



RAPD analysis of selected local Turkish grape cultivars (*Vitis vinifera*)

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ABSTRACT. Turkey is very rich in local grape varieties. The solution to the problem of identifying local cultivars, which is considered an important deficiency for the region, will only be possible when they can be defined with molecular markers. Forty-nine local grapevine cultivars from Şanlıurfa (Turkey) were characterized with RAPD markers. Twenty-five decamer primers selected from 60 primers were used in this analysis. A total of 171 bands were obtained with the 25 primers, of which 112 were polymorphic; the level of DNA polymorphism was 65.49% in these local cultivars. Among the selected primers, OPA-18, OPO-07 and P-123 gave the maximum number of polymorphic bands (seven). Genetic relationships among these cultivars were determined with a similarity index and using a dendrogram. Among the grape cultivars, the lowest similarity ratio (0.578) was observed among the Kūlahi-Kızılbanki cultivars and the highest similarity ratio (0.908) was observed for the Çilorut-Dökūlgen combination. The high similarity ratio among the grape cultivars of Şanlıurfa Province was also reflected in the dendrogram.

In general, no relationships were encountered between the genetic identification of the cultivars and their ampelographic properties.

Key words: *Vitis vinifera* L.; Molecular characterization; RAPD; Similarity index; Dendogram

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the oldest and most important perennial crops in the world. Turkey is an important center of origin of the cultivated grapevine *V. vinifera* L. Cultivation of grapes in Anatolia began approximately 7-8000 years ago and seeds of domesticated grapes dating from ca. 8000 BP have also been found in the region (Arroyo Garcia et al., 2006; This et al., 2006). In Turkey with its large grape germplasm, so far, approximately 1200 cultivars, including synonymous cultivars, have been transferred from the different ecological zones of the country to the National Germplasm Repository Vineyard in Tekirdağ (Ergül and Ağaoğlu, 2001).

Turkey is very rich in local grape varieties. The naming of local grapevines is a major problem in Turkish viticulture. There are many grape cultivars with similar morphological characters, which are cultivated in different locations under different names in Anatolia. There is much confusion about the names of the grape cultivars grown in this province, which has a very rich history of different civilizations and societies. The solution to the problem of the existence of cultivars, which is considered an important deficiency for the region, is only possible by creating genetic definitions for all the cultivars with molecular markers [random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), etc.].

The RAPD technique is fast and easy, since it does not require knowledge of the sequences of the markers and can produce abundant polymorphic fragments. RAPD analysis is one of the techniques that has been used successfully to reveal genetic variations (Kocsis et al., 2005). Notwithstanding the limitations, RAPD markers have proven to be a highly effective and efficient method for the genetic analysis (Gogorcena et al., 1993; Büscher et al., 1994; Ye et al., 1998; Vidal et al., 1999; Luo et al., 2002; Ulanovsky et al., 2002). Large numbers of data sets can be generated because different RAPD primers are commercially available (Fanizza et al., 2000).

The objective of this study was to identify the 49 local (Şanlıurfa-Turkey) grape cultivars. This study presents the genotyping of local (Şanlıurfa) grapevine cultivars, using the RAPD-PCR (polymerase chain reaction) technique. The determination of genetic diversity and relationships between cultivars, as well as the application of the data obtained for cultivar identification, are discussed.

MATERIAL AND METHODS

Plant material

Forty-nine local grapevine cultivars (*V. vinifera* L.) were studied for characterization. Forty genotypes of these plant samples were taken from vineyards in the Turkish city of Şanlıurfa. Nine genotype samples were taken from the National Germplasm Repository Vineyard of Tekirdağ. The cultivars used in this study are listed in Table 1.

Table 1. Grapevine cultivars studied with RAPD markers.

N	Cultivar name	Collection	Berry color	Use (table/raisin/wine)
1	Hönüsü	Ş	Red	Table
2	Şire	Ş	White	Wine
3	Sergi karası	Ş	Red	Raisin
4	Cibin	Ş	Red	Raisin
5	Tilgören	Ş	Red	Table, Wine
6	Çiloreş	Ş	White	Table, Wine
7	Çilorut	Ş	White	Table, Wine
8	Simore	Ş	White	Table, Wine
9	Gülgülü	Ş	Pink	Table, Wine
10	Zeyni	Ş	White	Table, Wine
11	Tahannebi	Ş	White	Table
12	Zeynebi	Ş	White	Table, Raisin
13	Bastikkabarcığı	Ş	White	Wine
14	Kabarcık	Ş	White	Wine
15	Külahi	Ş	White	Table
16	Yediveren-a*	Ş	Red	Table, Wine
17	Horoz karası	Ş	Red	Table, Wine, Raisin
18	Hatunparmağı (white)	Ş	White	Table, Raisin
19	Azezi	Ş	White	Table, Wine
20	Kayısı	Ş	White	Table
21	Şarabi	Ş	Red	Wine
22	Danabumu	Ş	Red	Table, Wine
23	Keçiemceği	Ş	Red	Raisin
24	Dimışkı	Ş	White	Table, Raisin
25	Muhammediye	Ş	White	Table
26	Yediveren-b*	Ş	Red	Table, Wine
27	Hatunparmağı (black)	Ş	Red	Table, Raisin
28	Elmaüzümü	Ş	White	Table, Wine
29	Karamikeri	Ş	Red	Table
30	Köseni	Ş	Pink	Table
31	Çördüğü	Ş	White	Table, Wine
32	Serpenekıran	Ş	White	Table, Wine
33	Halebi	Ş	White	Table, Wine
34	Avderi	Ş	White	Table, Wine
35	Kızılbanki	Ş	Pink	Table
36	Antepüzümü	Ş	Red	Table
37	Dusuzu	Ş	White	Wine
38	Siyahüzüm	Ş	Red	Wine
39	Kızlartahtası	Ş	White	Table, Wine
40	Şefafi	Ş	White	Wine
41	Simore*	T-Ş	White	Wine
42	Çilorut*	T-Ş	White	Wine
43	Siverek*	T-Ş	White	Wine
44	Ruhali*	T-Ş	White	Table
45	Tilgören*	T-Ş	Red	Wine
46	Kayısı*	T-Ş	White	Table
47	Zerik*	T-Ş	White	Raisin
48	Çiloreş*	T-Ş	White	Table, Wine
49	Çörtük*	T-Ş	White	Table

Ş = Şanlıurfa; T-Ş = National Germplasm Repository Vineyard samples that were previously brought from Şanlıurfa. *Yediveren-a = for vineyard type; Yediveren-b = for pergola trellis type.

DNA extraction

DNA from young, healthy leaves was extracted following the protocol of Lodhi et al. (1994). DNA concentration and purity were measured using a NanoDrop ND-1000 model spectrophotometer. An initial screening with 60 primers (10-mers) from the kits OPA, OPB, OPF, OPD, UBC, P, B, and S from Operon Technologies Inc. (Huntsville, AL, USA) was carried out on 5 cultivars. Only 25 informative primers were selected due to their ability to

produce polymorphic, unambiguous and stable RAPD markers.

RAPD analysis and gel electrophoresis

RAPD amplification was performed in a 25- μ L reaction volume containing 200 ng genomic DNA, 10X buffer (25 mM MgCl₂, 2.5 mM dNTPs, 200 ng primer (Operon Technologies Inc.), and 0.3 U Tag DNA polymerase (Promega, Madison, WI, USA). The thermocycler was programmed as follows; initial cycle of 5 min at 94°C followed by 35 cycles of 30 s at 94°C, an annealing temperature of 30° to 38°C for 1 min, elongation step of 1 min 45 s at 72°C, and a final extension step of 8 min at 72°C.

PCR products were separated by gel electrophoresis on 1.5% agarose gels with 1X TBE (Trisma base, boric acid, EDTA) buffer, using a volt range of 100 V/cm for 4 h. The gel was stained with 0.25 μ g/mL ethidium bromide and photographed black and white on Polaroid type 665 film. RAPDs have often been criticized for low reproducibility; in order to avoid this phenomenon we used highly constant conditions and all reactions were repeated at least twice (Kocsis et al., 2005).

Data analysis

In order to ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designed by their primer code and their size in base pairs. Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. Jaccard's coefficient was used to calculate the genetic distance (Dps) between cultivars. However, the MVSP software (Kowach, 1999) was used to calculate the similarity index. These indices were converted into a dendrogram by using "UPGMA" cluster.

RESULTS AND DISCUSSION

Forty-nine local cultivars were studied by RAPD markers for genotyping studies. Twenty-five informative primers were selected and used due to their ability to produce polymorphic RAPD markers. According to This et al. (1997), some primers seem to be more efficient than others in producing stable and reproducible DNA fingerprints.

A total of 171 bands were evaluated from the 25 primers used; 112 polymorphic bands were found and the level of DNA polymorphism established among local cultivars was 65.49%. From the selected primers OPA-18, OPO-07 and P-123, the maximum number of polymorphic (7 bands) bands were produced. The lowest number of polymorphic bands (3 bands) was obtained using OPA-01, OPA-04, OPA-07, OPA-15, OPF-08, OPO-11, P-437 primers. When the ratios of polymorphic bands are examined on the basis of primers, the highest ratio (87.50%) was determined with OPA-18 and P-123 and the lowest ratio (37.50%) was obtained with OPF-08. Figure 1 shows the sample gel images of RAPD patterns obtained with primer P-166.

Primer selection is essential for discrimination analysis. Obviously, the more bands scored and plants studied, the higher the statistical significance of the calculation will be. About 100 bands should be enough to obtain statistically significant results (Kocsis et al., 2005).

When the dendrogram determining the rate of relativity between genotypes on the basis of coefficient of similarity was examined, it was observed that the similarity ratio among

the local grape cultivars of Şanlıurfa Province was high. The dendrogram generated by RAPD analysis showed 5 distinct groups (1 = 3 cultivars; 2 = 5 cultivars; 3 = 20 cultivars; 4 = 8 cultivars, and 5 = 4 cultivars; Figure 2).

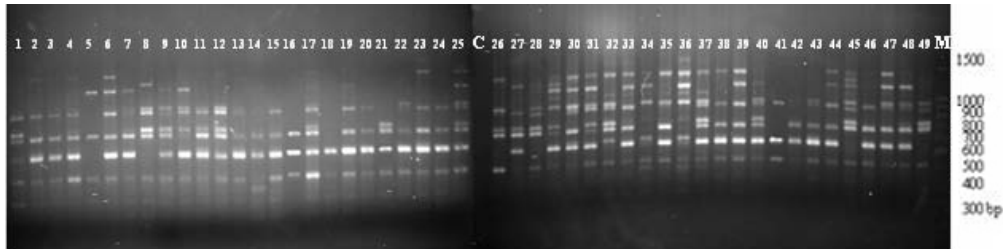


Figure 1. RAPD profiles of leaf extracts of grapevine cultivars from Şanlıurfa Province using primer P 166 [M = molecular marker - 100-bp DNA ladder (Promega)] C: Control. Lane 1 = Höntüsü; lane 2 = Şire; lane 3 = Sergi karası; lane 4 = Cibin; lane 5 = Tilgören; lane 6 = Çiloreş; lane 7 = Çilurut; lane 8 = Simore; lane 9 = Gülgülü; lane 10 = Zeyni; lane 11 = Tahannebbi; lane 12 = Zeynebi; lane 13 = Bastıkkabarcığı; lane 14 = Kabarcık; lane 15 = Külahi; lane 16 = Yediveren-a; lane 17 = Horoz karası; lane 18 = Hatunparmağı (white); lane 19 = Azezi; lane 20 = Kayısı; lane 21 = Şarabi; lane 22 = Danaburnu; lane 23 = Keçiemeceği; lane 24 = Dımışkı; lane 25 = Muhammeddiye; lane 26 = Yediveren-b; lane 27 = Hatunparmağı (black); lane 28 = Elmaüzümü; lane 29 = Karamikeri; lane 30 = Köseni; lane 31 = Çördüğü; lane 32 = Serpenekıran; lane 33 = Halebi; lane 34 = Avderi; lane 35 = Kızılbanki; lane 36 = Antepüzümü; lane 37 = Dusuzu; lane 38 = Siyahüzüm; lane 39 = Kızıltahtası; lane 40 = Şefafi; lane 41 = Simore*; lane 42 = Çilurut*; lane 43 = Siverek*; lane 44 = Ruhali*; lane 45 = Tilgören*; lane 46 = Kayısı*; lane 47 = Zerik*; lane 48 = Çiloreş*; lane 49 = Çörtük*. *Cultivars collected from the National Germplasm Repository Vineyard in Tekirdağ Province.

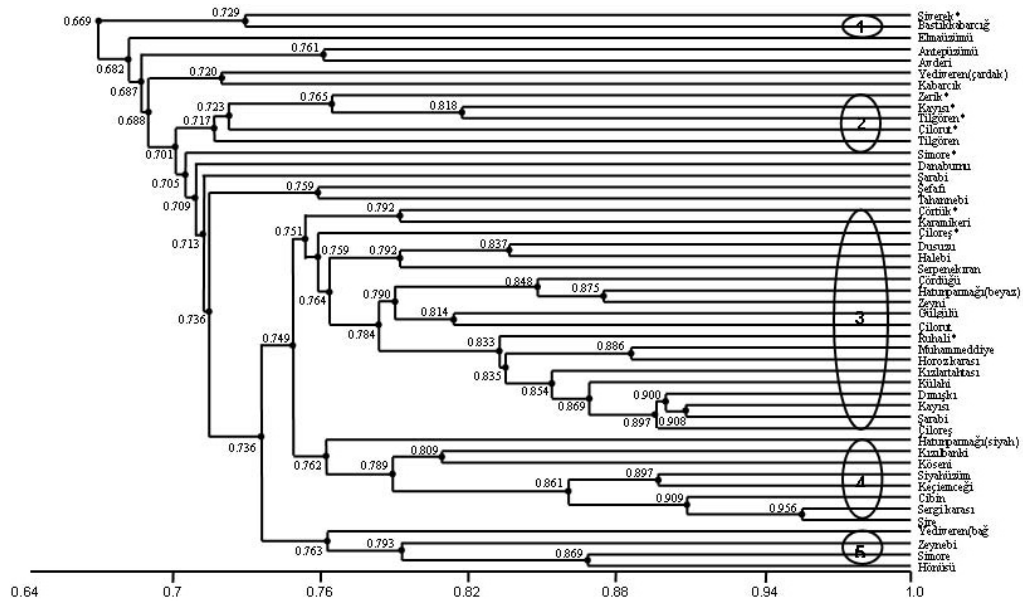


Figure 2. Dendrogram of 49 grape cultivars based on similarity index from RAPD data, showing the genetic relationships of tested cultivars. *Cultivars collected from Tekirdağ (National Germplasm Repository Vineyard of Tekirdağ).

The values of genetic distance ranged from 0.578 for the most closely related cultivars (Külahi-Kızılbanksi) to 0.908 for the most distantly related cultivars (Çilorut-Dökülgen).

In this study, the general similarity ratios among the cultivars were found to be between 0.650 and 0.900, showing a parallelism with the other RAPD analyses performed by other researchers, 0.553-0.952 (Ergül et al., 2002; Karataş, 2005; Ağaoğlu et al., 2006; Karataş and Ağaoğlu, 2008).

The similarity ratios of the grape cultivars obtained from Tekirdağ and the grapes with the same name obtained from Şanlıurfa Province are given below. Despite the fact that there is a high similarity ratio among them, they are different from each other. RAPD analyses determined that the grape cultivars grown in Şanlıurfa Province have a rich gene source in Turkey and the cultivars transferred to the National Germplasm Repository Vineyard of Tekirdağ from this province are cultivars or types, which are different from each other.

Different authors agree with the existing difficulties to detect intravarietal polymorphism in grapevines (Gogorcena et al., 1993; Sefc et al., 1998). The genetic intravarietal variability has been attributed to two factors: i) a probable polyclonal origin of the varieties and ii) an accumulation of somatic mutations over the centuries increases variability (Ulanovsky et al., 2002).

The RAPD method can solve one of the major problems of varietal identification in grapevines: the existence of homonyms and synonyms, particularly with regard to varieties that have been cultivated for centuries and are widely distributed (Borrego et al., 2002).

DNA fingerprint studies, based on agronomic characteristics, are most likely the cause of mistaken conclusions. Primer or probe screening regions are small compared to genome size in plants, including grapevines, which code the gene regions, not included within the amplified regions. Accordingly, genetic similarity degrees based on molecular markers of the cultivars could emerge independently from the agronomic similarities. On the other hand, the fact that generally there are ecological similarities and parallelism within the population composed of cultivars adapted to a certain geographical region, is another important factor, which should be taken into consideration (Ulanovsky et al., 2002; Ağaoğlu et al., 2006).

The similarity ratios of these local cultivars stated to be synonymous are among the lowest values on the dendrogram. In conclusion, it emerges that the region has a very rich and varied clonal structure. The fact that the cultivars stated to be synonymous by the local people and the cultivars having the same name obtained by us and from the National Germplasm Repository Vineyard of Tekirdağ have different genotype stems, most likely because they were, in fact, obtained from different types.

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