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Genetic diversity and population structure analysis of grapevine germplasm of the Aegean region (Türkiye) by SSR markers

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Abstract: The Aegean region makes a prominent contribution to Turkish viticulture. The region, which includes important grape culture centers such as Manisa, Denizli, and İzmir, has extremely favorable conditions for the cultivation of seedless grapes as well as table and wine grape varieties. In this region, which possesses rich grapevine genetic diversity together with its area and production potential, it is seen that there is variety confusion caused by different nomenclature and intravariety variations. In addition, some old grape varieties that are not used in cultivation are at risk of disappearing. In order to address these problems, the viticulture potentials of Manisa, Muğla, İzmir, Kütahya, Aydın, Denizli, Uşak, and Afyon provinces as populations were evaluated using simple sequence repeat (SSR) markers. In and research conducted using 15 SSR markers on 151 grape varieties including 149 native and 2 reference varieties, 3 similar (identical), 7 synonymous, and 13 homonymous cases were determined. The highest numbers of alleles were seen at the VVMD7 (14 alleles) and VVS2 (13 alleles) loci. The highest genetic similarity ratio among the genotypes was found to be 0.96 (96%) and the lowest value was 0.13 (13%). In population structure analysis, it was observed that there was significant gene flow among the Aegean region populations, while in Bayesian analysis, it was determined that the Aegean region populations (except the Aydın and İzmir populations) were highly admixed. The findings obtained in this research, which was carried out for the characterization of grape genetic resources at the SSR level on a regional scale, shed light for other genetic characterization studies. In addition, the results of this research may contribute to other viticulture activities (breeding, reproduction, variety registration, etc.) to be carried out in the region.

Key words: *Vitis vinifera* L., grapevine germplasm, simple sequence repeats, population structure, Aegean region, Türkiye

1. Introduction

Grapes (*Vitis vinifera* L.) are one of the most economically valuable horticultural crops in the world. Although wild grapevines spread over Central and West Asia and Southern Europe during the Neolithic period, historical evidence and archaeological records suggest that primary domestication of the cultivated grape, *Vitis vinifera* L. subsp. *vinifera*, began 6000–8000 years ago in the Near East from its wild progenitor, *Vitis vinifera* L. subsp. *sylvestris* (McGovern, 2003; Atak and Kahraman, 2014; Riaz et al., 2018). Moreover, some researchers have shown evidence supporting the idea of the existence of secondary domestication events along the Mediterranean basin (Myles et al., 2011; Arroyo-Garcia et al., 2013).

Türkiye, which has an important place in world agriculture with its ecological conditions in terms of the growth of different plant species, possesses very rich genetic potential in terms of plant genetic resource diversity. In this direction, studies on creating collections and the selection of individual plants with superior characteristics have been carried out for many years worldwide as well as in Türkiye to characterize current genetic resources. Many types of collections and partial selection studies were completed as a result of such studies, most of which were carried out within agricultural research institutes and some in faculties of agriculture (e.g., Tangolar et al., 2009; Ergül et al., 2011; Akçay et al., 2014; Eyduran et al., 2015; Ergül et al., 2021).

When the aforementioned studies on the identification and protection of plant gene resources are examined,

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definitions based on the morphological characters of vegetative and reproductive structures (ampelographic, etc.) are encountered. However, as it is known, the inadequacy of such definitions (affected by environmental conditions, investigators, scarcity of the criteria used in the definitions, etc.) has led to the gradual abandonment of these methods and the use of DNA identifiers, which yield definite results in research (Emanuelli et al., 2013; Fraga et al., 2016; Guan et al., 2019; Santos et al., 2020; Margaryan et al., 2021). DNA markers, which reveal differences at DNA level with varying sensitivity, have been used primarily with amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR)-microsatellite techniques in recent years (Emanuelli et al., 2013; De Lorenzis et al., 2015; Drabek et al., 2016; De Lorenzis et al., 2019; De Oliveira et al., 2020; Mihaljevic et al., 2020; Crespan et al., 2021). Among these DNA markers, SSR markers are short repeat sequences of 2–6 bases and show specificity to higher organisms' (eukaryotic) genomes (Taheri and Darzi, 2020). SSR markers relating allelic variations among genotypes with mutations in replication slippage (Schlotterer and Tautz, 1992) are extensively used in grape genetic characterization studies (Costantini et al., 2005; Şelli et al., 2007; Tangolar et al., 2009; Yılmaz et al., 2020) with the advantages of codominance, high polymorphism (Cipriani et al., 2010; Emanuelli et al., 2013), reproducibility, and hypervariability (Cretazzo et al., 2022) compared to other DNA-based markers.

Türkiye possesses vineyard area of approximately 390,221 ha and many provinces have at least 1% vineyard area within their agricultural product patterns. Almost one-third of the country's vineyard areas are located in the Aegean region, and approximately 50% of table and dried grape production is provided by this region (Turkish Statistical Institute, 2023).

Vineyards established with advanced systems are common in the region. In addition, irrigation, which is one of the most important conditions of quality and high yield, is carried out at optimum ratios. On the basis of variety, the cultivation of seedless grapes (Yuvarlak Çekirdeksiz and Sultani Çekirdeksiz) accounts for an important proportion in the Aegean region. Varieties such as Amasya Beyazı, Bozcaada Çavuşu, Buca Razakısı, Işıklı, Italia, İpek, Kozak Beyazı, Yalova İncisi, Alphonse Lavallee, Burdur Dimriti, Cardinal, Kozak Siyahı, Pembe Gemre, and Siyah Germe as table grape varieties and Bornova Misketi, Clairette, Adakarası, Cinsaut, Gamay, Karalahana, and Karasakız as wine grape varieties are recommended to be cultivated (Yüksel, 2008; Atak and Kahraman, 2014). Considering the area and production potential of the region, it is obvious that the region has very rich diversity. However, from among the gene resources in the region, the same varieties are grown with different names or some different varieties

carry the same name, and there is some confusion due to variations within cultivars. In addition, the prominence of some grape varieties in cultivation tends to increase the risk of the disappearance of varieties that have adapted to the conditions of the region and have been grown for hundreds of years.

In this study, genetic characterization of a total of 151 cultivars was performed using 15 SSR markers. The cultivars included 149 grape varieties transferred to the National Collection Vineyard (Tekirdağ Viticulture Research Institute, Tekirdağ, Türkiye) from important producing provinces including İzmir, Kütahya, Aydın, Denizli, Uşak, Afyon, and especially Manisa (located in the Aegean region and providing approximately 80% of Türkiye's dried grape production), and also 2 reference varieties (Cabernet Sauvignon and Merlot). Identical (same names and same SSR profiles), synonymous (identical genotypes called by different names), and homonymous (different genotypes called by the same name) cultivars were determined and genetic relationships among and within the populations were revealed.

Additionally, possible genetic relationships among 8 different populations of the Aegean region were determined by different structural analyses including factorial correspondence analysis (FCA), Bayesian analysis of population structure (BAPS), and unknown reconstructed panmictic populations (RPPs).

2. Materials and methods

2.1. Plant material

This study was conducted in 2010. Shoot tips and young leaves of 151 cultivars (149 from the Aegean region and 2 references) used as plant material in the research were obtained from the Tekirdağ Viticulture Research Institute National Collection Vineyard, Tekirdağ, Türkiye. Genotype numbers on the basis of provinces included 22 from Manisa, 32 from Muğla, 14 from İzmir, 11 from Kütahya, 5 from Aydın, 29 from Denizli, 17 from Uşak, and 19 from Afyon as Turkish varieties (Figure 1), while the reference varieties, Cabernet Sauvignon and Merlot, were French varieties. Cultivar numbers, cultivar names, and the provinces of the grapevine cultivars and references used in this study are given in Supplementary Table 1.

2.2. DNA isolation

DNA isolation was performed according to Lefort et al. (1998). Determination of DNA concentration and quality was performed according to Akçay et al. (2014) and Burak et al. (2014) using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and agarose (1%, w/v) gel electrophoresis. Isolated DNA samples were stored at –20 °C until polymerase chain reaction (PCR) applications were performed.

Table 1. Genetic parameters of Aegean region grape cultivars: allele number (*N*), expected heterozygosity (*He*), observed heterozygosity (*Ho*), and identity probability value (*PI*).

SSR Loci	<i>N</i>	<i>He</i>	<i>Ho</i>	<i>PI</i>
VrZAG79	9	0.817	0.841	0.107
VVIH54	12	0.766	0.549	0.133
VVMD24	6	0.552	0.556	0.288
VVMD7	14	0.793	0.867	0.128
VVMD28	12	0.798	0.774	0.125
VVMD27	9	0.757	0.761	0.155
VMC2H4	10	0.815	0.867	0.091
VVIB01	7	0.600	0.655	0.379
VrZAG83	6	0.623	0.728	0.368
VVS2	13	0.840	0.887	0.077
VVMD5	8	0.813	0.741	0.107
VrZAG62	11	0.716	0.755	0.183
VVMD31	9	0.750	0.768	0.167
VMC2C3	10	0.724	0.662	0.197
VVS1	5	0.503	0.549	0.447
Total	141	10.874	10.966	-
Average	9.4	0.725	0.731	-

2.3. SSR PCR and capillary electrophoresis

A total of 15 SSR primers including VVS2 (Thomas and Scott, 1993), VVMD5, VVMD7, VVMD24, VVMD27, VVMD28, VVMD31 (Bowers et al., 1999), VrZAG62, VrZAG79, and VrZAG83 (Sefc et al., 2000) and also VMC2H4, VMC2C3 (Goto-Yamamoto et al., 2006), VVIB01, VVIH54 (Merdinoglu et al., 2000), and VVS1 (Thomas and Scott, 1993) as microsatellite loci were used for genetic analysis. Information on the 15 SSR loci used (locus name, primer sequence, expected range (bp), chromosome location (Chr.), and references) is given in Supplementary Table 2. PCR optimization reactions were performed as indicated by Yilmaz et al. (2020), with (in a total of 10 µL of PCR mix) 15–200 ng of DNA, 5 pmol forward primer, 5 pmol reverse primer, 0.5 mM total dNTP, 0.5 U of Go Taq DNA Polymerase (containing 1.5 mM MgCl₂; Promega, Madison, WI, USA), and 1 µL of buffer (10X). In the PCR procedures, fluorescent-labeled D4 (blue), D3 (green), and D2 (black) forward primers belonging to each SSR locus were used. The presence of possible contamination in the PCR procedures was checked using a negative control (distilled water instead of DNA). Samples for DNA amplification were subjected to PCR for 3 min at 94 °C, 1 min at 94 °C, 1 min at 48–66 °C depending on the degree of primer binding (annealing temperature), and 2 min at 72 °C and then kept at 72 °C for 10 min during the last cycle with a total of 35 cycles.

The products of the SSR loci for which the PCR steps were completed were subjected to electrophoresis on a 2% agarose gel and amplification control with a 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was performed. PCR products were stored at –20 °C until the capillary electrophoresis step.

After diluting the PCR products with sample loading solution in appropriate proportions (20 µL), Genomelab DNA Standard Kit 400 (0.4 µL; Beckman Coulter, Fullerton, CA, USA) was added to the mixture and electrophoresis was performed with the CEQ 8800XL capillary DNA analysis system (Beckman Coulter). Peak sizes (bp) were determined using the fragment analysis software of the system. The Cabernet Sauvignon and Merlot cultivars were used as references. The reactions were repeated at least twice to ensure the accuracy of the data. At the end of the capillary electrophoresis, PCR fragments were visualized as heterozygous or homozygous considering the types and colors of each SSR locus peak.

2.4. Genetic analyses

2.4.1. SSR analyses

In this study, allele number (*N*), allele frequency, expected (*He*) and observed (*Ho*) heterozygosity, and detection probability (*PI*, probability of identity) values were determined as genetic parameters using the IDENTITY 1.0 program (Wagner and Sefc, 1999). The IDENTITY software program was used to detect the same genotypes.

Table 2. Identical, synonymous, and homonymous cultivars identified using SSR analysis for the Aegean region.

No	Identical (cultivar name / (cultivar number), province)	Synonymous (cultivar name / (cultivar number), province)	Homonymous (cultivar name / (cultivar number), province)
1	Morrazdağı (114), Uşak - Morrazdağı (115), Uşak - Morrazdağı (122), Uşak	Kara Büzgülü (52), Muğla - Büzgülü (73), Kütahya - Siyah Parmak (143), Afyon	Tek Çekirdekli (2), Manisa - Tek Çekirdekli (74), Kütahya
2	Siyah Misket (20), Manisa - Siyah Misket (21), Manisa	Aydın Üzüümü (118), Uşak - Hacı Eyüp (120), Uşak	Bulama (4), Manisa - Bulama (14), Manisa
3	Yediveren (65), İzmir - Yediveren (102), Denizli	Siyah Dilmit (47), Muğla - Yerli Kara (49), Muğla - Çalkarası (106), Denizli - Eski Kara (109), Denizli - Keçi Memesi (148), Afyon - Söbü Dimrit (149), Afyon	Beyaz Şam (5), Manisa - Beyaz Şam (31), Muğla
4		Siyah Misket (22), Manisa - Sikkara (68), İzmir -	Siyah Gemre (15), Manisa - Siyah Gemre (16), Manisa
5		İstanbul Dimliti (45), Muğla - Kayasar Dimriti (46), Muğla - Veysioğlu (136), Afyon	Ekşi Üzüm (28), Muğla - Ekşi Üzüm (61), İzmir
6		Misket (54), Muğla - Ziraat Üzüümü (98), Denizli	Tavşan Böbreği (32), Muğla - Tavşan Böbreği (94), Denizli
7		Beyaz Misket (19), Manisa - Kadın Parmağı (53), Muğla - Tek Çekirdeksiz (93), Denizli	Sıksarı (34), Muğla - Sıksarı (57), İzmir
8			Paşalar (69), Kütahya - Paşalar (72), Kütahya
9			Nuribey Üzüümü (71), Kütahya - Nuribey Üzüümü (77), Kütahya
10			Devegözü (110), Denizli - Devegözü (129), Uşak
11			Kadın Parmağı (111), Denizli - Kadın Parmağı (112), Denizli
12			Razakı (144), Afyon - Razakı (145), Afyon
13			Tilki Kuyruğu (146), Afyon - Tilki Kuyruğu (147), Afyon

The proportion of shared alleles was determined by the “ps (option 1 – (ps))” method described by Bowcock et al. (1994) and the similarity ratio index was calculated with the MICROSAT program (Minch et al., 1995).

Genetic similarities among the total 151 studied genotypes were determined with the NTSYS program (version 2.02g, Exeter Software, Setauket, NY, USA) using the unweighted pair-group method using arithmetic means (UPGMA) (Rohlf, 1988).

2.4.2. Population genetic analyses

Population genetic parameters of the grape cultivar groups were estimated using Arlequin software version 5.3 according to Excoffier and Lischer (2010). The genetic relationship dendrogram of the cultivars was created on the basis of the genetic distance of Nei using the NTSYS-pc analysis program (Rohlf, 2004). FCA was performed to find the presence of any population structure among the 8 grape populations using Genetix 4.05 (Belkhir et al.,

1996–1998). Gene flows (*nm*) among populations were estimated with Genetix 4.05 and genotypes were analyzed using the STRUCTURE 2.3 program. For each run, 100,000 replicates of burn-in were conducted, followed by an additional 100,000 Markov chain Monte Carlo (MCMC) replicates of data collection. A linkage model based on known distances among microsatellite loci based on a model of correlated allele frequencies was used and data were analyzed with STRUCTURE 2.3. Bayesian population genetic analysis was also applied using BAPS software to visualize population structures and admixtures (Corander et al., 2008).

We also analyzed population structure with STRUCTURE software to estimate the possible RPPs. The grape populations were analyzed according to Pereira-Lorenzo et al. (2018) based on population structure using STRUCTURE software (Pritchard et al., 2000) with the same computing parameters except that the K level was

calculated for $K = 2-9$ unknown RPPs with 25 replicates. We also used STRUCTURE-HARVESTER (Earl and von Holdt, 2012) to estimate the best K value supported by the current data (Evanno et al., 2005). Similar to Ergül et al. (2021), the number of accessions strongly assigned to each RPP was determined based on qI probabilities (probability of membership) of more than 80%.

3. Results

3.1. SSR analysis

Genetic characterization of a total of 151 cultivars, including 149 grape cultivars from Manisa, Muğla, İzmir, Kütahya, Aydın, Denizli, Uşak, and Afyon provinces in the Aegean region and Cabernet Sauvignon and Merlot as reference varieties, was performed using 15 SSR markers. A total of 141 alleles were detected. The average number of alleles for each SSR locus was 9.4. The lowest number of alleles (N) was 5, observed in VVS1. In contrast, the highest number of alleles (N) was 14, observed in the VVMD7 locus. This was followed by the VVS2 locus with 13 alleles. VVS2 and VMC2H4, with the lowest PI values of 0.077 and 0.091, respectively, were chosen as the loci with the highest discrimination power (Table 1). Considering the He and Ho values of each SSR locus, He was found to be slightly higher than Ho in the VVIH54, VVMD28, VVMD5, and VMC2C3 loci, while Ho was found to be high in the VrZAG79, VVMD24, VVMD7, VVMD27, VMC2H4, VVIB01, VrZAG83, VVS2, VrZAG62, VVMD31, and VVS1 loci. Accordingly, the highest He value was determined for VVS2 (0.840) and the highest Ho values were detected for VVS2 (0.887) and also the VMC2H4 and VVMD7 (0.867) loci, respectively. He and Ho values were found to be close to each other in all

SSR loci except VVIH54. The average values of He and Ho were 0.725 and 0.731, respectively (Table 1).

3.1.1. Genetic relations among grape cultivars

A dendrogram based on genetic similarity values is given in Supplementary Figure 1. Cultivars with similar genetic origins (3 different alleles, 90% similarity) and cultivars with clonal similarity (1 or 2 different alleles, 93%–96% similarity) are presented using genetic similarity values in Supplementary Table 3.

In this study, 3 identical (same names and same SSR profiles), 7 synonymous (identical genotypes called by different names), and 13 homonymous (different genotypes called by the same name) cases were determined (Table 2). It was to be expected that the genotypes of cultivar numbers 114, 115, and 122 of Morrazdağı, which are called “identical genotypes,” gave the same allele size. The same situation was found for the genotypes of cultivar numbers 20 and 21 of Siyah Misket and also the genotypes of cultivar numbers 65 and 102 of Yediveren as identical accessions. Considering examples of the “synonymous” varieties, Aydın Üzüümü (cultivar number 118) and Hacı Eyüp (cultivar number 120) from Uşak proved to be synonymous. Similarly, the Siyah Dilmit (cultivar number 47) and Yerli Kara (cultivar number 49) genotypes from Muğla and the Çalkarası (cultivar number 106) and Eski Kara (cultivar number 109) genotypes from Denizli were almost the same as synonymous accessions. Among the homonymous accessions, particularly those such as Bulama (cultivar numbers 4 and 14), Siyah Gemre (cultivar numbers 15 and 16), Kadın Parmağı (cultivar numbers 111 and 112), and Tilki Kuyruğu (cultivar numbers 146 and 147), it was observed that they belonged to the same provinces.



Figure 1. Ecogeographical distribution of Aegean region grape cultivars.

Table 3. Expected and observed heterozygosity, polymorphic loci at both 95% and 99% probability levels, and mean number of alleles per locus in 8 grape populations.

Population	Heterozygosity		Polymorphic locus		Mean # of Alleles / locus
	H _{exp}	H _{obs}	P (0.95)	P (1.00)	
Manisa	0.746 ±0.080	0.718±0.132	1.000	1.000	7.13
Muğla	0.709±0.108	0.721±0.110	1.000	1.000	6.73
İzmir	0.695±0.126	0.690±0.182	1.000	1.000	6.27
Kütahya	0.646±0.106	0.733±0.111	1.000	1.000	5.33
Aydın	0.647±0.098	0.693±0.183	1.000	1.000	4.07
Denizli	0.696±0.125	0.726±0.160	1.000	1.000	6.80
Uşak	0.681±0.123	0.733±0.186	1.000	1.000	5.87
Afyon	0.710±0.098	0.765±0.151	1.000	1.000	6.60
References	0.525±0.143	0.733±0.320	1.000	1.000	2.60

3.2. Genetic structure analysis among the grape cultivars' populations

Table 3 summarizes the genetic parameters estimated for the 8 grape populations and references. Expected and observed heterozygosity, polymorphic loci for 95% and 99%, and mean number of alleles per locus are given for each population. Accordingly, the mean numbers of alleles were determined to range between 2.60 (references) and 7.13 (Manisa population), while the expected and observed heterozygosity values were found to be close to each other in all populations except the references.

Genetic differentiation (*Fst*) values and their significance levels and *Nm* values are given in Table 4. *Fst* values among the populations were moderate, and the Afyon population showed significant genetic differentiation, particularly compared to Manisa (0.013, $p < 0.01$), Muğla (0.019, $p < 0.01$), İzmir (0.044, $p < 0.001$), and Aydın (0.023, $p < 0.05$). Table 4 also indicates that the İzmir population showed significant genetic differentiation compared to Kütahya (0.023, $p < 0.05$), Denizli (0.023, $p < 0.01$), and Uşak (0.017, $p < 0.05$). The *Fst* values explain the large values of *Nm* among the populations.

It was observed that *Nm* values among all populations were greater than 1 and a large amount of gene flow occurred among the populations. The highest *Nm* value (93.00) was observed between the İzmir and Manisa populations, while the lowest *Nm* value (5.33) was observed between the Afyon and İzmir populations (Table 4).

Genetic distance values are given in Table 5. The reference populations gave high genetic distance values with the 8 Aegean populations, and the highest genetic distance values among the Aegean populations were observed between the Afyon and Aydın (0.213) and Afyon and İzmir (0.209) populations, respectively. The lowest genetic distance values were observed between the

Muğla and Manisa (0.049) and Denizli and Muğla (0.052) populations, respectively. Genetic distance analysis results revealed high genetic diversity among the grape accessions of different populations.

Linkage disequilibrium (LD) comparisons were made based on 15 loci and a total of 105 allelic comparisons. Hardy-Weinberg equilibrium (HWE) was also checked for 15 loci used for evaluating the genetic variation of grape populations in the Aegean region of Türkiye (Supplementary Table 4). Several significant ($p < 0.05$) LDs were detected among the allele pairs of different loci. Different populations displayed various numbers of allele pairs having significant numbers of LDs. The highest LD was observed in the Denizli (LD: 57) and Muğla (LD: 54) populations and the lowest in the Aydın (LD: 8) population and the references (LD: 1) (Supplementary Table 4). All loci were polymorphic and the highest HWE was observed in the Aydın (HWE: 15) and reference (HWE: 15) populations and the lowest in the Manisa (HWE: 8) population (Supplementary Table 4).

FCA partially showed substructuring among the grape populations. Accordingly, three components explained 64.48% of the total variance, of which 34.09% was explained by axis 1, which clearly distinguishes the reference populations from the Aegean populations. Moreover, the genetic distance between the Afyon population and the İzmir and Aydın populations, as specified in Table 5, can be justified based on axis 2 and 3 (Figure 2).

The 8 grape populations from the Aegean region and the reference population were grouped according to provinces and subjected to BAPS analysis to observe the presence of any kind of population structure. All populations except Aydın, İzmir, and the references were found to be highly admixed with each other (Figure 3). The heterogeneous structure features of the populations,

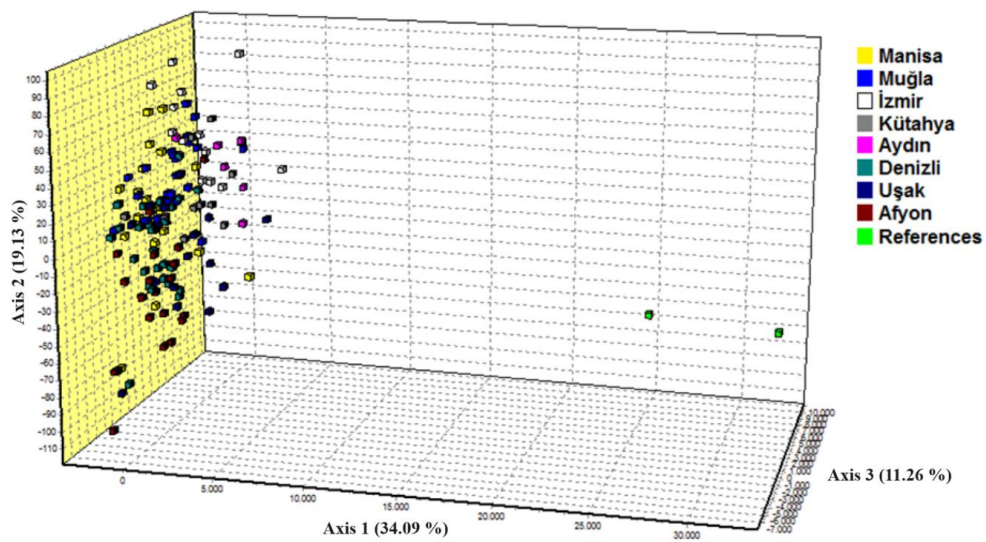
Table 4. Pairwise *Fst* values and gene flow (*Nm*) among the 8 grape populations.

Population (<i>Fst</i> / <i>Nm</i>)	Manisa	Muğla	İzmir	Kütahya	Aydın	Denizli	Uşak	Afyon
Manisa	-							
Muğla	-0.001ns/ Inf	-						
İzmir	0.004ns/93.00	0.005ns/48.68	-					
Kütahya	0.016ns/15.44	0.006ns/31.80	0.023*/10.60	-				
Aydın	-0.004ns/ Inf	-0.013ns/ Inf	-0.013ns/ Inf	-0.009ns/ Inf	-			
Denizli	0.006ns/43.81	0.005ns/47.48	0.023**/10.55	0.010ns/21.65	0.011ns/21.32	-		
Uşak	0.009ns/28.52	0.010ns/22.33	0.017*/14.09	0.005ns/32.31	0.003ns/54.02	0.004ns/51.52	-	
Afyon	0.013**/18.68	0.019**/12.66	0.044***/5.33	0.009ns/21.51	0.023*/9.71	0.006ns/35.06	0.001ns/115.92	-
References	0.075**/3.43	0.105***/2.03	0.079*/3.12	0.136*/1.45	0.086ns/2.74	0.078***/2.09	0.105**/2.00	-

ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Inf: infinite

Table 5. Genetic distance among the grape populations based on Nei (1972).

Population	Manisa	Muğla	İzmir	Kütahya	Aydın	Denizli	Uşak	Afyon	References
Manisa	-								
Muğla	0.049	-							
İzmir	0.090	0.076	-						
Kütahya	0.117	0.080	0.142	-					
Aydın	0.140	0.100	0.126	0.134	-				
Denizli	0.067	0.052	0.124	0.089	0.159	-			
Uşak	0.089	0.079	0.121	0.091	0.151	0.064	-		
Afyon	0.109	0.105	0.209	0.100	0.213	0.070	0.069	-	
References	0.554	0.607	0.521	0.682	0.614	0.579	0.486	0.636	-

**Figure 2.** Factorial correspondence analysis (FCA) of Aegean region populations with references.

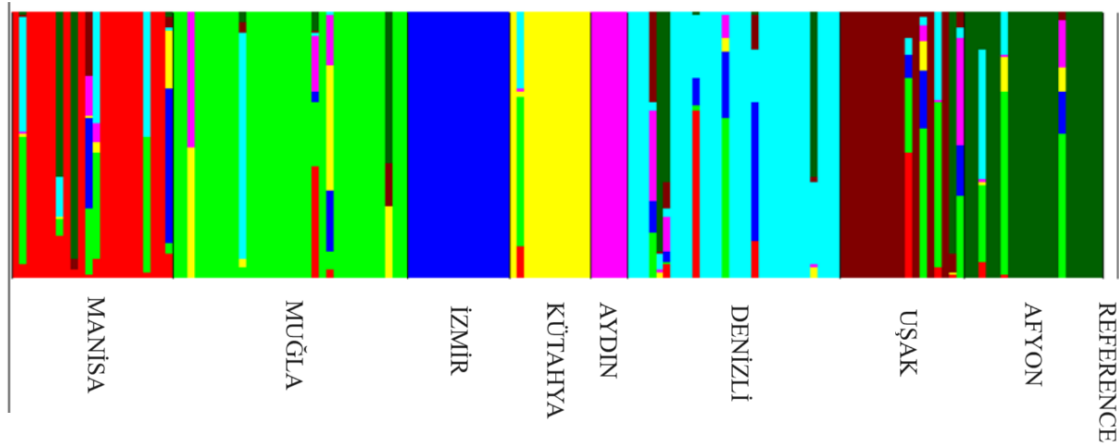


Figure 3. Bayesian analysis of population structure (BAPS) of the Aegean and reference cultivars, treating all populations as predefined. Each vertical bar represents one individual genotype and populations are separated by black vertical lines.

which showed admixture with each other according to FCA, were also revealed by BAPS analysis, and in contrast to the FCA results the Aydın and İzmir populations interestingly showed homogeneous (monocolored) structures according to BAPS analysis (Figures 2 and 3).

In STRUCTURE-HARVESTER analysis, the best K value was obtained as 3 ($K = 3$) when the reference cultivars were included. The graph showing the best K value is given in Supplementary Figure 2. Reconstructed panmictic populations were realized according to Bayesian model-based clustering methods (the best K representing the RPP genotypes was $K = 3$ (RPP1 – RPP3)) and a total of 3 main RPP groups (RPP1, RPP2, and RPP3) were defined (Figure 4). Only one reference cultivar was included in RPP1 and the rest of the populations were scattered across three RPPs. Overall results showed that 81 (54%) genotypes were assigned to each RPP with higher than 80% probability and 46% did not belong to the representative RPPs or showed low probability of membership. Table 6 and Figure 4 summarize these results. The RPP3 group had the highest genotype number with 61 genotypes from 8 different populations, of which 37 genotypes (61%) were found to have qI of $>80\%$, and the highest accession numbers were from the Muğla (9) and Manisa (7) populations, respectively. There were 53 genotypes in the RPP1 group as the group with the second highest genotype number and 29 genotypes were determined to have qI of $>80\%$, including all populations except Aydın. In the RPP2 group with the lowest genotype number (36), the Afyon (6) and Denizli (4) populations constituted the majority of the 15

genotypes determined to have qI of $>80\%$. In all RPPs, a total of 81 genotypes were identified as admixed genotypes ($qI > 80\%$) (Figure 4; Table 6).

4. Discussion

4.1. SSR analysis

Microsatellites as molecular markers have been used and accepted by many researchers for the characterization of varieties and identification of synonymous and homonymous cases (Regner et al., 2001; This et al., 2004; Popescu et al., 2017). Furthermore, microsatellites have also been used for parental analysis (Vouillamoz et al., 2004; Cipriani et al., 2010; Cao et al., 2020), genetic difference studies, molecular analysis of evolutionary development and origin determination (Sefc et al., 2000; Vouillamoz et al., 2003; Arroyo-Garcia et al., 2004), and genetic mapping studies (Fischer et al., 2004; Riaz et al., 2004) with positive results.

A survey of 15 *Vitis*-based SSR markers was conducted in this study for a subset of 151 grape cultivars to determine the most useful ones for discriminating the regional germplasm and successful amplifications (expected allele sizes, bp) were achieved for all 15 SSR loci.

In different studies, it has been explained that the main reason why SSR markers are highly discriminatory is that they give high allele counts per locus (El-Esawi et al., 2016; Rodolfi et al., 2018). In this study, the VVMD7 locus with 14 alleles involved the highest number of alleles, showing results similar to those of previous studies (Fatahi et al., 2003; De Souza-Leão et al., 2013). This was followed by

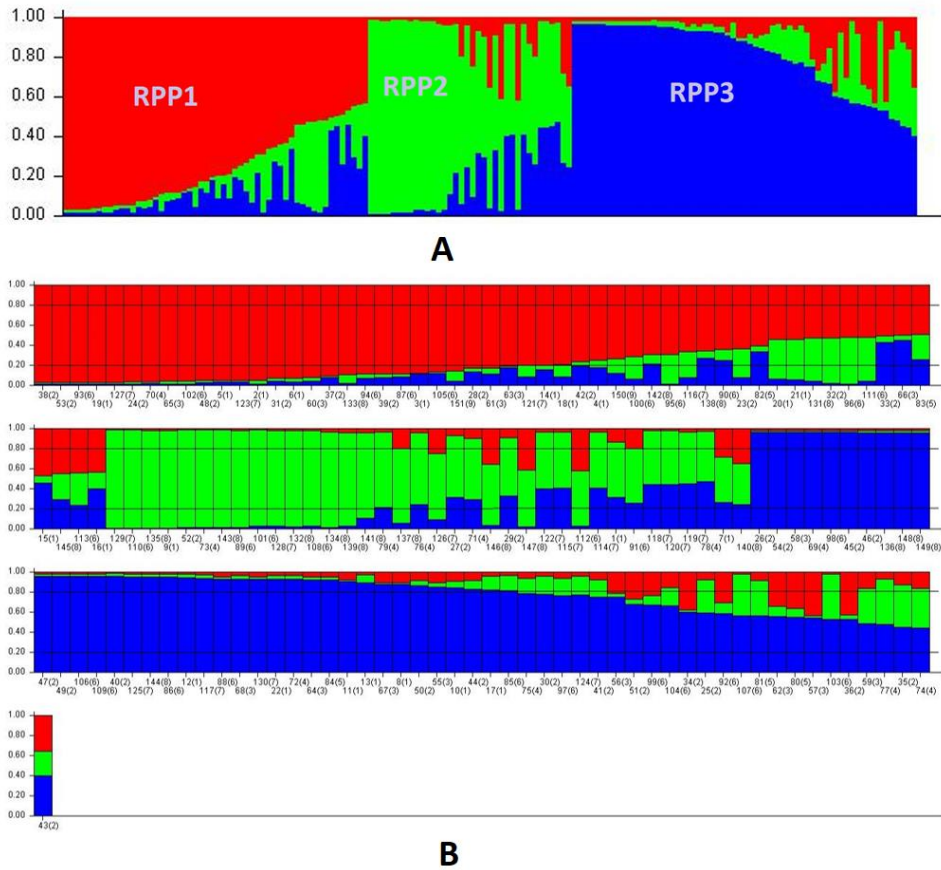


Figure 4. Reconstructed panmictic population (RPP) groups: general distribution (A) and individual representations of RPPs showing the percent probabilities of each individual in the RPP groups (B). RPPs are given as accession no. (population no.). Population numbers are as follows: (1) Manisa, (2) Muğla, (3) İzmir, (4) Kütahya, (5) Aydın, (6) Denizli, (7) Uşak, (8) Afyon. Supplementary Table 1 can be consulted for cultivar numbers.

Table 6. Reconstructed panmictic populations (RPPs) of 151 grape cultivars by STRUCTURE-HARVESTER using 15 loci for K = 3.

RPP Group	Number of genotypes	$qI > 0.8$	The most representative populations (the number of individuals)	$qI < 0.8$	The most representative populations (the number of individuals)
RPP1	53	29 (54%)	Manisa (6), Muğla (8), İzmir (4), Kütahya (1), Denizli (5), Uşak (3), Afyon (1), Reference (1)	24 (46%)	Manisa (6), Muğla (4), İzmir (1), Aydın (2), Denizli (6), Uşak (1), Afyon (4)
RPP2	36	15 (42%)	Manisa (2), Muğla (1), Denizli (4), Uşak (2), Afyon (6)	21 (58%)	Manisa (2), Muğla (2), Kütahya (4), Denizli (2), Uşak (7), Afyon (4)
RPP3	61	37 (61%)	Manisa (7), Muğla (9), İzmir (5), Kütahya (2), Aydın (1), Denizli (6), Uşak (3), Afyon (4)	24 (39%)	Muğla (8), İzmir (4), Kütahya (3), Aydın (2), Denizli (6), Uşak (1)
Overall	150	81 (54%)	All populations	69 (46%)	All populations

qI : coefficient coancestry

the VVS2 locus with 13 alleles (Goto-Yamamoto et al., 2006; Schuck et al., 2014; Yilmaz et al., 2020). The lowest number of alleles was observed in VVS1 with 5 alleles (Ladoukakis et al., 2005). The average number of alleles per locus found in the present work is supported by previously reported studies on grapes (Santana et al., 2010; Boz et al., 2011; Yilmaz et al., 2020). In a different SSR study conducted on 36 autochthonous cultivars from the Aegean region of Türkiye, the highest allele number was observed in the VVMD7 locus (6 alleles), similar to our study, while the lowest was detected in the VVS2 locus (2 allele) (İşçi and Dilli, 2014).

The probability of identity (*PI*) value is defined as the probability that two randomly selected unrelated genotypes will display the same SSR profile (Doulati-Baneh et al., 2013). The *PI* value was higher than 0.05 at all loci (Sefc et al., 2001). This shows that the microsatellite markers used in this study are highly polymorphic and indicates high heterozygosity levels in the grapevines (Table 1).

He was found to be slightly higher than *Ho* in the VVIH54, VVMD28, VVMD5, and VMC2C3 loci. A high *He* value may indicate the presence of a null (silent) allele, and investigation of the null allele frequencies (*r*) of these loci revealed that the values were either positive or negative (data not shown). However, the small values of the positive values reduce the risk of null alleles at the loci, as stated by other researchers (Costantini et al., 2005; Santana et al., 2007). In the study by Daler and Cangi (2022), *He* values were found to be higher than *Ho* values for 8 of 9 SSR markers, while in some other studies (Kupe et al., 2021; Li et al., 2021), the *He* values were found to be slightly higher than the *Ho* values for the VVMD28 locus, similar to this study. Interestingly, in the study of Huerta-Acosta et al. (2022), the *He* value was found to be significantly higher than the *Ho* value for both the VVMD5 and VVMD28 loci.

Considering the average *He* and *Ho* values, unlike some other studies on grape SSRs (Moreno-Sanz et al., 2011; Khadivi et al., 2019), the mean *Ho* (0.731) and *He* (0.725) values were determined to be close to each other in this study. This can be explained by the low degree of random union of gametes among the population features (Hvarleva et al., 2004). Nevertheless, the average *Ho* value of Aegean grapes (0.731) in this study was higher than the value obtained for Central Anatolian grape cultivars (0.704) and close to that reported for Eastern Anatolian grapes (0.739) (Eyduan et al., 2015; Yilmaz et al., 2020) (Table 1).

4.2. Genetic relations among grape accessions

In previous studies, the prevalence of synonymous and homonymous genotypes of Turkish cultivars was emphasized by various researchers (Ergül et al., 2006; Vouillamoz et al., 2006; Yilmaz et al., 2020).

The presence of 13 homonymous cases in this study revealed the rich diversity of genetic resources in the

region. In addition, the scarcity of synonyms and the emergence of homonymous varieties within and among the provinces confirmed this diversity. On the other hand, the high rate of homonymous cultivars also indicates that there is varietal complication in the region. The presence of homonymous varieties with the same name that are not identical to each other could be described as misnaming, as well as the occurrence of variations (clone, type, etc.) that emerged over time within each variety. The detection of 13 homonymous cases in this study suggests that a natural gene flow occurred in the region many years ago. For “homonym” varieties, it has been proven by molecular studies that most of them are cultivars with clonal variation (Ergül et al., 2006). As per the synonymous cultivars, the idea arises that the same genotypes may have been incorrectly named differently. It was also observed that the numbers of homonymous and synonymous cases determined in this study were higher than those reported by some other grape SSR studies conducted in Türkiye. Accordingly, in a study of the Dimrit and Gemre cultivars with scanning of 8 SSR markers, only 1 case of synonyms and 7 cases of homonyms were identified (Şelli et al., 2007), while in another study carried out with 51 cultivars from the Mediterranean region, 5 cases of synonyms and 8 cases of homonyms were detected (Tangolar et al., 2009).

The reference cultivars Cabernet Sauvignon and Merlot used in this study were also analyzed by other researchers (Bowers et al., 1999; This et al., 2004; Hizarci et al., 2012; Yilmaz et al., 2020) and parallel results were obtained.

In this study, when the genetic similarities of the genotypes were examined, the highest similarity rate besides that of the synonymous cultivars (with 100% genetic similarity) was determined to be 96.7% for 25 genotypes and 93.3% for 19 genotypes. The similarity rates of the other cultivars were found to be 86.7% or lower (Supplementary Figure 1). The lowest similarity rate was observed between the reference (Cabernet Sauvignon and Merlot) and Turkish cultivars.

Similar to this study, in another SSR study conducted with 36 autochthonous grapevine cultivars from the Aegean region of Türkiye, some homonymous cases were detected for Dimrit (also known as Dilmit) cultivars from different provinces (Afyon, Denizli, and Manisa) (İşçi and Dilli, 2014). Similarly, in our study, one homonymous case was observed among the Dimrit cultivars (Siyah Dilmit, Al Dilmit, Akdimrit, İstanbul Dimrit, Kırmızı Dimrit, Söbü Dimrit, etc.) (Table 2).

This situation may be a result of vegetative reproduction effects caused by different environmental factors and somatic mutations (İşçi and Dilli, 2014). Such findings support our results, and it is thought that the genotypes of every homonymous variety are clones and types of a particular variety that have undergone genetic variation.

4.3. Genetic structure analysis among grape cultivar populations

Structural analyses are of great importance for a more clear understanding of genetic diversity and are used extensively in population genetic studies (Dido et al., 2022). In this study, the observed heterozygosity values were higher than expected in all populations except for Manisa and to some extent the İzmir population. The higher value of observed heterozygosity rather than the expected heterozygosity can be attributed to isolation-breaking effects (i.e., the mixing of two previously isolated populations) or high levels of cross-pollination and random pollination among grape populations from different regions (Hvarleva et al., 2004). Low values of expected and observed heterozygosity along with the lowest mean number of alleles, which reflects little genetic variability, were also observed in the Aydın population. In such cases, we might suspect forces such as inbreeding or a lack of gene flow. Nevertheless, the mean values of *He* and *Ho* among the studied Aegean populations (*He*: 0.69, *Ho*: 0.72) were higher than the corresponding mean values of wild French grapevine populations (*He*: 0.65, *Ho*: 0.65) (Barnaud et al., 2010) and lower than those of Iranian (*He*: 0.78, *Ho*: 0.83) and Russian (*He*: 0.75, *Ho*: 0.90) (Ramezani et al., 2009) and also Italian (*He*: 0.81, *Ho*: 0.74) (Emanuelli et al., 2013) grape cultivars.

There is significant gene flow among all 8 studied Aegean grape populations but not the references. Considering the values of genetic distance and gene flow of the Aegean populations, it is concluded that the highest amount of gene flow and consequently the lowest genetic distance occurred between the Manisa and Muğla populations. The lowest levels of gene transfer were recorded between the Afyon population and the populations of İzmir and Aydın.

The highest values of genetic distance were seen between the Aegean populations and the references, which varied in a range between 0.486 (Uşak and references) and 0.682 (Kütahya and references). Gene flow rates between the Aegean and reference populations ranged from 1.45 to 3.43, indicating higher levels of gene flow compared to the corresponding values of Central Anatolian grapevine populations (1.15–1.76) and the same reference genotypes (Cabernet Sauvignon and Merlot) (Yilmaz et al., 2020). The low *Fst* values among the populations of the Aegean region could be attributed to the effect of genetic exchanges over the years as well as the geographic proximity of the provinces of the evaluated populations.

LD is influenced by many factors, including selection, rates of genetic recombination, mutation rates, genetic drift, mating systems, population structures, and genetic linkage (Slatkin, 2008; Fricano et al., 2012; Lucek and Willi, 2021). As seen in Supplementary Table 4, all loci were polymorphic and the highest HWE values were

observed in the Aydın and reference populations while the lowest rate occurred in the Manisa population. Natural selection and the presence of significant gene flow have been identified as contributing factors to LD in natural grape populations (Yilmaz et al., 2020).

Due to the three-dimensional distribution of the populations in the FCA diagram (Figure 2), there is an overlapping situation in all populations. For this reason, the genetic distance between the Afyon population and the İzmir and Aydın populations is not clearly visible. Moreover, the scattering of the blue dots that represent the accessions of the Muğla population and the yellow dots representing the Manisa population are remarkable. One of the most important reasons for the clear separation of the reference group is the considerable geographical separation of the cultivated areas. Similar results were obtained in previous studies (Riaz et al., 2018; Yilmaz et al., 2020).

BAPS analyses showed the same results, except for the Aydın and İzmir populations, as all of the grape populations in the Aegean region were highly admixed and were almost not separated from each other at all while the reference varieties formed a separate cluster based on 15 microsatellite loci. Although the different Aegean populations have different genotypes, the multiplicity of these groups (similar accessions) was found to be lower in the Aydın region, followed by İzmir (Figure 3). Evaluations of the genetic distance values of the populations of these areas (Aydın and İzmir) with other populations also confirmed this situation. It should be noted that although gene flow of the Aydın and İzmir populations was recorded with other populations, according to the homogeneous structures of these populations the occurrence of gene flow from these regions to other regions can be inferred.

Interestingly, in RPP analysis, one of the reference samples was clustered in RPP1 along with most of the Aegean populations included in this work, with a membership coefficient above 80% (Table 6). Pollen fluxes or exchanges of clonally propagated materials over time across the distinct populations have challenged the morphological identification of grape genotypes and may have had a significant impact on the evolution of the populations (Arroyo-Garcia et al., 2013). These results are consistent with those of Maul and Töpfer (2020) and also Maras et al. (2020), who found that two grapevine cultivars (Cabernet Franc and Sauvignon) reported as progenitors of a high number of cultivars were clustered with most of the studied cultivars.

These results underline a relationship among some Aegean accessions and the reference samples, emphasizing the possible occurrence of hybridization. Indeed, the Cabernet Sauvignon and Merlot cultivars, used here as reference cultivars, have been crossed with many other relevant regional varieties.

5. Conclusion

The Aegean region of Türkiye has an important role in Turkish viniculture, accounting for approximately 50% of Turkish grape production together with approximately 35% of the vineyards of the country. This study, conducted using microsatellite markers on Turkish grapevine genotypes, showed considerable genetic diversity existing among the populations. Evaluation of germplasm genetic diversity is crucial for utilizing genetic potential to improve traits needed for higher quality. Additionally, characterization of the cultivars and identification of their relationships could support genetic conservation and the management of breeding programs and would also contribute to other viniculture activities (plant propagation, etc.). To better

preserve genetic diversity, we suggest that new natural conservation habitats be established at the origins of germplasms, and we recommend that protection and management of grapevine species populations with high allelic richness and heterosis be prioritized.

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Supplementary

Table 1. Cultivar numbers, cultivar names, and provinces of the grapevine cultivars from the Aegean region.

Cultivar Number	Cultivar Name	Province
1	Tek Çekirdekli	Manisa
2	Kırmızı Şam	Manisa
3	Bulama	Manisa
4	Beyaz Şam	Manisa
5	Antep Şamı	Manisa
6	Gode Banma	Manisa
7	Dikilitaş	Manisa
8	Pembe Üzüm	Manisa
9	Hacı Hıdır	Manisa
10	Duman	Manisa
11	Hasan Üzüümü	Manisa
12	Nar Üzüümü	Manisa
13	Bulama	Manisa
14	Siyah Gemre	Manisa
15	Siyah Gemre	Manisa
16	İri Kara	Manisa
17	Beyaz Misket	Manisa
18	Beyaz Misket	Manisa
19	Siyah Misket	Manisa
20	Siyah Misket	Manisa
21	Siyah Misket	Manisa
22	Buçuk Arşın	Manisa
23	Kütür Üzüümü	Muğla
24	Beylerce	Muğla
25	Bandırma	Muğla
26	Buzağı Boku	Muğla
27	Ekşi Üzüm	Muğla
28	Köfte Üzüümü	Muğla
29	Pek	Muğla
30	Beyaz Şam	Muğla
31	Tavşan Böbreği	Muğla
32	Kızıl Üzüm	Muğla
33	Sıksarı	Muğla
34	Seyrek Sıkça	Muğla
35	Pembe Çekirdeksiz	Muğla
36	Alyanak	Muğla
37	Zeynel	Muğla
38	Koz Üzüümü	Muğla
39	Kara İbrahim	Muğla
40	Siyah Bandırma	Muğla

Table 1. (Continued)

41	Kınalı	Muğla
42	Tek Çekirdekli	Muğla
43	Pembe Çavuş	Muğla
44	Akdilmit	Muğla
45	İstanbul Dimliti	Muğla
46	Kayasar Dimriti	Muğla
47	Siyah Dilmit	Muğla
48	Sofra Karası	Muğla
49	Yerli Kara	Muğla
50	Aydın Karası	Muğla
51	Katı Kara	Muğla
52	Kara Büzgülü	Muğla
53	Kadın Parmağı	Muğla
54	Misket	Muğla
55	Gelin Dudağı	İzmir
56	Bolat	İzmir
57	Sıksarı	İzmir
58	Bademiye Üzüm	İzmir
59	Zeytin Üzümü	İzmir
60	Osmancık	İzmir
61	Ekşi Üzüm	İzmir
62	Hacı Azman	İzmir
63	Efe Püskülü	İzmir
64	Kayacık	İzmir
65	Yediveren	İzmir
66	Ak Üzüm	İzmir
67	Kara Üzüm	İzmir
68	Sıkkara	İzmir
69	Paşalar	Kütahya
70	Ahmet Üzümü	Kütahya
71	Nuribey Üzümü	Kütahya
72	Paşalar	Kütahya
73	Büzgülü	Kütahya
74	Tek Çekirdekli	Kütahya
75	Feslihan	Kütahya
76	Kıkırdak Üzüm	Kütahya
77	Nuribey Üzümü	Kütahya
78	Kokulu Misket	Kütahya
79	Akbüzgülü	Kütahya
80	Foça Karası	Aydın
81	Kınalı Belerce	Aydın
82	Güvercin Gözü	Aydın
83	Sulu Belerce	Aydın

Table 1. (Continued)

84	Bombalak	Aydın
85	Sultaniye	Denizli
86	Kınalı Sultaniye	Denizli
87	Kınalı Tırnak	Denizli
88	Muhammediye	Denizli
89	Fesliken	Denizli
90	Gül Üzüm	Denizli
91	Hırsız Çalmaz	Denizli
92	Kokarca	Denizli
93	Tek Çekirdeksiz	Denizli
94	Tavşan Böbreği	Denizli
95	Yanal Üzümü	Denizli
96	İri Kızıl	Denizli
97	Nazik Üzüm	Denizli
98	Ziraat Üzümü	Denizli
99	Boz Üzüm	Denizli
100	Kara Yaprak	Denizli
101	Büzgülü	Denizli
102	Yediveren	Denizli
103	Akdimrit	Denizli
104	Siyah Dimrit	Denizli
105	Pembe Gemre	Denizli
106	Çalkarası	Denizli
107	Aşıkara Sultaniyesi	Denizli
108	Ekşi Kara	Denizli
109	Eski Kara	Denizli
110	Devegözü	Denizli
111	Kadın Parmağı	Denizli
112	Kadın Parmağı	Denizli
113	Misket	Denizli
114	Morrazdağı	Uşak
115	Morrazdağı	Uşak
116	Mor Üzüm	Uşak
117	Şahin Tırnağı	Uşak
118	Aydın Üzümü	Uşak
119	Beylace	Uşak
120	Hacı Eyüp	Uşak
121	Kokulu Beyaz	Uşak
122	Morrazdağı	Uşak
123	Şambaba	Uşak
124	Terzi Nasuf	Uşak
125	Unknown	Uşak
126	Gelin Üzümü	Uşak

Table 1. (Continued)

127	Unknown	Uşak
128	Siyah Razakı	Uşak
129	Devegözü	Uşak
130	Kırmızı Dimrit	Uşak
131	Günyüzü	Afyon
132	Hevenk	Afyon
133	Sıdalak	Afyon
134	Manda Gözü	Afyon
135	Sakarya	Afyon
136	Veyisoğlu	Afyon
137	Unknown	Afyon
138	Algöynek	Afyon
139	Isparta	Afyon
140	Unknown	Afyon
141	Unknown	Afyon
142	Tel Göynek	Afyon
143	Siyah Parmak	Afyon
144	Razakı	Afyon
145	Razakı	Afyon
146	Tilki Kuyuğu	Afyon
147	Tilki Kuyruğu	Afyon
148	Keçi Memesi	Afyon
149	Söbü Dimrit	Afyon
150	Cabernet Sauvignon (Reference)	
151	Merlot (Reference)	

Table 2. SSR loci information (locus name, primer sequence (F: forward primer, R: reverse primer), expected range (bp), chromosome location (Chr.), and references).

Locus name	Primer Sequence (5' to 3')	Expected range (bp)	Chr.	References
VrZAG79	F:agattgtggaggagggaacaaacg, R:tgcctccattttcaaacctctcc	237 - 261	5	Sefc et al., 1999; Margaryan et al., 2019; Riaz et al., 2020
VVIH54	F:ccgcactgtgttgaatttcag, R:caaaccgtttttacaccagcag	139 - 177	13	Merdinoglu et al., 2005; Abbal et al., 2008; Margaryan et al., 2019
VVMD24	F:gtggatgatggagtagtcacgc, R:gatttttaggttcattgttggtgaagg	200 - 220	14	Bowers et al., 1996; Riaz et al., 2008; Margaryan et al., 2019
VVMD7	F:agagttgcggagacaggat, R:cgaaccttcacacgcttgat	233 - 265	7	Bowers et al., 1996; Margaryan et al., 2019; Riaz et al., 2020
VVMD28	F:aacaattcaatgaaaagagagagagaga R:tcattcaattctgtatctatttgcgtg	218 - 282	3	Bowers et al., 1996; Margaryan et al., 2019; Riaz et al., 2020
VVMD27	F:gtaccagatctgaatacatccgtaagt, R:acgggtatagacaaacgggtg	176 - 198	5	Bowers et al., 1996; Margaryan et al., 2019; Riaz et al., 2020
VMC2H4	F:accaggtgtgcctataagaatc, R:tctctggaacatccaatcaac	197 - 235	12	Goto - Yamamoto et al., 2006; Durán et al., 2011; Regner et al., 2016
VVIB01	F:tgaccctcgaccttaaatctt, R:tggtgagtgcaatgatagtaga	285 - 311	2	Merdinoglu et al., 2005; Regner et al., 2016; Margaryan et al., 2019
VrZAG83	F:ggcggaggcggtagatgagaggcg, R:acgcaacggctagtaataacaacgg	180 - 201	4	Sefc et al., 1999; Margaryan et al., 2019
VVS2	F:cagcccgtaaatgtatccatc, R:aaattcaaaattctaattcaactgg	123 - 155	11	Thomas and Scott, 1993; Guo et al., 2016; Margaryan et al., 2019
VVMD5	F:ctagagctacgcaatccaa, R:tataccaaaaatcataatcctaaa	226 - 266	16	Bowers et al., 1996; Margaryan et al., 2019; Riaz et al., 2020
VrZAG62	F:ggtgaaatgggcaccgaacacacgc, R:ccatgtctctctcagcttctcagc	182 - 206	7	Sefc et al., 1999; Margaryan et al., 2019; Riaz et al., 2020
VVMD31	F:cagtgtgttttcttaagtttcaagg, R:ctctgtgaaagaggaagagacgc	196 - 224	7	Bowers et al., 1996; Leão et al., 2011; Riaz et al., 2020
VMC2C3	F:tgcaatcccatattatctctt, R:aatattgtagaatgggtctttt	158 - 192	14	Goto - Yamamoto et al., 2006; Durán et al., 2011; Regner et al., 2016
VVS1	F:acaattggaacgcgtggag, R:cttctcaatgatattctaaacctatg	205 - 227	13	Thomas and Scott, 1993; Regner et al., 2016; Taheri and Darzi Ramandi, 2020

Table 3. Aegean grape cultivars with varying high degrees of genetic similarity identified based on SSR analysis.

Cultivar with similar genetic origin	
No	Cultivars with 90% genetic similarity (Cultivar number)
1	Antep Şamı (5) - Tavşan Böbreği (31)
2	Duman (10) - Nazik Üzüm (97)
3	Hacı Hıdır (9) - Sakarya (135)
4	Hasan Üzümlü (11) - Kırmızı Dimrit (130)
5	Beylerce (24) - Unknown (127)
6	Buçuk Arşın (22) - Feslihan (75)
7	Bandırma (25) - Tek Çekirdekli (74)
8	Kızıl Üzüm (32) - İri Kızıl (96)
9	Paşalar (69) - Unknown (125)
10	Gül Üzüm (90) - Algöynek (138)
Cultivars with clonal similarity	
No	Cultivars with clonal similarity 93% genetic similarity (Cultivar number)
1	Gode Banma (6) - Tavşan Böbreği (94)
2	Hasan Üzümlü (11) - Muhammediye (88)
3	Hacı Hıdır (9) - Devegözü (129)
4	Siyah Misket (19) - Beylerce (24)
5	Buzağı Boku (26) - Siyah Bandırma (40)
6	Beylerce (24) - Kadın Parmağı (53)
7	Buzağı Boku (26) - Paşalar (69)
8	Bandırma (25) - Kınalı Belerce (81)
9	Beylerce (24) - Tek Çekirdeksiz (93)
10	Buzağı Boku (26) - Razakı (144)
11	Seyrek Sıkça (34) - Sıksarı (57)
12	Siyah Bandırma (40) - Paşalar (69)
13	Siyah Bandırma (40) - Razakı (144)
14	Misket (54) - Unknown (125)
15	Akdilmit (44) - Paşalar (69)
16	Gül Üzüm (90) - Tel Göynek (142)
17	Ziraat Üzümlü (98) - Unknown (125)
18	Siyah Razakı (128) - Hevenk (132)
Cultivars with clonal similarity 96% genetic similarity (Cultivar number)	
19	Antep Şamı (5) - Şambaba (123)
20	Ekşi Üzüm (27) - Pek (29)
21	Buzağı Boku (26) - Misket (54)
22	Buzağı Boku (26) - Ziraat Üzümlü (98)
23	Siyah Misket (19) - Unknown (125)
24	Siyah Bandırma (40) - Unknown (125)
25	Siyah Bandırma (40) - Misket (54)
26	Siyah Bandırma (40) - Ziraat Üzümlü (98)
27	Kınalı (41) - Sultaniye (85)
28	Misket (54) - Paşalar (69)

Table 3. (Continued)

29	Kadın Parmağı (53) - Unknown (127)
30	Kara Büzgülü (52) - Siyah Razakı (128)
31	Kara Büzgülü (52) - Hevenk (132)
32	Misket (54) - Razakı (144)
33	Paşalar (69) - Ziraat Üzüümü (98)
34	Büzgülü (73) - Hevenk (132)
35	Muhammediye (88) - Kırmızı Dimrit (130)
36	Fesliken (89) - Manda Gözü (134)
37	Tek Çekirdeksiz (93) - Unknown (127)
38	Ziraat Üzüümü (98) - Razakı (144)
39	Büzgülü (101) - Ekşi Kara (108)
40	Siyah Razakı (128) - Siyah Parmak (143)
41	Devegözü (129) - Sakarya (135)
42	Hevenk (132) - Siyah Parmak (143)

Table 4. Linkage disequilibrium (LD) comparisons and Hardy–Weinberg equilibrium (HWE) loci ($p < 0.05$).

Population	LD (105 allelic comparison)	HWE loci (15 loci)
Manisa	31	8
Muğla	54	12
İzmir	13	14
Kütahya	18	14
Aydın	8	15
Denizli	57	14
Uşak	42	13
Afyon	35	12
References	1	15

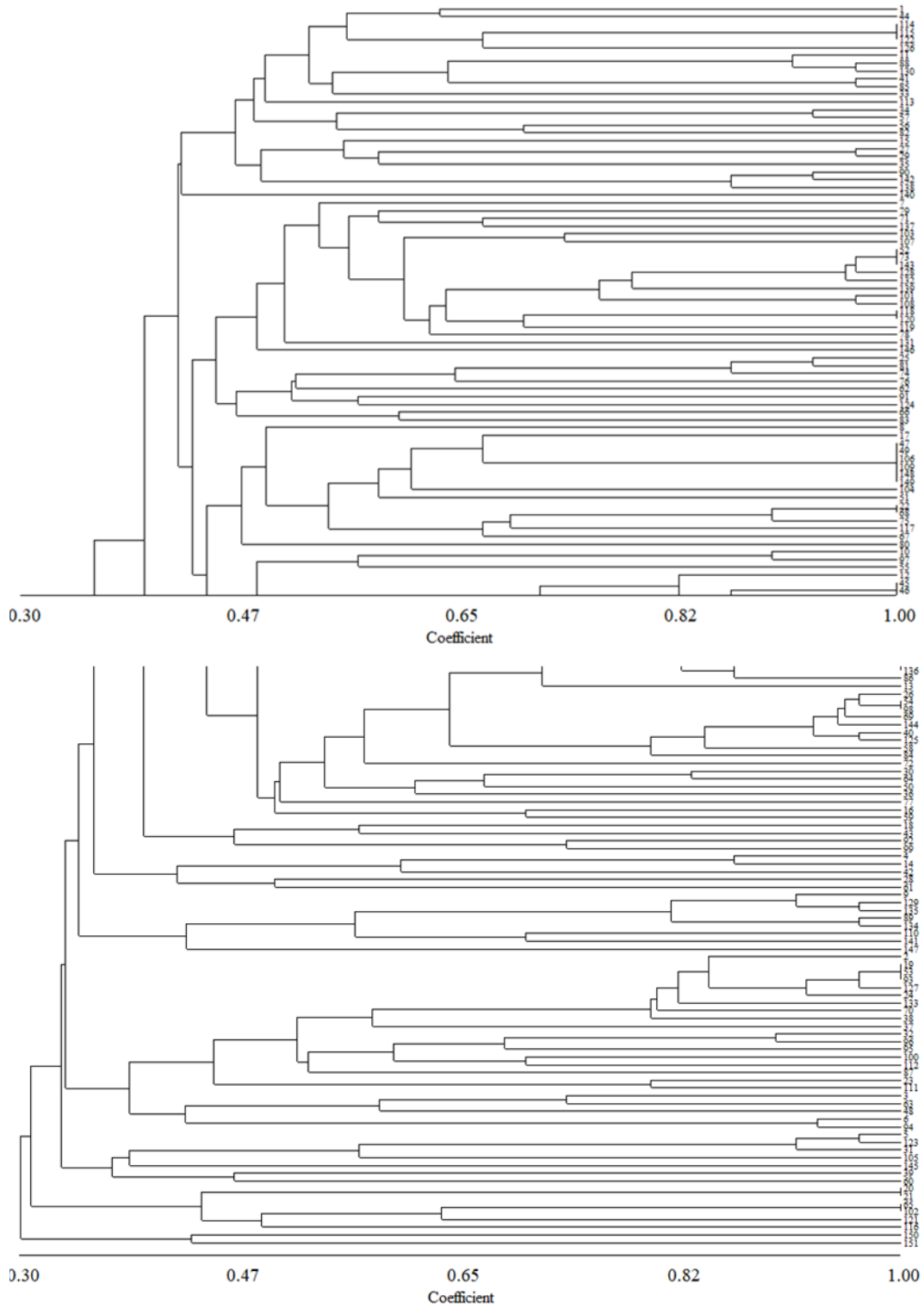


Figure 1. Dendrogram of 151 cultivars constructed from analysis based on 15 SSR markers (dendrogram was drawn in two parts for clear visibility).

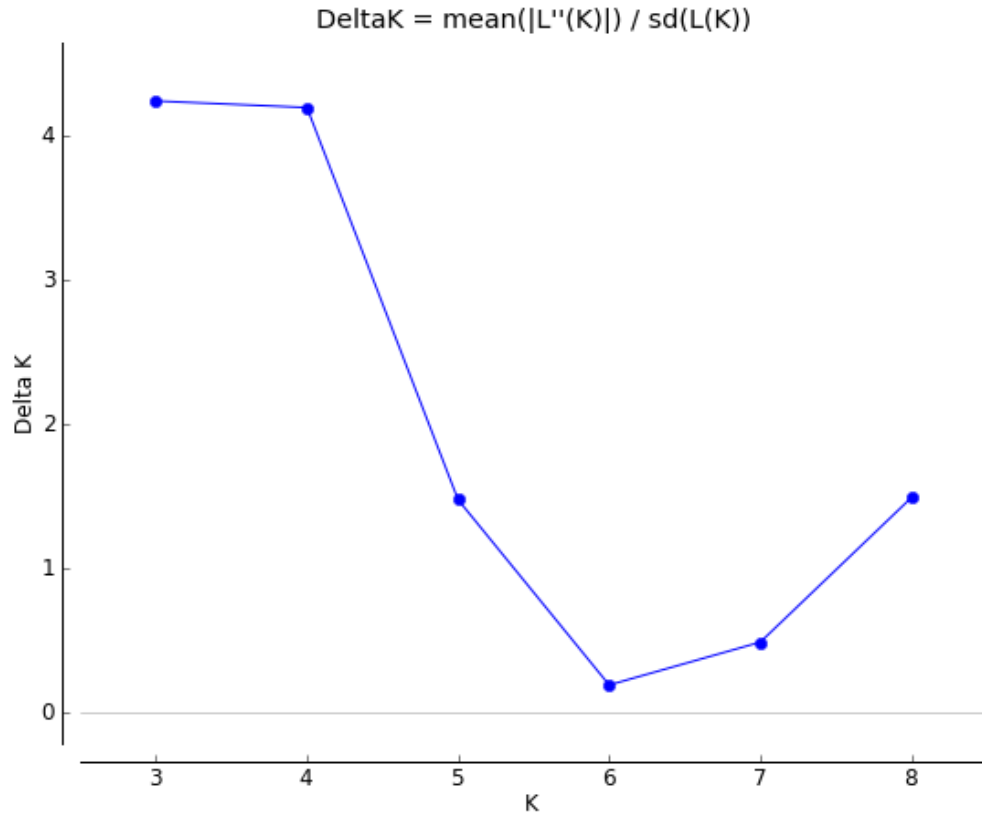


Figure 2. Graph showing the best K value of 3 (the highest peak) when references are excluded.