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Analysis of Genetic Diversity of Some *Vitis labrusca and Vitis spp.* with Molecular Markers

Bazı *Vitis labrusca ve Vitis* türlerinin Genetik Çeşitliliğinin Moleküler Markörlerle Analizi

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ANALYSIS OF GENETIC DIVERSITY OF SOME VITIS LABRUSCA AND VITIS SPP. WITH MOLECULAR MARKERS

ABSTRACT

Türkiye has a very suitable climate for viticulture, and different grape varieties have been grown in almost every region since ancient times. Although these grape varieties have different names, there are also grape varieties with the same genetic structure. In particular, in the Black Sea Region, *Vitis labrusca*, which is resistant to heavy rainfall and humidity, and hybrids of this species grow. However, it is known that different genotypes of this species are grown in the region. Genetic confusion exists in *Vitis labrusca* L. and its hybrids due to natural pollen transfer and crossing. This study evaluated the genetic similarities and differences among *Vitis* varieties and genotypes, mainly *Vitis labrusca* genotypes, with 18 different SSR primers. In the genetic relationship dendrogram, the varieties/genotypes showed similarity at rates ranging from 0.65% to 0.98%. The highest similarity rate between the varieties and genotypes used in the study was determined between the genotypes 5 '57 Gerze 04' and 6 '61 Of 04' with 92%. The results obtained have revealed different grape gene pools, especially in the Black Sea Region, and are expected to contribute to disease resistance breeding studies in the future significantly. Increasing consumer awareness of climate change will increase the interest in disease-resistant or tolerant grapes, such as *Vitis labrusca* species, in the coming years.

Keywords: Grape Cultivars/Genotypes, Ssr Markers, Similarity Index, Natural Cross, Breeding.

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BAZI VİTİS LABRUSCA VE VİTİS TÜRLERİNİN GENETİK ÇEŞİTLİLİĞİNİN MOLEKÜLER MARKÖRLERLE ANALİZİ

ÖZ

Türkiye bağcılık için oldukça uygun bir iklime sahip olup, eski çağlardan beri ülkenin hemen her bölgesinde farklı üzüm çeşitleri yetiştirilmektedir. Bu üzüm çeşitleri arasında farklı isimlere sahip olanlar bulunsa da aynı genetik yapıya sahip üzüm çeşitleri de bulunmaktadır. Özellikle Karadeniz Bölgesi'nde yoğun yağışlar ile neme dayanıklı *Vitis labrusca* türüne giren üzüm tip ve melezleri yetişmektedir. Ancak bölgede bu türün farklı genotiplerinin yetiştirildiğide bilinmektedir. *Vitis labrusca* L. ve melezlerinde doğal polen transferi ve melezlemeden dolayı genetik karışıklık söz konusudur. Bu çalışmada *Vitis labrusca* genotipleri başta olmak üzere *Vitis* çeşitleri ve genotipleri arasındaki genetik benzerlik ve farklılıklar 18

farklı SSR primeri ile değerlendirilmiştir. Genetik ilişki dendrogramında çeşitler/genotipler %0,65 ile %0,98 arasında değişen oranlarda benzerlik göstermiştir. Çalışmada kullanılan çeşit ve genotipler arasındaki en yüksek benzerlik oranı %92 ile 5 '57 Gerze 04' ve 6 '61 Of 04' genotipleri arasında tespit edilmiştir. Elde edilen sonuçların özellikle Karadeniz Bölgesi'nde farklı üzüm gen havuzu olduğunu ortaya çıkarmış olup, gelecekte hastalık direnci ıslahı çalışmalarına önemli katkı sağlaması beklenmektedir. İklim değişikliği ve tüketicilerde farkındalığının artması ile birlikte, önümüzdeki yıllarda *V. labrusca* türüne mensup üzümler gibi hastalığa dayanıklı veya tolerant olan üzümlere talebin artması beklenmektedir.

Anahtar Kelimeler: Üzüm Çeşitleri/Genotipleri, Ssr Markörler, Benzerlik İndeksi, Doğal Melezleme, Islah.

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1. INTRODUCTION

Vitis vinifera subsp. *vinifera* is the most cultivated vine species, especially in the temperate climate zone, and is economically very important (Li and Gschwend, 2023). It was domesticated from wild Vitis species in Eurasia in the past. (De Lorenzis, 2024; Dong et al., 2023; Zhou et al., 2017; Xiao et al., 2023). Due to the highly heterozygous nature of genotypes belonging to the *V. vinifera* species, cultivated grapevines are commonly propagated clonally so that they do not lose their desired fruit quality characteristics (Franks et al., 2002; Riaz et al., 2002; Vondras et al., 2019). More than seventy species of *Vitis* grow worldwide, most of which are native to North America and China but are also grown in different climates. Many of these species have adapted to different climate conditions (Ma et al., 2018; Walker et al., 2019).

Since most of the wild *Vitis* species are tolerant or resistant to different abiotic and biotic stress conditions, they are frequently preferred as genetic resources in grapevine breeding programs and studies on the development of new grape varieties (Atak, 2023; Reynolds and Reisch, 2015). Different varieties and genotypes of *Vitis labrusca*, which grows naturally, especially in the north and east of the United States, are easily distinguished from other species by their disease resistance, cold tolerance, and unique, intense foxy-flavored berries. Many new interspecific hybrid varieties have been developed by crossing *V. labrusca* and other wild grapevines, due to their resistance to different stress conditions, with *V. vinifera* varieties because of their superior berry quality characteristics in breeding programs (Wen et al.,2020).

First, ampelographic identification studies were carried out to reveal the grapevine genetic potential in Türkiye and to identify grape varieties suitable for dif-

ferent evaluation purposes from the existing population (Daler and Cangi, 2022; Hizarci et al., 2012; Karataş et al., 2007; Karataş et al., 2014; Yılmaz et al., 2020). Later, isoenzyme studies and, finally, DNA markers were used in identification studies due to their superiority over the ampelographic method (Ergül et al., 2011).

Different methods examine genetic variations in other plant species at the genome, gene, transcriptomic, proteomic, and metabolomic levels. The light of these studies and the results obtained enable the development of an ecoprotection strategy that includes maximum allelic variation for the relevant species and the determination of suitable functional alleles for special studies. Among the most critical molecular markers used in grapes are SSR(microsatellites) (Bowers et al., 1996; Grassi et al., 2003).

In Türkiye, different grape genotypes that have a unique foxy aroma, thick skins, seeds, and slip-skin that are easily separated from the flesh and show the characteristics of the *Vitis labrusca* L. species are grown mainly in the coastal areas of the Black Sea Region. These grape genotypes, generally called 'Isabella' or foxy black grapes, strawberry grapes, black grapes, or American grapes, are resistant to fungal diseases and can grow naturally in places with cool and humid climates (Cangi et al., 2006; Çelik et al., 2008; Köse, 2014).

No previous study has used molecular markers on such a large number of *Vitis labrusca* L. genotypes grown, especially in the Black Sea Region. In this study, the genetic similarities of a total of 71 genotypes collected both from the Black Sea region and from different origins and showing *V. labrusca* characteristics were compared with each other using 18 simple sequence repeat (SSR) loci.

2. MATERIAL AND METHOD

2.1. Materials

The study material consists of grape varieties and genotypes collected in the Yalova Atatürk Horticulture Central Research Institute collection vineyard, collected within the scope of TÜBİTAK Project Number 1130641. Some of these varieties and genotypes were collected from the Marmara and Black Sea Regions through selection within the project framework above. Some materials were obtained from the collection vineyard of the Horticulture Department of the Faculty of Agriculture of Ondokuz Mayıs University. Other materials were brought by cargo from the USDA Grape Genetic Collection located in the Geneva Campus of Cornell University. Information on the 71 varieties and genotypes characterized with the help of SSR markers in the project is given in Table 1. A total of 69 *V. labrusca*/interspecific hybrids and 2 *V. vinifera* varieties (Red Globe and Italy) were used in the study.

2.2. Methods

2.2.1. DNA Isolation

Leaf samples of the genotypes were taken into 2.0 mL Eppendorf tubes and stored at -80 ˚C until the samples were analysed. For DNA isolation, leaf tissues were first thoroughly homogenized. The samples were shredded using a Qiagen brand Tissuelyser-II shredder until they became powder. DNA isolation of the powdered samples was carried out with the Qiagen brand DNeasy Plant mini kit by the kit protocol. The isolated DNAs were stored at -20 °C until use.

2.2.2. SSR and Genetic Analysis

A total of 18 primer pairs were used in PCR reactions, including the VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79 microsatellite loci, which are included in the grapevine variety identification list and are now accepted as the minimum standard set all over the world. Base sequences and TM temperatures of the SSR primers are given in Table 2.

Table 1. Species and origins of the grape varieties and genotypes. (SOMU: Samsun Ondokuz Mayıs University; TVRI: Tekirdağ Viticulture Research Institute; YAHCRI: Yalova Atatürk Horticulture Central Research Institute; USDA: Geneva USDA Grape Repository; CFDT: Collected from Different part of Türkiye; IGBG: Institute for Grapevine Breeding Geilweilerhof

Variety/Genotype Name or Code	Speices	Origin	Variety/Genotype Name or Code	Speices	Origin
57 Ayancık 01*	*V.labrusca*	SOMU	Özer Karası	Interspecies	TVRI
Batum 4	Interspecies	SOMU	FX1-10	Interspecies	TVRI
57 Erfelek 03	*V.labrusca*	SOMU	55 Merkez 09	*V.labrusca*	SOMU
57 Gerze 04	*V.labrusca*	SOMU	57 Gerze 01	*V.labrusca*	SOMU
61 Of 04	*V.labrusca*	SOMU	57 Merkez 07	*V.labrusca*	SOMU
61 Sürmene 01	*V.labrusca*	SOMU	Bluebell	Interspecies	SOMU
61 Sürmene 02	*V.labrusca*	SOMU	Buffalo	Interspecies	SOMU
57 Merkez 02	*V.labrusca*	SOMU	Vanessa Seedless	Interspecies	SOMU
61 Yomra 04	*V.labrusca*	SOMU	Niagara	Interspecies	SOMU
53 Merkez 02	*V.labrusca*	SOMU	Ontario	Interspecies	SOMU
Rizessi	*V.labrusca*	SOMU	Steuben	Interspecies	SOMU
Rizpem	*V.labrusca*	SOMU	Kyoho	Interspecies	YAHCRI
Çeliksu	*V.labrusca*	SOMU	Rize geççi	Interspecies	YAHCRI

Rizellim	*V.labrusca*	SOMU	Marechall Foch	Interspecies	USDA
55 Merkez 06	*V.labrusca*	SOMU	Valiant	Interspecies	USDA
55 Merkez 12	*V.labrusca*	SOMU	Orion	Interspecies	IGBG
55 Merkez 11	*V.labrusca*	SOMU	Regent	Interspecies	IGBG
53 Güneysu 01	*V.labrusca*	SOMU	Kay Grey	Interspecies	YAHCRI
08 Arhavi 01	*V.labrusca*	SOMU	Muscat Bailey A	Interspecies	YAHCRI
Ülkemiz	*V.labrusca*	SOMU	Price	Interspecies	USDA
53 Pazar 02	*V.labrusca*	SOMU	Edelweiss	Interspecies	USDA
55 Çarşamba 01	*V.labrusca*	SOMU	Canadice	Interspecies	USDA
28 Tirebolu 02	*V.labrusca*	SOMU	Himrod	Interspecies	SOMU
28 Merkez 01	*V.labrusca*	SOMU	Lakemont	Interspecies	SOMU
Concord	*V.labrusca*	SOMU	Newyork muscat	Interspecies	USDA
Yerli İsabella	*V.labrusca*	TVRI	Seneca	Interspecies	USDA
Mercan	*V.labrusca*	TVRI	Sheridan	Interspecies	USDA
Favli	Interspecies	TVRI	Van Buren	Interspecies	USDA
Gürcü	*V.labrusca*	TVRI	Yates	Interspecies	USDA
S1 Isabella	*V.labrusca*	CFDT	Venus	Interspecies	SOMU
S2 Isabella	*V.labrusca*	CFDT	Glenora	Interspecies	USDA
S3 Isabella	*V.labrusca*	CFDT	Hendrickson seedless	Interspecies	USDA
Sunbelt	Interspecies	USDA	Concord seedless	Interspecies	USDA
Isabella (Yalova)	*V.labrusca*	YAHCRI	Italya	*V. vinifera*	YAHCRI
Köfteci üzümü	*V.labrusca*	YAHCRI	Red globe	*V. vinifera*	YAHCRI
FX1-1	Interspecies	TVRI			

Table 2. Base sequences and TM temperatures of the SSR primers.

No	Locus	Primer Sequence (5'3')	Tm (°C)
1	VVS1-F VVS1-R	ACAATTGGAAACCGCGTGGAG CTTCTCAATGATATCTAAAACCATG	53
2	VVS2-F VVS2-R	CAGCCCGTAAATGTATCCATC AAATTCAAAATTCTAATTCAACTGG	55
3	VVMD5-F VVMD5-R	CTAGAGCTACGCCAATCCAA TATACCAAAAATCATATTCCTAAA	55
4	VVMD7-F VVMD7-R	AGAGTTGCGGAGAACAGGAT CGAACCTTCACACGCTTGAT	55
5	VVMD21-F VVMD21-R	GGTTGTCTATGGAGTTGATGTTGC GCTTCAGTAAAAAGGGATTGCG	55

6	VVMD24-F VVMD24-R	GTGGATGATGGAGTAGTCACGC GATTTTAGGTTCATGTTGGTGAAGG	55
7	VVMD27-F VVMD27-R	GTACCAGATCTGAATACATCCGTAAGT ACGGGTATAGAGCAAACGGTGT	55
8	VVMD28-F VVMD28-R	AACAATTCAATGAAAAGAGAGAGAGAGA TCATCAATTTCGTATCTCTATTTGCTG	55
9	VVMD31-F VVMD31-R	CAGTGGTTTTTCTTAAAGTTTCAAGG CTCTGTGAAAGAGGAAGAGACGC	55
10	ZAG21-F ZAG21-R	TCATTCACTCACTGCATTCATCGGC GGGGCTACTCCAAAGTCAGTTCTTG	65
11	ZAG47-F ZAG47-R	GGTCTGAATACATCCGTAAGTATAT ACGGTGTGCTCTCATTGTCATTGAC	55
12	ZAG62-F ZAG62-R	GGTGAAATGGGCACCGAACACACGC CCATGTCTCTCCTCAGCTTCTCAGC	55
13	ZAG64-F ZAG64-R	TATGAAAGAAACCCAACGCGGCACG TGCAATGTGGTCAGCCTTTGATGGG	55
14	VRZAG79-F VRZAG79-R	AGATTGTGGAGGAGGGAACAAACCG TGCCCCCATTTTCAAACTCCCTTCC	66
15	ZAG83-F ZAG83-R	GGCGGAGGCGGTAGATGAGAGGGCG ACGCAACGGCTAGTAAATACAACGG	66
16	ZAG112-F ZAG112-R	CGTTTAAAGCCAGCTGAATCTTGGG TGGCTCCATACTGCTTCACGTAGGC	55
17	VMC2h4-F VMC2h4-R	ACCAGGTGTGCCTATAAGAATC TCTCTGGAACATCCAATCAAC	50
18	VMC2c3-F VMC2c3 –R	TGCAATCCCATTATTATCTCTT AATATTTGTAGAATGGTGCTTTT	48

Isolated DNA samples were amplified in the Bio-Rad T100 Thermal Cycler device and amplified bands were obtained. Amplification conditions for Polymerase Chain Reaction DNA: 20-100 ng, Primer pair: 20-50 pmol, dNTP: 200-500 mM, $MgCl_2$: 1.5-3.5 mM, DNA Polymerase: 0.5-1.5 Unit,10X Buffer: 2.5 µl Total volume: 25 µl. The PCR program was set as 3 min at 94 C, 1 min at 94 C, 1 min at 50–60 C depending on the binding degree of the primer, 2 min at 72 C, and 10 min at 72 C in the final stage. Additionally, steps 2, 3 and 4 were repeated a total of 35 times. After PCR products belonging to the loci were checked in 2% agarose gel, the numerical bath sizes of the amplified samples were determined by capillary electrophoresis.

Fragment analyses of the genotypes used were performed on the Bioptic Q sep 100 Genetic Analysis System. The scored band images were entered into this program and as a result, the genetic similarities of the varieties and types used in the study were revealed and their molecular identifications were made. The polymorphism information content (PIC) coefficient of each primer is PIC = 1-Σpi2 (pi frequency of alleles) Smith et al. (1997) method. PIC values were obtained for each of the 18 SSR primers used. The ratio of shared alleles was calculated by genetic uniqueness and these data were converted into a similarity matrix in Excel.

Dendrogram data based on the genetic similarities of the varieties and genotypes used in the study were used to create an Unweighted Pair Group of Arithmetic Average (UGCMA cluster) analysis and similarity index and Principle Component Analysis (PCA) tables using the NTSYSpc (Numerical Taxonomy and Multivation Analysis System) statistical program developed by Rolf (2000).

3. RESULTS AND DISCUSSION

In this study, the genetic similarities of 35 *V. labrusca*, 34 interspecific hybrids, and 2 *V. vinifera* varieties or genotypes were evaluated with the help of 18 different SSR markers. The results obtained, the amplified band sizes are given in Table 3, and the total number of polymorphic bands and average polymorphism coefficient are shown in Table 4. Additionally, frequency values at SSR loci are given in Figure 1, and the Genetic similarity dendrogram of 71 varieties and genotypes are given in Figure 2.

When we look at the band sizes obtained from a total of 18 SSR loci, it is seen that the widest band size range is obtained from the VrZAG64 primer with a range of 121-255 bp. It is followed by the VVS2 primer with a band size range of 112-225. The narrowest band size range was obtained from the VrZAG79 primer with a band size range of 246-271 (Table 3).

A total of 201 alleles were obtained in studies conducted with 18 SSR primers. While the highest number of alleles was obtained from the VrZAG62 primer with 15 alleles, the lowest number of alleles was obtained from the VVMD7 primer with 8 alleles. VrZAG79, VVS1, VVMD28 and VrZAG21 l primers were determined to be the other highest allele yielding loci with 13 alleles each. The SSR primers with a high rate of polymorphism (difference) and the highest PIC values were VVMD31 (0.98), VrZAG47 (0.97) and VrZAG21 (0.96), respectively. The lowest polymorphism (difference) was obtained from VVMD7 (0.79) and VVMD24 (0.89) primers, respectively (Table 4).

Table 3. Amplified band size of the SSR primers

SSR Primer	Minimum and Maximum Band size (bp)
VrZAG62	185-226
VrZAG79	246-271
VVMD24	210-255
VVMD7	250-320
VVMD27	187-223
VVMD5	239-278
VVS2	112-225
VVMD21	225-294
VVS1	160-245
VVMD28	217-275
VVIB01	295-364
VVMD31	189-220
VrZAG21	202-274
VrZAG47	167-202
VrZAG64	121-255
VrZAG83	167-256
VVIH54	240-278
VrZAG112	152-212

Table 4. Total Number of Polymorphic Bands and Average Polymorphism Coefficients of SSR Primers

SSR Primer	Total Polymorphic Band Number	Average Polymorphism Coefficient
VrZAG62	15	0.93
VrZAG79	13	0.90
VVMD24	12	0.89
VVMD7	8	0.79
VVMD27	12	0.96
VVMD5	9	0.95
VVS2	11	0.97
VVMD21	12	0.95
VVS1	13	0.92
VVMD28	13	0.96
VVIB01	12	0.95
VVMD31	10	0.98
VrZAG21	13	0.96
VrZAG47	11	0.97
VrZAG64	11	0.95
VrZAG83	12	0.94
VVIH54	12	0.93
VrZAG112	11	0.95

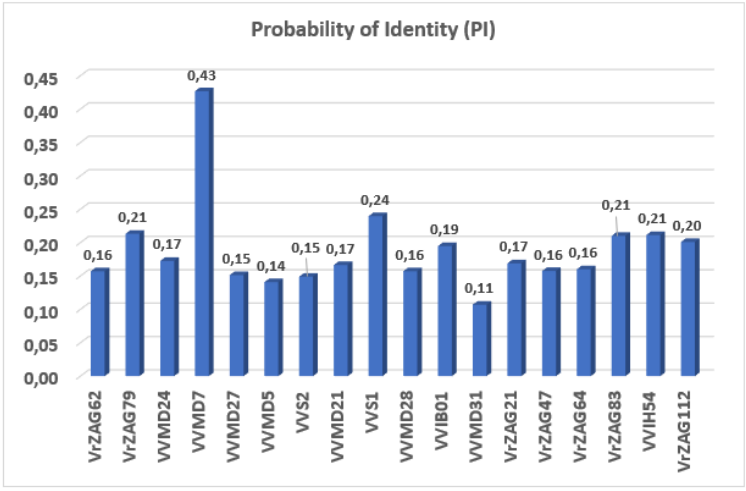

Figure 1. Frequency values at SSR loci

In terms of probability of identification (PI: Probability of Identity), all primers used were from Sefc et al. (2000) gave results above the 0.05 threshold value. The highest value was obtained from primers VVMD7 (0.43), VVS1 (0.24), VrZAG83 (0.21), VrZAG79 (0.21) and VVIH54 (0.21), respectively (Figure 1).

When the genetic relationship dendrogram is examined, it shows that the varieties/genotypes are not the same and show similarity at rates ranging from 0.65% to 0.98%. Among the varieties and genotypes used in the study, the highest similarity rate, 92%, was determined between genotypes 5 '57 Gerze 04' and 6 '61 Of 04'. These were followed by genotypes 43, '55 Merkez 09', and 44, '57 Gerze 01', with a similarity rate of 91%. In the dendrogram, varieties/genotypes belonging to the *V. labrusca* species are shown with a blue line on the right side, while interspecific hybrids are shown with a green line and *V. vinifera* ones with a red line. When the genetic relationship dendrogram is examined, it has been determined that although the varieties/genotypes of the same species are sometimes in the same branch, some of them are in the same branch with other species. This shows that there may be traces of parents from different species in the origin of the varieties/genotypes, or in other words, there is a possibility of cross-pollination (Figure 2).

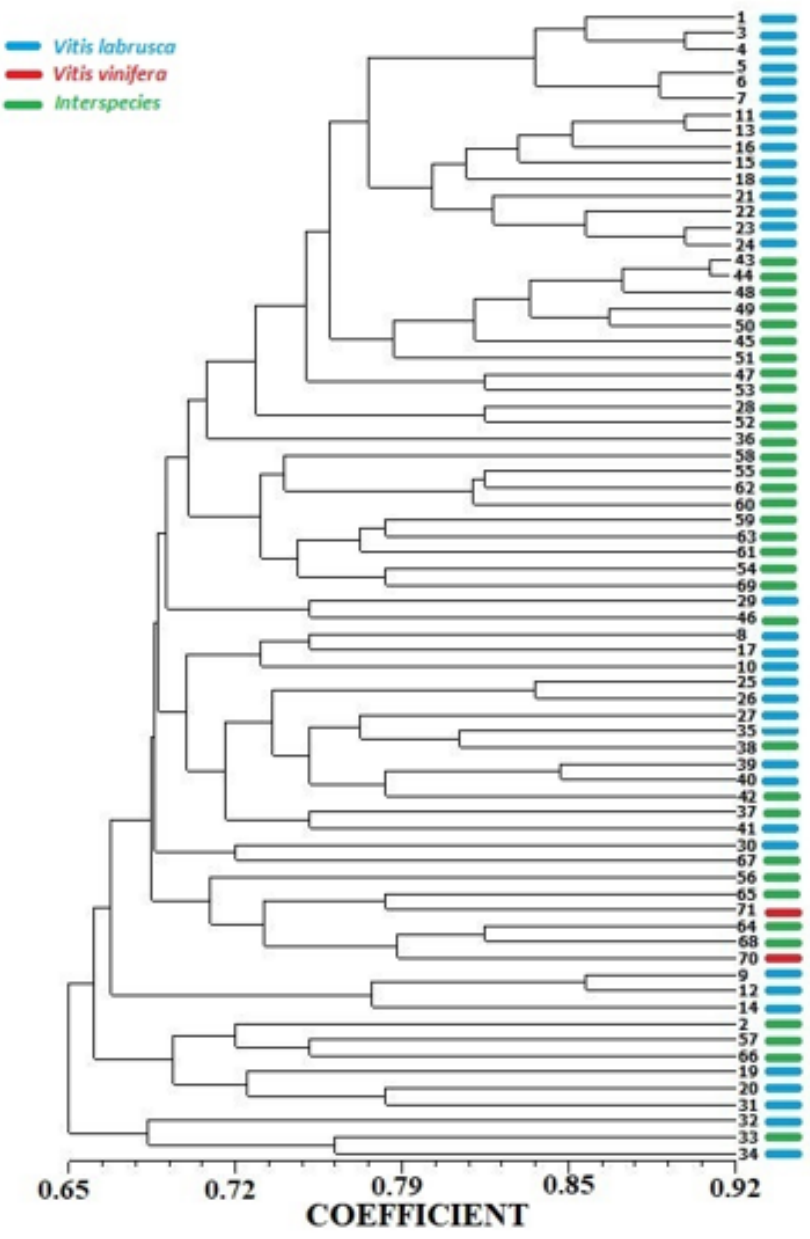

Figure 2. Genetic similarity dendrogram of 71 varieties and genotypes

Many researchers have used SSR or microsatellite markers successfully, as in our study, to determine the similarities of grape varieties and genotypes to each other because they are highly polymorphic, show a co-dominant mode of inheritance, and allow simple data interpretation. (Bowers et al., 1999; İkten et al., 2024; Margaryan et al., 2023).

In research on the identification of international grapevine genetic resources, as in this study; Using SSR markers, different levels of homonymous (genotypes called by the same name but genetically distinct from each other) and synonymous (genotypes called by different names but genetically identical) genotypes are frequently encountered (Ibáñez et al., 2003; Martínez et al., 2006; This et al., 2004). Similarly, studies conducted with Turkish grape varieties have revealed the prevalence of homonymous and synonymous groups (Karataş et al., 2014; Şelli et al., 2007; Vouillamoz et al., 2006).

This study revealed that many grape genotypes grown in the Black Sea Region with the same name actually show genetic differences. In this study, the allele numbers obtained with a total of 18 primers are largely similar to studies conducted by different researchers in terms of values such as allele sizes and polymorphism rates (Atak et al., 2012; Dangi et al., 2001; Ibanez et al., 2003; Şelli et al., 2007; Vouillamoz et al., 2006). Additionally, this study contains similar results to other studies reporting that the VVS2 locus has 10 or more alleles (Fatahi et al., 2003; Sefc et al., 2000; Vouillamoz et al., 2006).

In their study, Tahmaz Kahraman et al. (2022) examined the phenolic compound and antioxidant capacity contents of grape varieties/genotypes similar to this study. According to their results, they confirmed their differences by obtaining different results for all genotypes and varieties. This reveals the differences between similar grape varieties grown with the same names, in line with the results obtained in our study.

Also, Çelik and Köse (2018) ampelographically identified 109 grape varieties/genotypes collected from the Black Sea Region, which they thought belonged to the *Vitis labrusca* species. According to the ampelographic identification dendrogram formed from their study, they reported that the Central and Eastern Black Sea Regions have two main groups. They also noted that all genotypes were similar to each other at different rates. The same genotypes from the Black Sea Region were studied at the molecular level in our study. According to the results, some genotypes were in the same group, while some were in different groups. This situation shows that some genotypes may be varieties that emerged due to pollination with different varieties at flowering time.

4. CONCLUSION

Grapevine genetic resources are protected in many countries for use in later years. The varieties and genotypes of these resources found in different regions within the country should be screened regularly, and those that differ should be included in the collections. Especially *Vitis labrusca* species and the varieties/genotypes obtained by crossing these species with different species are very valuable due to their disease resistance and high phenol content. In the coming years, especially with the effects of climate change, it is expected that varieties or interspecific hybrids of this species will be used more both in breeding studies and production. For this reason, it is important that all grape varieties of this species within the country are protected in at least one location.

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Conflict of Interest

The authors declare that they have no competing, actual, potential or perceived conflicts of interest.

Author contributions

Y.D. and A.A. contributed equally to the manuscript. The authors read and approved the final version of the manuscript. The authors verify that the text, figures, and tables are original and that they have not been published before.

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