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MOLECULAR FINGERPRINTS OF SOME OLIVE (*Olea europaea* L.) CULTIVARS IN EGYPT

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ABSTRACT

The present investigation was conducted to develop molecular genetic fingerprints for ten olive cultivars using RAPD polymorphism and to elucidate relationships among these cultivars. Fresh and young leaf samples from each cultivar were used separately for DNA extraction. Randomly amplified polymorphic DNA, based on polymerase chain reaction (RAPD-PCR) was used. Fifteen 10-*mer* random primers were tested. However, only five of which (OPA-03, OPA-04, OPA-07, OPA-14 and OPA-16) amplified reproducible fragments. The results of RAPD analysis completely discriminated among the ten olive cultivars by one or more unique bands or unique combined class pattern. Some of the random primers (OPA-03) were more successful in identifying the cultivars than others where they generated higher number of unique bands. The similarity coefficients for all pairs of the ten olive cultivars were scored from polymorphism of RAPD analyses and were used to identify the genetic relationships among these cultivars. The obtained dendrogram separated the studied

cultivars into two categories. The first category included olive cvs. Aggezi Akssi, Aggezi Shamy, Pecual, Tofahi, Frantoio and Arbiqueen. The second category comprised olive cvs. Manzanillo, Chemlaly, Kalamata and Koronaiki. The similarity coefficient reached the highest value of 0.699 between olive cv. Aggezi Akssi and Aggezi Shamy and reached a minimum value of 0.281 between olive cv. Koronaiki and Aggezi Akssi.

Key words : *band, dna, olive cvs . polymorphism, primer, rapd.*

1. INTRODUCTION

Olives (*Olea europaea* L.) are among the oldest known cultivated trees in the world and it has been part of the Mediterranean civilization before recorded history and regarded as a symbol of peace. Olive is a typical woody species in the Mediterranean region. It belongs to the family Oleaceae. The olive is a diploid species (2n = 46 chromosomes), which comprises the cultivated type (var. *sativa*) and the wild type (var. *oleaster*). Olive trees were cultivated in Egypt thousands years ago.

Identification of plant taxa based on the phenotypic assay *i.e.* morphological characters requires extensive observation of individuals. The major limitation of these procedures is the lack of polymorphism among closely related cultivars (Wrigley *et al.*,1987).Varieties of many economically important plants including cultivated olives are numerous, but many mislabeling have occurred and many synonymous and homonymous have emerged. Also, the existence of groups of of classification and cultivar discrimination difficult(Wrigley *et al.*, 1987).

Recently, the random amplified polymorphic DNA (RAPD) cultivars with similar morphological characteristics has made the work approach was demonstrated by Williams *et al.*(1990). It is also, called AP-PCR (Arbitrarily Primer PCR) by Welsh and McClelland (1990). DNA-based genetic markers have been recently integrated into several plant systems and are expected to play a very important role in the future of molecular genetics and plant breeding. These markers, which are not affected by environmental factors or by the different phenological stages of the plant, would allow for a more correct evaluation of

genetic relationships among different cultivars and would permit genetic discrimination. In this regard, polymorphism detected by RAPD analyses facilitates identification and classification of geographically related genotypes of olive-trees (Gonzalo-Claros *et al.*, 2000), as well as analyzing genetic relationships between cultivated and wild olives (Bronzini de Caraffa *et al.*, 2002). Also, RAPD markers were used to develop genetic database for identifying olive cultivars (Guerin *et al.*, 2002). DNA polymorphism has been successfully used by many authors to identify cultivars in various fruit and crop species, including peach (Quarta *et al.*, 2001); *Prunus armeniaca* (Mariniello *et al.*, 2002); olive (Mekuria *et al.*, 2002; Roselli *et al.*, 2002; Khadari *et al.* 2003; Said 2004); and barley (Ibrahim, 2004).

This study was planned to establish molecular genetic fingerprints for ten olive cultivars based on RAPD polymorphism and elucidate the relationships among these cultivars.

2. MATERIALS AND METHODS

Ten olive cultivars representing different genomes were used in this investigation. The cultivars are Tofahi, Aggezi Shamy, Aggezi Akssi, Pecual, Frantoio, Arbiqueen, Koronaiki, Manzanillo, Kalamata and Chemlaly. Samples for each cultivar were kindly supplied by the Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Young fresh leaves were collected from the ten olive cultivars and immediately ground in a mortar using liquid nitrogen. Then about 0.2 g of these leaves were used for DNA extraction following the Dellaporta method (Dellaporta *et al.*, 1983) as follows:

About 0.3 g (fresh weight) of plant tissues was ground to fine powder in liquid N_2 in a mortar. Before the tissue thawed, 1 ml extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA and 0.5 M NaCl) and 0.2 ml 20% SDS were added. The mixture was incubated at 65 ^{O}C in water bath for 20 minutes. Then 1 ml of phenol, chloroform and isoamyl alcohol (25 : 24 : 1) was added. Centrifugation was performed at 10,000 rpm for 10 minutes. The supernatants of each sample was transferred separately to a new tube, then 1 ml of chloroform and isoamyl alcohol (24 : 1) was added .Centrifugation was performed at

10,000 rpm for 10 minutes. The supernatants of each sample were transferred separately to a new tube, then 1 ml of isopropanol was added and then kept overnight in a freezer. Centrifugation was performed at 10,000 rpm for 10 minutes. The resulted pellets containing DNA were re-suspended in 1 ml ethanol. Centrifugation was performed at 10,000 rpm for 2 minutes. The DNA pellets were re-suspended in 200 µl TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) buffer. DNA was quantitated by spectrophotometer and gel electrophoresis.

Fifteen 10-*mer* random DNA oligonucleotide primers were independently used in the PCR reactions according to Williams *et al.* (1990). The primers were from Operon Kit (Operon Tech. Inc., USA). Only Five primers generated reproducible polymorphism in the DNA profiles. Each experiment was repeated twice and only stable products were scored. The code and sequences of these primers were as follows:-

Primer code	Sequences
OPA-03	5`- AGT CAG CCA C - 3`
OPA-04	5`- AGT CAG CCA C - 3`
OPA-07	5`- CAG CAC TGA C- 3`
OPA-14	5`- AGC ATG GCT C- 3`
OPA-16	5`- TCG GCG GTT C- 3`

Amplification was performed in 25µl reaction volume containing the following: 2.5µl Primer, 2.3µl Template DNA, 7.7µl Sterile water, 12.5 µl 2 x Ready Mix RED *Taq* PCR Reaction mix. It consists of the follwing: 20 mM Tris-HCl (pH 8.3), 100 mM KCl, 3 mM $MgCl_2$, 0.002 % gelatin, 0.4 mM dNTBs mix (dATP, dCTP, dGTP and dTTP), stabilizers, 60 units Taq DNA polymerase /ml.

Each of the reaction mixtures was overlaid with a drop of light mineral oil per sample. Amplification was carried out in Perkin Elmer Gene Amp PCR thermocycler. The optimal conditions for PCR amplification were as follows: an initial 4 minutes denaturation step at 95 °C followed by 35 cycles of 45 second at 94 °C, 1 minute at 37 °C and 2 minutes at 72 °C, with a final extension step at 72 °C for 12 minutes.

A volume of 10µl of the RAPD products was electrophoresed in 1.4 % agarose gel. The gel was prepared by adding 1.4 g agarose to 100 ml of 1 X TBE (0.04 M Trisacetate,

1 mM EDTA, pH 8), followed by boiling in water bath. Then 0.5 µg/ml ethidium bromide was added to the melted gel. The melted gel was poured in the tray of mini-gel apparatus and the comb was inserted immediately. The comb was removed when the gel becomes hardened. The electrophoresis buffer (1X TBE) covered the gel. About 10 µl of DNA amplified product was loaded in each well and run at 60 V for about (45-75) minutes. The gels were visualized and photographed by gel documentation system (GelDoc BioRad 2000) under UV transilluminator.

2.1. Data analysis

Polymorphism was scored on the basis of presence (denoted 1) or absence (denoted 0). The data gained across RAPD-PCR analysis for each cultivar were pooled together to estimate the similarity coefficients among the studied cultivars on the basis of the number of shared bands according to Dice (1945) equation as implemented in the computer program SPSS version 10. A dendrogram based on similarity coefficients was generated by using the unweighted pair group method of arithmetic means (UPGMA) as revealed by the same computer program.

3. RESULTS AND DISCUSSION

The RAPD-PCR markers have proved to be a valuable addition to classical morphological markers for cultivar identification and for the estimation of genetic similarities among genotypes. In addition, the use of these markers for genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. DNA markers have the potential to enhance the operation of a plant breeding program through a number of ways, ranging from fingerprinting of elite genetic stocks, assessment of genetic diversity and increasing the efficiency of selection for difficult traits (Terzi, 1998).

Fifteen 10- *mer* arbitrary oligonucleotide primers were initially used to establish RAPD-PCR fingerprints of the studied olive

cultivars and discriminate among them. Only five primers (OPA-03, -04, -07, -14 and -16) were successful in generating reproducible and polymorphic products. Each of the five primers displayed a strong amplification with distinct bands. The fingerprints generated by the five primers revealed characteristic profiles for each of the olive cultivars, in terms of the number and position of RAPD bands. Tables (1, 2 and 3) and Figures (1a, b, c, d and e) demonstrate the results of RAPD-PCR profiles of the studied genotypes. The total number of reproducible fragments amplified by the 5 primers reached 156, from which 154 were polymorphic fragments with a polymorphism percentage ranging from 97.06 % to 100 % (Table 1). Similar results were obtained by Asante and Offei (2003) who used RAPD technique to study fifty cassava (*Manihot esculenta* Crantz) clones. Genetic diversity of these genotypes was studied using four primers, OPK-01, OPR-02, OPR-09 and OPJ-14. Levels of polymorphic fragments detected by the four primers ranged from 90% to 100%.

Table (1): Number and types of the amplified DNA bands as well as the percentage of the total polymorphism generated by the primers OPA-03, 04, 07,14 and 16 in the ten olive cultivars.

Primer code	Monomorphic bands	Polymorphic bands		Total bands	Polymorphism %
		Nonunique bands	Unique bands		
OPA.03	1	35	5	41	97.56
OPA.04	0	13	2	15	100.00
OPA.07	0	29	3	32	100.00
OPA.14	0	30	4	34	100.00
OPA.16	1	33	0	34	97.06
Total	2	142	12	156	98.92

The primer OPA-03 revealed clear variations in RAPD products between the studied olive cultivars. Figure (1a) illustrates the DNA polymorphism obtained with this primer. Fourty polymorphic bands out of 41 were scored in the studied genotypes (Table 1). Only one monomorphic band was recorded at about 298 bp. Five unique bands were identified out of the polymorphic ones (Tables, 1 and 2). Three

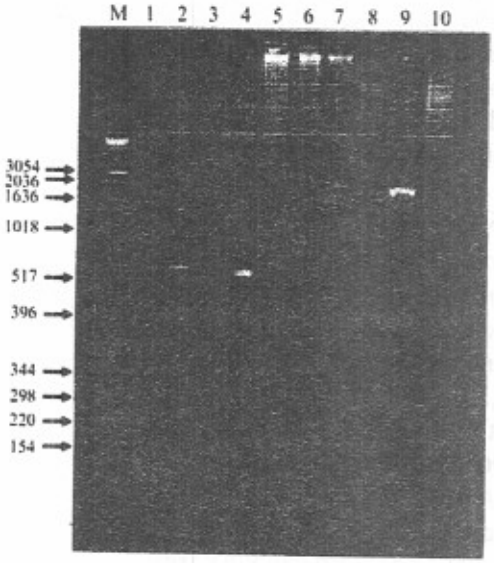
unique DNA bands (at about 2036, 490 and 240 bp) were detected in olive cv. Koronaiki (lane 7). The fourth unique band was identified in olive cv. Kalamata at about 220 bp (lane 9) while the fifth one was recorded at about 200 bp in olive cv. Shemlaly. These bands clearly discriminate between the three olive cultivars Koronaiki, Kalamata and Shemlaly as well as from the other studied cultivars (Table 2). Primer 03 showed a high potentiality for generating unique bands than the other primers.

The primer OPA-03 generated 35 non-unique polymorphic bands. The most pronounced are those identified at apparent molecular size of 517, 396 and 260 bp (Table 3 and Figure 1a). Comparing these bands with each other leads to a partial discrimination among the different cultivars under study. According to the presence or absence of these bands, the studied cultivars were categorized into four categories. The first category was characterized by the presence of the band at about 396 bp and includes the three cultivars Tofahi, Aggezi Shamy and Aggezi Akssi. The second category was distinguished by the presence of the two bands at about 396 and 260 bp. It comprised olive cv. Pecual . The third category was distinguished by the presence of the two bands at about 517 and 260 bp and included the three olive cultivars Frantoio, Arbiqueen and Koronaiki. The three olive cultivars Manzanillo, Kalamata and Shemlaly (lanes 8, 9 and 10) comprised in the fourth category and they were characterized by the presence of the three bands at about 517, 396 and 260 bp.

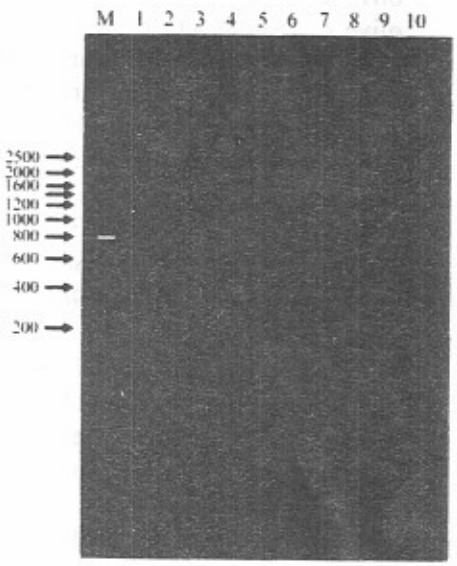
Analysis of the RAPD banding pattern of the studied olive cultivars generated by the primer OPA-04 revealed a total of 15 polymorphic bands, which were not necessarily present in all the studied genotypes (Tables 1 and 2 and Figure 1b). The bands were detected at a molecular weight ranging from approximately 2600 to 100 bp. No monomorphic bands were detected in the resulted profile. Two polymorphic bands were scored as unique ones. The first unique band was recorded at about 210 bp in Aggezi Akssi (lane 3) while the second one was scored at about 100 bp in Shemlaly (lane 10).

The primer OPA-04 generated thirteen non-unique polymorphic bands. The clearest of them are the bands identified at about 1500, 800 and 250 bp (Table 3 and Figure 1b). These bands grouped the studied

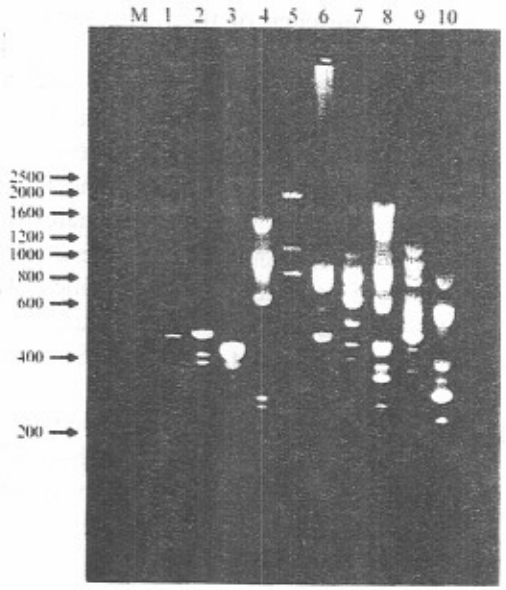
Primer O3



Primer O4



Primer O7



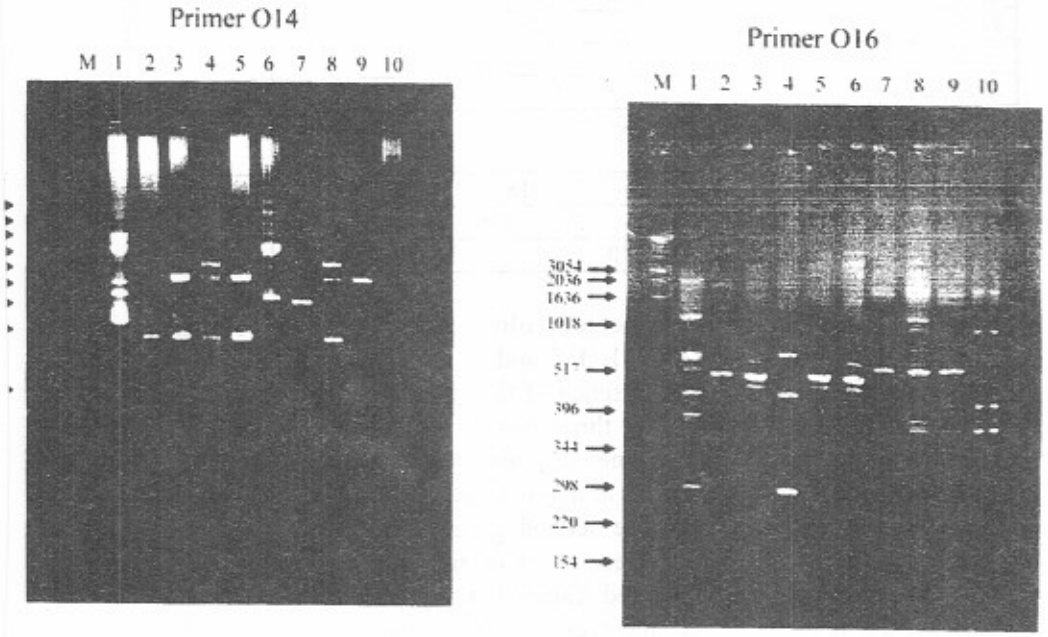


Figure (1): RAPD fingerprints of the ten olive cultivars generated by the five primers (a) OPA-03, (b) OPA-04, (c) OPA-07, (d) OPA-14 and (e) OPA-16. Short arrows indicate unique bands while long arrows indicate the most pronounced polymorphic bands.

Lane : M	DNA Marker	Lane : 4	Pecual	Lane : 8	Manzanillo
Lane : 1	Tofahi	Lane : 5	Frantoio	Lane : 9	Kalamata
Lane : 2	Aggezi Shamy	Lane : 6	Arbiqueen	Lane : 10	Shemlaly
Lane : 3	Aggezi Akssi	Lane : 7	Koronaiki		

A S E 0 5 10 15 20 25

Label Num +---------+---------+---------+---------+---------+

Table (2): Molecular size in bp of the amplified polymorphic (unique) DNA bands generated by the five DNA random primers used for identifying ten olive cultivars

Olive cvs.	OPA-03	OPA-04	OPA-07	OPA-14	OPA-16
Tofahi	-	-	-	-	-
Aggezi Shamy	-	-	-	-	-
Aggezi Akssi	-	210	-	-	-
Pecual	-	-	-	-	-
Frantoio	-	-	-	-	-
Arbiqueen	-	-	-	2600-400	-
Koronaiki	2036-490-240	-	-	-	-
Manzanillo	-	-	185-175	320-290	-
Kalamata	220	-	1700	-	-
Shemlaly	200	100	-	-	-

cultivars into four groups. The four olive cultivars Tofahi, Pecual, Frantoio and Arbiqueen (lanes 1, 4, 5 and 6) were grouped in the first group and identified by the presence of the two polymorphic bands at about 1500 and 800 bp. The three olive cultivars Aggezi Shamy, Aggezi Akssi and Shemlaly (lanes 2, 3 and 10) were distinguished by the presence of the three bands at about 1500, 800 and 260 bp. These genotypes were grouped in the second group. The third group was identified by the absence of these polymorphic bands. It includes the two olive cultivars Koronaiki and Kalamata (lanes 7 and 9). The fourth group was characterized by the presence of the band at about 260 bp and involved only olive cv. Manzanillo (lane 8).

Figure (1c) represents RAPD banding pattern of the examined olive cultivars generated by the primer OPA-07. The obtained patterns exhibited a molecular weight range of about 2000 – 175 bp. A maximum number of 32 polymorphic bands was recorded, which were not necessarily present in all the studied cultivars (Table 1). No monomorphic bands were identified in the resulted RAPD profiles. Three polymorphic bands were identified as unique bands (Table 2). The first and second unique bands were observed in Manzanillo olive cv. (lane 8) at apparent molecular size of 185 and 175 bp while the third unique one was detected at about 1700 bp in Kalamata (lane 9). These two unique bands could be used to discriminate olive cvs. Manzanillo and Kalamata from the remaining

studied genotypes.

The remaining non-unique polymorphic bands produced by primer OPA-07 provide further discriminatory power to identify each of the studied olive cultivars by a unique pattern (Table 3 and Figure 1c). The most pronounced ones are the five bands observed at about 640, 540, 490, 420 and 300 bp. The presence or absence of these bands completely discriminates the studied cultivars by a group of unique class patterns. Olive cv. Tofahi was characterized by the presence of the bands at about 640 and 490 bp, while olive cv. Aggezi Shamy was recognized by the presence of the bands at about 490 and 420 bp. Similarly, olive cv. Aggezi Akssi was distinguished by the presence of the bands at about 540, 420 and 300. Pecual was identified by 640, 490 and 300 bp, Frantoio by 640 bp, Arbiqueen by 540 and 490 bp, Koronaiki by 460, 540, 490 and 300 bp, Manzanillo by 420 and 300 bp, Kalamata by 640, 540, 490, 420 and 300 bp and Shemlaly by 300 bp.

Primer OPA-14 produced 34 scorable polymorphic bands among the studied olive cultivars with a molecular weight range of about 2600 to 140 bp (Tables 1 and 2 and Figure 1d). Four bands were identified as unique ones. According to the presence of these bands, two cultivars were discriminated from the remainder of the studied cultivars. Arbiqueen was characterized by the presence of two unique bands with a molecular size of about 2600 and 400 bp (lane 6), while Manzanillo was identified by the presence of the two unique bands at about 320 and 290 bp (lane 8).

Considering the non-unique polymorphic bands generated by the primer OPA-14, the most scoreable ones are those recorded at about 1050, 850, 490 and 370 bp (Table 3 and Figure 1d). These bands provide further capability to discriminate among the studied olive cultivars. Depending on the presence or absence of these bands, the studied genotypes were sorted in five groups. Tofahi was involved in the first group. It was identified by the presence of the polymorphic band at about 490 bp (lane 1). The four olive cultivars Aggezi Shamy (lane 2), Aggezi Akssi (lane 3), Frantoio (lane 5) and Shemlaly (lane 10) were placed together in the second group. This group was identified by the presence of the three bands at about 850, 490 and 370 bp. The third group was identified by the presence of the bands with apparent molecular size of about 1050, 850 and 370 bp. It comprised

Pecual and Manzanillo (lanes 4 and 8). The fourth group includes olive cv. Arbiqueen (lane 6). This group was characterized by the presence of the bands at about 1050 and 490 bp. Koronaiki and Kalamata grouped together in the fifth group (lanes 7 and 9). They were discriminated by the presence of the two bands at about 850 and 370 bp.

Table (3): Molecular size in bp of the amplified polymorphic (non-unique) DNA bands generated by five DNA random primers used for identifying ten olive cultivars.

Olive cvs.	OPA-03	OPA-04	OPA-07	OPA-14	OPA-16
Tofahi	396	1500-800	640-490	490	580-540-470-440
Aggezi Shamy	396	1500-800-260	490-420	850-490-370	580-470-440
Aggezi Akssi	396	1500-800-260	540-420-300	850-490-370	850-470
Pecual	396-260	1500-800	640-490-300	1050-850-370	470-440
Frantoio	517-260	1500-800	640	850-490-370	850-470
Arbiqueen	517-260	1500-800	540- 490	1050-490	850-470
Koronaiki	517-260	-	460-540-490-300	850-370	540
Manzanillo	517-396 – 260	260	420-300	1050-850-370	580-540-440
Kalamata	517-396-260	-	640-540-490-420-300	850-370	
Shemlaly	517-396-260	1500-800-260	300	850-490-370	580-540-440

Primer OPA-16 produced 34 scorable bands among the studied olive cultivars. A total of 33 bands was scored as polymorphic ones (Table 1 and Figure 1e). Only one monomorphic band was recorded at apparent molecular size of 375 bp. The most observed polymorphic bands are those identified at about 580, 540, 470 and 440 bp. Comparing these bands led to further discrimination among the different genotypes under study. According to the presence of these bands, the studied cultivars were classified into seven categories (Table

3).The first group was characterized by the presence of the four bands 580, 540, 470 and 440 bp and comprised Tofahi (lane 1). The second group involved Aggezi Shamy (lane 2), and was characterized by the presence of the three bands 580, 470 and 440 bp. The third group included the three cultivars Aggezi Akssi (lane 3), Frantoio (lane 5) and Arbiqueen (lane 6) This group was discriminated by the presence of the two bands 850 and 470 bp bands. The fourth group was distinguished by the presence of the two bands 470 and 440 bp and involved Pecual (lane 4). The fifth group includes Koronaiki (lane 7). This group was characterized by the presence of the band at about 540 bp. The sixth group involved . Manzanillo (lane 8) and Shemlaly (lane 10) and was identified by the presence of the three bands at about 580, 540 and 440 bp. The seventh group involved Kalamata (lane 9). This cultivar was discriminated from the other studied cultivars by the presence of the two bands at molecular size of about 540 and 440 bp.

Recently, techniques that utilize the polymerase chain reaction have allowed a more representative assessment of genetic variation in plants by screening multiple loci distributed throughout the genome. The analyses reveal sufficient polymorphism for the examination of fine-scale genetic differences among individuals. In this study, only five out of fifteen primers succeeded to generate polymorphic and reproducible amplification products. Many authors reported the use of a large number of primers to identify and characterize many plant genotypes, but a limited number of primers succeeded to generate distinct and reproducible profiles with sufficient polymorphism. In this concern, Vierling *et al.* (1994) used 73 random primers to study genetic diversity among elite sorghum lines by RAPD analysis, but only 57 primers produced clear amplification products. Also, Baum *et al.* (1997) used 33 primers to analyze genetic diversity in 88 genotypes from 20 populations of wild barley (*Hordeum spontaneum*) from Israel, Turkey and Iran, but only 22 primers yielded informative products. Also, Emam *et al.* (2000) used nine decamer primers to fingerprint three rice cultivars, but five of these primers generate good and informative amplification. Hassan *et al.* (2002) investigated genetic polymorphism in ten accessions representing five *Populus* species from different locations by RAPD-PCR analyses. RAPD analysis revealed that all the studied poplar samples were identified by one or more unique bands or a group of banding pattern. The obtained

dendrogram showed that poplar samples of each species collected from different geographical locations are grouped together in one cluster. Khadari *et al.* (2003) used molecular markers to characterize 100 accessions of olive and to study genetic relationships between them. A total of 497 olive trees was genotyped using 32 RAPD markers. The authors identified 114 RAPD profiles and detected several cases of mislabeling, synonymy and homonymy. This study led to the construction of a molecular database for the reference collection and to analyze genetic diversity for further prospecting, and for introducing new olive accessions. Ibrahim (2004) used six random primers to discriminate 14 barley genotypes including 7 cultivars and 7 landraces. The obtained results allowed to discriminate between landraces and cultivars.

3.1. Similarity coefficient

Based on the combined data obtained through the polymorphism of RAPD profiles, the similarity coefficient values among the studied olive cultivars were calculated according to Dice (1945) equation (Table 4). High levels of intercultivar polymorphism were detected among the studied genotypes. The similarity coefficient reached a highest value of 0.699 between olive cvs. Aggezi Akssi and Aggezi Shamy and reached a minimum value of 0.281 between olive cvs. Koronaiki and Aggezi Akssi.

The similarity coefficients were used to generate a dendrogram (Figure 2) by UPGMA analysis as implemented in the computer program SPSS version-10. The obtained dendrogram revealed two main groups. The first main group was subdivided into two subgroups, which are distinguished from each other at a distance of 21. The first subgroup was further subdivided into two categories, the first category includes Aggezi Akssi, Aggezi Shamy and Pecual whereas, the second category involved only olive cv. Tofahi. The second subgroup comprises, Frantoio and Arbiquecn. Similarly, the second subgroup was further subdivided into two categories. The three olive cultivars (Manzanillo, Shemlaly and Kalamata) were grouped in one category. The second category involves only Koronaiki (Figure 6).

Many authors used RAPD analyses to elucidate similarity indices of many plant species or varieties. In this regard, Ramser *et al.* (1996) used RAPD analysis to assess intraspecific variability and

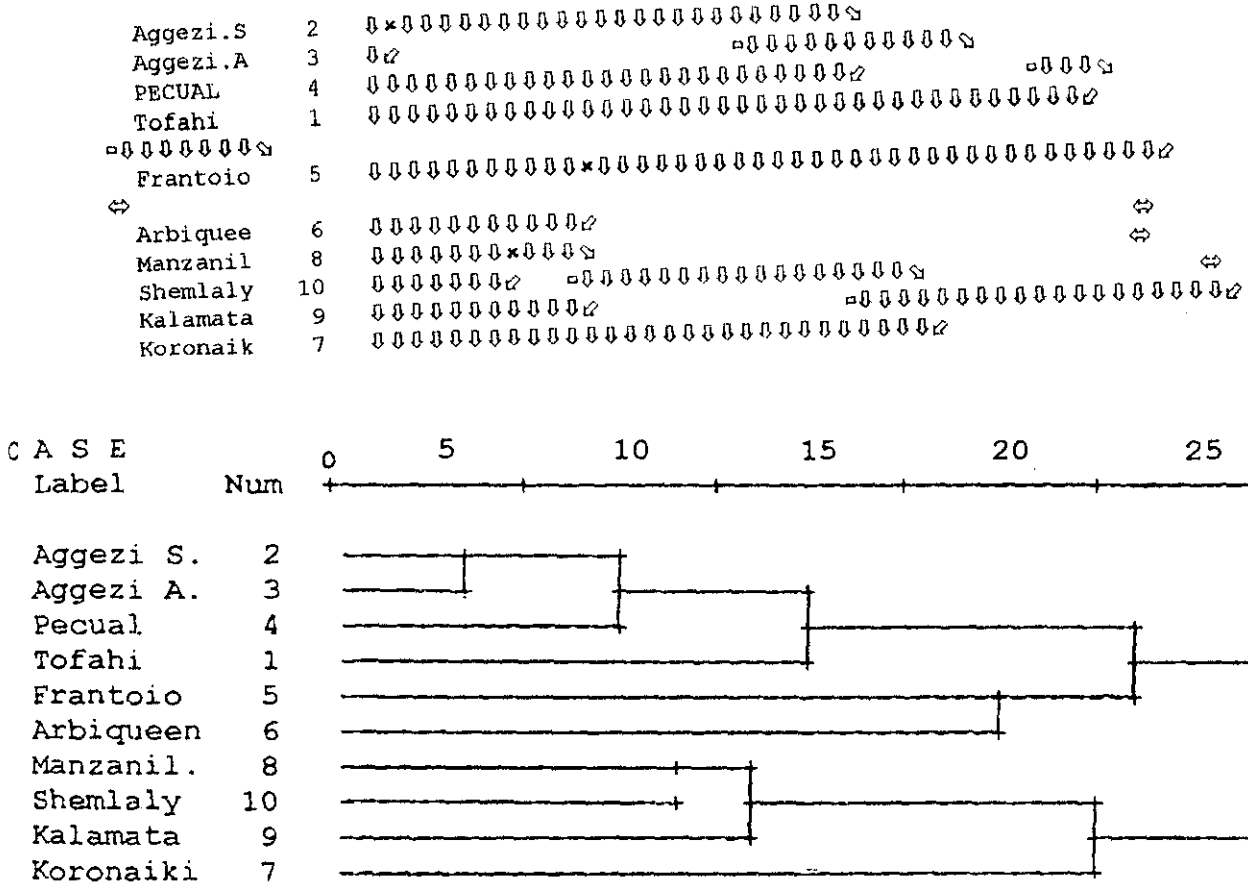


Figure (2): Dendrogram illustrating genetic distance between the ten olive cultivars based on RAPD analysis

Table(4): Similarity coefficients of the ten olive cultivars obtained by RAPD analysis.

	Matrix file input								
Olive cvs.	Tofahi	Aggezi Shamy	Aggezi Akssi	Pecual	Frantoio	Arbiqueen	Koronaiki	Manzanillo	Kalamata
Aggezi Shamy	0.459								
Aggezi Akssi	0.444	0.699							
Pecual	0.460	0.505	0.553						
Frantoio	0.421	0.514	0.500	0.380					
Arbiqueen	0.400	0.473	0.385	0.317	0.626				
Koronaiki	0.433	0.383	0.281	0.321	0.467	0.397			
Manzanillo	0.365	0.364	0.300	0.375	0.429	0.346	0.485		
Kalamata	0.359	0.375	0.306	0.291	0.410	0.407	0.553	0.620	
Shemlaly	0.382	0.413	0.336	0.393	0.412	0.364	0.496	0.657	0.642

relationships in 23 accessions of aerial yam (*Dioscorea bulbifera*) from different geographical locations in Africa, Asia and Polynesia. Cluster analysis was found to be in accordance with the geographical origin of the samples. Ruas *et al.*, (2001) studied genetic relationships among 18 accessions, including 16 of *Ananas* and two of *Pseudananas*, using RAPD molecular markers. From the total of 148 markers scored, 132 (89.2%) were polymorphic. The similarity matrix was used for cluster analysis. The phenogram developed from the RAPD bands showed that for most of the cases, the accessions within a species were grouped together. Nevertheless, a moderate infraspecific genetic variation was observed. Bronzini de Caraffa *et al.* (2002) studied olives grown on two Mediterranean islands, Corsica and Sardinia. A dendrogram was constructed using the UPGMA method, which separates olive trees into two main clusters. The results of the analysis showed the existence of a genetic divergence between the oleasters and the cultivated varieties. They suggested that some of the Corsican varieties were probably selected from local wild forms, contrary to the Sardinian varieties. They also showed that there are feral (uncultivated) forms growing on both islands, which result from hybridization between oleasters and varieties. Sharma and Jana (2002)

used RAPD markers to distinguish between 28 different accessions belonging to 14 species and two sub-species of *Fagopyrum.* of the 75 random 10-mer primers tested, only 19 generated clear and easily interpretable amplification products. A total of 364 bands was observed with an average of 19.15 bands per primer, of which 99.45% were polymorphic. Cluster analysis using the unweighted paired group method of arithmetic means (UPGMA) showed four main clusters. Abdel-Tawab *et al.* (2003) used 10-mer random primers to fingerprint ten tomato (*Lycopersicon esculentum* Mill) cultivars. The obtained dendrogram divided the cultivars into two groups. Some RAPD markers were linked to some yield related traits. Such markers could be used in marker-assisted selection in breeding program to predict both yield and quality traits in tomato. Said (2004) used five random primers to fingerprint ten olive cultivars. The obtained dendrogram separated the studied cultivars into two categories.

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البصمة الوراثية لبعض أصناف الزيتون المنزرعة فى مصر

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ملخص

استهدف هذا البحث التعرف على البصمة الوراثية ودرجات التشابه لعشرة من أصناف الزيتون باستخدام التباين فى التضاعف العشوائى لأجزاء من الدنا فى جهاز سلسلة تفاعلات إنزيم البلمرة (RAPD-PCR). إستخدمت الاوراق الغضة صغيرة السن لكل صنف على حده لإستخلاص الدنا. عمل تضاعف عشوائى فى جهاز السلسلة لأجزاء من الدنا بإستخدام خمسة من البادئات العشوائية وهى (OPA-03, OPA-04, OPA-07, OPA-14 and OPA-16). أدت نتائج التضاعف العشوائى للدنا الى التمييز الكامل لكل أصناف الزيتون بواسطة حزمة متفردة او أكثر أو بواسطة مجموعة من الطرز المجمعة. وكانت بعض البادئات (OPA-03) أكثر قدرة من البادئات الأخرى فى تميز اصناف الزيتون ، فقد اعطت حزم متفردة اكثر بالتضاعف العشوائى للدنا. أستخدمت نتائج التباين فى التضاعف العشوائى للدنا لتحديد معامل التشابة بين أصناف الزيتون تحت الدراسة للتعرف على العلاقات الوراثية بينها. قسمت شجرة القرابة لأصناف الزيتون العشرة الى مجموعتين. اشتملت المجموعة الاولى على أصناف البيكوال والعجيزى الشامى و العجيزى العقصى والأربكوين والفرانتويو والتفاحى بينما اشتملت المجموعة الثانية على المنزنيللو والكوروناكى والكلاماتا والشملالى. وكانت أعلى قيمة لمعامل التشابه هى ٠,٦٩٩ بين الصنفين العجيزى الشامى والعجيزى العقصى وكانت أقل قيمة لمعامل التشابه هى ٠,٢٨١ بين الصنفين كوروناكى وعجيزى عقصى.

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