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Genetic and Biologic Characterization of Some Olive (*Olea europaea* L.) Cultivars Grown in Turkey

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Abstract: There are many olive (*Olea europaea* L.) varieties grown in Anatolia, which have not been characterized (morphologic and biologic characteristics), yet. Some of them that have been identified by morphologic and biological characteristics, but still have discriminate some variations due to unknown genotypic or phenotypic causes. Genetic diversity studies using Random Amplified Polymorphic DNA (RAPD) technique were carried out in a set of 10 olive cultivars (Ayvalik, Derik Halhali, Domat, Gemlik, Kilis Yaglik, Manzanilla, Memecik, Nizip Yaglik, Sari Ulak and Tavsan Yuregi) from the Turkish Olive Germplasm Bank of the Olive Research Institute in Izmir, Turkey. Analysis of PCR products was achieved by using the simple matching coefficient and UPGMA clustering. Primers were identified and used in combination to discriminate between different varieties. The diversity of RAPD patterns demonstrated the biodiversity among varieties. Also some pomological and biochemical analysis were done in order to support the results of the genetic variability. Among cultivars, Derik Halhali showed the big differences, according to the genetic and biochemical results.

Key Words: olive, Anatolia, RAPD-PCR, DNA, genotypic, phenotypic, germplasm

Türkiye'de Yetiştirilen Bazı Zeytin Çeşitlerinin Genetik ve Biyolojik Özelliklerinin Karşılaştırılması

Özet: Anadolu'da yetiştiriciliği yapılan bir çok zeytin (*Olea europaea* L.) çeşidi bulunduğu halde bunların morfolojik ve biyolojik özellikleri henüz tam olarak belirlenmis değildir. Bunlardan çok az bir kısmının özellikleri belirlenmiş olduğu halde, bunların da içlerinde genotipik veya fenotipik nedenlere bağlı varyasyonların olduğu gözlenmektedir. RAPD-PCR tekniği ile genetik farklılıkların belirlenmesi üzerine yapılan bu çalışmada İzmir-Bornova Zeytincilik Araştırma Enstitüsü, Kolleksiyon Bahçesinden elde edilen 10 zeytin çeşidi (Ayvalık, Derik Halhalı, Domat, Gemlik, Kilis Yağlık, Manzanilla, Memecik, Nizip Yağlık, Sarı Ulak and Tavşan Yureği) kullanılmıştır. Diğer yandan elde edilen verileri destekleyebilmek amacıyla pomolojik ve biyokimyasal gözlem ve analizler de yapılmıştır. Mevcut çeşitler arasında, Derik Halhalı genetik ve biyokimyasal olarak en farklı sonuçlar gösteren çeşit olmuştur.

Anahtar Kelimeler: zeytin, Anadolu, RAPD-PCR, genotipik, fenotipik, kolleksiyon

Introduction

The olive tree (*Olea europaea* L.) originated from Upper Mesopotamia and Southwest Asia, including a part of the South East Anatolia Region of Turkey and Syria. The Semitic people in this region first did cultivation and improvement as early as 3000 BC. From this region, olive cultivation spread northward into Anatolia, then the Aegean Sea islands, Greece, Italy and Spain, southward into Egypt, then Tunisia and Morocco (Ertem 1987, Hehn 1998, Anonymous 1996, Garibagaoglu and Baysal 1998).

In most producing countries, there are hundreds of cultivars, which have adapted to various microclimates and soil types. Bartolini et al (1993) have ascertained the existence throughout the world of roughly 1,200 named cultivars with over 3,000 synonyms. There are 97 selected varieties or types, originating from Turkey and 33 varieties, originating from other countries, in the Olive Research Institute (ORI), Turkey collection. ORI has completed a study to identify the most common and important 27 local cultivars based on traditional methods, which are based on morphological and agronomic characteristics (Anonymous 1991). The Ministry of Agriculture registered these cultivars. However the remaining cultivars or types need further study to be characterized.

The traditional technique for identifying olive cultivars is based on morphological and agronomic characters. There is much confusion and uncertainty concerning the identity of the olive trees in a region. In view of the considerable variability in performance and phenotypic development observe by growers among, the traditionally identified, a variety (Wiesman et.al. 1998).

Identification of olive varieties, based on the analysis of gene products, such as isozymes, has been used (Pontikis et al. 1980, Loukas and Krimbas 1983), but the disadvantage of this method is common and may make interpretation of results difficult; because of the relatively small number of polymorphisms that are produced, and the possibility that isozyme expression can also be

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altered by environmental conditions (Trujillo et al. 1995.). Random Amplified Polymorphic DNA (RAPD) analysis, described by Williams et al. (1990) and Welsh, McClelland (1990) and Newburry and Ford-Lloyd (1993), has proven to be a useful tool for genetic typing and mapping. They have reported Random Amplified Polymorphic DNA (RAPD) markers, which involve the direct analysis of DNA extracted from samples of leaves, are independent of the environmental conditions and present a greater degree of polymorphism. Bogani et al. (1994), Fabrri et al. (1995), Cresti et al. (1996) and Wiesman et al (1998) about advantages of using RAPDs for identification of olive varieties.

In olive cultivation, the intensive modes of production favor the use of a few varieties with a stable and regular yield over a wide area associated with acceptable organoleptic characteristics. This selection leads to genetic erosion due to the abandonment of numerous locally adapted olive varieties (Bronzini de Caraffa et.al. 2002).

The present study represents a first attempt to characterize important olive varieties grown in Turkey at the molecular level using the RAPD system, with particular emphasis on the 9 officially registered (by Ministry of Agriculture in Turkey) olive varieties "Ayvalik, Domat, Gemlik, Halhali (Derik), Kilis Yaglik, Memecik, Nizip Yaglik, Sari Ulak, Tavsan Yuregi", originating in Turkey, and "Manzanilla", originating in Spain (Table 1). They have been obtained from the Turkish Olive Germplasm Bank in Olive Research Institute, Izmir, Turkey. They had been traditionally identified and some morphological and biological traits were summarized in a table.

Material and Methods

Ten olive (*Olea europea* L.) cultivars were analyzed by using RAPD-PCR technique. Plant material was obtained from a collection maintained in the experimental orchards at the Olive Research Institute, Izmir, Turkey (Table 1).

DNA extraction: Total DNA was extracted from the leaf tissue by using the CTAB method of Doyle and Doyle (4) with some modifications. Young leaves of 0.2 g were ground in a mortar and mixed with 1 ml extraction buffer containing 100 mM Tris-HCl pH 8, 10 mM EDTA, 1 M NaCl, 2 % CTAB, 2 % PVP 40, 0.1 % β-mercaptoethanol. Samples were incubated for 15 min. at 65°C mixed with 0.5 ml of Chloroform/Isoamyl alcohol (24:1) and centrifuged at 5000xg for 5 min. The aqueus phase was recovered and mixed with 2/3 volume of isopropanol.

Table 1. Olive cultivars included in this study and their region and town of origin

No	Cultivars	Town	Region
1	Ayvalık	Edremit	Aegan
2	Domat	Akhisar	Aegan
3	Gemlik	Gemlik	Marmara
4	Halhalı	Derik	South East
5	Kilis	Kilis	South East
6	Manzanilla	Cordoba	-
7	Memecik	Mugla	Aegan
8	Nizip	Nizip	South East
9	Sarı Ulak	Tarsus	Mediterranean
10	Tavsan Yuregi	Fethiye	Mediterranean

The mixture was centrifuged at 14000xg for 5 min. The pellet was washed with 1 ml Ethanol/Ammonium acetate (%76 Ethanol, 10 mM Ammonium acetate) and centrifuged at 14000xg for 5 min. The pellet was resuspended in 0.2 ml H_2O and the DNA quantity was determined by spectrophotometer reading at 260 nm.

RAPD analysis: PCR amplification was carried out in a total volume of 25 µl containing 200 µM dNTP, 2 mM $MgCl_2$, 1 µM primer (Operon), 1U Taq DNA polymerase (Promega), 1x Reaction Buffer (Promega) and 25 ng template DNA. Fourteen decamer oligo nucleotides (Operon Technologies, Alameda, California) were used. The PCR program was started at 94°C for 1 min, followed by 50 cycles of denaturation at 94°C for 20 sec, annealing at 35°C for 20 sec, extension at 72°C for 30 sec, and final extension at 72°C for 5 min. Amplification products were separated on 1.5% agarose gels (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).

The amplified DNA fragments were scored by presence and absence. The degree of genetic similarity between each pair of cultivars was calculated by using Similarity Index Method (S.I.M.) (10). For dendrogram construction Numerical Taxonomy and Multivariate Analysis System software (Version 1.8) was used (9).

DNA extraction: Total DNA was extracted from the leaf tissue by using the CTAB method of Doyle and Doyle (1987) with some modifications. Young leaves of 0.2 g were ground in a mortar and mixed with 1 mL extraction buffer containing 100 mM Tris-HCl pH 8, 10 mM EDTA, 1 M NaCl, 2 % CTAB, 2 % PVP 40, 0.1% β-mercaptoethanol. Samples were incubated for 15 min. at 65°C mixed with 0.5 mL of Chloroform/Isoamyl alcohol (24:1) and centrifuged at 5000xg for 5 min. The aqueus phase was recovered and mixed with 2/3 volume of isopropanol. The mixture was centrifuged at 14000xg for 5 min. The pellet has been washed with 1 mL Ethanol/Ammonium acetate (% 76 Ethanol, 10 mM Ammonium acetate) and centrifuged at 14000xg for 5 min. The pellet was resuspended in 0.2 mL H_2O and the DNA quantity was evaluated by reading at 260 nm.

RAPD analysis: Fourteen decamer oligo nucleotides (Operon Technologies, Alameda, California) were used for PCR amplification with the following conditions; PCR was performed in 25 µl volume containing 200 µM dNTP, 2 mM $MgCl_2$, 1 µM primer (Operon), 1U Taq DNA polymerase (Promega), 1x Reac. Buffer (Promega) and 25 ng template DNA. For amplification reaction, a thermal cycler (PTC 200, MJR) was used with the following program; One cycle of denaturation at 94°C for 1 min. followed by 50 cycles at 94°C for 20 sec, at 35°C for 20 sec, at 72°C for 30 sec for denaturing, annealing and extension respectively. The last cycle was followed by 5 min. incubation at 72 °C. Amplification products were analysed by gel electrophoresis in 1.5 % agarose (SeaKem LE, FMC) in TAE buffer, stained with ethidium bromide, recorded and analysed by using UVP Gel Analysis System. Molecular sizes of amplification products were

estimated using ϕX174 DNA/*Hae* III marker (Promega). Data generated by RAPD-PCR was analysed and a dendrogram was constructed using a windows program TFPGA v1.3 by Mark P. Miller.

The cultivars were also examined for some morphological and biological characteristics and oil composition. The characteristics were observed according to the Catalogue of Turkish Standard Olive Cultivars (Anonymous 1991). The oil analysis was established by using Gas chromatography (HP 6890 Gas Choromatograph) methods according to Boas et al. (1994).

Results and Discussion

Each of the 14 decamer oligo nucleotides successfully amplified DNA fragments. Some information about 10 olive cultivars and an example of primer C-11 are shown in Fig.1. Amplification products of fourteen primers were selected for further analysis because these primers generated polymorphic amplification fragments (Table 2). These primers revealed 57 reproducible polymorphic amplification fragments. The number of reproducible polymorphic fragments per primer ranged between 2 and 5, and fragment size ranged between 271 and 1353 base pairs. Genetic similarity among 10 olive cultivars are summarized in Table 3 and genetic relationships among them are shown in a dendrogram (Fig 2).

According to the dendrogram, five cultivars (Domat, Gemlik, Kilis Yaglik, Manzanilla and Nizip Yaglik) were clustered in group one which shows more than 60% genetic similarity. Derik Halhali was separated from all other cultivars.

Three cultivars, Ayvalık, Domat and Memecik, originated from the Aegean Region. Gemlik originated from the Marmara region. Derik Halhali, Kilis yaglik and Nizip yaglik originated from the South East Anatolia region. Sari Ulak and Tavsan Yuregi originated from the Mediterranean Region (Table 1, Fig 1 and Table 4).

While Domat is used mainly for green table olive, Ayvalik and Memecik are used for oil production. According to oil quality both cvs are good. Gemlik is the main black table olive cvs in Turkey. While Kilis and Nizip yaglik are used mainly for oil production, Derik Halhali is used for table olive. Sari Ulak is mainly used for table olive in the area of Adana and Mersin (Eastern Mediterranean), Tavsan Yuregi is also used for table olive in the area of Antalya (Western Mediterranean).

The most advanced and accurate methods available for olive cultivar identification have been the combination of morphological, phenological and biochemical techniques (Table 4 and Table 5) used by the world's research institutes and nurseries. Technological advances are now allowing the industry to combine these techniques with the use of Random Amplified Polymorphic DNA (RAPD) analysis.

As a conclusion, although information available for the cultivars and the area of cultivation of cultivars (Table 4) and the dendrogram (Fig 2) shows that there is no relationship regarding the geographic origin of the olive cultivars, detailed researches should be done in near future. Also specifically analysis of oil composition (Table 5) shows that there is no relation regarding the geographic origin and morphological and biological differences. Further, researches by using other primers should be conducted will help to establish the relationship, among cultivars.

Table 2. List of the 14 RAPD Oligonucleotide (primers) and their sequences which produced polymorphic markers among cultivars studied.

Primers (Oligo)	Sequence (5'-3')	# polymorphic loci
A-04	A A T C G G G C T G	4
A-11	C A A T C G C C G T	5
A-16	A G C C A G C G A A	4
C-11	A A A G C T G C G G	5
C-15	G A C G G A T C A G	5
D-03	G T C G C C G T C A	5
E-20	A A C G G T G A C C	3
K-19	C A C A G G C G G A	5
Q-15	G G G T A A C G T G	2
Q-17	G A A G C C C T T G	5
S-03	C A G A G G T C C C	4
X-01	C T G G G C A C G A	3
Z-10	C C G A C A A A C C	3
Z-11	C T C A G T C G C A	4
Total		57

Polymorphic loci: Total number of DNA fragments shared in 10 *Olea europea* cultivars.

Table 3. Genetic similarity value of 10 standard Turkish olive cultivars.

Cultivars	1	2	3	4	5	6	7	8	9	10
Ayvalık	-									
Domat	0,5762	-								
Gemlik	0,5167	0,6738	-							
Derik Halhali	0,4000	0,5429	0,4762	-						
Kilis Yaglık	0,4707	0,6310	0,6667	0,3857	-					
Manzanilla	0,3667	0,6071	0,6714	0,3214	0,6262	-				
Memecik	0,3857	0,5024	0,6190	0,4381	0,5071	0,4874	-			
Nizip Yaglık	0,4881	0,4670	0,6667	0,3119	0,6452	0,6810	0,4762	-		
Sari Ulak	0,4252	0,4310	0,5500	0,3190	0,5690	0,4670	0,4060	0,4952	-	
Tavsan Yureği	0,5422	0,5964	0,6071	0,5000	0,5136	0,4480	0,5476	0,4898	0,4255	-

Table 4. Morphological and biological traits of 10 standard Turkish olive cultivars

Cluster No		Fruit size	Fruit weight (gr)	Oil (%)	# of flower per cluster	Avg. # of flower	Seed weight (gr)	Seed % in fruit	Leaf shape	Evaluation
1	Manzanilla	middle	3.73	20,39	8-16	12	0.45	11,97	medium long-wide elliptic	Good for green table olive production.
1	Nizip yağlık	very small	2.18	27,36	10-30	17	0.41	18,69	medium long-wide elliptic	Include high % of oil. Good quality oil. Third after Halhali and Kilis Yaglik
2	Domat	big	5.30	20,57	10-23	14	0.86	16,24	short-wide elliptic	high quality olive for black table olive production
2	Gemlik	middle	3.73	29,98	8-27	16	0.53	14,14	very long-narrow elliptic	good for green table olive production. Advised for irrigated orchards.
3	Kilis Yağlık	very small	1.77	31,82	14-30	20	0.31	17,75	medium long-wide elliptic	include high % of oil. Good quality oil. Very small fruit is problem.
4	Memecik	big	4.78	24,50	6-15	11	0.56	11,72	medium long-medium wide elliptic	high quality for oil, but also used for black and green table olive production
4	Tavşan Yüreği	very big	6.08	20,20	8-15	11	0.83	13,57	long-very narrow elliptic	good for green and black table olive production for local consumption
5	Ayvalık	middle	3.65	24,72	14-34	20	0.54	14,74	long-narrow elliptic	High quality (chemical and organoleptic) olive oil has
6	Sarı Ulak	middle	3.77	18,84	1-28	17	1.06	28,15	medium long-wide elliptic	good for green and black table olive production for local consumption
7	Halhalı	middle	3.83	21,11	9-25	12	0.66	17,21	long-narrow elliptic	generally used for green table olive

Table 5. Oil composition of 10 standard Turkish olive cultivars

%		Domat	Gemlik	Derik halhalı	Kilis yağlık	Manzanilla	Nizip yağlık	Sarı ulak	Tavşan yüreği	Ayvalık	Memecik	TSE	IOOC 1984
Myristic acid	(C 14:0)	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,02	0,02	<=0,250	0,0-0,1
Palmiti acid	(C 16:0)	13,64	12,97	7,96	13,51	15,03	15,52	13,29	12,63	12,64	11,72	7,5-20,0	7,5-20,0
Palmitoleic acid	(C 16:1)	0,59	1,09	0,57	0,87	1,00	0,99	0,48	0,92	0,66	0,81	0,3-3,5	0,3-3,5
Heptadecanoic acid	(C 17:0)	0,26	0,13	0,15	0,13	0,04	0,05	0,19	0,17	0,16	0,04	<=0,3	<0,5
Heptadecanoic acid	(C 17:1)	0,23	0,24	0,23	0,16	0,07	0,05	0,21	0,31	0,20	0,06	<=0,3	<0,6
Stearic acid	(C 18:0)	5,46	2,86	2,57	4,17	2,29	5,83	4,03	2,56	3,57	2,41	0,5-5,0	0,5-5,0
Oleic acid	(C 18:1)	64,20	75,42	82,29	70,51	70,50	64,74	60,54	70,02	70,24	75,99	55,0-83,	56,0-83,0
Linoleic acid	(C 18:2)	13,63	5,93	4,92	8,93	9,60	11,35	19,43	11,57	10,73	7,18	3,5-21,0	3,5-20,0
Linolenic acid	(C 18:3)	0,83	0,56	0,59	0,55	0,68	0,56	0,71	0,88	0,62	0,74	<=0,9	0,0-1,5
Arachidic acid	(C 20:0)	0,65	0,39	0,30	0,63	0,38	0,58	0,57	0,41	0,58	0,47	<=0,6	<0,8
Eicoseneic acid	(C 20:1)	0,29	0,26	0,32	0,27	0,24	0,16	0,30	0,35	0,36	0,37		
Behenic acid	(C 22:0)	0,14	0,10	0,07	0,17	0,11	0,12	0,17	0,10	0,16	0,14	<=0,2	<0,2
Lignoceric acid	(C 24:0)	0,06	0,05	0,02	0,08	0,05	0,05	0,07	0,06	0,07	0,07	<=0,2	<1,0

TSE: Turkish Standardization Institute.
IOOC: International Organization of Olive Council.

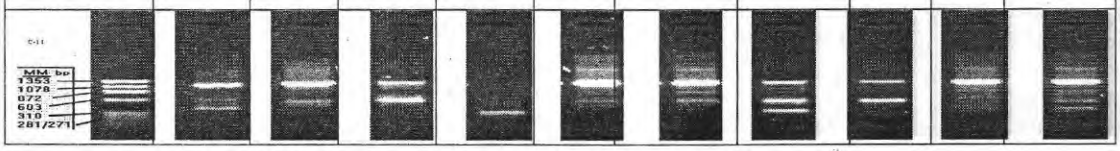
	AYVALIK	DOMAT	GEMLİK	HALHALI	KİLİS YAĞLIK	MANZANİLLA	MEMECİK	NİZİP YAĞLIK	SARI ULAK	TAVŞAN YÜREĞİ
SYNONYM	Edremit Yağlık, Şakran, Midilli, Ada Zeytini	-	Trilye, Kaplık, Kıvırcık, Kara	-	-	Manzanillo	Taş arası, Aşıyeli,Tekir, Gülümbe, Şehir,Yağlık	-	-	Ters yaprak
ORIGIN	Edremit	Akhisar	Gemlik	Derik	Kilis	Cordoba	Muğla	Nizip	Tarsus	Fethiye
CULTIVATED AREA	Çanakkale, Körfez area of Aegan Sea Region, İzmir, İçel, Antalya, Adana, K.maraş, Mardin	Manisa, İzmir, Aydın	Bursa, Tekirdağ Kocaeli, Bilecik, Kastmonu, Zonguldak, Sinop, Samsun, Trabzon, Balıkesir, İzmir, Manisa, Aydın, İçel, Adana, Antalya, Adıyaman,	Hatay, Gaziantep, K.Maraş, Mardin	Kilis, Gaziantep, Şanlıurfa K.Maraş, Mardin	Spain, USA, Argentina, Israel Australia	İzmir, Aydın, Manisa, Denizli, Muğla, Antalya, Sinop, K.Maraş, Kastamonu	Nizip, K.Maraş, Mardin	İçel, Adana	Muğla, Antalya, Karaman
C-11										

Fig.1. Some information about ten olive cultivars and RAPD patterns of them with primer C-11.

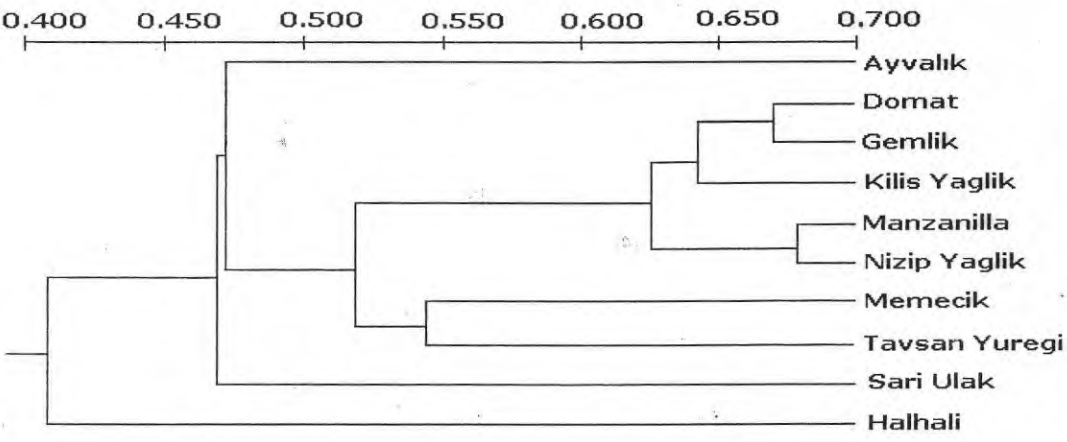


Fig. 2. Dendrogram of ten olive cultivars based on similarity index calculated from RAPD data.

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