Assessment of inter- and intra-variations in some olive (*Olea europaea*) cultivars using molecular markers (ISSR and SCoT)

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ABSTRACT

Genetic variations among and within 15 cultivated olive (Olea europaea L.) genotypes distributed in three farms in El-Arish, Egypt were screened using two PCR-based molecular markers; Inter Simple Sequence Repeat (ISSR) and Start codon targeted (SCoT). Nine ISSR primers and 15 SCoT primers successfully produced 170 (89%) and 347 (91.1%) polymorphic bands. Fourty five and 108 positive unique cultivar-specific bands were generated by ISSR and SCoT markers, respectively. ISSR 1 and ISSR 18 could characterize genotypes within Chemlali population. While, within Manzanillo population, genotypes were differentiated using ISSR 20. On the other hand, SCoT2, SCoT4 and SCoT13 could successfully differentiate genotypes within Picual population. Moreover, Coratina population was identified by SCoT14 and SCoT22 primers. PIC values, Nei's gene diversity and Shannon index were 0.21, 1.27 and 0.31, respectively for ISSR marker compared to 0.20, 0.17 and 0.29 respectively for SCoT marker. Picual genotypes had the highest values of Na (1.29), Ne (1.17), H (0.10) and I (0.16) and Koroneiki population showed the lowest values for these parameters (1.20, 1.11, 0.07 and 0.11), respectively. Analysis of molecular variance (AMOVA) showed significant genetic variations among (48%) and within (52%) olive populations. Dendrogram obtained by UPGMA clustering confirmed the genetic relationships among as well as between olive cultivars. This study suggests that combining genetic variations data resulting from ISSR and SCoT have a higher potentiality in assessing intra- and inter- varietal variations in olive genotypes supporting continued progress in olive breeding strategies in Egypt.

Keywords: *ISSR, SCoT, inter- intra variations, Olea europaea, Egypt.*

INTRODUCTION

Olive (*Olea europaea* L.) is one of the most economically important subtropical fruit tree species which is located mainly in Mediterranean basin (FAO, 2018). The unique group of Egyptian olive genotypes had been cultivated more than 1800 BC. While some cultivars are of known origin, others were not assigned to certain groups (Newton *et al*. 2006). Although Egypt has a comparative production advantage of olive crop generating a distinct economic return, Egypt ranked tenth in the global ranking of the countries producing olive oil. Since the beginning of olive domestication, breeders propagated it vegetative to keep the best combination of genes that arose by random

crosses or mutations, which led to genetic erosion. Also, the long history of olive with little information is offered on the genetic background of domestic olive genotypes, in addition to numerous cases of homonymy (one denomination for several genotypes) and synonymy (one genotype with several denominations) (Gomes *et al*., 2012). This limitation decreased the value and the usefulness of olive genetic resources and their use (Rashed, 2017). Therefore, an efficient and rapid characterization method is essential prerequisite to a proper and wide utilization of the olive genetic resources, this is the first step toward sustainable production, through the direct use or in breeding programs.

Classical olive certification system is based on morphological and agronomic procedures which are affected by environmental conditions, and mislabeling accessions can negatively affect certification of olive products (Hannachi *et al*., 2008). Molecular marker systems are efficient and rapid discriminatory methods to determine the inter and intra-variation of olive cultivars. (Muzzalupo *et al*., 2010; Ipek *et al*., 2012; Salameh *et al*., 2018).

Molecular markers have been successfully used in cultivars characterization (Noor Mohammadi *et al.,* 2012; Chegini *et al*.,2016). Among the polymerase chain reaction (PCR)-based marker techniques, inter-simple sequence repeats (ISSR) are one of the simplest and widely used markers., Linos *et al*., 2014. Also, Start Codon Targeted (SCoT) (Collard and Mackill, 2009) is dominant DNA markers with higher polymorphism due to SCoT primers are based on conserved regions flanking the initiation codon sequences of genes. A combination of diverse molecular marker types is the best to provide an accurate assessment of the extent of intra-and inter-population genetic diversity especially that are at risk of decline, besides prevent misidentification of olive cultivars and to minimize intra-cultivar variation. Therefore, certified olive propagation materials by markers should be used during the establishment of new olive orchards.

Main objectives of the present investigation were: to assist the extent of inter- and intra-cultivar variations among and between 15 genotypes belonging to the 5 endemic cultivars in North Sinai, El Arish and to develop cultivar- specific molecular markers characterizing each cultivar and genotype to figure out the genetic relationship between them to put an essential step towards optimized conservation of these cultivars that located in remote regions to support olive breeding program for newly reclaimed regions

MATERIALS AND METHODS

Plant material

For this study, we selected 15 olive genotypes (20 – 25 years old) belonging to the five most important endemic olive cultivars that are cultivated in El-Arish city, North Sinai, Egypt. (Table 1). A random sample of 50 healthy fresh young leaves were collected from each of the selected genotypes. The collected leaf samples were carried out in spring 2018.

Table (1): Number of samples,olive cultivar name, geographic origin and use of fruit.

No.	Cultivar	Geographic origin	Use of fruit
1	Coratina	Italy	Oil
2	Coratina	Italy	Oil
3	Coratina	Italy	Oil
4	Picual	Spain	Table
5	Picual	Spain	Table
6	Picual	Spain	Table
7	Koroneiki	Greece	Oil
8	Koroneiki	Greece	Oil
9	Koroneiki	Greece	Oil
10	Manzanillo	Spain	Table & Oil
11	Manzanillo	Spain	Table & Oil
12	Manzanillo	Spain	Table & Oil
13	Chemlali	Tunisia	Oil
14	Chemlali	Tunisia	Oil
15	Chemlali	Tunisia	Oil

DNA isolation

Total genomic DNA was extracted from 100 mg fresh leaves after ground in liquid nitrogen using QIAGEN™ DNeasy plant mini kit. Thermo Scientific™ NanoDrop 2000 used to quantify and assess the purity of DNA.

ISSR and SCOT analysis

A total of nine ISSR primers and fifteen SCOT primers (Table 2 and 3) were used to characterize inter and intra genetic variations of the selected fifteen Olive genotypes.

PCR Reaction

Amplification reactions were carried out using GoTaq® Master Mix-Promega Corporation in 25 µl volume containing 30 ng DNA, 1 µM single primer, 1x PCR reaction buffer, 1.7 mM $MgCl_2$, 200 µM dNTPs mix and 1.5 units GoTaq® DNA polymerase. Amplification was carried out in a Gene Amp® PCR System 9700 thermal cycler (Applied Biosystems). The following steps were used for ISSR reaction: 1 initial denaturation cycle for 4 min at 94°C, followed by 45 cycles of 40 s at 94°C, 50 s at 55°C and 60 s at 72°C, and a final cycle of 10 min at 72°C. The SCoT amplifications were carried out with a preliminary cycle of 4 min at 94°C, followed by 40 cycles of 40s at 94°C, 50s at 50°C, and 60s at 72°C, and a final cycle of 10 min at 72°C. The used ISSR and SCOT primers and their sequences are shown in Tables 2 and 3. PCR products were separated by electrophoresis on1.5% agarose gel in 1x TAE buffer, stained by ethidium bromide and visualized under UV light. 1k DNA ladders were used to fragment size. Imaging for gels was carried out by Bio-Rad™ Molecular Imager® Gel Doc™ XR+ System. The amplification products were analyzed by Image Lab™ Software.

Statistical analysis

Molecular markers (ISSR and SCoT) results were scored as a binary data matrix presence (1) or absent (0) using Image Lab™ Software. Then they were used to estimate genetic similarities using Dice coefficient (Nei and Li, 1979). The dendrograms were constructed using similarity coefficients by mean of the unweighted pair group method of arithmetic means (UPGMA) implemented in SPSS v16. POPGENE software v1.32 (Yeh *et al*., 1997) were used to estimate of observed number of alleles (Na) and effective number of alleles (Ne) (Hartl and Clark,1989); Nei's gene diversity (H) and Shannon's information index

(Shannon, 1949) for loci and also for each population separately. Inter-population genetic diversity was determined by Nei's gene diversity (H). H was calculated at the population (Hpop) and species levels (Hsp) (Nei, 1973). 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. Analysis of molecular variance (AMOVA) was performed to reveal significant genetic differences between populations using GenAlex 6.4 (Peakall and Smouse, 2006). The Polymorphic Information Content (PIC) was calculated by Power Marker program v 3.25 according to Botstein *et al*. (1980).

RESULTS AND DISCUSSION

ISSR polymorphism

ISSR primers produced a total of 191 bands, of which 170 were polymorphic with fragment sizes ranged from 250 to 2500 (Fig. 1). The number of polymorphic bands ranged from 6 (ISSR 2) to 37 (ISSR 11) with an average number of 18.9 bands per primer (Table 2). These results agree with Kaya (2015); Mohamed *et al*. (2017); Rashed (2017) and Sesli and Yegenoglu (2017). The polymorphism percentage ranged from 56.2% with primer (ISSR 20) to 100% with primer (ISSR 18) with an average of 89% across all loci. This result reveals the high degree of inter-varietal genetic polymorphism at olive cultivars tested that stated. These results coordinate with that of Martins-Lopes *et al*. (2007) (88%); Gomes *et al*. (2009) (79%); Hegazi *et al*. (2012); Noormohammadi *et al*. (2012) (81.74%); Zhan *et al.* (2015) (91.8%) and Abood *et al*. (2017) (86.44%). The differences of polymorphism rates would be attributed to germplasm origin as well as to the number of primers used in the corresponding study. The high level of diversity between olive cultivars is probably due to the diverse germplasm origin, that most likely results from cross between wild and cultivated olives resulting in new cultivars in different parts of the Mediterranean, and low breeding pressures (Martins-Lopes *et al*., 2007; Kaya *et al*., 2015 and Ben Mohamed *et al*., 2016).

Unique banding patterns obtained by ISSR markers gave unique fingerprinting or genetic profiling that used to characterize the studied 15 genotypes. Interestingly, Contreras and Tapia, (2016) obtained the genetic profile of the INIA Sevillana variety (Azapa) compared to six olive varieties based on the ISSR marker that confirming the validity of the method to differentiate the Sevillana variety (Azapa). Moreover, eight ISSR markers successfully generated 45 positive unique bands that differentiated between the 15 olive genotypes studied. These unique markers are useful as cultivar-specific ones. The highest number of unique markers (10) was detected by both ISSR 1 and ISSR18 while genotype 14 (Chemlali) had the highest number (7) of unique markers. While only two ISSR primers (ISSR 5 and ISSR 18) could characterize twelve out of the fifteen cultivars. Also, ISSR12 is a cultivar-specific marker (cultivar 13). Moreover, ISSR 14 is a cultivar-specific positive marker (cultivar 6). Also, ISSR1and ISSR18 each could characterize intra genetic variations between the three Chemlali genetypes. Manzanillo genotypes were differentiated using ISSR 20. Thus, ISSR marker is a powerful tool for olive inter and intra varietal identification and selection of optimal genotypes to encourage continued development in olive breeding strategies, which is consistent with other studies of genetic polymorphism in different olive cultivars using ISSR markers, (Gemas *et al*., 2004; El Saied *et al*., 2012; Zhan *et al*., 2015; Chegini *et al*., 2016 and Sesli and Yegenoglu, 2017).

Table (2): ISSR Primers used for analysis with total number of bands, polymorphic bands, and % of polymorphism.

Primer	Primer´s Sequence (5´- 3´)	Total no. of bands	Polymorphic bands	% Polymorphism	Unique markers	genotype
ISSR 1	(AG)8YC	29	26	89.6	10	3,7,10,13,14,15
ISSR 2	(AG)8YG	9	6	66.6	-	
ISSR 5	(GT)8YG	20	19	95	7	1,2,4,10,11,12
ISSR 11	(AC)8YA-	39	37	94.4	4	1,4,14
ISSR 12	(AC)8YC	14	12	85.7	1	13
ISSR 13	(AG)8YT	22	20	90.9	3	4,8,9
ISSR 14	(CTC)5TT	8	7	87.5	6	6
ISSR 18	HVH(CA)7T	34	34	100	10	4,6,8,10,13,14,15
ISSR 20	HVHT(GT)7	16	9	56.2	4	5,10,11,12
		191	170	89%	45	

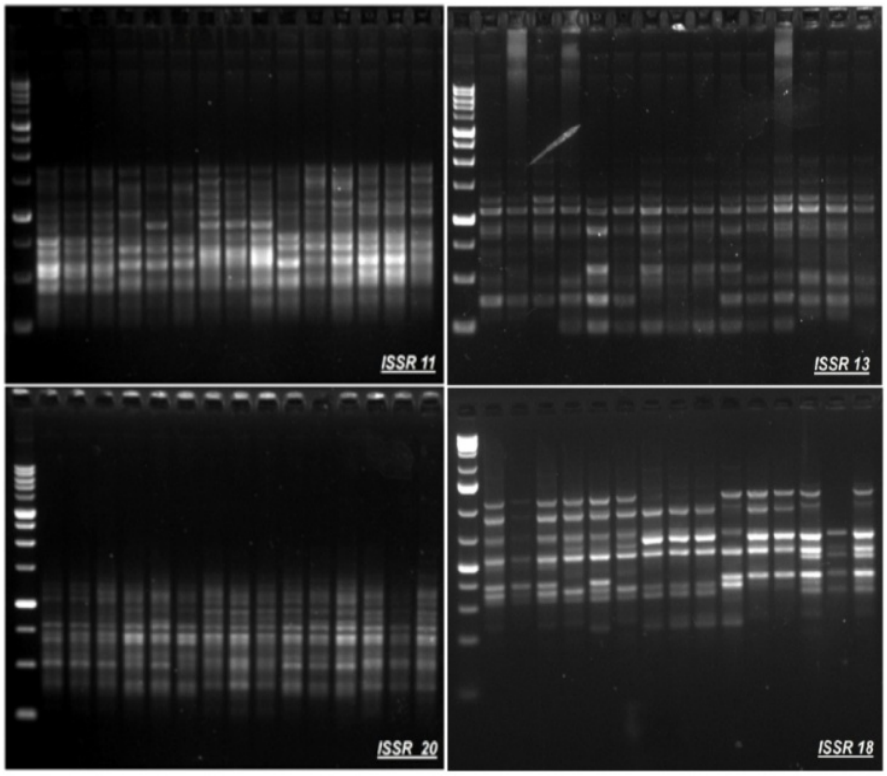

Fig. (1): ISSR marker profiles produced by primers ISSR 11, ISSR 12, ISSR 18 and ISSR 28 among 15 olive genotypes listed in Table 1.

SCoT polymorphism

A total of 381 bands were detected among fifteen olive genotypes using fifteen SCoT markers Table (3). The total number of amplified SCot bands varied from 8 (SCOT 11) to 48 (SCOT 13) with an average of 25.4 bands per genotype which is quite similar to the results obtained working on different plant species; olive (Mohamed *et al*., 2017), mango (Luo *et al*., 2010), grape (Zhang *et al*., 2015), Cicer species (Amirmoradi *et al*., 2012), date palm (Adawy *et al*., 2014) as well as on wild Salvia (Etminan *et al*., 2018). In this study, the total polymorphic bands were 347 (91.1%) with an average number of 23.13 bands per primer. Along with the obtained results, similar results were reported by Zhang *et al*., (2015) (91.91%) working on *Elymus sibiricu* and higher than that obtained by Shahlaei *et al*., (2014) (36.14%) working on tomato, but lower than that of Mohamed *et al*., (2017) (97.52 %) from studying the 9 olive cultivars, Que *et al*., (2014) (92.85%) working on Chinese sugarcane, Elframawy *et al*., 2016 (97.10%) studying *Atriplex halimus*. Results showed a minimum of 5 and a maximum of 48 bands (primers SCoT 11 and SCoT 13) respectively. Two primers (SCoT 13 and SCoT 22) showed a polymorphism of 100%, six revealed higher levels of polymorphism (>90%), four were very informative (>80%) and two were comparatively less informative (62.5 and 72.7%) with primers SCOT 11 and SCoT 36. The overall size of the amplified products ranged from 200 to 3000bp. A representative amplification profile generated is represented in Figure 2 (SCoT2, SCoT13, SCoT14 and SCoT4). These findings agree with that obtained studying different plant species (Guo *et al*., 2012; Shahlaei *et al*., 2014; Mohamed *et al*., 2017; Agarwal *et al*., 2018 and Etminan *et al*., 2018).

The unique markers were used in genotype identification and in generating a unique fingerprint for each genotype. Results presented in Table (3) showed that SCoT primers used revealed a total of 108 positive unique markers as cultivar-specific for the 15 olive genotypes. They successfully gave a unique fingerprint for each genotype. SCoT 13 gave the highest unique number (24 unique markers) and SCoT 11and SCoT 39 each as the lowest number (1) was cultivar-specific for genotype 4 (Picual) genotype 1 (Coratina) and genotype 9. While, genotype 4 (Picual) was characterized by the highest number of unique markers (13) but genotype 12 was characterized by only one unique marker. Interestingly, among the 5 olive populations, SCoT2, SCoT4 and SCoT13 successfully differentiated tree olive genotypes (No.4, 5 and6) belonging to Picual cultivar by three different unique markers at different molecular weight. Also, other three different unique markers identified three genotypes (No.1, 2 and 3) belonging to Coratina cultivar by SCoT14 and SCoT22. Moreover, the three Chemlali cultivars could be discriminated by two primers (SCoT10 and SCoT14). These specific unique markers proved the presence of intra-specific variations in olive cultivars that would assist in selection and identification of different cultivars. These results are in accordance with Sheidai *et al.,* (2010).

Table (3): SCOT primers used for analysis with total number of bands, polymorphic bands, % of polymorphism, No. of positive unique marker and genotypes.

Primer	Primer´s Sequence (5´- 3´)	Total bands	polymorphic bands	% of Polymorphism	No. positive Unique marker	genotype
SCOT 01	CAACAATGGCTACCACCA	42	37	88.1	8	1,4,5,7,9,15
SCOT 02	CAACAATGGCTACCACCC	19	17	89.4	6	3,4,5,6,9,10
SCOT 04	CAACAATGGCTACCACCT	47	44	93.6	8	4,5,6,9,13,14
SCOT 10		21	14	66.6	5	9,13,14,15
SCOT 11	AAGCAATGGCTACCACCA	8	5	62.5	1	4
SCOT 12	ACGACATGGCGACCAACG	19	16	84.2	2	3,4
SCOT 13	ACGACATGGCGACCATCG	48	48	100	24	1,3,4,5,6,7,11,12,14, 15
SCOT 14	ACGACATGGCGACCACGC	41	40	97.5	23	1,2,3,4,5,7,8,10,11,1 3,14,15
SCOT 16	ACCATGGCTACCACCGAC	11	10	90.9	1	1
SCOT 20	ACCATGGCTACCACCGCG	40	39	97.5	10	4,8,11,13,14
SCOT 22	AACCATGGCTACCACCAC	21	21	100	10	1,2,3,5,6,7,10,15
SCOT 24		14	13	92.8	2	4,11
SCOT 33	CCATGGCTACCACCGCAG	17	15	88.8	2	5,15
SCOT 35	CATGGCTACCACCGGCCC	22	20	90.9	5	6,9,10
SCOT 36	GCAACAATGGCTACCAC	11	8	72.7	1	9
Total		381	347	91.1	108	

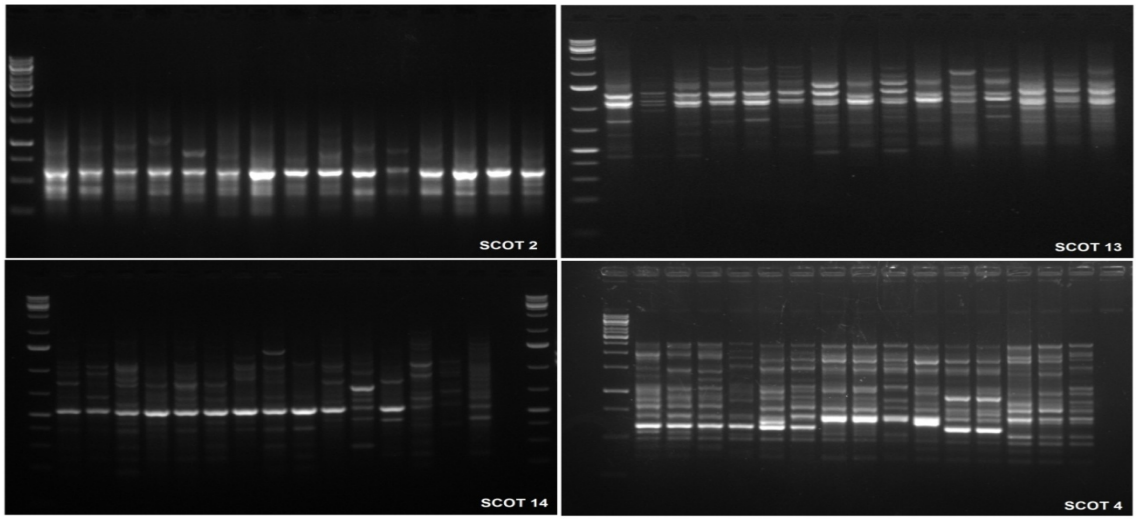

Fig. (2): SCoT marker profiles were amplified bySCoT 2, SCoT4, SCoT13 and SCoT14 among 15 olive genotypes listed in Table 1.

Intra- and inter-population diversity

The mean values of the observed alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H) and Shannon index (I) was determined within and among populations (Tables 4 and 5). ISSR marker gave the mean values of Na, Ne, H and I for all the primers among populations (1.93, 1.27, 1.18 and 0.31, respectively compared to scot marker (1.90, 1.25, 1.17 and 0.29, respectively). While,

mean PIC values of ISSR and SCoT were 0.21 and .0.20, respectively. The low genetic variation obtained in this study could be due to the predominant propagation method for olive is grafting is self pollinated cultivar. Therefore, the maintenance of cultivar is expected and could lead to minimize the genetic diversity within olive trees. These findings are similar to previous reported by Salameh *et al.,* (2018).

Within population genetic diversity for ISSR and SCOT, Picual population had the highest value of Na, Ne, H and I and the Koroneiki population had the lowest values for these parameters. Similarly, the highest (29.02%) and lowest (19.58 %) polymorphism were also detected for these two populations. Based on combined ISSR and SCoT markers, the highest value of Hpop/Hsp as the indication of genetic diversity within populations was found for Picual population, Table 5. The highest genetic diversities within (Hpop/Hsp) and among (Hsp-Hpop/Hsp) populations for ISSR data were also observed in Picual and Koroneiki populations, respectively. Some specific ISSR bands were observed in the populations; ISSR1and ISSR18 markers were specific for Chemlali population. While, Manzanillo population was differentiated by ISSR 20. On the other hand, SCoT2, SCoT4 and SCoT13 successfully differentiated Picual population and Coratina population were identified by SCoT14 and SCoT22. As well, Chemlali cultivars could be discriminated by two primers (SCoT10 and SCoT14). Nei's genetic distance determined between pairs of five olive populations studied for ISSR and SCoT data (Table 7) revealed that Coratina and Chemlali populations had the greatest genetic distances (0.61) at both molecular markers, while Picual and Koroneiki populations had the shortest genetic distances (0.34).

Analysis of molecular variance (AMOVA) carried out for ISSR and SCoT data (Table 6) showed significant differences ($P < 0.001$) among and between the populations studied for both molecular markers which, identified significant variability among populations (48%) along with (52%) within-population genetic variability, which proved intra-variation within populations. Previous studies by Contreras *et al*. 2018 obtained Low levels of genetic variability (21%) between populations of Huasco and Azapa centennial trees using AMOVA analysis for ISSR and RAPD while, the total genetic diversity was attributed to differences between individuals within these populations (79%).

These results indicated that both markers enabled discrimination between closely related cultivars. The previous studies (Hannachi *et al*., 2008; Muzzalopo *et al*., 2010; Kaya, 2015; Ben Mohamed *et al*., 2016) reported that, intra cultivar variation was probably due to the somatic mutations, cross-pollination; the presence of a high level of homonym.

Table (4): Genetic variability parameters among 15 samples olive listed in Table (1) detected by SCoT and ISSR.

Parameters	**ISSR**	**SCoT**
Number of assay units	9 primers	15 primers
Total no. of bands	191	381
No. of polymorphic bands	170	347
No. of bands per assay unit	21.2	25.4
Percentage of polymorphic bands	89	91.1
Observed number of alleles ($n_a \pm SD$)	1.93±0.26	1.90±0.30
Effective number of alleles ($n_e \pm SD$)	1.27±0.27	1.25±0.25
Nei´s gene diversity (H ± SD)	1.18±0.14	1.17±0.13
Shannon´s index (I ± SD)	0.31±0.19	0.29±0.18
polymorphic information content (PIC)	0.21	0.20

Table (5): Genetic diversity parameters among 5 cultivars populations based on ISSR and ScoT loci. Na = number of different alleles; Ne = number of effective alleles; I = Shannon's information index; P%= polymorphism %; Hpop and Hsp = Nei's genetic diversity for population and species levels, respectively; numbers in parentheses = standard deviation.

Cultivars	**No. of genotypes**	**Na±SE**	**Ne±SE**	**I±SE**	**He±SE**	**P%**	**Hpop/ Hsp**	**(Hsp - Hpop)/Hsp**
Coratina	3	1.2045	1.1353	0.1161	0.08	20.45 %	1.116	0.944
Picual	3	1.2902	1.1701	0.1562	0.10	29.02%	1.095	0.926
Koroneiki	3	1.1958	1.1141	0.1051	0.07	19.58 %	1.123	0.950
Manzanillo	3	1.2185	1.130	0.118	0.08	21.85 %	1.115	0.943
Chemlali	3	1.1976	1.1259	0.1103	0.07	19.76 %	1.119	0.947
Mean		1.218	1.135	0.12124	0.08	22.123%		

Table (6): Variability in olive populations under study revealed by AMOVA.

Source	df	SS	MS	Est. Var.	%
Among Pops	4	645.333	161.333	39.400	48%
Within Pops	10	431.333	43.133	43.133	52%
Total	14	1076.667		82.533	100%

Table (7): Nei's genetic distance determined between pairs of five olive populations studied for ISSR and SCoT data.

Binary data template	D (2)	Pairwise Population PhiPT Values			
Pop1	Pop2	Pop3	Pop4	Pop5	
0.000	0.107	0.080	0.104	0.101	Pop1
0.388	0.000	0.104	0.101	0.096	Pop2
0.541	0.341	0.000	0.098	0.102	Pop3
0.537	0.361	0.441	0.000	0.116	Pop4
0.607	0.491	0.551	0.438	0.000	Pop5

PhiPT Values below diagonal

Genetic relationships

Valuable bands obtained as a result of ISSR and SCoT markers were used to calculate genetic similarities using the Dice coefficient (Dice, 1945) that was subjected to the UPGMA clustering method in order to determine the grouping of different cultivars and genotypes. In the Present study, based on ISSR, SCoT and combined dendrograms, the 15 olive genotypes were split into 2 clusters and Coratina genotypes were separated in the first cluster. The second cluster was divided to two groups, Picual and Koroneiki were separated in first group, and the other group composed the genotypes related to Manzanillo, Chemlali cultivars. These findings indicated the two markers showed clear separation among genotypes that had a high degree of genetic similarities among them which is agree with reports by Hegazi *et al*. (2012) showed that a high degree of genetic similarities among the Egyptian cultivars. Also, ISSR and SCoT markers be used for cultivar identifications. In this regard, several authors reported on the usefulness of ISSR and SCoT markers for olive cultivar identifications (Essadki *et al*. 2006; Hegazi *et al*., 2012; El Saied *et al*., 2012; Zhan *et al*., 2015; Chegini *et al*., 2016; Mohamed *et al*., 2017 and Sesli and Yegenoglu, 2017).

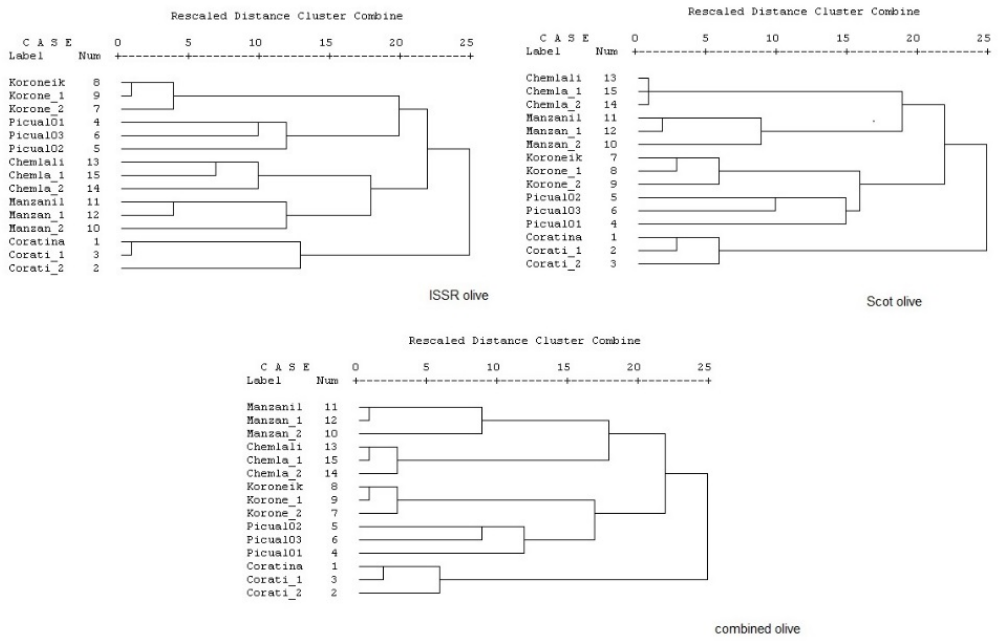

Fig. (3): Dendrograms obtained from ISSR, SCoT and combined data, respectively using the UPGMA clustering method for 15 samples olive listed in Table (1).

CONCLUSION

Molecular markers (ISSR and SCoT) are very efficient to study the variability and to assist of the extent of inter- and intra-cultivar variations in olive and determine the genetic relationship between them. intra-cultivar variation was observed for the populations studied. combined data compared to Scot and ISRR successfully split the 15 genotypes into 5 groups represents the five-cultivar population so the two markers showed clear separation among and between populations (Coratina, Picual, Koroneiki, Manzanillo, Chemlali). These findings proved inter and intra-variation within populations through genetic diversity parameters and AMOVA analysis. Such information may prove useful in the selection of optimal varietals and help promote continued progress in olive breeding strategies.

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الملخص العربي

تقييم التباين الوراثي مابين وداخل بعض أصناف الزيتون باستخدام المعلمات الجزيئية (ISSR and SCoT)

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تم دراسة التباين الوراثي بين ١٥ من الطرز الوراثية المزروعة والتي تنتمي إلى ٥ أنواع من الزيتون ، (*Olea europaea* L.) وهي Coratina، Picual، Koroneiki، Manzanillo و Chemlali والموزعة في ثلاث مناطق مختلفة في العريش، مصر بواسطة اثنين من الواسمات الجزيئية المعتمدة على تفاعل البلمرة المتسلسل؛ وهي (ISSR) و (SCoT). أنتجت ٩ من بادئات ISSR و ١٥ بادئاً من SCoT عدد ١٧٠ (٨٩٪) و ٣٤٧ (٩١.١٪) معلمات متعددة الأشكال لكل من ISSR و SCoT مما يؤكد وجود تباينًا جينيًا عاليًا بين هذه المجموعة. بالنسبة إلى ISSR ، بلغ متوسط PIC ومؤشرالتنوع الجيني Nei ومؤشر شانون ٠.٢٠٨ و ١.٢٧ و ٠.٣١ ، على التوالي مقارنة بتلك الخاصة ب SCoT والتي بلغت 0.205و ٠.١٧ و ٠.٢٩ على التوالي. بالإضافة إلى ذلك ، تم الحصول علي عدد ٤٥ و ١٠٨ معلمات موجبة مميزة بواسطة علامات ISSR و SCoT ، على التوالي و من الممكن التي تستخدم كمعلمات مميزة داخل الصنف نفسه. تم الحصول علي بعض المعلمات المميزة بواسطةISSR. كانت البادئات ISSR1 و ISSR18 مميزة لصنف الشملالي، بينما تم التمييز بين الصنف مانزانيللو بواسطة ISSR 20، من ناحية أخرى، امكن للبادئات SCoT2 و SCoT4 و SCoT13 التمييز بنجاح بين الصنف Picualوتم التمييز بين الصنف Coratina بواسطة SCoT14 و SCoT22 ، أيضًا ، استنادًا إلى المعلمات المميزة للتنوع الوراثي ضمن ال ١٥ نمطًا وراثيًا التي تنتمي إلى ال ٥ أصناف من الزيتون، كانت التراكيب الوراثية للصنف بيكوال الأعلى قيمة في Na و Ne و H و I كما سجل الصنف Koroneiki القيم الادني لهذه المعلمات. كما لوحظت أعلى تنوعات وراثية داخل (Hpop / Hsp)وبين (Hsp-Hpop / Hsp) بين الاصناف لبيانات ISSR في اصناف Picual و Koroneiki ، على التوالي. وقد حدد تحليل التباين الجزيئي (AMOVA) التباين الكبير بين الاصناف (٤٨ ٪) إلى جانب (٥٢ ٪) التباين الوراثي داخل الاصناف. كما أظهر dendrogram الناتج عن UPGMA العلاقات الوراثية بين وكذلك بين الأصناف ، بالإضافة إلى الفصل الواضح بين الاصناف. تشير هذه الدراسة إلى أن الجمع بين مزايا ISSR وتقنية SCoT يمكن أن يكون له إمكانات أعلى في تحديد أصناف الزيتون وللتباينات الموجودة داخل الأصناف والتي قد تثبت فائدتها في اختيار الأصناف الأمثل ودعم التقدم المستمر في استراتيجيات التربية للزيتون في مصر.