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*Full Length Research Paper*

# Comparison of Manzanilla and wild type olives by RAPD-PCR analysis

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**The object of this study was to detect genetic similarities and distances between cultivated Manzanilla and wild type olives by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. In this study, the cultivated type olive Manzanilla was supplied from Olive Research Institute of Turkey and the wild type olives from the villages of Manisa, Izmir and Mugla provinces. Genomic DNA's were extracted from young leaves and PCR was used to generate RAPD bands. Sixty random primers obtained from Operon technology were tested by RAPD-PCR (OP-A, OP-I, OP-Q). Thirty of 60 primers used in the study provided 150 considerably polymorphic bands and the average number of bands was defined as 5. Comparisons of cultivated and wild type olives are important for understanding genetic relation. It was determined that the genetic distance values varied between 0.0665 and 0.2863 and genetic similarity values varied between 0.9356 and 0.7511. It was observed that the samples most close to each other were wild 8 and wild 3 and the samples most distant from each other were wild 5 and Manzanilla, in accordance with such genetic distance and similarity values.**

**Key words:** Manzanilla, RAPD, PCR, wild olives.

## INTRODUCTION

The olive is one of the most ancient cultivated fruit trees of mankind because it is a rich plant of poor lands. It is known that wild olive trees emerged from the eastern part of Mediterranean basin, that is, Mesopotamia and spread to the countries in the north and south coats of the Mediterranean Sea over Southeast Anatolia and then to America and Australia continents (MOARA, 2006). Olive adapts easily to the grown area and it is suitable to hot, sometimes windy conditions. Breeding the olive tree is ideal for the valley regions because they can survive practically in all weather conditions, except the heavy cold. The *Olea europaea* L. has two subspecies; these are wild olives (*Olea europaea oleaster*) and the cultivated olives named as *Olea europaea sativa* (Mendilcioglu, 1999).

Wild type olives are important in terms of improvement since the resistance of wild forms against diseases and adaptation of environmental conditions are higher. Therefore, wild types of olive are generally indispensable resources in improving the mechanism of resistance against diseases (Sehirali and Ozgen, 1987).

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Genetic identification can be performed by examining morphological or phenotypical characteristics but such characteristics are affected by environmental conditions (Chawla, 2002). However, DNA based techniques allow scanning the genome directly without being environmentally affected (Doveri et al., 2008; Martins-Lopes et al., 2007; Essadki et al., 2006). Random amplified polymorphic DNA (RAPD), which is polymerase chain reaction (PCR) based, was developed by Williams et al. (1990) and Welsh and McClelland (1990). Today, genetic variety or similarity can be revealed in a short time and easily and the populations can be examined rapidly through RAPD-PCR (La Rosa et al., 2003, Wu et al., 2004). One of the genetic markers used commonly in determining the genetic variety in olives is RAPD markers (Fabbri et al., 1995; Besnard et al., 2001; La Rosa et al., 2003; Belaj et al., 2003; Wu et al., 2004; Ganino et al., 2007; Martins-Lopes et al., 2007).

There are approximately 900 million olive trees on an area of 10 million hectares in the world. It is estimated that olives are cultivated on an area of 7 million hectares in the Mediterranean basin (Khadari et al., 2003). Since the Mediterranean region provides ideal conditions for breeding, a very large portion of world's olives are

**Table 1.** Origins and supplying places of Manzanilla and wild olives.

| Type of olive | Origin | Place of supply | Province |
|---|---|---|---|
| Manzanilla | Cordoba, Spain | ORI* | Izmir, Bornova, Turkey |
| Wild 1 | Caglak Village | Natural | Akhisar, Manisa, Turkey |
| Wild 2 | Haskoy Village | Natural | Akhisar, Manisa, Turkey |
| Wild 3 | Harlak Village | Natural | Akhisar, Manisa, Turkey |
| Wild 4 | Sabancilar Village | Natural | Akhisar, Manisa, Turkey |
| Wild 5 | Yayakirildik Village | Natural | Akhisar, Manisa, Turkey |
| Wild 6 | Pinarcik Village | Natural | Milas, Mugla, Turkey |
| Wild 7 | Karacakas Village | Natural | Soma, Manisa, Turkey |
| Wild 8 | Bornova County | Natural | Izmir, Bornova, Turkey |

*ORI: Olive Research Institute of Turkey.

available in Mediterranean countries such as Spain, Greece, Italy, Tunisia, Turkey, Syria, Portugal, Morocco and Algeria (Aegean Union of Olive and Olive Oil Exporters, 2009). The country having the most olive trees in the world is Spain which has about 308.758.000 trees on an olive breeding area of 2.423.841 ha. Olives are cultivated in a total area of more than 300.000 ha in the Cordoba region of Spain, which is the origin of Manzanilla (Aegean Union of Olive and Olive Oil Exporters, 2007).

Turkey also has a significant position among countries cultivating olives. The total number of fruit bearing trees in the Aegean Region is 81.869.363 and 9.252.943 in Manisa, 13.851.990 in Izmir and 12.929.068 in Mugla. It is estimated for 2008-2009 that 72.408 tons would be reserved as table olives and 52.869 tons for oil production and 8.988 tons of olive oil would be produced in average with an olive productivity of 13.5 kg per tree in Manisa region. It was also estimated for Izmir region that 15.362 tons would be reserved as table olives and 134.762 for oil production with an olive grain of 10.8 kg per tree and that 27.097 tons of olive oil would be produced. For Mugla region, it was estimated that the average productivity per tree would be 9.5 kg; that 10.994 tons would be reserved as table olives and 111.738 tons for oil production and 22.348 tons of olive oil would be produced (Aegean Union of Olive and Olive Oil Exporters, 2009).

In Turkey, there are cultivated type olives supplied from foreign countries. Manzanilla is one of the varieties cultivated mainly in Aydin 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 climatic conditions. It is a medium strong tree and its fruits are medium sized and are almost round, transversely and longitudinally symmetric shaped and in addition, its rooting ratio in production with cutting is high. It has spread to the Mediterranean and Aegean Regions. It provides yield regularly when maintained well. It is processed and consumed as table olives and it is sufficiently self-pollinating (MOARA, 2006).

Determination of genetic distances and similarities between Spanish origin Manzanilla (cultivated type) and the wild type olives are an issue that require consideration in terms of improvement studies as the sensitivity of cultivated types against diseases are higher (Sehirali and Ozgen, 1987). For instance, in the struggle against *Verticillium* wilt, being one of the diseases, cause significant economical losses in olive breeding, the varieties resistant and sensitive against this disease should be determined in addition to cultural precautions. In the light of this information, the genetic distances and similarities of the cultivated type Manzanilla and the wild type olives have been examined through RAPD-PCR method.

## MATERIAL AND METHODS

### Plant material

Wild olives as used in the present study were supplied from the villages of Manisa, Izmir and Mugla provinces and the Manzanilla was supplied from Olive Research Institute of Turkey. A large number of samples were enumerated and the most healthy and uniform ones among the same were examined. Fresh leaves were taken from a total of 9 plants including 1 Manzanilla and 8 wild olives and they were kept in liquid nitrogen until DNA extraction. Table 1 shows the Manzanilla and wild olives used in this study, their origins and supply regions.

### DNA extraction

Genomic DNA was extracted from young leaves by using Doyle and Doyle method (1987). Pre-chilled mortar and pestle was used to ground fresh leave tissues. Powderized tissues were immediately transferred to 1.5 ml Eppendorf tubes. 700 µl preheated CTAB extraction buffer (CTAB 2%, 20 mM EDTA, 1.4 M NaCl, 100 mM Tris-HCl pH: 8.0, 2% β- mercaptoethanol) was added onto frozen ground leaves, mixed several times by gentle inversions. Samples with CTAB buffer were incubated for 30 min in 65°C. Tubes were mixed by inversions in every 5 min during incubation time. After removing from the hot bath, tubes were allowed to cool and then added to 700 µl of cold chloroform: Isoamyl Alcohol (24:1). Tubes were centrifugated at 10.000 rpm/min for 10 min at + 4°C. Supernatants were transferred into new tubes into which 600 µl of

**Table 2.** Genetic distance-similarity matrix obtained from OP-A, OP-Q, OP-I primer sets (Nei, 1972).

| Olives | Manzanilla | Wild 6 | Wild 1 | Wild 2 | Wild 3 | Wild 4 | Wild 8 | Wild 5 | Wild 7 |
|---|---|---|---|---|---|---|---|---|---|
| Manzanilla | **** | 0.7811 | 0.7811 | 0.7983 | 0.8326 | 0.8069 | 0.8197 | 0.7511 | 0.8026 |
| Wild 6 | 0.2470 | **** | 0.8798 | 0.8283 | 0.8627 | 0.8455 | 0.8584 | 0.7897 | 0.8412 |
| Wild 1 | 0.2470 | 0.1280 | **** | 0.8627 | 0.8970 | 0.8712 | 0.8927 | 0.8240 | 0.8755 |
| Wild 2 | 0.2253 | 0.1883 | 0.1477 | **** | 0.8970 | 0.8798 | 0.8927 | 0.8155 | 0.8755 |
| Wild 3 | 0.1832 | 0.1477 | 0.1087 | 0.1087 | **** | 0.9056 | 0.9356 | 0.8584 | 0.9185 |
| Wild 4 | 0.2146 | 0.1678 | 0.1378 | 0.1280 | 0.0992 | **** | 0.9013 | 0.8240 | 0.8927 |
| Wild 8 | 0.1988 | 0.1527 | 0.1135 | 0.1135 | 0.0665 | 0.1039 | **** | 0.8712 | 0.9142 |
| Wild 5 | 0.2863 | 0.2361 | 0.1935 | 0.2040 | 0.1527 | 0.1935 | 0.1378 | **** | 0.8712 |
| Wild 7 | 0.2199 | 0.1729 | 0.1329 | 0.1329 | 0.0851 | 0.1135 | 0.0897 | 0.1378 | **** |

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

cold chloroform: isoamyl alcohol (24:1) was added and mixed by gentle inversions for 5 min. Samples were centrifugated at 10.000 rpm/min for 10 min at + 4°C. Supernatants were transferred to fresh tubes including 10 M ammonium acetate and 3 M sodium acetate. 500 µl cold Isopropanol was added and mixed by shaking very gently for DNA precipitation. Precipitated DNA was removed with pipette and washed with 70% ethanol. DNA's were dried and re-suspended in 50 µl EDTA. The crude DNA sample was treated with 1 ml RNase (10 mg/ml stock) for 10 min at 37°C.

#### Spectrophotometric analysis

The determination of DNA quality and concentration of DNA in samples were performed by 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-PCR 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 was performed on an eppendorf mastercycler thermal cycler in a total volume of 25 µl. PCR mix included 25 ng template DNA, 2.42 µl. 10 X PCR reaction buffer (with $MgCl_2$, Sigma), 0.44 µl. dNTP (Sigma), 1 µM primer, and 0.13 µl Taq DNA polymerase (Sigma). The initial denaturation was carried out for 60 s at 94°C. The PCR program comprised 35 cycles with 20 s at 94°C; 20 s at 35°C; 30 s at 72°C and final extension performed at 72°C for 5 min.

#### Agarose gel electrophoresis

Amplification products loaded onto 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 (Sigma, Fermentas) was loaded on the first lane of each gel. After the separation of PCR products by agarose gel electrophoresis, gels were visualized with Photo Print (Vilber Lourmat, France) imaging system and analyzed by BioOne D++ software (Vilber Lourmat, France). The RAPD bands were scored as 1 for the presence or 0 for absence, only clear bands were scored for the construction of the 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 hierarchical clustering analysis method (Sneath and Sokal, 1973; Nei, 1972; Yeh et al., 1997).

## RESULTS

A total of 60 primers (OP-A, OP-Q, OP-I) supplied from Operon technology (Alameda, California) were used in the study. A total of 38 bands were obtained from OP-A (1-20) primer set. No evaluable bands were obtained from OP-A 7, OP-A 8, OP-A 17 primers. 11 bands were obtained from OP-Q (1-20) primer set. Evaluable bands were obtained from OP-Q 1, OP-Q 2, OP-Q 3, OP-Q 4 primers. 101 bands were obtained from OP-I (1-20) primer set. No evaluable bands were obtained from OP-I 1, OP-I 2, OP-I 3, OP-I 4, OP-I 5, OP-I 6, OP-I 8, OP- 10, OP-I 15, OP-I 18, OP- I 19 primers. Table 2 shows the genetic distance and similarity matrix as obtained from primer sets.

It was concluded that the total number of evaluable bands obtained from three primer sets were 150. Maximum bands were observed in OP-I (1-20) primer set with 101 bands and least bands were observed in OP-Q (1-20) primer set with 11 bands. It was concluded that the average number of band per primer was 5. The matrix shown in Table 2 was obtained by using Nei's genetic distance coefficient in POPGEN32 software for determining the genetic similarities and distances between Manzanilla and wild type olives. As a result of cluster analysis produced by using UPGMA method, the dendrogram of olive types is shown in Figure 1.

When the matrix and dendrogram were examined, genetic distance values were between 0.0665 (wild 3 and wild 8) and 0.2863 (Manzanilla and wild 5). Thus, samples closest to each other (wild 3 and wild 8) as well the samples most distant to each other were (Manzanilla and wild 5) based on the genetic distance values. Genetic similarity values were between 0.9356 (wild 8 and wild 3) and 0.7511 (wild 5 and Manzanilla). Thus, the samples with closest genetic similarities were (wild 8 and wild 3) and the samples with most distant genetic similarities were (wild 5 and Manzanilla). Genetic similarity values were totally in compliance with the genetic distance

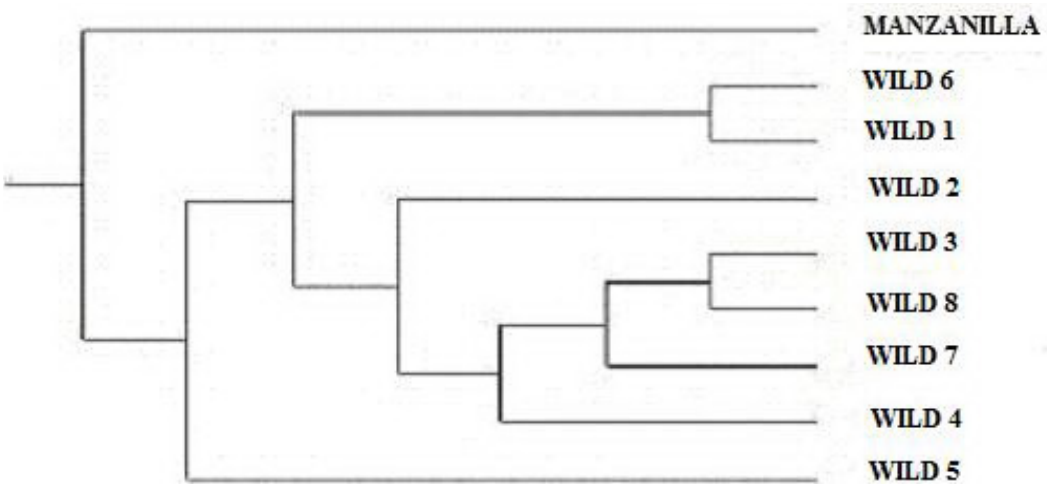


**Figure 1.** Genetic distance dendrogram obtained from Manzanilla and wild olive types using OP-A, OP-Q and OP-I primer sets (Nei, 1972).

values.

## DISCUSSION

Primer sets yielding evaluable bands vary from study to study. In this study, OP-A (1-20), OP-Q (1-20) and OP-I (1-20) primers produced scorable bands in wild olives and Manzanilla sample. Present bands were recorded as one and non-present bands were recorded as zero (Banilas et al., 2003). RAPD markers generated in this study showed a highly polymorphic conformation as mentioned by other researchers. The obtained high polymerphism rate indicates a high genetic diversity in varieties. These results indicated a distinct potential in selection and availability as a genetic source (Fabrri et al., 1995; Bandelj et al., 2002).

Wu et al. (2004) specified that RAPD markers had been useful as the first step to produce a genomic map in plants with unknown or much less known genetic series. The examination of genetic relations between wild types and cultivated types led to the preparation of genetic map of olive tree and to find suitable genetic markers for olive improvement studies.

In conclusion it was determined that the samples most distant to each other based on the genetic distance and similarity values were Manzanilla (of Spanish origin) and Wild 5 (from Manisa, Turkey) olives. As shown in this research, comparing the local wild olives with cultivated types and discovering the genetic relations (distances and similarities) between same can lead the way to selection and improvement studies to be performed towards resistance against diseases.

Manzanilla and wild 5 olives have been determined as the subspecies whose sensitivity and resistance against *Verticillium* pathogen would be tested in further studies to be conducted based on the dendrogram obtained.

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### REFERENCES

Aegean Union of Olive and Olive Oil Exporters (2007). 2006-2007 Study Report. pp. 22-26.

Aegean Union of Olive and Olive Oil Exporters (2009). 2008-2009 Study Report. pp. 24-48.

Bandelj D, Jakše J, Javornık B (2002). DNA fingerprinting of olive varieties by microsatellite markers. Food Technol. Biotechnol. 40: 185-190.

Belaj A, Caballero JM, Barranco D, Rallo L, Trujillo I (2003). Genetic characterization and identification of new accessions from Syria in an olive germplasm bank by means of RAPD markers. Euphytica, 194(3): 261-268

Besnard G, Baradat P, Bervillé A (2001). Genetic relationships in the olive (*Olea europaea* L.) reflects multilocal selection of cultivars. Theor. Appl. Genet. 102: 251-258.

Chawla HS (2002). Introduction to plant biotechnology. Science publishers, USA. pp. 329-330.

Doveri S, Sabino Gil F, Diaz A, Reale S, Busconi M, da Camara Machado A, Martin A, Fogher C, Donini P, Lee D (2008). Standardization of a set of microsatellite markers for use in cultivar identification studies in olive (*Olea europaea* L.). Sci. Hortic. (Amsterdam) 116: 367-373.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

Essadki M, Ouazzani N, Lumaret R, Moumni M (2006). ISSR variation in olive-tree cultivars from Morocco and other western countries of the Mediterranean Basin. Genet. Resour. Crop. Evol. 53(3): 475-482.

Fabbri A, Hormaza JI, Polito VS (1995). Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. J. Am. Soc. Hortic. Sci. 120: 538-542.

Ganino T, Beghè D, Valenti S, Nisi R, Fabbri A (2007). RAPD and SSR

markers for characterization and identification of ancient cultivars of *Olea europaea* L. in the Emilia region, Northern Italy. Genet. Resour. Crop Evol. 54(7): 1531-1540.
Khadari B, Breton C, Moutier N, Roger JP, Besnard G, Bervillé A, Dosba F (2003). The use of molecular markers for germplasm management in a French olive collection. Theor. Appl. Genet. 106: 521-529.
La Rosa R, Angiolillo A, Guerrero C, Pellegrini M, Rallo L, Besnard G, Bervillé A, Martin A, Baldoni L (2003). A first linkage map of olive (*Olea europaea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. Theor. Appl. Genet. 106: 1273-1282. Martins-Lopes P, Lima-Brito J, Gomes S, Meirinhos J, Santos L, Guedes-Pinto H (2007). RAPD and ISSR molecular markers in *Olea europaea* L.: Genetic variability and *molecular* cultivar identification. Genet. Res. Crop Evol. 54(1): 117-128.
Mendilcioglu K (1999). Subtropical Climate Fruits. (Olive), Publications of Faculty of Agriculture, Ege University. pp. 1- 8, Bornova-İzmir.
MOARA (2006). Ministry of Agriculture and Rural Affairs of Republic of Turkey, Farmers Training series 14, Olive Breeding Ankara-2006. p. 7.
Nei M (1972). Nei's Original Measures of Genetic Identity and Genetic Distance. Am. Nat. 106: 283-292.
Sehirali S, Ozgen M (1987). Plant Genetic Resources. Ankara University Agricultural Faculty Publishing. 1020: 212.
Sneath PHA, Sokal, RR (1973). Taxonomic Structure. Numerical Taxonomy, pp. 230-234, W.H. Freeman and Co., San Francisco, CA, USA.
Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.
Wu S, Collins G, Sedgley MA (2004). Molecular linkage map of olive (*Olea europea* L.) based on RAPD, microsatellites and SCAR markers. Genome, 47: 26-35.
Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1997). POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.