F. De Oliveira, A. Borem and G.M.A. Cançado, 2012. Genetic diversity of Brazilian and introduced olive germplasm based on microsatellite markers. Genetics and Molecular Research, 11(1): 556-571.
5. Cadulen, T., M. Morchen, C. Blassiau and A. Clabaut, 2010. Development of SSR markers and construction of a consensus genetic map for chicory (*Cichorium intybus* L.). Mol. Breed., 25: 699-722.
6. Junghans, A. and M. Metzlatt, 1990. A simple and rapid method for the preparation of total plant DNA. Biotechniques, 8: 176.
7. Rohlf, F.J., 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology, 47: 147-158.
8. Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy- the principles and practice of numerical classification. (W.H. Freeman: San Francisco.), pp: 573.
9. Terzopoulos, P.J., B. Kolano, P.J. Bebeli, P.J. Kaltsikes and I. Metzidakis, 2005. Identification of *Olea europaea* L. Cultivars using Inter-Simple Sequence Repeat Markers. Sci. Hortic., 105: 45-51.
10. Vergari, G., M. Patumi and G. Fontanazza, 1999. Varietals Discrimination of 15 genotypes of olive (*Olea europaea* L.) belonging To CV. Manzanilla. Act. Hort., 474: 141-145.
11. Lavee, S., N. Avidan and Wiesman, 1999. Genetic variation within the nabali baladi cultivars of west bank. Acta. Hort., 474: 129-132.