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; Mendilcioğlu, 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...
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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>
<thead>
<tr>
<th>Type of olive</th>
<th>Province</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild 1</td>
<td>Pinarcık 1, Milas, Mugla, Turkey</td>
</tr>
<tr>
<td>Wild 2</td>
<td>Pinarcık 2, Milas, Mugla, Turkey</td>
</tr>
<tr>
<td>Wild 3</td>
<td>Caglak 1, Akhisar, Manisa, Turkey</td>
</tr>
<tr>
<td>Wild 4</td>
<td>Caglak 2, Akhisar, Manisa, Turkey</td>
</tr>
<tr>
<td>Wild 5</td>
<td>Harlak, Akhisar, Manisa, Turkey</td>
</tr>
<tr>
<td>Wild 6</td>
<td>Sabancılar 1, Akhisar, Manisa, Turkey</td>
</tr>
<tr>
<td>Wild 7</td>
<td>Sabancılar 2, Akhisar, Manisa, Turkey</td>
</tr>
<tr>
<td>Wild 8</td>
<td>Bornova 1, Bornova, İzmir, Turkey</td>
</tr>
<tr>
<td>Wild 9</td>
<td>Bornova 2, Bornova, İzmir, Turkey</td>
</tr>
<tr>
<td>Wild 10</td>
<td>Yaykuralık 2, Akhisar, Manisa, Turkey</td>
</tr>
<tr>
<td>Wild 11</td>
<td>Dikili, Bademli, İzmir, Turkey</td>
</tr>
<tr>
<td>Wild 12</td>
<td>Karacakas 2, Soma, Manisa, Turkey</td>
</tr>
</tbody>
</table>

Table 1. Provinces where wild olives were supplied.

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-1 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) (Ope- ron 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 MgCl₂, 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.

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.

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.
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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REFERENCES


