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Full Length Research Paper

Comparative molecular analysis of old olive (*Olea europaea* L.) genotypes from Eastern Mediterranean Region of Turkey

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Olive is an important Mediterranean tree species having many different ways of utilizations. The olive grove is an important farming sector in Turkey and dates back to thousands years, particularly in Anatolia. An historical culture of olive resulted in a broad genetic base for olive which is a long-lived tree. We compared the genetic profiles of six old olive cultivars from an Eastern Mediterranean Region of Turkey to 15 modern Turkish olive cultivars from different geographical origins. The RAPD profiles successfully clarified the molecular relationships among the genotypes tested. Seventeen RAPD primers generated 153 reproducible bands, 81% of which were polymorphic. The data were subjected to cluster and principle coordinates analyses. Cluster analysis supported three small groups based on geographical origins and these groups did not include any of the old cultivars. The old cultivars scattered around the ungrouped accessions and formed four subgroups. Principle coordinate resulted in similar overall patterns. Our results revealed that although sampled from a relatively narrow region, the old olive cultivars have broad genetic basis and are closely related to some of present-day cultivars/clones.

Key words: Genetic resources, genetic variability, olive, *Olea europaea* L., RAPD.

INTRODUCTION

Olive culture is centered on the Mediterranean Basin including Turkey (Hagidimitriou et al., 2005; Tabatabaei, 2006). Anatolia is considered as a part of the genetic center for olive, although exact origin and the initiation of the domestication period are still controversial (Zohary and Hopf, 1994; Besnard et al., 2001, 2002; Breton et al., 2006). Olive cultivation is ancient (Galili et al., 1997) but mainly developed during the roman period (Lenoir and Akerraz, 1984; Loumou and Giourga, 2003).

Several studies showed that cultivated olive trees have been selected from spontaneous populations named oleasters in different Mediterranean areas (Besnard et al., 2001, 2002; Breton et al., 2006). Olive is one of the most ancient cultivated fruit tree species in the Mediterranean Basin, with great socioeconomic impact for the countries present in the area. Description and characterization of the genetic resources of such an important clonally propagated crop should provide the extent of its diversity, geographical spread and domestication and help with decision making in breeding programs. Furthermore, cultivar certification is required for nursery propagated clones to maintain and control quality of oil and table olives and for identifying synonyms (Hagidimitriou et al., 2005).

Olive trees are distinct from most other horticultural species since they are much longer-lived trees as compare to many others. There are many olive trees reported to be more than 1000 years old from different parts of Mediterranean Basin. Olive trees are increasing their popularity among Turkish growers. The Ministry of Agriculture has several supporting programs for olive culture. As a result, there is a significant increase for the olive orchard plantations. For example, more than 3 million olive trees have been planted in the Hatay province during the last three years.

The objective of this study was to compare the molecular variation using RAPDs (Random Amplified Polymorphic DNA) between six local olive genotypes and modern cultivars from Turkey. We also aimed to set a base for

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Figure 1. An example (PK) of old trees from Payas districts of Hatay, Turkey which are estimated to be more than 500 years old.

further genetic studies for the olive genotypes of the region.

MATERIALS AND METHODS

Plant materials

There are numerous olive genetic resources scattered in the Eastern Mediterranean Region of Turkey. In particular, Dörtyol, Erzin and Payas districts of Hatay province has many populations. Some of these olive trees are very old, estimated to be more than 500 (Dağli et al., 2004; Dankoff, 2004). An example of these trees, PK, is given in Figure 1. Six of these old genotypes were sampled from the region: PK, PM2, PM3, PM4, PM5 and PM6. PM2 – PM5 were estimated to be between 100 – 500 years old. Along with these old trees, one wild (Wild) and one present local clone ('Yerli') was included in the study. Fifteen additional cultivars/clones were chosen with the aim of evaluating the genetic relatedness of the old cultivated genotypes utilized in the study. These individuals included 14 Turkish genotypes ('Adana Topagi', 'Akilli asisi', 'Ayvalik', Barrevi, 'Derik', 'Domat', 'Ensititu', 'Erkence', 'Gemlik', 'Girit zeytini', 'Hallali 1', 'Hallali 2', 'Memecik', 'Sari hasabi') and one cultivar from Israel ('Barnea').

Molecular analysis

For the DNA extractions, young leaves were collected from a single tree for each accession and immediately frozen in liquid nitrogen and stored at –80°C. It was possible to extract high molecular weight genomic DNA from the leaf samples following the protocol for Minipreps by using CTAB (Dellaporta et al., 1983). DNA concentration was measured using a NanoDrop, ND 100 spectrophotometer (NanoDrop Technologies, Inc.) and gel electrophoresis. DNA was diluted in water to a final concentration of 50 ng/µl and stored at –20°C.

A hundred RAPD primers from Operon Series (Operon Technologies, Almeda, CA, USA) were screened initially on a sample of the accessions with 12 individuals. Primers that produced reproducible, polymorphic bands were used to amplify the rest of the accessions. Seventeen 10-mer primers which were found to be polymorphic were used to generate RAPD markers. Amplification conditions were similar to Durgac et al. (2008). In short, reactions were performed in 10 µl volumes containing 2x PCR Mastermix (Fermantas K0171), 1 unit of Taq DNA polymerase (Fermentas EP0402), $MgCl_2$, 30 ng of the primer and 20 ng of sample DNA. The mixtures were assembled at 0°C and then, transferred to thermal cycle, precooled at 4°C. The amplification was carried out in a model Master Gradient thermal cycler (Eppendorf) using a program consisting of an initial denaturation step of 2 min at 94°C and then, 55 cycles of 2 min at 94°C, 1 min at 37°C, 2 min 72°C, followed by a 10 min elongation step at 72°C. PCR products were stored at 4°C before analysis.

The separations of the amplification products were conducted by electrophoresis in 2% agarose gels and 0.5 µg/ml Ethidium bromide in 1x TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH: 8.0) for 3 h at 70 volts. The fragment patterns were photographed under UV light for further analysis. A 1 kb DNA ladder was used as the molecular standard in order to confirm the appropriate RAPD markers.

Table 1. List of arbitrary oligonucleotide primers, size range of amplified fragments and number of RAPD bands.

Primer	Size (bp)	No. of band		
	Min - Max	Total	Polymorphic	Polymorphism (%)
OPAH-01	500 - 1500	8	5	63
OPAH-02	500 - 1400	4	4	100
OPAH-09	250 - 1500	12	11	92
OPAH-11	400 -1600	7	7	100
OPAH-12	400 - 2000	11	9	82
OPAH-17	300 - 1400	10	8	80
OPAH-18	300 - 2000	7	5	71
OPAH-19	400 - 1100	7	7	100
OPAI-08	200 - 1400	12	10	83
OPAI-11	200 - 1400	12	6	50
OPAI-12	300 - 1000	8	6	75
OPAI-14	300 - 1100	9	9	100
OPC-16	250 - 1800	9	9	100
OPK-16	150 - 1200	10	3	30
OPX-01	250 - 1500	9	7	78
OPX-03	250 - 1500	13	13	100
OPX-09	700 - 1400	5	5	100
Total/Mean		153	124	81

Statistical analysis

RAPD data were recorded either as 1 for the presence of a band or 0 for its absence to generate a binary matrix. Only reproducible bands were scored. The data set was used to perform Principle Coordinate (PCoA) and cluster analyses using NTSYS (Rohlf, 1997). In this analysis, a similarity matrix was generated using Jaccard coefficients. This matrix was then used for PCoA. For cluster analysis, the UPGMA (Unweighted Pair Group Method using Arithmetic Average) method was used to construct cladogram. The bootstrap values for the clusters were calculated by 1000 replicates using PAUP (Swofford, 1998).

RESULTS AND DISCUSSION

All RAPD primers generated successful and consistent banding patterns. Seventeen primers tested generated 153 bands, 124 of which were polymorphic (Table 1). Thus, the overall average polymorphism was 81%. The sizes of the amplified fragments ranged from ~150 bp (OPK 16) to ~2000 bp (OPAH-12 and OPAH-18). The number of bands obtained ranged from four (OPAH-02) to 13 (OPX-03). The same number of bands and the primers accounted for the ranges in polymorphic bands. All of the bands generated by OPAH-02, OPAH-11, OPAH-19, OPAI-14, OPC-16, OPX-03 and OPX-09 were polymorphic while the percentage of polymorphic bands were only 30 and 50% for OPK-16 (3/10) and OPAI-11 (6/12), respectively.

All individuals display a specific RAPD profile confirming that all individuals are genetically different. UPGMA cladogram of the olive accessions tested was presented in Figure 2. As it can be seen from the Figure, three were three small groups supported by bootstrap analysis. The first group was formed by the cultivars/clones ‘Ayvalık’, ‘Gemlik’, ‘Memecik’ and ‘Akilli asici’. It should be noted that this group was formed by the cultivars from Marmara Region. The second group included ‘Halhali 1’, ‘Halhali 2’, ‘Ensititu’ and ‘Sari Hasabi’. These cultivars/clones are from Eastern Mediterranean Region. ‘Derik’ and ‘Girit zeytini’ formed the last group supported by the bootstrap analysis. These cultivars/clones are from Southeastern (‘Derik’) and Aegean (‘Girit zeytini’) Region of Turkey. The three group supported by the bootstrap analysis had different geographical origins. Therefore it seems that there is a correlation between molecular data created by RAPD markers and the geographical origin for these cultivars as already shown in other previous studies (Besnard et al., 2001; Khadari et al., 2003).

The separations of the other groups were not supported by the bootstrap analysis. Thus, the separation of the groups should be considered as subgroups within the ungrouped genotypes. As expected, the wild accessions as well as Israel cultivar ‘Barnea’ were different from the rest of the cultivars/clones. ‘Adana Topagi’ stood alone in the analysis as well. The rest of the genotypes, having the six old genotypes from the Eastern Mediterranean Region, formed four subgroups. In the first one, ‘Domat’ and PM6 grouped together. PK and PM4 grouped in the other one. The last two groups had the genotypes of the question (PK and PM2-PM6) and some clones. In the one of these subgroups, Yerli, ‘Erkence’ and PM2 grouped together while ‘Barrevi’, PM3 and PM5

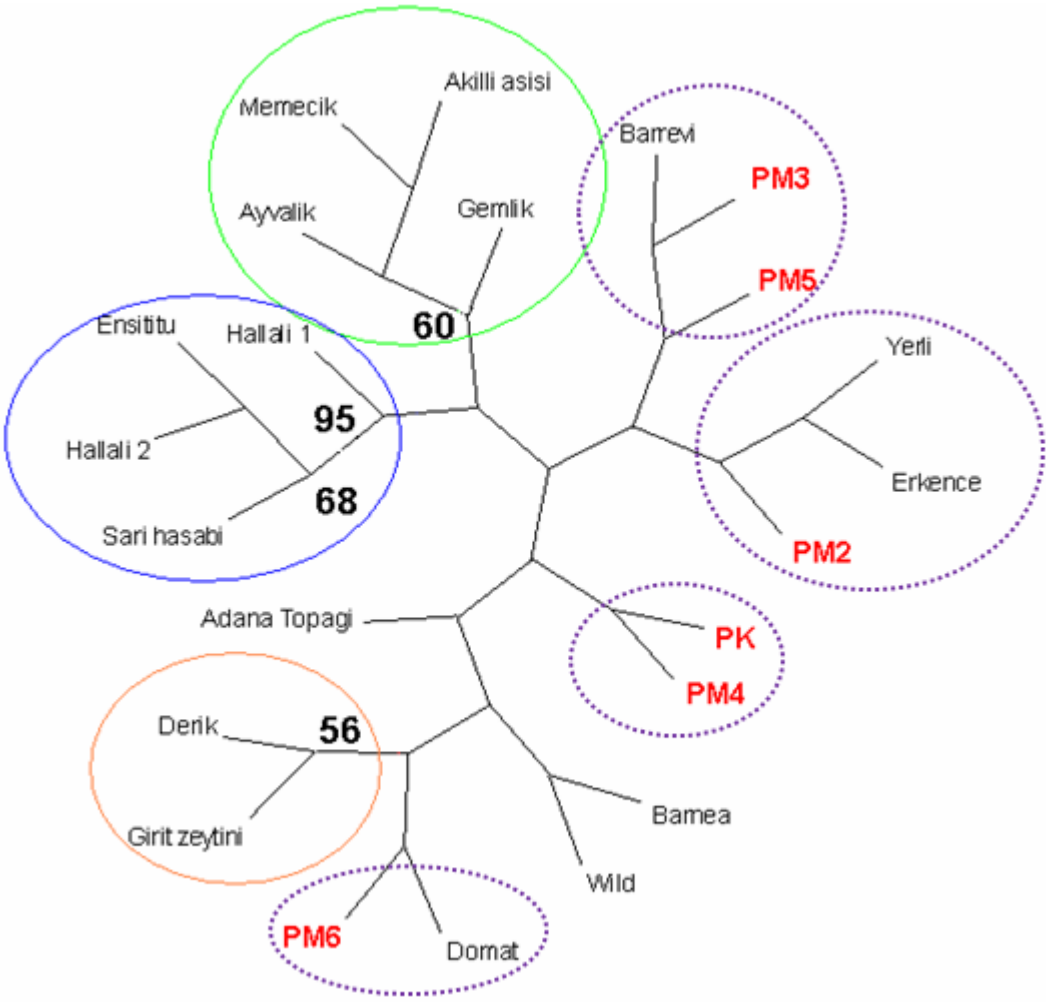

Figure 2. UPGMA cladogram of 23 olive accessions with the bands generated by 17 arbitrary RAPD primers. Bootstrap values were indicated for each node if they were higher than 50. Solid lines, in different colors, indicates the groups while dotted lines indicated subgroups (unsupported by bootstrap values).

grouped at the last one.

The results of the PCoA were presented in Figure 3. The first three dimensions explained the 12, 9 and 8% of the variation making a total of 29%. The comparative analysis of six old cultivars from Eastern Mediterranean Region to the 17 other olive genotypes with PCoA gave similar results to those of cluster analysis. The two of the three groups of the cluster analyses (1 = 'Ayvalık', 'Gemlik', 'Memecik' and 'Akilli asici'; 2 = 'Halhali 1', 'Halhali 2', 'Ensititu' and 'Sari Hasabi') were formed similar groupings by PCoA as well. However, the two genotypes ('Derik' and 'Girit zeytini') were placed differently. 'Girit zeytini' was among the other ungrouped genotypes. However, despite from this exceptions and overall pattern similarity was present as the rest of the genotypes.

The ungrouped genotypes Israel cultivar, 'Barnea' was located distantly from other accessions in the three-dimensional graph (Figure 2). The old genotypes from Eastern Mediterranean Region did not group together, but were at proximity of other genotypes than in the cluster analysis. For example, PM4 and PK were closely located on the three-dimensional graph. However, there were slight discrepancies as well. 'Domat' was closer to PM5 when compared to its relatedness to PM6 although 'Domat' and PM6 grouped together in the cluster analysis.

Olive is one of the most important Mediterranean crops. Its production has been scattered throughout the regions with Mediterranean climates. Because it can be used for many different ways, it has been in the diet of the man for thousands years. Along with the general germplasm preventions and utilizations, there has been an interest on characterization of local and regional genotypes. Molecular markers have been a preferable tool to serve this purpose because of their advantages such as high polymorphism, low cost, independence from environmental factors (Staub et al., 2002). For example, Ganino

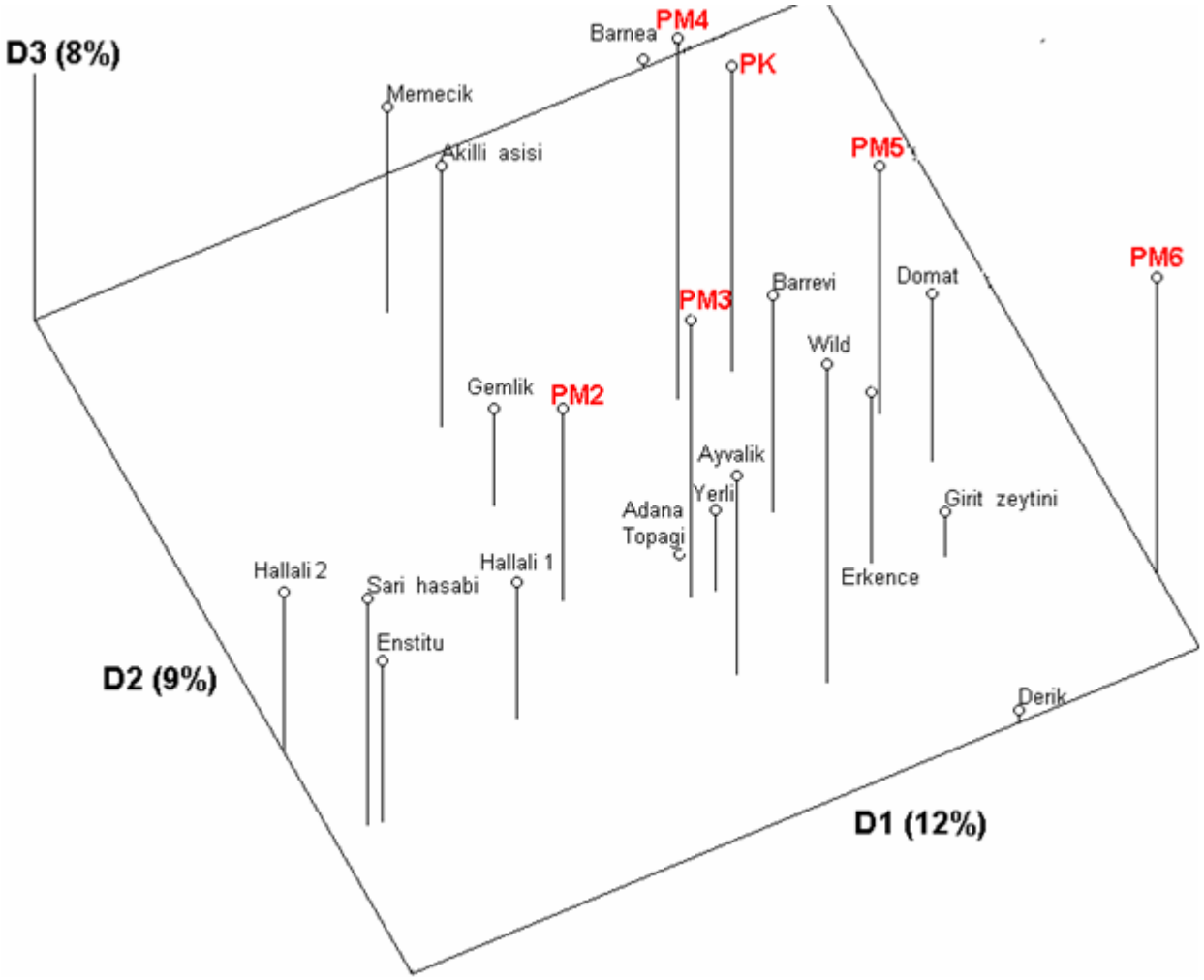

Figure 3. The distribution of 23 olive accessions on the first three dimensions (D) after the principle coordinate analysis of the bands generated by 17 arbitrary RAPD primers.

et al. (2007) studied olive clones from the Emilia region (Northern Italy) along with eight standard cultivars by 20 RAPD primers and 3 SSR primers. Their molecular analyses showed the genetic relationship among accessions. Their results also indicated the reliability of RAPDs and SSRs to identify all studied olive cultivars and to reveal the degree of their relatedness to each other. Similarly, Poljuha et al. (2008) characterized 27 olive accessions grown in Istria (Croatia) using 12 microsatellite markers revealing a total of 81 alleles. Their analysis shed light into the genetic relationships of varieties of Croatian Istria with introduced olive varieties, along with the neighboring Slovene Istria region. Once again, the results confirmed that the efficiency of the molecular markers characterizing olive genotypes from a relatively small region.

In our study, six old olive cultivars with the aging 100 – 500 years old along with 15 Turkish cultivars and two outgroups were characterized by 17 RAPD primers. The RAPD profiles clarified the molecular relationship among the genotypes tested successfully. Among the local material tested, the most interesting clone was PK as it was the oldest among the tested genotypes. However, no clear relationship was found with any of the other cultivar tested to PK. Such a relationship would indicate descent from this old material. However, several cultivars/clones sampled from different parts of Turkey did not have close relationships.

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