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RAPD ANALYSIS OF GENETIC RELATIONS BETWEEN BÜZGÜLÜ GRAPE CULTIVARS (*VITIS VINIFERA*) GROWN IN DIFFERENT PARTS OF TURKEY

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The genetic relations between Büzgülü grape ecotypes *(Vitis vinifera* L.) grown in different regions of Turkey were investigated by RAPD analysis. Of 50 decamer primers tested, 20 were used in the study, and 17 yielded clear and reproducible polymorphic bands. Differences between ecotypes were analyzed statistically. The similarity index was lowest (0 .601) between Etli Büzgülü (collection no. 619, from Antalya province) and Büzgülü (no. 572, Eskisehir prov.), and highest (0.879) between Sık Büzgülü (no. 516, Konya prov.) and Büzgülü (no. 738, Kütahya prov.). The data matrix indicated genetic distances ranging up to 0.038, although most were under 0.03. Phylogenetic trees obtained by UPGMA and neighbor-joining methods basically resembled each other, with some differences. The results from RAPD analysis correlated with some morphological characteristics. This study of Turkish grape germplasm suggests that the geographical and ecological distribution of the plants contributes to higher genetic similarity.

Key words: *Vitis vinifera* L., Büzgülü, polymorphism, RAPD, Turkey.

INTRODUCTION

With its rich grape germplasm, Turkey is one of the world's leading producers of grapevine. It is one of the regions where grapevines are suggested to have been first taken under cultivation (Winkler et al., 1974), and viticulture forms one of its most important agricultural branches. A large number of economically important cultivars have been developed throughout the country's history. Grape is utilized in the commonly known ways and in many other ways unique to Turkey: grape-leaf brine used for pickling food, grape molasses, thin sheets of sun-dried fruit pulp, sweets made of starch and boiled-down grape juice, etc. The true number of grape varieties is not known, but country-wide surveys revealed 1600 different types, of which only some 40–60 are used for commercial viticulture (Agaoglu, 1999).

The National Germplasm Repository Vineyard accommodates approximately 1200 accessions collected from different ecological zones of the country. Up to now, in Turkey the cultivars have been distinguished and identified by ampelographic means, and a number of isoenzyme studies have examined genetic diversity in some grapevine cultivars (Ergül and Agaoglu, 2001, Söylemezoglu et al., 2001; Türkben et al., 2002). Studies employing PCR-based molecular markers are few (Ergül et al., 2002). Genetic identification of important grapevine cultivars is a task for the future. To confirm the trueness of type of the grapevine varieties of Turkey, ampelographic studies must be confirmed by molecular marker analyses. The current study was conducted to identify 14 accessions of the Büzgülü cultivar complex, transferred from different regions of Turkey to the National Germplasm Repository Vineyard in Tekirdag.

This study used RAPD technique to assess genetic relations in the Büzgülü group. RAPD technique has been employed by many researchers for discrimination of *Vitis vinifera* L. cultivars (Büscher et al., 1994; Ye et al., 1998; Flourier et al., 1998; Vidal et al., 1999; Regner et al., 2000; Luo and He, 2001). This is the first study of molecular markers in the Büzgülü group, and is focused on determining genetic distances and identifying the genotypes of all the ecotypes present in the collection.

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TABLE 1. Some characteristics of the varieties from the Büzgülü group

	Variety name (Collection No)	Province	Bunch Shape	Berry Shape	Berry Color	Taste	Inside	Crop maturity
B1	Etli Büzgülü (619)	Antalya	Conical	Long elliptic	Black	Medium	Juicy	Middle of October
B2	Gode Büzgülü (613)	Antalya	Conical	Fragmented	Black	Medium	Juicy	Middle of October
B3	Büzgülü (360)	Antalya	Conical	Long elliptic	Black	Medium	Fleshy crisp	End of September
B4	Akbüzgülü (747)	Kütahya	Branched	Elliptic	White	Less sweet	Fleshy	
B5	Büzgülü (347)	Antalya	Conical	Elliptic	White	Sweet	Fleshy crisp	End of September
B6	Büzgülü (518)	Konya	Conical	Elliptic	Black	Sweet	Juicy	End of September
B7	Büzgülü (572)	Eskisehir	Dense conical	Elliptic	Black (Red)	Medium	Juicy	End of September
B8	Siyah Büzgülü (568)	Eskisehir	Dense cylindrical	Long elliptic	Black (Red)	Sour	Juicy crisp	End of September
B9	Büzgülü (519)	Konya	Cylindrical	Rounded	Black	Good	Fleshy	End of September
B10	Büzgülü (553)	Denizli	Cylindrical	Elliptic	Black			End of September
B11	Akbüzgülü (826)	Ankara	Cylindrical	Long elliptic	White	Sweet	Crisp	End of September
B12	Kara Büzgülü (643)	Mugla	Conical	Long elliptic	Black			End of August
B13	Büzgülü (738)	Kütahya	Branched conical	Elliptic	Black	Less sweet	Fleshy	Middle of September
B14	Sık Büzgülü (516)	Konya	Long conical Cylindrical	Ellipsoidal	Black	Sweet	Fleshy juicy Crisp	End of September

MATERIALS AND METHODS

PLANT MATERIAL

Fourteen Büzgülü grape accessions were obtained from the National Grapevine Germplasm Vineyard at the Institute of Viticulture in Tekirdag. Some important characteristics of the varieties are presented in Table 1. Young leaves and shoots of these plants were used for DNA extraction.

RAPD CONDITIONS AND GEL ELECTROPHORESIS

DNA extraction was performed according to Lodhi et al. (1994). Fifty different decamer primers used by different researchers for RAPD analyses of *V. vinifera* L. cultivars were screened, and 20 primers were selected for the reactions. Seventeen of them amplified clear and reproducible polymorphic bands.

PCR was performed in a reaction volume of 25 µl containing 200 ng genomic DNA, 2.5 µl 10 × reaction buffer, 3 mM $MgCl_2$, 4 mM total dNTP, 50 ng primer and 1 unit of Taq polymerase (Promega). PCR was performed in a Techne Cyclogene Thermal Cycler. The PCR program had an initial cycle of 3 min at 94˚C followed by 35 cycles of 30 sec at 94˚C, 1 min at 34˚C and 1 min 45 sec at 72˚C. The final extension was performed at 72˚C for 8 min. Amplified samples were loaded on 2% agarose gels (mixture of 1% agarose and 1% Nu Sieve GTG agarose, FMC Corporation), and run at 100 V for 4 h. For detection of any other kinds of DNA contaminants, a negative control of PCR-mix without any template DNA was used. The sequences of the 20 decamer primers used in the study are shown in Table 2.

TABLE 2. RAPD primers and degree of polymorphism

Primer	Sequence (5'-3')	Total amplified bands	Polymorphic bands	Polymorphic percentage (%)
P123	GggATTCgAC	23	15	65.2
OPA 18	AggTgACCgT	14	8	57.1
OPA 10	GTg ATCgCAG	27	19	70.3
OPA 11	CAATCgCCgT	17	10	58.8
OPA 09	GggTAACgCC	19	8	42.1
OPA 15	TTC CgAACCC	15	5	33.3
OPA 17	GACCgCTTgT	10	8	80,0
OPA 05	AggggTCTTg	14	11	78.5
P166	GTgACggACT	12	9	75.0
P232	CCgCTTgTTg	20	14	70.0
P394	CgACTCCAAC	17	10	58.8
OPO 04	AAgTCCgCTC	20	9	45.0
OPO 07	CAgCACTgAC	18	10	55.5
OPO 19	GgTgCACgTT	17	9	52.9
OPF 06	GggAATTCgg	17	7	41.1
OPF 07	CCgATATCCC	9	5	55.5
OPO 02	AcgTAgCgTC	15	8	53.3
OPA 1	CAggCCGTTC	21	0	0.0
OPO 03	CTgTTgCTAC	15	0	0.0
OPA 03	AgTCAgCCAC	13	0	0.0
Total		333	165	49.5

DATA ANALYSIS

RAPD assays were repeated twice for each primer, and only clear, reproducible bands were scored, with particular attention to sharp bands. Faint ones were ignored. The RAPD data matrix was used to compute the genetic distances of accessions according to Jaccard's coefficient. MVSP ver. 3.1 (Kovach, 1999) was used to calculate Jaccard's (1908) similarity coefficients. These coefficients were used to construct a dendrogram by the unweighted pair-group method of arithmetic average (UPGMA). Principal coordinate analysis (PCO) was

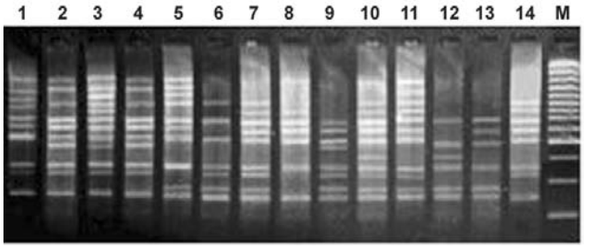

Fig. 1. RAPD patterns with the use of the primer OPA 05 (5' AggggTCTTg 3'). M – Molecular weight marker (100 bp ladder). 1 – Etli Büzgülü (no. 619, Antalya prov.); 2 – Gode Büzgülü (no. 613, Antalya prov.); 3 – Büzgülü (no. 360, Antalya prov.); 4 – Akbüzgülü (no. 747, Kütahya prov.); 5 – Büzgülü (no. 347, Antalya prov.); 6 – Büzgülü (no. 518, Konya prov.); 7 – Büzgülü (no. 572, Eskisehir prov.); 8 – Siyah Büzgülü (no. 568, Eskisehir prov.); 9 – Büzgülü (no. 519, Konya prov.); 10 – Büzgülü (no. 553, Denizli prov.); 11 – Akbüzgülü (no. 826, Ankara prov.); 12 – Kara Büzgülü (no. 643, Mugla prov.); 13 – Büzgülü (no. 738, Kütahya prov.); 14 – Sık Büzgülü (no. 516, Konya prov.).

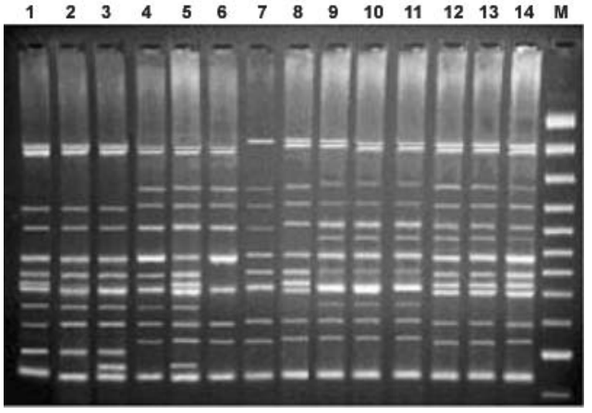

Fig. 2. Amplification of DNA for RAPD analysis with primer OPO 02 (AcgTAgCgT). M – Molecular weight marker (100 bp ladder). Sample numbering as in Figure 1.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
B1	1,000													
B2	0,867	1,000												
B3	0,795	0,832	1,000											
B4	0,724	0,773	0,804	1,000										
B5	0,750	0,761	0,791	0,824	1,000									
B6	0,669	0,699	0,699	0,717	0,795	1,000								
B7	0,601	0,631	0,661	0,697	0,711	0,743	1,000							
B8	0,670	0,699	0,735	0,766	0,767	0,756	0,847	1,000						
B9	0,642	0,701	0,701	0,732	0,714	0,695	0,719	0,765	1,000					
B10	0,686	0,740	0,727	0,758	0,722	0,709	0,727	0,785	0,854	1,000				
B11	0,696	0,737	0,754	0,792	0,737	0,689	0,737	0,813	0,785	0,860	1,000			
B12	0,657	0,681	0,692	0,740	0,711	0,698	0,690	0,760	0,764	0,765	0,799	1,000		
B13	0,677	0,701	0,719	0,744	0,732	0,676	0,699	0,777	0,748	0,756	0,845	0,789	1,000	
B14	0,705	0,722	0,733	0,790	0,765	0,716	0,721	0,804	0,770	0,796	0,858	0,817	0,879	1,000

Fig. 3. Similarity index (Jaccard's coefficient) of the tested accessions.

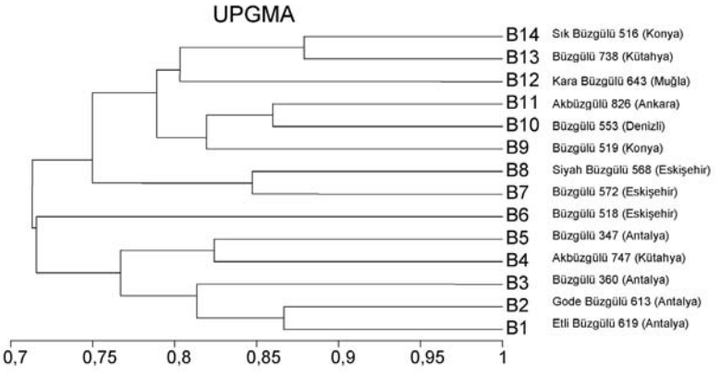

Fig. 4. Dendrogram (UPGMA) showing the genetic relationships between the 14 accessions of Büzgülü grape cultivar.

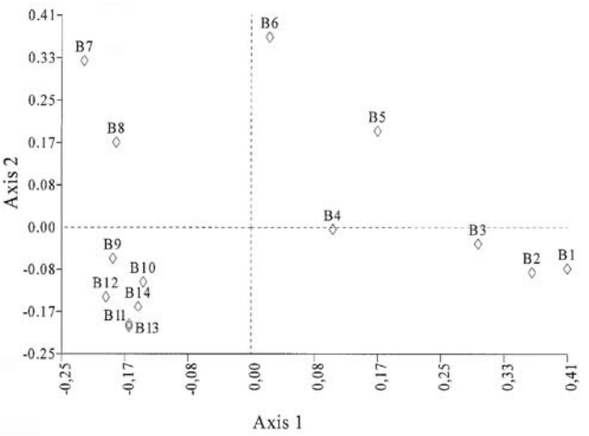

Fig. 5. Associations between the 14 accessions of the Büzgülü grape cultivar as found by principal coordinate analysis (PCO). Numbers in the plot represent the accessions listed in Figure 1.

performed to show the multiple dimensions of the distribution of the genotypes in a scatter plot.

Distance matrices were also constructed using the Nei-Li (1979) similarity index. Neighbor-joining analysis was done with PAUP ver. 4.0 (Swofford, 2002).

RESULTS AND DISCUSSION

The RAPD profiles obtained with OPA 05 and OPO 02 primers are shown in Figures 1 and 2, respectively. Of the 330 bands obtained at the end of RAPD analyses, 165 were determined as polymorphic. Amplified fragments ranged from 100 to 2000 bp, and the number of bands for each primer varied from 9 to 21. OPA 01, OPA 03 and OPO 03 gave only monomorphic bands. Other primers gave high ratios of polymorphic bands in PCR reactions: 70.3% for OPA 10, 80% for OPA 17, 78.5% for OPA 05, 75% for P 166 and 70% for P 232. Primers P 123, OPA 18, OPA 11, OPA 09, P394, OPO 04, OPO 07, OPF 06, OPF 07, OPO19 and OPO 02 displayed polymorphism between 41.1% and 65.2%. OPA 15 gave the lowest ratio, 33.3% (Tab. 2).

Each RAPD band was treated as a separate character and scored 1 (present) or 0 (absent), and a rectangular binary data matrix was obtained. A similarity matrix was obtained using Jaccard's (1908) coefficient and converted to distances (Fig. 3). The distance matrix was then used in cluster analysis, and a dendrogram was constructed using the UPGMA procedure (Fig. 4).

Distance matrix was also constructed using the Nei-Li (1979) similarity index (Fig. 6), and neighbor-joining analysis was done with PAUP ver. 4.0 (Swafford, 2002) (Fig. 7).

According to the similarity index by Jaccard's coefficient and cluster analysis by UPGMA (Figs. 3, 4), the lowest similarity found was between Etli Büzgülü (collection no. 619, from Antalya province) and Büzgülü (no. 572, Eskisehir prov.), with a value of 0.601. The Sık Büzgülü (no. 516, Konya prov.) and Büzgülü (no. 738, Kütahya prov.) ecotypes gave the highest ratio, 0.879.

Cluster analysis by UPGMA suggests the existence of geographic and ecological groups with higher genetic similarities (Fig. 4). The Büzgülü grape cultivars used in the study are listed in Table 1, with some of their characteristics and locations. In the dendrogram, the Büzgülü accessions formed two main clus-

B1 Etli Büzgülü (619) Antalya													
Gode Büzgülü (613) Antalya	0.01144660												
Büzgülü (360) Antalya	0.01782730	0.01674808											
Ak Büzgülü (747) Kütahya	0.02403061	0.01800330	0.01582445										
B5 Büzgülü (347) Antalya	0.02647914	0.02504243	0.01790884	0.01549507									
Büzgülü (518) Konya	0.03868007	0.03447058	0.03263212	0.02876403	0.01826094								
Büzgülü (572) Eskişehir	0.03529612	0.03133484	0.02860439	0.02886889	0.02138251	0.01528676							
Siyah Büzgülü (568) Eskişehir	0.02958980	0.02702347	0.02354689	0.02288647	0.01945391	0.01923017	0.00920783						
Büzgülü (519) Konya	0.03328841	0.02531186	0.02674660	0.02406088	0.02346826	0.02859901	0.02355948	0.02150005					
Büzgülü (553) Denizli	0.02931934	0.02180977	0.02519017	0.02164575	0.02393029	0.02794720	0.02009434	0.01725875	0.01163352				
Ak Büzgülü (826) Ankara	0.02674660	0.02153145	0.02108609	0.01866098	0.02264688	0.02945240	0.02176127	0.01623255	0.01997741	0.01233252			
Kara büzgülü (643) Muğla	0.03257212	0.02987484	0.02824608	0.02276604	0.02602501	0.02908873	0.02717315	0.02217791	0.02235790	0.01903537	0.01525847		
Büzgülü (737) Kütahya	0.02983697	0.02822325	0.02659909	0.02588779	0.02531964	0.03270159	0.02647914	0.02432639	0.02457675	0.02303758	0.01433022	0.01934399	
Sık Büzgülü(516) Konya	0.02618141	0.02477124	0.02329806	0.01895063	0.02111794	0.02689576	0.02406088	0.01832315	0.02126625	0.01707722	0.01168282	0.01640083	0.01181211

Fig. 6. Similarity indexes (Nei and Li, 1979) of the tested accessions.

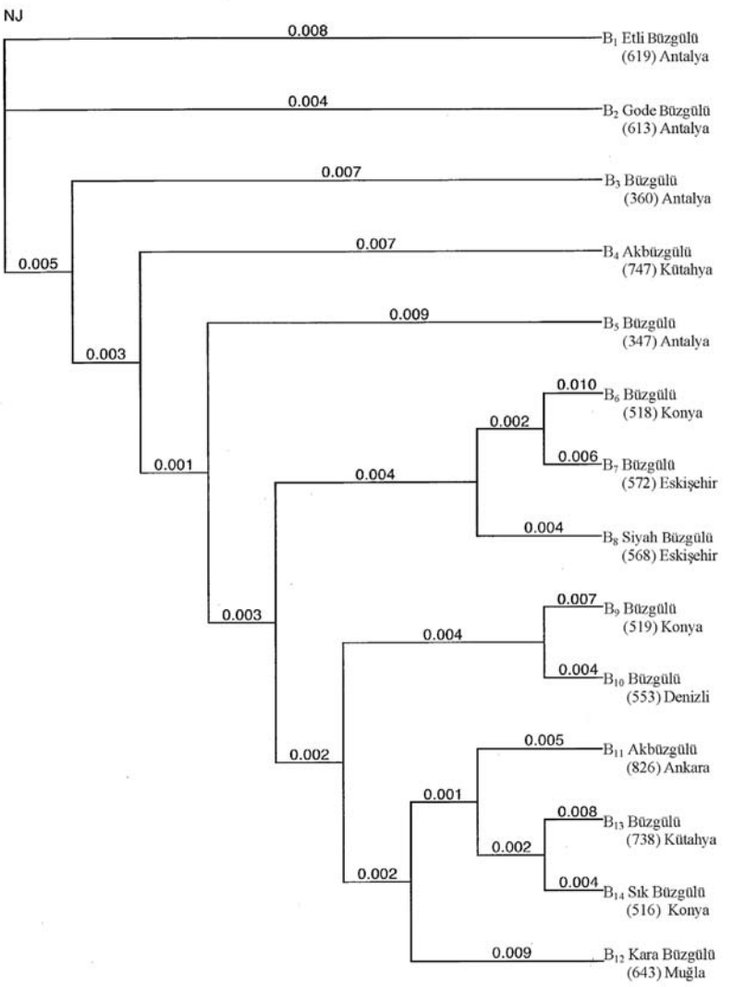

Fig. 7. Dendrogram of Büzgülü grape cultivar genotypes based on RAPD polymorphism. Neighbor-joining cluster analysis.

ters. In the first main group are 6 varieties, 4 of which are from Antalya province (South Anatolia). The second main group contains 8 varieties, 6 of which are located in the middle Anatolian region of Turkey. Two samples from Antalya province (no. 619, Etli Büzgülü; no. 613, Gode Büzgülü) showed one of the highest similarity ratios (0.867) and formed a group under the first branch. The 8th sample (no. 568, Siyah Büzgülü) and 7th sample (no. 572, Büzgülü) from Eskisehir province (Middle Anatolia) also showed high similarity (0.847) and formed a group within the second branch. In most cases the higher similarity ratios among geographic groups suggest genomic relatedness, perhaps the result of having common ancestors growing in the same region, but in some cases this interpretation does not seem valid: for example, the 6th sample (no. 518, Büzgülü, Konya prov.) which is included in the first group, and the 12th sample (no. 643, Kara Büzgülü, Mugla prov.) from the second group of the dendrogram, may have been introduced from outside.

Principal coordinate analysis was performed in order to determine the genetic relationships among the accessions with minimum distortion. The accessions were plotted on principal coordinates 1, 2 and 3, accounting for 23.2%, 15.6% and 10.6% of the variation, respectively, and together explaining 49.4% of the total variation (Fig. 5). The Büzgülü (no. 572) and Siyah Büzgülü (no. 568) cultivars in the upper left quarter of the plot are both from Eskisehir province. Three samples from Antalya province are grouped in the lower right quarter of the plot (Fig. 5). B4 (no. 747, Akbüzgülü, Kütahya prov.) is between the upper right and lower right quarters. B1 (no. 619, Etli Büzgülü, Antalya prov.) and B2 (no. 613, Gode Büzgülü, Antalya prov.) are in the lower right quarter; they also form a group in the dendrogram and resemble each other more than

the other accessions from Antalya province. These accessions are also quite similar to each other morphologically compared to the other accessions from Antalya province (Tab. 1). Sample B9 (no. 519, Büzgülü, Konya prov.), B10 (no. 553, Büzgülü, Denizli prov.), B11 (no. 826, Akbüzgülü, Ankara prov.), B12 (no. 643, Kara Büzgülü, Mugla prov.), B13 (no. 738, Büzgülü, Kütahya prov.) and B14 (no. 516, Sık Büzgülü, Konya prov.) formed a group in the lower left quarter. Konya, Ankara and Kütahya provinces are located in the middle Anatolian part of Turkey. The two accessions from Kütahya province (B13 and B4), showing relatively low similarity (74.4%), are also morphologically distant from each other.

A distance matrix was also made on the basis of Nei-Li (1979) similarity indexes (Fig. 6) and clustered by neighbor-joining analysis (Fig. 7). According to this matrix the genetic distances range up to 0.038, although most are under 0.03. In this cluster analysis, the Büzgülü accession from Antalya province (no. 619) and Kara Büzgülü (no. 643) from Mugla province differed the most, and were also relatively different according to UPGMA clustering. Büzgülü from Kütahya province (no. 737) and Sık Büzgülü from Konya province (no. 516) formed a sister group by neighbor-joining analysis, and were also paired by the UPGMA method. Sample B9 (no. 519, Büzgülü, Konya prov.) and B10 (no. 553, Büzgülü, Denizli prov.) also formed a sister group by neighbor-joining analysis. B6 (no. 518, Büzgülü, Konya prov.), which formed a single group by UPGMA clustering, was part of a group with B7 (no. 572, Büzgülü, Eskisehir prov.) and B8 (no. 568, Siyah Büzgülü, Eskisehir prov.) according to neighbor-joining clustering.

Although B1 (no. 619, Etli Büzgülü, Antalya prov.), B2 (no. 613, Gode Büzgülü, Antalya prov.), B3 (no. 360, Büzgülü, Antalya prov.), B4 (no. 747, Akbüzgülü, Kütahya prov.) and B5 (no. 347, Büzgülü, Antalya prov.) were nearest neighbors, they did not compose a group by neighbor-joining, but did by UPGMA analysis.

The results presented here are the first data on the Büzgülü cultivar complex, and are a contribution to the characterization of Turkish grape germplasm. The RAPD technique was shown to be adequate for discrimination of the 14 Büzgülü accessions, although the accuracy of these results should also be confirmed with studies using markers such as microsatellites and AFLP.

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