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Study of Genetic Diversity in Some Olive (*Olea europaea* L.) Cultivars by Using RAPD Markers

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Abstract: RAPD markers variations were studied in eleven olive cultivars. Seventeen RAPD primers out of 27 produced 610 bands in total. Four hundred forty four bands were polymorphic bands (72.70 %) and 166 bands were monomorphic (27.20 %). Primer C05 produced the highest number of bands (56), while primer C03 produced the lowest number of bands (7). Specific bands were observed in some of the cultivars, which may be used in the cultivars discrimination. Different similarity coefficients determined among the cultivars studied, showed the highest value of similarity between cultivars Kroneiki and Manzanila. Different clustering methods showed distinctness of the olive cultivars studied. The grouping of the cultivars did not correlate completely with their place of origin or fruit characteristics.

Key words: Olive cultivars, genetic diversity, RAPD markers, Iran

INTRODUCTION

The olive-tree (*Olea europaea* L.) is one of the most characteristic species of the Mediterranean area and now a days it is the only cultivated representative of the genus *Olea*. The olive tree is the oldest known cultivated tree in history. Olives were first cultivated in Africa and then spread to Morocco, Algiers and Tunisia by the Phoenicians. *O. europaea* was first cultivated in Crete and Syria over 5000 years ago. Around 600 BC olive tree cultivation spread to Greece, Italy and other Mediterranean countries. Olive is one of the most important cultigens and played in exceptionally significant rôle in the ancient civilizations around the Mediterranean sea: Egyptians, Phoenicians, Greeks and Romans knew and valued olive oil. Olives are grown in the whole Mediterranean region and are a most important part of the diet in all Mediterranean countries. Olive oil is ubiquitously used as a cooking medium and pickled olives are popular both a spice and as a snack.

Accurate and rapid identification of clones, varieties, or species is especially important in vegetatively propagated plants like olive-trees (*Olea europaea* L.). Hundreds of cultivars have been selected over the centuries for their adaptation to microclimate and soil types. As a result, there is considerable uncertainty about the names of many olive cultivars, becoming synonymous and homonymous a long-standing problem in olive producing countries. Differentiation of olive-tree cultivars is traditionally performed by phenotypic traits of trunk, leaf, flower and fruit shape, however recently is lozymes have been found useful markers for cultivar identification (Bautista *et al*., 2002). However weakness of these classifications have been shown by the evidence that chemical and morphological changes in olive-trees as well as other plants are influenced by domestication and agronomic selection.

Studying the genetic diversity as well as cultivar identification by using various molecular markers including RAPD (Random Amplified Polymorphic DNA) has been performed in several plant species. These molecular markers provide an opportunity for direct comparison and identification of different genetic material independent of any influences (Harvey and Botha, 1996, Bautista *et al*., 2003).

Olive cultivars are grown in different parts of Iran and cultivation of the same cultivars for long period of time may lead to the genetic erosion confining the subsequent breeding programs. Therefore it is necessary to study the available diversity and introduce new variability as well. For this reason, the present study considers molecular analyses of some imported olive cultivars of Iran for the first time.

MATERIALS AND METHODS

Eleven olive cultivars were used for molecular studies. For RAPD analysis, fresh leaves were selected

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randomly from 3-5 plants of each cultivar and DNA extraction was done by use of NucleoSpin Plant kit (Macherey-Nagel, Germany). The PCR reaction mixture consisted of template DNA, 1×PCR buffer (10 mM Tris-HCJ pH 8.8, 250 mM KC12), 200 μM dNTPs, 0.80 μM 10-base random primers and 1 unit of Taq polymerase, in a total volume of 25 μL. DNA amplification was performed on a palm cycler GP-001 (Corbet, Australia). Template DNA was initially denatured at 94°C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92°C, primer annealing for 1 min at 36°C and primer extension for 2 min at 72°C. A final incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion. The PCR amplified products were separated by electrophoresis on a 2% agarose gels using 0.5×TBE buffer (44.5 mM Tris/Borate, 0.5 mM EDTA, pH = 8) or 12% polyacrylamide geles. The geles were stained with ethidium bromide and visualized under UV light or silver stained for added sensitivity. RAPD markers were named by primer origin, followed with the primer number and the size of amplified products in base pairs. Twenty-seven random primers of Operon technology (Alameda, Canada) were used.

RAPD bands were treated as binary characters and coded accordingly (presence = 1, absence = 0). simple matching coefficient and Nei and Li coefficients were determined among the cultivars studied and grouping of the genotypes was determined by using different clustering methods and ordination based on Principal Coordinate Analysis (PCO) (Chatfield and Collins, 1995; Ingrouille, 1986.). Cophenetic correlation was determined for different clustering methods. NTSYS Ver. 2.02 (1998) was used for statistical analyses.

RESULTS AND DISCUSSION

Seventeen RAPD primers out of 27 produced 610 bands in total. Four hundred forty four bands were polymorphic bands (72.70%) and 166 bands were monomorphic (27.20%, Fig. 1). Primer C05 produced the highest number of bands (56), while primer C03 produced the lowest number of bands (7).

The primers R01, H07 by producing 90.60 and 92.10% of polymorphic bands showed the highest level of polymorphism while, primers I16 and B05 produced 42.80 and 40.00% of polymorphic bands showing the lowest level of polymorphism.

Specific bands were observed in some of the cultivars, which may be used in the cultivars discrimination. For example bands C03-2, 3, 4, 5, 6 and 7, as well as bands R02-33, R02-43, B07-11 were specific for the cultivar Cyprus. Bands R02-43, B07-7, I16-41 and C05-26 were specific for the cultivar Mostoides. Some bands were present in all the cultivars except one, which also may be of use in olive cultivar differentiation. For example band C02-1 was present in all except in the cultivar Amphissis, band C02-2 was absent in the cultivar Amigdalolia, band C02-9 was absent only in the cultivar Manzanila.

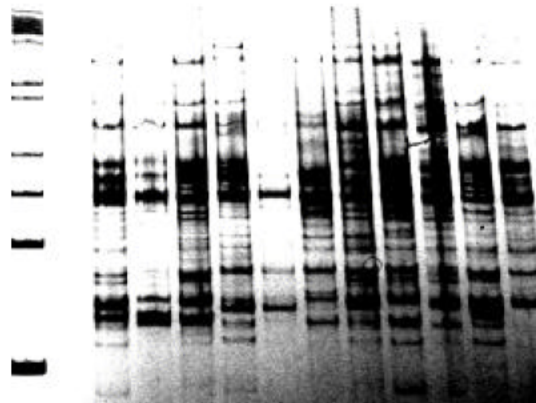

Fig. 1: RAPD profile of olive cultivars by primer C-08. (Columns from left to right: Molecular marker, No DNA, Cyprus, Belladi, Manzanila, Kroneiki, Mostoides, Amphissis, Amigdalolia, Sevilana, Konservolia, Kalamon and Mission

Different similarity coefficients determined among the cultivars studied, showed the highest value of similarity between cultivars Kroneiki and Manzanila (for example r = 0.687 in simple matching coefficient), followed by the cultivars Konservolia and Sevilana (r = 0.685). The lowest value of similarity occurred between the cultivars Cyprus and Amficis (r = 0.497) (Table 1).

Different clustering methods including UPGMA (unweighted paired group with arithmetic average), single linkage and complete linkage performed on molecular data by using the simple matching similarity and Nei and Li coefficients produced similar results. PCO ordination of the cultivars also supported the clustering results. The cophenetic correlation determined showed the highest value for UPGMA method (r = 0.71). Therefore the result of UPGMA clustering along with PCO ordination is discussed bellow.

In general four major clusters or groups are formed (Fig. 2 and 3). The first major cluster is comprised of three sub-clusters. The cultivars Mission, Konservolia and Sevilana form the first sub-cluster in which the last two cultivars show more similarity and are placed close to each other. In PCO plot also these cultivars along with the cultivar Cyprus are place close to each other (cultivars

Table 1: Simple matching coefficient among olive cultivars studied

	1	2	3	4	5	6	7	8	9	10	11
1	1.000										
2	0.638	1.000									
3	0.653	0.600	1.000								
4	0.662	0.610	0.685	1.000							
5	0.626	0.623	0.637	0.626	1.000						
6	0.539	0.551	0.569	0.584	0.597	1.000					
7	0.540	0.560	0.607	0.628	0.597	0.596	1.000				
8	0.584	0.575	0.617	0.648	0.592	0.577	0.675	1.000			
9	0.526	0.598	0.615	0.630	0.642	0.580	0.620	0.687	1.000		
10	0.626	0.614	0.628	0.643	0.648	0.560	0.627	0.633	0.678	1.000	
11	0.580	0.545	0.647	0.580	0.555	0.497	0.564	0.617	0.560	0.644	1.000

Cultivars abbreviations: 1- Mission, 2- Kalamon, 3- Konservolia, 4- Sevilana, 5- Amigdalolia, 6- Amphissis, 7- Mostoides, 8- Kroneiki, 9- Manzanila, 10- Belladi and 11- Cyprus

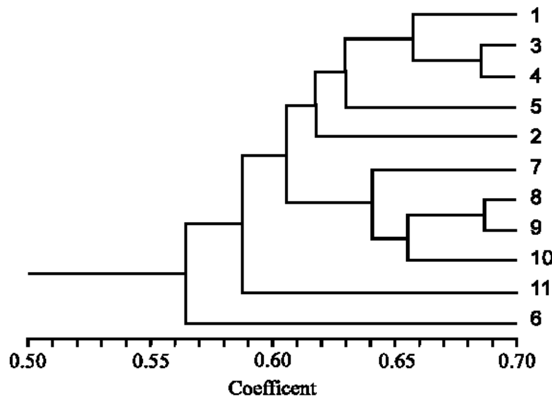

Fig. 2: UPGMA clustering of olive cultivars based on RAPD markers. Cultivars abbreviations: 1-Mission, 2-Kalamon, 3-Konservolia, 4-Sevilana, 5-Amigdalolia, 6-Amphissis, 7-Mostoides, 8-Kroneiki, 9-Manzanila, 10-Belladi and 11- Cyprus

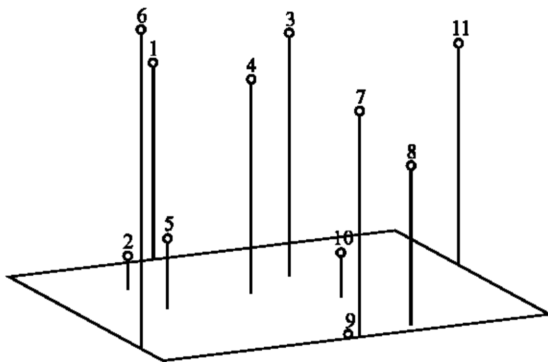

Fig. 3: PCO ordination of olive cultivars based on RAPD markers. Cultivars abbreviations: 1-Mission, 2-Kalamon, 3-Konservolia, 4-Sevilana, 5-Amigdalolia, 6-Amphissis, 7-Mostoides, 8-Kroneiki, 9-Manzanila, 10-Belladi and 11-Cyprus

No. 11, 1, 3 and 4 in Fig. 3). All of these cultivars possess ovoid-shape, slightly asymmetric to asymmetric fruit with pointed apex and nipple absent.

The cultivars Kalamon and Amigdalolia form the second and third sub-clusters and are joined to the members of the first sub-cluster with some distance. These two cultivars share some of their fruit characteristics including elongated and asymmetric fruits, but differ in fruit apex and the presence of nipple.

The second major cluster is comprised of the cultivars Mostoides, Kroneiki, Manzanila and Belladi, in which the first two show more similarity and are place close to each other. These cultivars also show in part similarities in their fruit characteristics and differ in fruit apex and the presence of nipple.

Two cultivars of Cyprus and Amphissis show much difference in their molecular characteristics and stand alone far from the other cultivars forming the third and fourth major clusters. It is interesting to note that Amphissis is a known synonym for the cultivar Konservolia, which are placed very far from each other in both clustering and PCO plot. Therefore there is either a mistake in labeling of this imported cultivar or it is due to high genetic variation occurred in this cultivar and their morphological characteristics should be investigated in depth. This statement is supported by the fact that Amphissis possesses some specific RAPD bands like C02-11 and also lacks some bands common in the other cultivars (for example bands C02-1 and C04-19) including its supposed synonym Konservolia.

RAPD markers have been shown to be very powerful in revealing genetic polymorphism in olive tree cultivars (Trujillo *et al*., 1999; Khadari and Bervillé, 2001; Bautista *et al*., 2002; De la Rosa *et al*., 2002; Banilas *et al*., 2003 and Lazovic *et al*., 2002).

Khadari and Bervillé (2001) while evaluating the genetic diversity of Moroccan olive germplasm could identify 68 multi locus RAPD profiles and each of 9 traditional denominations was identified by one specific multi locus profile. They could identify more genotypes than previously known in the cultivated Moroccan olive by using RAPD markers possibly due to high potential of cultivar selection.

A similar study was performed by Banilas *et al.* (2003) using RAPD markers to reveal the genetic diversity among accessions of an ancient olive variety Ladolia of Cyprus. RAPD analysis produced 70 distinct electrophoretic profiles, showing a high level of genetic diversity in Ladolia cultivar. RAPD profiles obtained could identify all the accessions grouping them in to distinct clusters, which were correlated to the fruit size.

The earlier report of RAPD analysis in 11 local olive cultivars of Iran also showed a high degree of genetic diversity among the cultivars studied and their groupings in cluster analysis was correlated to their geographical distribution (Samaee *et al.*, 2003). It also revealed that some of these cultivars might be divided in to sub-types both on morphological and molecular characteristics.

The present study, which considers the imported olive cultivars grown in Iran, also shows the usefulness of RAPD analysis in distinguishing these cultivars. Particularly identification of the cultivar specific bands may be considered important for the olive cultivar separation. However the grouping obtained by clustering and ordination methods neither correlate with the origin of these cultivars nor their fruit characteristics. For example except two cultivars of Mission (from USA) and Belladi (from Syria), all the other cultivar's origin is Greece and Spain cultivars are not place close to each other in clustering and PCO plot. More over as stated earlier, in some of the cases the cultivars present in a single cluster or group do not share similar fruit characteristics.

The present study, which is a part of molecular, cytogenetic and morphometric study of olive germplasm in Iran shows distinctness of the olive cultivars in their molecular characteristics. A similar result is obtained from cytogenetical study of the same cultivars considering the chromosome pairing and segregation as well as the occurrence and effect of B-chromosomes (Unpublished data). Some of these cultivars are also capable of producing unreduced pollen grains, which will be presented in the coming paper. By performing similar studies on the other olive genotypes available, a better breeding program may be planned for the olive cultivars of the country.

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