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Full Length Research Paper

RAPD-PCR analysis of cultured type olives in Turkey

Meltem Sesli[1]* and E. Dilşat Yeğenoğlu[2]

[1]College of Tobacco Expertise, Turkish Republic, Celal Bayar University 45210, Akhisar, Manisa, Turkey
[2]Akhisar Vocational College, Turkish Republic, Celal Bayar University 45210, Akhisar, Manisa, Turkey.

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The aim of this study was to detect genetic similarities and distances among cultured type olive trees by RAPD-PCR technique. Olives are raised in a high range from the Aegean, Mediterranean, Marmara and Black Sea to Southeast Anatolia regions of Turkey. Olive breeding had a rapid increase in Turkey during recent years, among the agricultural products. Finding the genetic relationships between cultured type olives may help to improve genetic resources and our knowledge of their evolutionary background and to determine genetic relationships with wild type relatives. In this study, samples were obtained from the Olive Production Research Institute (Manzanilla, Domat, Gemlik and Memecik) and sapling producers in Manisa, Akhisar (Uslu, Edremit). Genomic DNA's were extracted from young leaves and PCR was used generate RAPD bands. Sixty random primers obtained from Operon Tech. were tested by RAPD-PCR (OP-A, OP-I, OP-Q). A total of 33 primers among 60 Operon random primers (Kit OP-A, OP-I, OP-Q) yielded clear and firm bands. The electrophoretic patterns of olive samples showed that 159 highly polymorphic loci. Averages of 4.81 scorable bands per primer were determined from RAPD-PCR analysis.

Key words: RAPD, PCR, culture olives.

INTRODUCTION

Olive, originating from Anatolia, is a valuable plant being cultivated for thousands of years in the Aegean and Mediterranean basin. The olive tree likes the Mediterranean climate; it has been cultivated in the Aegean and Mediterranean basin since the ancient age after its value had been appreciated; it also has been referred to as sanctity and considered as a symbol for many virtues such as peace (Bülbül, 2007; Anonymous, 2006). It is distinguished from other cultivated trees because of being a perennial tree. It is a member of *Oleaceae* family (*Olea europaea* L.). It is estimated that more than 2000 species are cultivated around the worldwide (Dülger 2004). It is known that olive has two types of subspecies. These subspecies are the wild type (oleaster) *Olea europaea oleaster*, and the cultivated type *Olea europaea sativa*, which are widely seen in the Aegean Region, Turkey.

Since Mediterranean climate creates ideal conditions for the cultivation, vast majority of world's olive presence is in Mediterranean countries such as Turkey, Spain, Greece, Portugal, Tunisia, Morocco and Algeria (Sesli et al., 2007). Olive cultivation has become an agricultural activity with a high economical value realized in 35 provinces in Turkey. It has a wide dispersal from Artvin in the north to Hatay in the south and Mardin in Southeast Anatolia. The regions having the most suitable olive cultivation conditions are the Aegean, Marmara, Mediterranean, and Black Sea due to their climates.

In areas where olive cultivation is available in our country, Mediterranean climate is seen where the winters are warm and rainy, and the summers are dry and hot. Olive groves are generally located in areas close to the shores in places where the mountains laying in parallel to the sea and in areas running along valleys to 100 - 150 km inside in places where the mountains are steep. Olive trees are grown mostly in sloping lands where other products cannot be grown. However, new olive groves are also being established in flat areas in the recent years. (Mendilcioğlu, 1999; Anonymous, 2006).

900 million olive trees are grown on over areas of 10 million hectares in the world; and 98 % of these olive trees are located in the countries of Mediterranean Basin. Turkey is in a significant position among countries that

*Corresponding author. E-mail: meltem.sesli@bayar.edu.tr. Tel: (+90) 236 412 68 96. Fax: (+90) 236 413 70 58.

cultivate olives. 2.8% of approximately 23 million hectare agriculture area is covered with olive trees in Turkey. There are totally 107 million olive trees, 95 million of which is bearing fruits, in a closed area of 644 thousand hectares in Turkey. This number is equivalent to 10 % of olive trees in the world (Sesli et al., 2007; Tunalıoğlu, 2002; Tunalıoğlu, 2003 a, b).

It is determined that the increase in number of olive trees in Turkey was 14, 6 million within the last decade. This figure shows that there is an increase of approximately 1.5 million every year. It is certain that there will be notable increases in future production due to the change in nutrition habits and the increases in consumption in the world (Yavuz, 2006).

Manisa province and its districts, Muğla province and its districts and Izmir province and its districts which are among provinces where olives are cultivated in the Aegean Region. Turkey has an important place in the sector. It is determined that the number of live trees in Manisa in 2006-2007 were totally 10.993.335 and that the average yield per tree was 15.5 kg. It is also determined that, in the same period, the number of olive trees in Muğla was 13.978.216 and the average yield per tree was 11.2 kg; and in İzmir, number of olive trees was totally 15.180.960 and the average yield per tree was 10.5 kg (Anonymous, 2007a, b, c).

Olive cultivation has become an economically high value agricultural activity especially in the Aegean and Mediterranean Regions; and some cultivated olive types grown in these regions include Edremit (Ayvalık), Gemlik (Trilye), Uslu, Domat and Memecik varieties. Also Spain originated Manzanilla is planted in addition to these varieties.

The origin of Edremit (Ayvalık) cultivated variety grown in the Aegean Region is Edremit area; and it is also called as Yağlık, Şakran, Midilli and Ada. This variety forms large trees; its trunk is strong. It has a medium size fruit structure; its yield is good, and it is used as table olives. It has self-pollination and it is also used as pollinator for Gemlik, Memecik, Erkence Edremit varieties. Domat which is one of the varieties peculiar to Aegean Region, originates from Akhisar district of Manisa province; its tree is strong, broad and large. Its fruit is large, cylindrical and symmetric; its yield is quite good. It is consumed as stuffed or seeded green olives. It has self-pollination and it is a good pollinator. Uslu, again a variety originating from Akhisar district, Manisa province, has a strong trunk. Its fruits are medium sized, and are oval and longitudinally asymmetric shaped, and it is a table olive variety generally used as pickled. Its pollination is not known. The origin of Memecik variety is Muğla, and it is also called as Taş Arası, Aşı Yeli, Tekir, Gülümbe and Yağlık. It is a variety having a wide geographic distribution in Turkey; its tree grows strongly under good maintenance conditions. Its fruits are large and oval, transversely symmetric shaped; it is processed for oil production. It is partially self-pollinating, and its Edremit, Gemlik, Çakır, Memeli, Erkence types may be used as pollinator for Memecik. Manzanilla variety, which is originating from Cordoba, Spain, is one of the varieties cultivated mainly in Aydın province in Aegean Region. It was brought to Turkey in 1974 and it has become a variety for production because it easily adapts to different climate conditions. It has a medium strong tree and its fruits are medium sized, and are almost round, transversely and longitudinally symmetric shaped. It is processed and consumed as table olives; it is sufficiently self-pollinating. The origin of Gemlik variety, which is an olive variety cultivated in Marmara Region, is Gemlik district of Kocaeli province. It is also called as Trilye, Kaplık, Kıvırcık, Kara; it has medium strong tree. Its fruits are almost round, longitudinally and transversely symmetric shaped, and medium sized; it is a quite productive variety; and it is used for oil production and as table olives; it is partially self-pollinating and it's Edremit, Çakır, Erkence types are used as pollinator for Gemlik (Bülbül, 2007; Anonymous, 2006; Canozer, 1991).

Determination of the genetic structure of plants is important for cultivation and improvement works. For this purpose, firstly the markers such as leaves, fruits, seeds, etc. are used in improvement studies; and the effects of environmental conditions on such markers have limited their use. Therefore, the use of genetic markers with DNA based has increased. DNA based techniques ensured determination of distances and similarities between the plants as well as the typing, varieties, types, clones in economically important plants easily. In addition, genetic markers have provided rapid and reliable results in selection studies to be performed for qualitative characteristics such as resistance to diseases (Gonzalo - Claros et al., 2000).

RAPD is a fast and commonly using molecular technique for screening genome of living organisms by arbitrary primers (Welsh and McClelland, 1990; Williams et al., 1990). RAPD was initially used for determining the genetic structure of plants such as wheat, corn, barley and grapes (D'Ovidio et al., 1990; Shattuck et al., 1990; Weining and Landridge, 1991; Gogorcena et al., 1993; Collins and Symons, 1993). RAPD is used successfully in detecting genetic polymorphism and similarity of both *oleasters* and cultured varieties of olives; and studies indicate that DNA-based markers are suitable for determining polymorphism as the genetic variety is high in olive trees. (Gemas et al., 2000; Gonzalo- Claros et al., 2000; Wu et al., 2004, Wiesman et al., 1998; Mekuria et al., 2004).

This study is supported by the Turkish Republic State Planning Organization; it aims at examining the genetic structure of olive samples obtained from the Olive Production Research Institute (Manzanilla, Domat, Gemlik, Memecik) and sapling producers in Manisa, Akhisar (Uslu, Edremit). These are first results of the study and it is planned to work with more arbitrary primers and to search olive genome more deeply and throughly by deve-

Table 1. Origins of cultivated varieties used in the study, and the places where they were supplied.

Type of Olive	Origin	Place of supply	Province
Manzanilla	Cordoba, Spain	Olive Production Research Institute	Izmir, Bornova, Turkiye
Gemlik	Gemlik, Kocaeli, Turkiye	Olive Production Research Institute	Izmir, Bornova, Turkiye
Domat	Akhisar, Manisa, Turkiye	Olive Production Research Institute	Izmir, Bornova, Turkiye
Memecik	Muğla, Turkiye	Olive Production Research Institute	Izmir, Bornova, Turkiye
Edremit	Edremit, Balikesir, Turkiye	Sapling Planters	Akhisar, Manisa, Turkiye
Uslu	Akhisar, Manisa, Turkiye	Sapling Planters	Akhisar, Manisa, Turkiye

loping SCAR markers in the next phase. Also, it planned to work for comparison of cultivated wild and cultural olive types in Turkey.

MATERIALS AND METHODS

Plant Material

Olive samples were supplied from the Olive Production Research Institute, and the sapling planters. Then, olive samples were transferred to the glasshouse; young leaves were collected and stored in liquid nitrogen until DNA extraction. A total of 6 samples were extracted by Doyle and Doyle method (1987). Table 1 shows the types of cultivated olives used in this study, their origins, and places where they were supplied.

DNA extraction

Genomic DNA was extracted from young leaves by Doyle and Doyle method (1987). Following steps of DNA extraction, for the determination of DNA quality and concentration of DNA samples, samples were subjected both spectrophotometric analysis and run in 0.8% agarose gels. In spectrophotometric analysis, each sample of DNA was calculated by their optical density values at 230, 260 and 280 nm.

Optical density ratios were evaluated and only good quality DNA samples were used in PCR (Wu et al., 2004).

RAPD analysis

A total of sixty primers from Kits OP-A, OP-I, OP-Q (Operon Technologies, Alameda, CA, USA) were used for RAPD-PCR analysis. PCR amplifications were carried out in a total volume of 25 µl. PCR mix including 25 ng template DNA, 2.42 µl 10X PCR reaction buffer (with $MgCl_2$, Sigma), 0.44 µl dNTP (Sigma), 1 µM primer, and 0.13 µl Taq DNA polymerase (Sigma). PCR cycles were performed with 60 s 94°C initial denaturation and 35 cycles of 20 s 94°C; 20 s 35°C; and 30 s 72°C. Final extension performed at 72°C for 5 min. PCR amplifications were carried out in 96 well Thermal cycler (Eppendorf Master Cycler) and all amplifications were carried out at two times. A PCR mixture without template DNA was put in each analysis as a control.

PCR products were separated 1.5 % agarose gels (Sigma) in 0.5 X TBE buffer with 0.5 µg/ml ethidium bromide at 100 V constant voltages. For evaluating the base pair length of bands, DNA ladder (Fermentas) was loaded on first lane of each gel.

Data analysis

Gels were visualized with Photo Print (Vilber Lourmat, France) imaging system and analysis of RAPD bands were performed by Bio-One D++ software (Vilber Lourmat, France). The RAPD bands (markers) scored as 1 if present and 0 if absence. Only clear and reproducible bands were used for binary data matrix. The dendrogram was constructed by POPGEN32 program according to Nei's coefficient and then UPGMA algorithm (Unweighted Pair-Group Method Using Arithmetic Averages) was chosen for hierar-chical clustering analysis method (Sneath and Sokal, 1973; Nei, 1972; Yeh et al., 1997).

RESULTS

A total of 33 primers among 60 Operon random primers (Kit OP-A, OP-I, OP-Q) yielded clear and firm bands. The electrophoretic patterns of olive samples showed that 159 highly polymorphic loci. An average of 4.81 scorable bands per primer was determined from RAPD-PCR analysis.

Totally 41 bands were obtained from OP-A primer set, and most bands were provided from OP-A 13 with 10 bands. It was determined that the molecular sizes of totally 41 bands varied between 5316 bp and 333 bp. Evaluable bands were not derived from primers OP-A 4, OP-A 7, OP-A 8, OP-A 10. For Operon Kit A primer set, 2.5 evaluable bands were determined per primer.

Totally 77 bands were obtained from OP-I primer set; and OP-I primer set was the primers producing most bands in cultivated varieties. Most bands were provided by primer OP-I 2 with 26 bands. It was determined that the molecular sizes of totally 77 bands varied between 1686 bp and 228 bp. Evaluable bands were derived from primers OP-I 1, OP-I 2, OP-I 3, OP-I 4, OP-I 8, OP-I 9, OP-I 10, OP-I 11 and OP-I 12, 8.5 evaluable bands were obtained per primer in the primer set; and primers OP-I were the primers providing most bands in the cultivated varieties that were researched.

Totally 41 bands were obtained from OP-Q primer set; and most bands were obtained from OP-Q 16 with 18 bands. It was determined that the molecular sizes of totally 41 bands varied between 5328 bp and 350 bp. Evaluable bands were derived from primers OP-Q 7, OP-Q 8, OP-Q 9, OP-Q 10, OP-Q 14, OP-Q 15, OP-Q 16 and OP-Q 20. Number of evaluable bands per primers in Q primers was determined as 5.1. In Table 2 shows the primers providing most bands among all primers, the series, and the ranges of base pairs.

Table 2. Primers where most bands are obtained, numbers of bands and band ranges.

Primer	Sequence (5' to 3')	Number of loci per primer	Base pair lengths
OP- A 13	CAGCACCCAC	10	5316 - 333 bp.
OP- Q 16	AGTGCAGCCA	18	5328 - 350 bp.
OP- I 2	GGAGGAGAGG	26	1686 - 228 bp.

Table 3. Genetic distance and similarity matrix obtained from OP-A, OP-Q, OP-I primers in culture type olives (Nei, 1972).

	Gemlik	Manzanilla	Domat	Memecik	Edremit	Uslu
Gemlik	****	0.8845	0.8788	0.9220	0.9364	0.9076
Manzanilla	0.1227	****	0.8465	0.8905	0.9015	0.8832
Domat	0.1292	0.1666	****	0.8938	0.8973	0.8767
Memecik	0.0812	0.1159	0.1122	****	0.9555	0.9144
Edremit	0.0657	0.1037	0.1084	0.0455	****	0.9384
Uslu	0.0969	0.1242	0.1316	0.0895	0.0636	****

****Values above the diagonal indicate genetic similarity, whereas values below the diagonal indicate genetic distance.

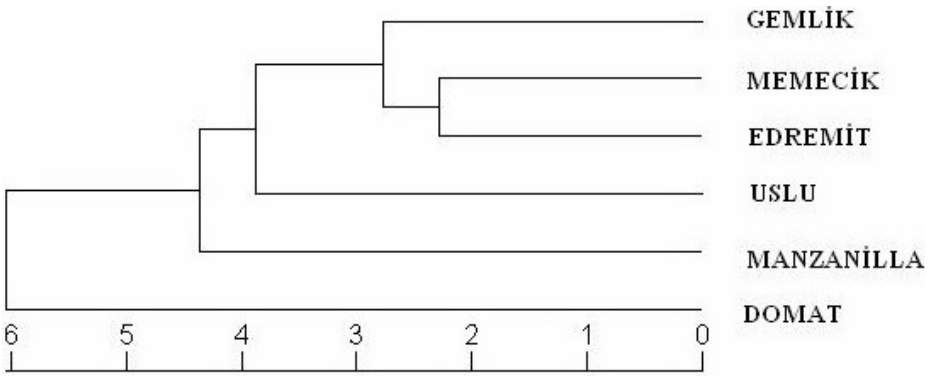

Figure 1. Genetic distance dendrogram produced by evaluable bands as obtained from OP-A, OP-Q, OP-I primers in culture type (Nei, 1972).

Hierarchical cluster analysis

The matrix shown in Table 3 was obtained by using Nei's genetic distance coefficient in POPGEN32 software for determining the genetic similarities and distances between culture olives in the study. As a result of cluster analysis conducted by using UPGMA method, the dendrogram of different culture olive types were developed as in Figure 1. The dendrogram of genetic distances conducted by using UPGMA algorithm was produced 5 clusters (Figure 1).

Table 3 shows genetic distance and similarity matrix as derived from primers OP-A, OP-I, OP-Q in cultivated types according to Nei (1972). Data matrix was developed for samples common in all evaluable primers yield-ing bands while developing the dendrogram and matrix of genetic distances and similarities. Genetic distance values were between 0.0455 (Memecik and Edremit) and 0.1666 (Manzanilla and Domat). Thus, samples closest to each other are (Memecik and Edremit); samples most distant to each other are (Manzanilla and Domat) based on their genetic distance values (Table 3).

Genetic similarity values are between 0.9555 (Edremit and Memecik) and 0.8465 (Domat and Manzanilla). Thus, samples with closest genetic similarities are (Edremit and Memecik); samples with most distant genetic similarities are (Domat and Manzanilla). Genetic similarity values and genetic distance values had been fully in compliance with each other.

DISCUSSION

Environmental factors affect morphologic features of culti-

vated plants. Therefore, it is difficult to determine varieties based on the phenotype because of the assocation between environmental conditions. RAPD-PCR was used in defining *oleaster* or their cultured types relatives based on genetic structure. DNA based markers are not affected by environmental conditions and it allows to directly determining the plant genotype.

Presently, olive is an agricultural product having highly significant economical yields. Studies on improving the olives are profoundly important. It is necessary to identify genetic structure of varieties correctly by using genetic markers in order to prevent confusion. In this study, DNA fragments were reproduced in different sizes from DNA samples isolated from cultured olive leaves with RAPD-PCR method with sixty Operon primers. When the fragments of RAPD analysis were evaluated at the end of study, it was observed that both OP-A, OP-Q and OP-I primers generated scorable bands in cultured olive types.

The amplificated bands (markers) determined in this study showed a highly polymorphic structure as mentioned by other researchers. The observed high polymorphism rate indicates a high genetic diversity and point to good potential in selection studies as a genetic source (Fabrri et al., 1995; Bandelj et al., 2002). This is an *a priori* advantage gained at the beginning for genetic improvement experiments. Phenotypic variations observed in field, despite of genetic similarities or differences, are most probably associated with genetic expression varying with changing environmental conditions and growing applications (Wiesmann et al., 1998). For instance, Edremit (Ayvalık), Memecik varieties develop strongly under good maintenance conditions; on the other hand, Uslu variety shows a very strong development based on different irrigation conditions (Bülbül, 2007, Anonymous, 2006).

Although Fabbri et al. (1995) noted that the differences between varieties of olives are not related with their geographical origin, Wiesman et al. (1998) determined a significant grouping difference between Manzanilla-IT and other samples as they examined in their studies, and specified that there may be a genetic difference classifiable in accordance with their geographic origin.

In our study, it has been observed that there was a significant difference between Manzanilla and Domat as a result of RAPD analysis we used in our study. Manzanilla, an olive variety originating from Spain, and Domat variety, originating from Akhisar, Manisa, were determined as varieties most distant to each other with a genetic distance value of 0.1666. Thus, there may be a genetic difference according to their geographic origin as specified by Wiesman et al. (1998). It was determined that genetic similarity of Uslu variety, originating from Akhisar district of Manisa province, to Edremit, Memecik, Gemlik varieties is close. Further studies with more primers and cultivated varieties would be useful for examining this result in more detail. Nevertheless, high genetic similarity between Edremit and Memecik varieties may be explained with the use of foregoing varieties grown in Aegean Region as inter-pollinators (male). Similarly, close genetic similarity between Gemlik variety, grown in Marmara Region, and Edremit and Memecik varieties may be explained with the case that one of the pollinators of Gemlik is Edremit, and that Edremit and Memecik may have been used as inter-pollinators. Pollination of Uslu variety is not known; however, its genetic similarity with Edremit, Memecik, Gemlik varieties shows the possibility that such varieties may have been pollinators for Uslu. The result obtained in self-pollinating variety Domat shows expressly the fact that no other varieties have been used as pollinator for the pollination of Domat.

RAPD markers are frequently used in studies aimed at the determination of genotyping and genetic diversity of olive trees. As specified by Wu et al. (2004), it was observed that RAPD marker applications had been useful as the first step to produce a genomic map in plants with unknown or much less known genetic series. Primer sets OP-A, OP-I, OP-Q, which provided evaluable bands, yielded successful results in the analysis of cultivated varieties.

It is very important for olive production to identify the varieties of olive, which is appreciated very well today in the economical sense, correctly. It is very important to define variety-specific genetic structure; to determine genetic distances and similarities between the varieties, and to specify and preserve genetic structures of local types peculiar to regions by way of variety identifications. In this respect, detailed determination of genetic characteristics of this plant, which is very valuable for human health, through similar studies would have huge contribution in olive cultivation.

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