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Molecular Characterization of Some Selected Wild Olive (*Olea oleaster* L.) Ecotypes Grown in Turkey

Mücahit Taha ÖZKAYA[1] Erkan ERGÜLEN[2] Salih ÜLGER[3] Nejat ÖZİLBEY[4]

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Abstract: The wild olive subspecies oleaster called "Karadelice" in Turkish is a small tree or bush of rather irregular growth, with thorny branches and oppositely positioned oblong pointed leaves, dark grayish-green on the leaf surface and, in the early growth state, hoary on the lower surface with whitish scales. Generally, it is used as a dwarf rootstock; however, it has some grafting incompatibility with certain important olive cultivars. Some wild olive plants were selected from the village Kayadibi, 20 km distant from the city of İzmir in Turkey. This region is a very unique place for this type of wild olive. These ecotypes were differentiated by molecular markers using RAPD-PCR. Since they can be used as a dwarf rootstock, the correlations with some important olive cultivars were analyzed. For that reason Ayvalık cv, which is the most important olive cultivar for olive oil production was used primarily. Since Ayvalık cv and KD-8 are 97% similar, it was expected that they may have grafting compability. In the second part of the study, the comparison were done with Memecik and Tavşan Yüreği cultivars which are important olive oil and table olive cultivars, respectively. Since Memecik and Tavşan Yüreği were 100% similar therefore, it was considered that they may have more grafting compability with oleasters KD-3 and KD-8. Both studies were carried out to find similarities in 9 olive oleasters growing at Kayadibi of İzmir province which are said to have confused and low level morphologic variations in their features.

Key Words: Wild olive, Olive, Oleaster, RAPD, ecotypes, Ayvalık, Memecik, Tavşan Yüreği, Karadelice

Türkiye'de Yetişen Bazı Seçilmiş Karadelice (*Olea oleaster* L.) Ekotiplerinin Moleküler Tanımlaması

Öz: Karadelice olarak adlandırılan yabani zeytin, alt tür oleaster; düzensiz büyüme gösteren dikenli dallara sahip bodur ağaç veya çalı formundadır. Genç yapraklar, koyu grimsi-yeşil renkli üst ve açık renkli alt yüzeye sahip, sürgün üzerinde bir birine karşılıklı olarak dizilmiştir. Genellikle bodur anaç olarak kullanılmaktadır ancak bazı önemli zeytin çeşitleri ile aşı uyuşmazlığı bulunmaktadır. Karadelicelerin ender bulunduğu yerlerden biri olan İzmir'e 20km mesafedeki Kayadibi köyünden bazı karadeliceler seçilmiştir. Bu ekotiplerin RAPD-PCR tekniği ile moleküler tanımlamaları yapılmıştır. Bodur anaç olarak kullanılabilirliği nedeniyle bazı önemli zeytin çeşitleri ile ilişkileri de analiz edilmiştir. Bu nedenle öncelikle zeytinyağı üretimi için en önemli zeytin çeşidi olan Ayvalık çeşidi kullanılmıştır. Ayvalık çeşidinin KD-8 ekotipi ile %97 oranında benzerlik göstermesi anaç-çeşit aşı uyuşabilirliğini de gösterebilir. Çalışmanın ikinci kısmında karşılaştırma önemli bir yağlık zeytin çeşidi olan Memecik ile önemli bir sofralık zeytin çeşidi olan Tavşan Yüreği ile yapılmıştır. Memecik ve Tavşan Yüreği çeşitleri %100 benzerlik göstermiş olmaları, her ikisinin de KD-3 ve KD-8 ekotipleri ile aşı uyuşabilirliğini gösterebilir. Her iki çalışmada da Kayadibi-İzmir'de yetişen dokuz karadelice ekotipi içinde, en düşük seviyede morfolojik farklılık gösteren ve karıştırılanlarda genetik bazda benzerlik bulunmuştur.

Anahtar Kelimeler: Delice, Oleaster, Karadelice, RAPD, Zeytin, Yabani zeytin, Ayvalık, Memecik, Tavşan Yüreği

Introduction

The olive and oleaster correspond to *Olea europaea* subsp. *europaea* L. var. *europaea* and var. *sylvestris*, respectively. Oleaster is the wild form, while the olive is the cultivated form. The oleaster is believed to have originated in the eastern Mediterranean, implying that those in the western Mediterranean basin could be feral. The genetic relationships between the two remain unclear, as it is difficult to identify trees on the basis of morphology in many (Besnard and Berville 2000, Lumaret and Quazzani 2001, Breton et al. 2006). According to Zohary and Spiegel Roy (1975), the oleaster type olives originated in the

[1] Ankara Univ., Fac. of Agriculture, Department of Horticulture, Ankara, Turkey
[2] Duzen Laboratory Groups, Ankara, Turkey
[3] Akdeniz Univ., Fac. of Agriculture, Department of Horticulture, Antalya, Turkey
[4] Ministry of Agriculture and Rural Affairs, Olive Research Institute, Bornova, Izmir, Turkey

eastern Mediterranean basin where it was domesticated about 5850 years ago, which infers an absence in the western end (Zohary 1994, Besnard et al. 2001, Belaj et al. 2002). Spennemann and Allen (2000) reported that the cultivars moved westward with human migration or birds through seed dissemination resulting in feral olive trees. So these trees may be considered as oleasters and therefore confused with genuine oleasters from the eastern Mediterranean. In every location where cultivated olives grow, wild olives called oleaster also exist. According to Vossen (2007), these plants may be seedlings of cultivated varieties spread by birds and other wildlife feeding on the fruit, or they could be more native forms of subspecies or ecotypes that already existed there before the introduction of the cultivated olive. All the Olea genera have the same chromosome number (2n=46), and crosses between many of them have been successful. Most scientists now use the nomenclature of Olea europaea L. sativa to distinguish it from the wild olive subspecies oleaster (Lavee, 1996). The olive trees which were accepted as feral olive trees may not differ from the eastern oleasters when using phenotypic description, but they may be differentiated by molecular markers. Bronzini de Caraffa et al, (2002) have performed a study of nuclear and mitochondrial DNAs of cultivated and wild olives from two Corsican and Sardinian Mediterranean islands by using RAPD and RFLP markers. The results have indicated that the combination of mitotype and RAPD markers can be used as a powerful tool for differentiating two groups in the wild forms: the Western true oleasters and the feral forms.

Rootstock characteristics have been less studied than those of the cultivars in olives; consequently, rootstock selection has been somehow neglected. Şeker et al. (2009) have assessed the level of genetic variation in the seedling population of 'Uslu' cultivar on the basis of isoenzymatic variability of six enzyme systems.

The wild olive subspecies oleaster which is called "Karadelice" in Turkish is a small tree or bush of rather irregular growth, with thorny branches and oppositely positioned oblong pointed leaves, dark grayish-green on the leaf surface and, in the early growth state, hoary on the lower surface with whitish scales. Generally, it is used as a dwarf rootstock; however, it has some grafting incompatibility with certain important olive cultivars, such as Memecik and Ayvalık (Ülger ve ark. 2001).

A study by Ozkaya et al. (2004) identified ten olive cultivars and was a first attempt to characterize important olive varieties grown in Turkey at molecular level using the RAPD system. According to the RAPD-PCR dendrogram, five cultivars (Domat, Gemlik, Kilis Yaglik, Manzanilla and Nizip Yaglik) were clustered in group one which show more than 60% genetic similarity. Derik Halhali, Sarı Ulak and Ayvalık were separated from all other cultivars. The cultivars Memecik and Tavşan Yüreği were placed in a separate group in which they had about 55% similarity.

Ayvalık originated from Edremit-Balıkesir and is cultivated in Balikesir, İzmir and some other areas for olive oil. Memecik originated from Muğla and is cultivated in Muğla, Antalya and Aydın for dual purposes (oil and table). Tavşan Yüreği originated from Fethiye-Antalya and is cultivated in Antalya and Muğla as table olive (Ozkaya et al. 2004).

The characterization of olive germplasm is an essential task in the modern olive culture. Genetic identification is important to protect and to preserve the genetic resources, to safeguard the typical oils (Busconi et al. 2003) and to certificate the propagation material.

In this study some wild olive plants were selected from village Kayadibi, 20 km distant from the city of İzmir in Turkey, which is a very unique place for this type of wild olives. These samples were differentiated by molecular markers using RAPD-PCR. Since they can be used as a dwarf rootstock, it should be better to see if they have a correlation with some important olive cultivars. For that reason Ayvalık cv was used primarily, as this is the most important olive cultivar for olive oil production. In the second part of the study, comparisons were done with Memecik and Tavşan Yüreği cultivars which are important olive cultivars for olive oil and table olive, respectively.

The present study represents a first attempt to characterize oleasters "Karadelice" which are the important dwarf rootstocks, in Turkey at molecular level using the RAPD system.

Material and Methods

Nine wild-olive eco-types (*Olea europaea oleaster*) which are referred by name as "Karadelice" and denoted as in Table 1. were collected from Kayadibi-İzmir province. The olive cvs. Ayvalık, Memecik and Tavşan Yüreği (*Olea europea* L.) from the National Olive Germplasm Collection, Bornova, Izmir, Turkey and were characterized using the RAPD -PCR technique.

These nine Karadelice ecotypes were analyzed together with olive cvs. Ayvalık, Memecik and Tavşan Yüreği. However two PCR reactions were conducted; one of them was nine Karadelice with cv. Ayvalık and the other one was nine Karadelice with cvs. Memecik and Tavşan Yüreği.

Table 1. Denotations of Karadelice in the dendograms and in the text and tables

No.	Code in the Dendograms	Code in the text and tables
1	Delice-1	KD-1
2	Delice-2	KD-2
3	Delice-3	KD-3
4	Delice-4	KD-4
5	Delice-5	KD-5
6	Delice-6	KD-6
7	Delice-7	KD-7
8	Delice-8	KD-8
9	Delice-9	KD-9

DNA extraction: Total DNA was extracted from leaf material by using modified CTAB method of Doyle and Doyle (1987). Young leaves (0.2 g) were ground using mortar and mixed with 1ml extraction buffer containing 100mM Tris-HCl pH 8, 10mM EDTA, 1M NaCl, 2% CTAB, 2% PVP 40, 0.1% β-mercapto-ethanol. Samples were incubated for 15 min. at 65ºC in 0.5 ml of chloroform/isoamyl alcohol (24:1), then centrifuged at 5,000×g for 5 min. The aqueous phase was recovered and mixed with a two-third volume of isopropanol. The mixture was centrifuged at 14,000×g for 5 min. The pellet was washed with 1 ml ethanol/ammonium acetate (76% ethanol, 10mM ammonium acetate) and centrifuged at 14,000×g for 5 min. The pellet was resuspended in 0.2 ml DNase- and RNase-free water, and the DNA concentration was determined by spectrophotometer at 260 nm.

RAPD analysis: Fourteen different RAPD oligonucleotide primers (A-04, A-11, A-16, C-11, C-15, D-03, E-20, K-19, Q-15, Q-17, S-03, X-01, Z-10, Z-11, Operon, Inc.) were used in each PCR and only eight of them produced polymorphic bands. Primers were amplified twice to ensure their reliability and reproducibility. For each PCR amplification was carried out in a total volume of 25 µl containing 200 µM dNTPs, 2 mM MgCl2, 1 µM primer (Operon), 1U Taq DNA polymerase (Promega), 1x Reaction Buffer (Promega) and 25 ng template DNA. The PCR reactions were performed with a initial step of denaturation at 94ºC for 1 min, followed by 50 cycles of denaturation at 94ºC for 20 sec, annealing at 35ºC for 20 sec, extension at 72ºC for 30 sec, and final extension at 72ºC for 5 min. Amplification products were separated on 1.5% agarose gel (SeaKem, FMC) in TAE buffer, stained with ethidium bromide, recorded and analyzed by using UVP Gel Analysis System. Molecular sizes of amplification products were estimated using φX174 DNA/Hae III marker (Promega).

Both data generated by both RAPD-PCR analyses were analyzed using the Nei similarity index (Nei and Li 1979). The dendrograms were constructed based on the similarity matrix data by applying an unweighted based on Nei's similarity index calculated from RAPD data pair group method with arithmetic averages (UPGMA) cluster analysis using the NTSYS program (Exeter Software, Setauket, N.Y.).

Results and Discussion

A total of 68 reproducible amplifications were obtained by use of 8 primers, of which 29 were polymorphic. The number of reproducible bands averaged 6 per primer.

RAPD-PCR for Nine Karadelice with Ayvalık: The Nei estimate of similarity showed similarity values ranging from 0.7895 for KD-4, KD-6 and KD-7 to 0.9474 for KD-8 and Ayvalık. The dendogram resulting from the UPGMA analysis is shown in Figure 1. The molecular analysis showed variability within nine oleasters and between Ayvalık cv and nine oleasters collected from Kayadibi. There were two main groups (KD-4, KD-6, KD-7 and the others) with subgroups. Ecotypes of oleasters KD-4, KD-6 and KD-7 were least similar (88%) type and located in a seperate group. This group was 79% similar with other groups. Ecotype KD-1 distinguished itself in a separate subgroup, showing a similarity of 91% with the others. The oleaster KD-8 and the cv. Ayvalık were 97% similar. Ecotypes KD-5 and KD-9 were 94% similar (Figure 2 and Table 2).

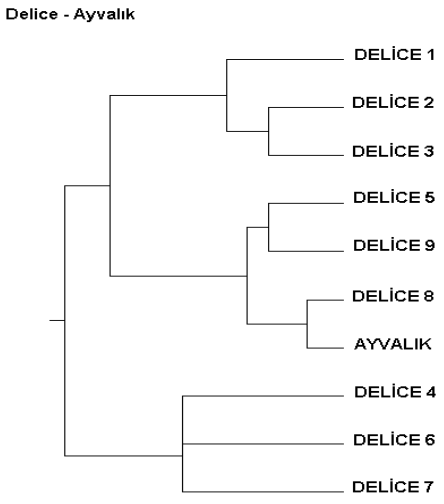

Figure 1. Dendogram of nine ecotypes from Kayadibi-İzmir province, with Ayvalık cultivars from the National Germplasm Collection, Izmir.

Table 2. Similarity matrix of nine ecotypes from Kayadibi-İzmir Province and Ayvalık cv. from the National Germplasm Collection, Izmir.

Pop ID	KD-1	KD-2	KD-3	KD-4	KD-5	KD-6	KD-7	KD-8	KD-9	Ayvalık
KD-1	****	0.8421	0.8421	0.7895	0.6842	0.6842	0.6842	0.6842	0.7895	0.6316
KD-2	0.1719	****	0.8947	0.6316	0.7368	0.7368	0.7368	0.6316	0.7368	0.5789
KD-3	0.1719	0.1112	****	0.6316	0.8421	0.6316	0.6316	0.7368	0.8421	0.6842
KD-4	0.2364	0.4595	0.4595	****	0.6842	0.7895	0.7895	0.6842	0.7895	0.7368
KD-5	0.3795	0.3054	0.1719	0.3795	****	0.5789	0.6842	0.8947	0.8947	0.8421
KD-6	0.3795	0.3054	0.4595	0.2364	0.5465	****	0.7895	0.4737	0.5789	0.5263
KD-7	0.3795	0.3054	0.4595	0.2364	0.3795	0.2364	****	0.6842	0.7895	0.7368
KD-8	0.3795	0.4595	0.3054	0.3795	0.1112	0.7472	0.3795	****	0.8947	0.9474
KD-9	0.2364	0.3054	0.1719	0.2364	0.1112	0.5465	0.2364	0.1112	****	0.8421
Ayvalık	0.4595	0.5465	0.3795	0.3054	0.1719	0.6419	0.3054	0.0541	0.1719	****

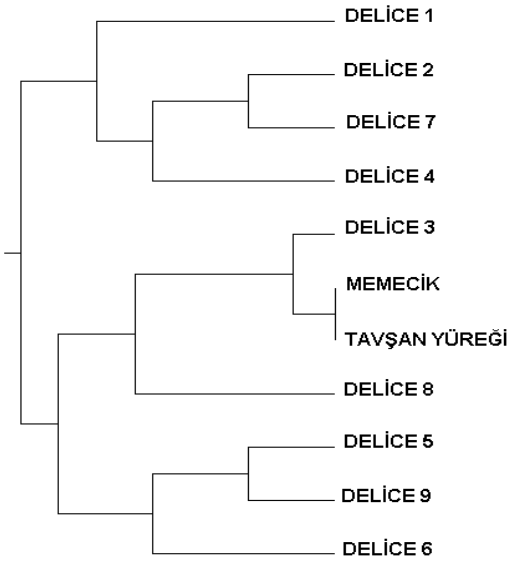

Figure 2. Dendogram of nine ecotypes from Kayadibi-İzmir province, with Memecik and Tavşan Yüreği cultivars from the National Germplasm Collection, Izmir.

This study was carried out to set forth similarities of nine oleasters growing in Kayadibi province of İzmir, claimed to confused with each other, although at least differing in morphological features. Ayvalık cv and KD-8 were 97% similar, showing that they may have grafting compatibility.

The results showed that Ayvalık was similar to the others and might be totally different from other ecotypes of oleasters. However, Ozkaya et al. (2004) found that Ayvalık cv was located in a separate group close to cv. Halhalı.

RAPD-PCR for Nine Karadelice with Memecik and Tavşan Yüreği: The Nei estimate of similarity showed similarity values ranging from 0.6522 for KD-1, KD-6 and KD-8 to 1.0000 for Memecik and Tavşan Yüreği. The dendogram resulting from the UPGMA analysis is shown in Fig 2. The molecular analysis showed variability within nine oleasters and between Memecik and Tavşan Yüreği cvs and nine oleasters collected from Kayadibi. There were two main groups (KD-1, KD-2, KD-7, KD-4 and the others) with subgroups. Ecotypes of oleasters KD-1, KD-2, KD-7, KD-4 were least similar (88%) type and located in a separate group. This group was 84% similar with other groups. Ecotypes KD-1 distinguished itself in a seperate subgroup, showing similarities of 88% with others. The oleaster KD-3 and the cvs. Memecik and Tavşan Yüreği were 98% similar, since Memecik and Tavşan Yüreği were 100% similar. Ecotypes KD-8, KD-3, Memecik and Tavşan Yüreği were 90% similar and located in a group (Figure 2 and Table 3).

This study was carried out to set forth similarities of nine oleasters growing in Kayadibi area of İzmir province, which are claimed to be confused with each other although at least differing in morphological features. Since Memecik and Tavşan Yüreği were 100% similar and may be it can have more grafting compatibility with KD-3 and KD-8.

Table 3. Similarity matrix of nine ecotypes from Kayadibi-İzmir Province and Memecik and Tavşan Yüreği cultivars from the National Germplasm Collection, Izmir.

Pop-ID	KD-1	KD-2	KD-3	KD-4	KD-5	KD-6	KD-7	KD-8	KD-9	Memecik	Tavşan Yüreği
KD-1	****	0.8696	0.6957	0.6957	0.7391	0.6522	0.7826	0.6522	0.6522	0.6522	0.6522
KD-2	0.1398	****	0.8261	0.8261	0.7826	0.6957	0.9130	0.6957	0.6957	0.7826	0.7826
KD-3	0.3629	0.1911	****	0.8261	0.7826	0.6957	0.7391	0.7826	0.6957	0.9565	0.9565
KD-4	0.3629	0.1911	0.1911	****	0.6957	0.6957	0.8261	0.6957	0.6957	0.7826	0.7826
KD-5	0.3023	0.2451	0.2451	0.3629	****	0.8261	0.7826	0.7391	0.9130	0.8261	0.8261
KD-6	0.4274	0.3629	0.3629	0.3629	0.1911	****	0.7826	0.8261	0.8261	0.7391	0.7391
KD-7	0.2451	0.0910	0.3023	0.1911	0.2451	0.2451	****	0.6957	0.6957	0.6957	0.6957
KD-8	0.4274	0.3629	0.2451	0.3629	0.3023	0.1911	0.3629	****	0.6522	0.8261	0.8261
KD-9	0.4274	0.3629	0.3629	0.3629	0.0910	0.1911	0.3629	0.4274	****	0.7391	0.7391
Memecik	0.4274	0.2451	0.0445	0.2451	0.1911	0.3023	0.3629	0.1911	0.3023	****	1.0000
Tavşan Yüreği	0.4274	0.2451	0.0445	0.2451	0.1911	0.3023	0.3629	0.1911	0.3023	0.0000	****

The results showed that Memecik and Tavşan Yüreği were similar to the others and might be totally different ecotypes of oleasters. However, Ozkaya et al. (2004) found that Memecik and Tavşan Yüreği were 55% similar, in RAPD-PCR molecular analysis.

The results showed that the estimates of a molecular genetic relationship can be helpful for organizing germplasm for conservation of genetic resources for the identification of cultivars for selection of parents for hybridization, for predicting favorable heterotic combinations. This also helps to reduce the number of samples required for sampling of genetically variable broad range of accessions in breeding programs. Accessions with the most distinct DNA profiles are likely to contain the greatest number of novel alleles, which are likely to uncover the largest number of unique and potentially agronomically useful alleles. It is hoped further studies using more discriminatory techniques such as AFLP and SSR will produce more conclusive results to quantify the variability among these ecotypes of oleasters. Bronzini de Caraffa et al. (2002) observed that the true oleasters were characterized by a Western mitotype and a Western RAPD pattern. Feral forms originate either from varieties or from hybridisation between a variety and an oleaster.

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Correspondence Address:

Mucahit Taha OZKAYA
Ankara University, Faculty of Agriculture
Department of Horticulture, Ankara, Turkey
Tel: 0-312-596 16 35
E-mail: ozkaya@agri.ankara.edu.tr