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Determination of the genetic relationships between wild olive (*Olea europaea oleaster*) varieties grown in the Aegean region

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ABSTRACT. The RAPD technique was used for determining genetic differences between 12 wild-olive varieties grown in the Aegean provinces of Izmir, Mugla, and Manisa in Turkey. Wild olives obtained from the same provinces were included in the same plot. Twenty of 25 operon primers (OP-I 4, OP-I 14, OP-I 15, OP-I 16, OP-I 17, OP-Q1, OP-Q2, OP-Q3, OP-Q4, OP-Q11, OP-Q12, OP-Q13, OP-Q14, OP-Q15, OP-Q16, OP-Q17, OP-Q18, OP-Q19, OP-Q20, OP-F1, OP-F2, OP-F3, OP-F6, OP-F7, OP-F8) yielded bands. The differences between the varieties were determined based on their genetic similarities, using principal coordinate analysis; genetic distances were determined using neighbor-joining analysis. The varieties wild 7 and wild 12 had the lowest genetic similarity (0.97, Jaccard similarity index); they also had the greatest genetic distance between them (0.3606, Nei's genetic distance). It was concluded that the RAPD technique is adequate for the evaluation of genetic relationships among wild olives. Principal coordinate analysis and neighbor-joining analysis gave results that support the

use of this type of analysis to help understand the genetic background of olives and for further genetic studies.

Key words: *Olea europaea oleaster*; RAPD; Neighbor-joining method; Principal coordinate analysis

INTRODUCTION

Olive is a generous tree with two different subspecies including wild olive (*Olea europaea oleaster*) within the *Olea europaea* L. species. Turkey holds an important position among olive growing countries. Pelletier suggested that the homeland of wild olive tree is Anatolia where many wild olives form dense forests (Olive and Olive Oil Publicity Committee, 2009). Wild olives are used as rootstock in olive cultivation, because wild and primitive samples are mostly used in transferring the genes controlling qualitative characters and typically the genes for resistance against diseases and in direct selections (Sehirali and Özgen, 1987; Mendilcioglu, 1999; Olive Research Institute, 2007). In this respect, wild olive types are very important in terms of breeding.

Molecular markers used commonly in the research of genetic characteristics of olives include randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism, simple sequence repeats, and sequence characterized amplified regions (Wu et al., 2004; Baldoni et al., 2006). In the examination of genetic structures of plants, the RAPD method based on polymerase chain reaction (PCR) is an easy method in which primers formed by random short nucleotide sequences and much less plant DNA are used (Williams et al., 1990; Welsh and McClelland, 1990). After obtaining the RAPD profile, family trees are developed with phylogenetic analyses such as UPGMA, neighbor-joining by using different indexes such as Nei's genetic distance, Jaccard's similarity or Dice's similarity (Jaccard, 1902; Dice, 1945; Nei and Li, 1979).

Neighbor-joining is one of the most widely used algorithms for constructing dendrograms from a distance matrix. Neighbor-joining is different from UPGMA in that the branch lengths for sister taxa can be different, and thus provides additional information on relationships between populations. Neighbor-joining is useful for large data sets and for bootstrap analysis, which involves the construction of hundreds of replicate trees (Allendorf and Luikart, 2007).

UPGMA and other pairwise alignment methods are older methods for producing trees. The newer and more accurate trees are created by neighbor-joining (Cristianini, 2006) and principal coordinate analysis (PCO), which provide estimates of genetic similarity between individuals, and have been used as an alternative way to represent inter-individual and inter-group relationships (Baldoni et al., 2006).

In studies with olives, Besnard et al. (2001) argued that RAPD profiles of 112 olives collected from the Mediterranean basin were collected in 24 groups, and that the distribution of varieties varied in accordance with their country, region and the use of olives. They suggested that this may be an indicator that variety selection is performed in different genetic pools and different regions. They supported their assertion through mitotic RFLP studies and noted that selection was available in many countries. Wu et al. (2004) studied the molecular linkage map of olive by using RAPD, microsatellite and sequence characterized amplified region markers. Mekuria et al. (1999, 2002), in their studies performed on olive types available in Australia, analyzed both the cultivated type produced commonly in that country and the isolated wild types. They determined the differences within and between the groups by using

RAPD markers in order to identify genetic polymorphism between and within olive groups in their study performed using the RAPD method on a wild population.

In consideration of neighbor-joining analysis applications in different plants, Joung et al. (2001) used this method in order to determine the characterization of *Acer griseum*. Mohammadi and Prasanna (2003) noted that the neighbor-joining method has been more commonly used for phylogenetic studies.

Baldoni et al. (2006), in their study, acquired two-dimensional PCO plots by using PCO analysis. They reported that the greatest distance between wild plants and cultivars was obtained in the Sardinian set of samples. Additionally, good differentiation was also shown by two Sicilian sets, while some of the Umbrian cultivars were intermixed with *oleasters*.

The purpose of this study was to determine the genetic distances of 12 wild olive types obtained from three different provinces of the Aegean region (Izmir, Mugla and Manisa) by using the RAPD method with PCO analysis over genetic similarities, and with neighbor-joining analysis over genetic distances.

MATERIAL AND METHODS

Plant material

The wild olives used in our study were supplied from the villages of Manisa, Izmir and Mugla provinces and wild 8 and wild 9 were supplied from the Olive Research Institute of Turkey. Fresh leaves were taken from a total of 12 wild olives and they were kept in liquid nitrogen until DNA extraction. Table 1 shows wild olives used in this study and their provinces.

Table 1. Provinces where wild olives were supplied.

Type of olive	Province
Wild 1	Pınarcık 1, Milas, Mugla, Turkey
Wild 2	Pınarcık 2, Milas, Mugla, Turkey
Wild 3	Caglak 1, Akhisar, Manisa, Turkey
Wild 4	Caglak 2, Akhisar, Manisa,Turkey
Wild 5	Harlak, Akhisar, Manisa, Turkey
Wild 6	Sabancılar 1, Akhisar, Manisa, Turkey
Wild 7	Sabancılar 2, Akhisar, Manisa, Turkey
Wild 8	Bornova 1, Bornova, İzmir, Turkey
Wild 9	Bornova 2, Bornova, İzmir, Turkey
Wild 10	Yayakırıldık 2, Akhisar, Manisa, Turkey
Wild 11	Dikili, Bademli, İzmir, Turkey
Wild 12	Karacakas 2, Soma, Manisa, Turkey

DNA extraction and RAPD-PCR analysis

Genomic DNA was extracted from young leaves using the Doyle and Doyle method (1987). Twenty-five different decamer primers were used for RAPD analyses of *Olea europaea oleasters*. A total of 25 primers from Kit OP-I, OP-Q, OP-F (OP-I 4, OP-I 14, OP-I 15, OP-I 16, OP-I 17, OP-Q1, OP-Q2, OP-Q3, OP-Q4, OP-Q11, OP-Q12, OP-Q13, OP-Q14, OP-Q15, OP-Q16, OP-Q17, OP-Q18, OP-Q19, OP-Q20, OP-F1, OP-F2, OP-F3, OP-F6, OP-F7, OP-F8) (Operon Technologies, Alameda, CA, USA) were used for RAPD-PCR analysis. PCR was performed on an Eppendorf MasterCycler Thermal Cycler in a total volume of 25 µL PCR mix including 25 ng template DNA, 2.42 µL 10X PCR buffer (with $MgCl_2$, Sigma), 0.44 µL dNTP (Sigma), 1 µM

primer, and 0.13 µL Taq DNA polymerase (Sigma). The amplification reactions were carried out for 60 s at 94°C as an initial denaturation. The PCR program comprised 35 cycles with 20 s at 94°C; 20 s at 35°C; 30 s at 72°C and a final extension performed at 72°C for 5 min.

Amplification products were loaded onto 1.5% agarose gels (Sigma) in 0.5X TBE buffer with 0.5 µg/mL ethidium bromide at a constant voltage of 100 V. For evaluating the base pair length of bands, a DNA ladder (Sigma) was loaded on the first lane of each gel. After the separation of PCR products by agarose gel electrophoresis, gels were visualized with the Photo Print (Vilber Lourmat, France) imaging system and analyzed by the BioOne D++ software (Vilber Lourmat).

Data analysis

Principal coordinate analysis was performed to show the similarities of the wild olives in a scatter plot. PCO analysis was done with FAMD version 1.2 (Fingerprinting analysis with missing data) using the Jaccard's similarity index (Schlüter and Harris, 2006). The RAPD data were used to compute the genetic distances of wild olives according to Nei's distance index and MEGA version 4.1 (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2007) was used to construct a neighbor-joining (Saitou and Nei, 1987) dendrogram.

RESULTS AND DISCUSSION

At the end of the RAPD analysis 109 bands were obtained of which 80.73% were polymorphic. The number of bands per individual is 11.17. Twenty-five primers were used in the study and 5 of the primers belonging to OP-F did not work (OP-F1, OP-F2, OP-F3, OP-F6, and OP-F7).

Each RAPD band was treated as a separate character and scored as either 1 (present) or 0 (absent), and a rectangular binary data matrix was obtained. A similarity matrix was obtained using Jaccard's (1902) coefficient and converted to similarities (Figure 1). The similarity matrix was constructed using the PCO procedure (Figure 2). A distance matrix was constructed using the Nei (1972) distance index (Figure 3), and neighbor-joining analysis was done with Mega version 4.1.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
W1	0.000											
W2	0.833	0.000										
W3	0.928	0.937	0.000									
W4	0.941	0.823	0.895	0.000								
W5	1.000	1.000	0.923	1.000	0.000							
W6	0.900	0.818	1.000	0.937	0.888	0.000						
W7	0.954	0.913	0.917	0.885	0.900	1.000	0.000					
W8	1.000	0.944	0.947	0.954	0.933	0.933	0.783	0.000				
W9	1.000	0.950	1.000	1.000	0.941	1.000	0.929	0.956	0.000			
W10	0.937	0.944	1.000	1.000	1.000	0.933	1.000	1.000	0.909	0.000		
W11	1.000	1.000	1.000	0.931	1.000	1.000	0.941	1.000	0.933	0.888	0.000	
W12	1.000	1.000	1.000	1.000	1.000	1.000	0.970	0.964	0.893	0.964	0.844	0.000

Figure 1. Similarity matrix (Jaccard's coefficient) of the wild olives. For W1-W12 identifications, see legend to Figure 3.

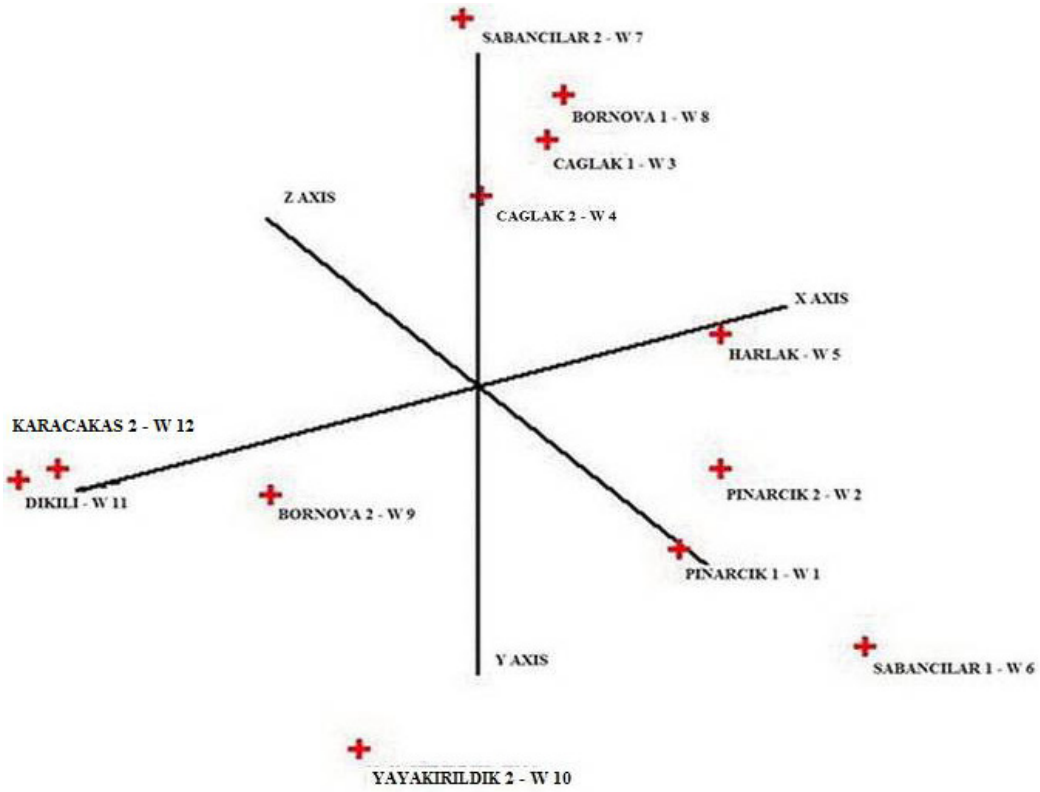

Figure 2. Similarities between the 12 wild olives as found by principal coordinate analysis.

	W 1	W 2	W 3	W 4	W 5	W 6	W 7	W 8	W 9	W 10	W 11	W 12
W 1	****											
W 2	0.0962	****										
W 3	0.1270	0.1481	****									
W 4	0.1587	0.1375	0.1696	****								
W 5	0.1064	0.1270	0.1166	0.1696	****							
W 6	0.0862	0.0862	0.1375	0.1481	0.0762	****						
W 7	0.2140	0.2140	0.2254	0.2370	0.1805	0.2254	****					
W 8	0.1696	0.1696	0.1805	0.2140	0.1375	0.1375	0.1805	****				
W 9	0.1915	0.1915	0.2254	0.2605	0.1587	0.1805	0.2725	0.2254	****			
W 10	0.1481	0.1696	0.2027	0.2370	0.1587	0.1375	0.2969	0.2254	0.2027	****		
W 11	0.2605	0.2846	0.2969	0.2846	0.2487	0.2487	0.3475	0.3219	0.2969	0.2487	****	
W 12	0.2487	0.2725	0.2846	0.3219	0.2370	0.2370	0.3606	0.2846	0.2605	0.2846	0.2846	****

Figure 3. Distance matrix (Nei, 1972) of the wild olives. W 1 = Pınarcık 1; W 2 = Pınarcık 2; W 3 = Caglak 1; W 4 = Caglak 2; W 5 = Harlak; W 6 = Sabancılar 1; W 7 = Sabancılar 2; W 8 = Bornova 1; W 9 = Bornova 2; W 10 = Yayakırıldık 2; W 11 = Dikili; W 12 = Karacakas 2.

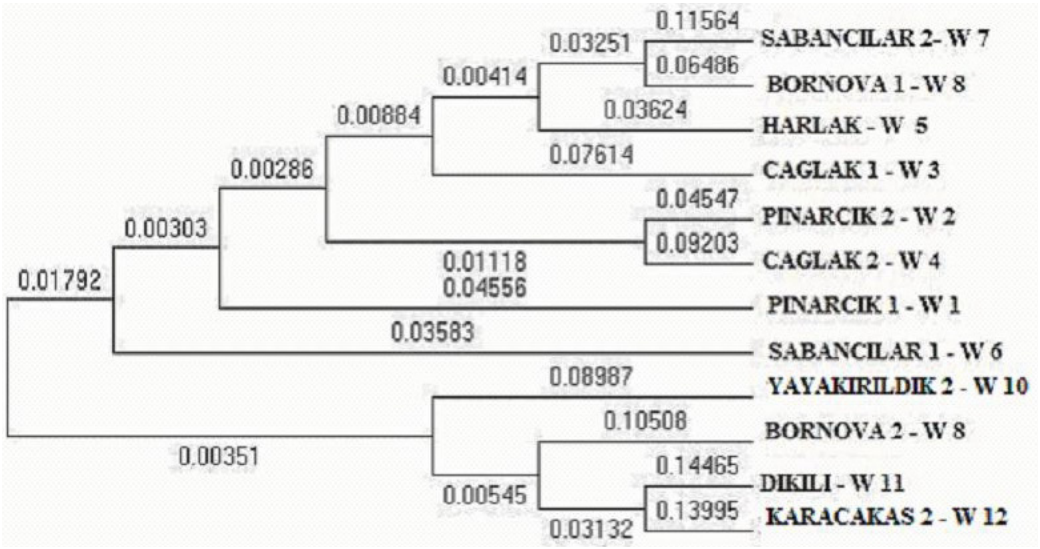

Figure 4. Dendrogram of wild olives based on RAPD polymorphism clustered by the neighbor-joining analysis. In accordance with the Jaccard's similarity matrix shown in Figure 1, the types presenting the closest genetic similarity are wild 7 and wild 8 with a value of 0.783. The types presenting the lowest genetic similarity are wild 7 and wild 12 with a value of 0.970. In accordance with the result obtained with principal coordinate analysis (Figure 2), the proportions obtained from the X-axis, Y-axis and Z-axis were 15.38, 13.64 and 11.51%, respectively. A total of 40.53% was found. Accordingly, it was determined that wild 11 and wild 12 samples are in the same plot, which are the wild types grown in districts that are closest to each other as the place of settlement. Wild 3 and wild 4 are in the same plot; and both are grown in Akhisar district. It was observed that wild 1 and wild 2 are located at a position not distant to each other in the plot, both were supplied from the Milas district.

A distance matrix was made on the basis of Nei (1972) (Figure 3) and clustered by neighbor-joining analysis (Figure 4). According to this matrix, genetic distance values were found to be between 0.0762 and 0.3606. Hereunder, it was determined that the samples closest to each other based on their genetic distance values are wild 5 and wild 6, and the samples most distant from each other are wild 7 and wild 12.

As a result, RAPD technique was shown to be adequate for the discrimination of wild-type olives. Furthermore, PCO and neighbor-joining analysis yielded results that support each other in the determination of genetic differences between wild olive types that are grown in different districts of the same region (the genetically most distant samples showed the same results in both indexes) and support the common use of such analyses.

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