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Characterization of grapevine (*V. vinifera* L.) varieties grown in Yozgat province (Turkey) by simple sequence repeat (SSR) markers

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Abstract: The study was conducted to characterise 50 grape varieties grown in Yozgat by molecular methods. Molecular definitions were realized using 9 simple sequence repeat (SSR) primers (VVS2, VVMD5, VVMD7, VVMD24, VVMD27, VVMD28, VVMD31, VrZAG62, and VrZAG79), including six microsatellite loci used for grapevine variety collections by the European Union funded project (GENRES 081), accepted as the minimum standard set (core set) by the world. According to the SSR analysis results, 451 alleles, 233 of which polymorphic, were obtained. The highest allele numbers were designated as 52 in the VVMD7, VVMD24, VVMD27, VrZAG62, and VrZAG79 loci. The mean number of alleles was recorded as 50.11 (± 1.306), while the average number of polymorphic alleles was 25.89 (± 1.896). The VVMD28 primer gave the highest number of polymorphic alleles (Na=36). The mean of the expected heterozygosity (He) value was calculated as 0.932 (± 0.005), while the average of observed heterozygosity (Ho) value was 0.481 (± 0.082). Polymorphism information content (PIC) values ranged from 0.908 (VVMD31) to 0.955 (VVMD28) with a mean PIC value of 0.927. The unweighted pair group method with arithmetic average (UPGMA) clustering technique was used to generate the dendrogram. Population structure analysis results showed that by compatible phylogenetic analysis, the varieties depicted into 2 main clusters. The genetic similarity rate among the varieties changed to ranging from 0% to 50%. The highest genetic similarity coefficient with a 0.50 was found between Horoz Üzümü and Karagevrek.

Key words: Characterization, simple sequence repeats, structure analysis, *Vitis vinifera* L., Yozgat province

1. Introduction

The grapevine is one of the oldest known plant groups of the earth according to geological findings (Çelik et al., 1998). Since ancient times, grapes have been used in different ways, both for table and as processed (black treacle, grape juice, raisins, wine, vinegar, mash, etc.). Grapes, being extremely important in terms of human health, contain important substances, vitamins, proteins, carbohydrates, and minerals, also flavonoids, proanthocyanidins, and anthocyanidins, along with phenols and polyphenols such as anthocyanin, flavanol, flavonol, phenolic acid, caffeic acid, catechin, quercetin, resveratrol (Xia et al., 2010; Lim, 2013).

The grapevine is a plant belonging to the "Vitaceae" family of the "Rhamnales" order. All the grape varieties cultivated in the world are included in the "*Vitis*" genus, which is the most significant member of this family, and most of these varieties are included in the "*Euvitis*" subgenus that also inclue the "*V. vinifera* L." species as pure or hybrid (Winkler et al., 1974; Antcliff, 1992).

Of the world's 10,000 known grapevine varieties provide more than 95% *V. vinifera* L. species (Çelik, 2011). According to the data of FAO 2020, 77.1 million tonnes of grape production has been conducted on an area of 6.9 million hectares in the world. Turkey ranks 5th with 400,000 hectares (5.85%) in terms of area and ranks 6th with 4.2 million tonnes (5.32%) concerning grape production[1] in the world.

The viticulture history of Yozgat, which has been one of the oldest settlements of Anatolia, dates back to 1800 – 1600 BC, and the archaeological excavations document that the viticulture and wine culture has a deep-rooted history in Yozgat and its surrounding (Wilson and Allen, 1937; Oraman, 1965; Çelik, 2011).

In Yozgat, which has a total agricultural area of 1.1 million ha, viticulture activities have been performed on 2.9 thousand hectares areas, and a total of 15.6 thousand tonnes of grapes (table, seeded) produced[2].

[1] Food and Agriculture Organization of the United Nations (2021). FAOSTAT [online]. Website http://www.fao.org/faostat/en/ [accessed 10.04.2021].

[2] Turkish Statistical Institute (2021). TURKSTAT [online]. Website https://www.tuik.gov.tr [accessed 05.04.2021].

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In Yozgat, where the continental climate is dominant, the common vegetation the steppe. The average altitude of the province above sea level is approximately 1500 m. According to climate data between the years 2000 and 2020, the difference between day and night temperatures has an average of 15.3 °C. Annually, the average temperature throughout the city is 11.29 °C, the average temperature of the summer months is 20.83 °C, the hottest month average temperature is 22.13 °C, the coldest month average temperature is –0.46 °C, and the average temperature of the development period is 16.90 °C.

The effective heat summation of the province is 1,559.69 degree days. However, an average of 89.4 days of the year is below zero. Frost days have not encountered only in July in the region, and the development period is limited to 149.71 days on average. The number of sunny hours annually is 2 528.29 h, and the average daily sunbathing time is 6 h 52 min. The average annual rainfall is 411.49 mm, and the distribution of precipitation according to the seasons is irregular. The annual average relative humidity is 63.97%. Annually average wind speed 2.61 m / s. The effective wind direction is northeast, the second dominant wind direction is north. Also, the local pressure average is 888.36 mbar[3].

So far, the most comprehensive study performed to reveal grapevine genetic resources has been the "Determination, Conservation and Identification of Grapevine Genetic Resources (National Collection Vineyard)" project launched by Tekirdağ Viticulture Research Institute in 1965, and, with this project, "National Collection Vineyard" established. Preliminary studies have given us the idea that Yozgat province may have a richer grapevine gene potential.

The grapevine genetic resources of Yozgat province have not been characterised by molecular methods until now. These varieties, which have been grown in Yozgat for many years and adapted to the cold climate conditions of the region, are preferred by the local people and are consumed fresh and used in the production of local products. In this research, Autochthonous grape varieties grown in Yozgat were identified with 9 SSR primers from molecular methods. The regional grapevine genetic diversity is a prerequisite for future grapevine – breeding studies.

Therefore, the works on the determination, conservation, and management of genetic resources are of great importance.

2. Materials and methods

2.1. Plant material

This research was conducted on 50 grape varieties grown in Çandır, Boğazlıyan, Şefaatli, Sarıkaya, and Sorgun districts of Yozgat in 2017–2020. All analyses on molecular descriptions were performed in Sivas Cumhuriyet University Advanced Technology Research and Application Centre. To collect the identity (passport) information of the varieties, the methods specified in The International Board for Plant Genetic Resources (IBPGR, 1997) were used. Coordinates and altitudes of varieties were tagged using the navigation application (Kraus und Karnath GbR 2Kit Consulting GPS & Maps-v2.8). Identity (passport) information of the grapevine varieties was presented in Table 1.

2.2. DNA extraction

As plant material, were used fresh leaves received from the tip of the shoots (1st and 3rd node) of 50 autochthonous grape varieties, and two reference varieties (Cabernet Sauvignon and Merlot).

Isolation of total genomic DNA from leaf samples received was performed according to the CTAB procedure, adapted with some modifications of the Doyle and Doyle (1990) method. The extracted total genomic DNAs were controlled in both 1% agarose gel electrophoresis and Nanodrop (Maestrogen, MN-913) to evaluate the quality and quantity. Checked DNAs were stored at –20 °C for PCR reactions.

2.3. SSR – PCR reactions

In the research, 9 SSR primers, VVS2 (Thomas and Scott, 1993), VVMD5, VVMD7 (Bowers et al., 1996), VVMD24, VVMD27, VVMD28, VVMD31 (Bowers et al., 1999b), VrZAG62 and VrZAG79 (Sefc et al., 1999) were used. VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79 loci have been accepted as the minimum standard set (core set) according to international norms and have been made mandatory to be used in molecular characterization studies in *Vitis* species (This et al., 2004). The other 3 SSRs (VVMD24, VVMD28, and VVMD31) were also preferred in this study, as they were frequently included in previous studies for molecular characterisation and determination of genetic relatedness degrees (Karauz, 2013; Agüero et al., 2003; Karaağaç, 2006; Vouillamoz et al., 2006; Yıldırım, 2008; Aslantaş, 2010; Yıldırım, 2010).

The forward primers of each locus were marked fluorescently. Optimal melting (Tm) and binding (Ta) temperature values for the amplification of SSR loci were determined by the Gradient PCR approach.

The PCR reaction was performed in a final PCR reaction volume of 25.125 µL containing 25 – 100 ng DNA, 10 × Taq buffer (KCl – $MgCl_2$), 25 mM $MgCl_2$, 2.5 mM total dNTP, 10 pmol labelled forward primer, 10 pmol reverse primer, 0.625 U Taq DNA polymerase (5 U / µl) and ddH_2O. PCR conditions of an initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 45 s (denaturation), the

[3] Turkish State Meteorological Service (2020). MGM Reports for the year 2000 – 2020.

Table 1. Identity (passport) information and some berry characteristics of the grapevine varieties.

No	*Variety Name*	*OIV 223*	*OIV 225*	*Location*	*Coordinates*		*Altitude (m)*
		Berry: shape	*Berry: color of skin*		*North*	*East*	
1	*Cam Üzümü*	Globose	Green yellow	Kozan/Çandır	39°15'07"	35°33'16"	1270
2	*Kırmızı Bulut*	Globose	Dark red violet	Kozan/Çandır	39°15'07"	35°33'17"	1269
3	*Zilifder*	Globose	Green yellow	Kozan/Çandır	39°15'06"	35°33'16"	1268
4	*Kara Üzüm*	Globose	Dark red violet	Kozan/Çandır	39°15'06"	35°33'17"	1268
5	*Karanlıkdere Beyazı*	Globose	Green yellow	Kozan/Çandır	39°15'05"	35°33'19"	1265
6	*Candır Üzümü*	Cylindric	Dark red violet	Çandır	39°14'39"	35°31'03"	1224
7	*Kara Bulut*	Globose	Dark red violet	Çandır	39°14'44"	35°30'54"	1231
8	*Çiğitsiz*	Broad ellipsoid	Green yellow	Çandır	39°14'45"	35°30'54"	1233
9	*Mor Üzüm*	Globose	Grey	Çandır	39°14'38"	35°31'04"	1222
10	*Gül Üzümü*	Broad ellipsoid	Rose	Çandır	39°14'38"	35°31'03"	1223
11	*Eldaş*	Globose	Green yellow	Çandır	39°14'37"	35°31'03"	1222
12	*Ak Üzüm*	Globose	Green yellow	Kozan/Çandır	39°14'55"	35°32'57"	1273
13	*Dirmit*	Broad ellipsoid	Dark red violet	Kozan/Çandır	39°15'12"	35°33'22"	1280
14	*Sarı Üzüm*	Globose	Green yellow	Kozan/Çandır	39°15'14"	35°33'25"	1289
15	*Şıralık*	Globose	Dark red violet	Kozan/Çandır	39°15'01"	35°33'06"	1275
16	*Gök Üzüm*	Globose	Grey	Çandır	39°14'37"	35°31'02"	1222
17	*Mis Üzümü*	Broad ellipsoid	Green yellow	Kozan/Çandır	39°15'05"	35°33'18"	1265
18	*Dağ Karası*	Broad ellipsoid	Dark red violet	Kozan/Çandır	39°15'15"	35°33'23"	1284
19	*Kuş Üzümü*	Globose	Green yellow	Çakmak/Boğazlıyan	39°18'03"	35°11'26"	1311
20	*Gelinparmağı*	Horn shaped	Green yellow	Çakmak/Boğazlıyan	39°18'03"	35°11'25"	1313
21	*Çavuş*	Broad ellipsoid	Green yellow	Çakmak/Boğazlıyan	39°18'03"	35°11'26"	1312
22	*Kabaeldaş*	Globose	Green yellow	Çakmak/Boğazlıyan	39°18'04"	35°11'27"	1312
23	*Bozdirge*	Globose	Green yellow	Çakmak/Boğazlıyan	39°19'28"	35°11'59"	1270
24	*Baldırıkızıl*	Broad ellipsoid	Dark red violet	Çakmak/Boğazlıyan	39°18'04"	35°11'28"	1312
25	*Beyaz Patpat*	Globose	Green yellow	Cankılı/Şefaatli	39°33'11"	34°41'15"	881
26	*Karaevlek*	Broad ellipsoid	Dark red violet	Cankılı/Şefaatli	39°33'12"	34°41'15"	872
27	*Mor Patpat*	Broad ellipsoid	Dark red violet	Cankılı/Şefaatli	39°33'12"	34°41'15"	874
28	*Hevenk*	Broad ellipsoid	Green yellow	Cankılı/Şefaatli	39°33'12"	34°41'15"	878
29	*Köftür*	Globose	Green yellow	Cankılı/Şefaatli	39°33'11"	34°41'15"	881
30	*Karaburcu*	Globose	Dark red violet	Cankılı/Şefaatli	39°33'10"	34°41'14"	890
31	*Şeker Üzümü*	Globose	Grey	Cankılı/Şefaatli	39°33'10"	34°41'13"	889
32	*Pembe Üzüm*	Broad ellipsoid	Rose	Cankılı/Şefaatli	39°33'12"	34°41'24"	870
33	*Cafer Üzümü*	Broad ellipsoid	Dark red violet	Cankılı/Şefaatli	39°33'11"	34°41'16"	878
34	*Kaya Üzümü*	Globose	Dark red violet	Cankılı/Şefaatli	39°33'12"	34°41'16"	875
35	*Alaca Üzüm*	Globose	Dark red violet	Cankılı/Şefaatli	39°33'12"	34°41'16"	872
36	*Ekşi Kara*	Globose	Dark red violet	Cankılı/Şefaatli	39°33'11"	34°41'16"	882
37	*Erik Üzümü*	Globose	Blue black	Cankılı/Şefaatli	39°33'12"	34°41'16"	875
38	*Yerli Kara*	Globose	Dark red violet	Babayağmur/Sarıkaya	39°22'04"	35°28'28"	1262
39	*Mor Bulut*	Globose	Grey	Babayağmur/Sarıkaya	39°22'03"	35°28'28"	1263
40	*Göğcek*	Globose	Green yellow	Babayağmur/Sarıkaya	39°22'03"	35°28'37"	1265
41	*Şahmuratlı Üzümü*	Broad ellipsoid	Green yellow	Şahmuratlı/Sorgun	39°44'42"	35°05'20"	1170
42	*Siyah Üzüm*	Broad ellipsoid	Dark red violet	Şahmuratlı/Sorgun	39°44'43"	35°05'20"	1172
43	*Köledoyuran*	Obloid	Green yellow	Şahmuratlı/Sorgun	39°44'30"	35°05'27"	1157
44	*Kirpi Üzümü*	Obloid	Green yellow	Şahmuratlı/Sorgun	39°44'43"	35°05'19"	1172
45	*Horoz Üzümü*	Narow ellipsoid	Grey	Şahmuratlı/Sorgun	39°44'33"	35°05'28"	1158
46	*Tatlı Kara*	Globose	Dark red violet	Şahmuratlı/Sorgun	39°44'41"	35°05'19"	1169
47	*Karagevrek*	Broad ellipsoid	Dark red violet	Şahmuratlı/Sorgun	39°44'38"	35°05'30"	1158
48	*Misket Üzümü*	Obloid	Green yellow	Şahmuratlı/Sorgun	39°44'37"	35°05'30"	1158
49	*Parmak Üzümü*	Horn shaped	Green yellow	Şahmuratlı/Sorgun	39°44'38"	35°05'31"	1158
50	*Bulut Üzümü*	Globose	Dark red violet	Şahmuratlı/Sorgun	39°44'37"	35°05'31"	1158

temperature specific to the primer pair for 30 s (annealing); and 72 °C for 30 s (extension), and a final extension at 72 °C for 3 min gave the best amplification for all the primer pairs. PCR products belonging to the loci were checked in a 2% agarose gel electrophoresis environment according to fragment sizes to determine whether amplification had occurred. Amplified samples were diluted with 20 µl SLS (Sample Loading Solution) in different proportions according to the fluorescent dyes used in labelling (D2, D3, and D4), and then 0.2 – 0.4 µL the standard – 400 was added. Allele types (homozygous and heterozygous) and allele sizes (bp) at all loci were analysed with Bioptic Qsep100 DNA / RNA Fragment Analyzer using a high – resolution cartridge.

2.4. Data analysis

After genotyping of grape varieties was completed, genetic diversity and differentiation indices at both population and locus levels were calculated using GenAIEx 6.51b2 software, according to Nei (1987)'s unbiased genetic similarity and genetic difference coefficients. Coordinate graphs based on SSR allele sizes of varieties were created using GenAIEx 6.51b2 programme.

NTSYSpc v.2.10e programme was used to determine phylogenetic relationships between loci (Rohlf, 1998). Genetic Similarity Matrix was calculated according to the SM (Simple Matching) parameter of Sokal and Michener (1958). The dendrogram was drawn according to the SM coefficient based on UPGMA (Unweighted Pair Group Method with Arithmetic Average). Populations are structured into genetically distinct subpopulations (Intarapanich et al., 2009). Analysis of population structure involves grouping individuals into subpopulations based on common genetic variations.

The population structure was investigated through clustering based on the Bayesian model in which the Markov Chain Monte Carlo (MCMC) algorithm was applied in Structure v.2.3.4 software (Pritchard et al., 2000). In this model, a number of populations (K) are assumed to be present that are characterised by a set of allele frequencies at each locus. The MCMC process begins with the random assignment of individuals to a predetermined number of populations (clusters), then variable frequencies are estimated in each group, and individuals are reassigned based on these frequency estimates. This process involved the burning process that results in progressive convergence towards reliable allele frequency estimates in each population and membership probabilities of individuals to a population. Delta K (ΔK) method (Evanno et al., 2005) was used in the Structure Harvester programme to determine the best K cluster (Earl & von Holdt, 2012). LnP(D) (logarithm probability for each K) values were calculated, and the logarithm probability curve L(K) was drawn. The simulations were created with 10 independent repetitions for each K (the number of inferred genetic clusters) value ranging from 1 to 10, with a burn-in of 100 000 and 1 000 000 MCMC. Delta K, based on the second-order ratio of change in LnP(D), was calculated (ΔK = 2 to ΔK = 10) and the graph drawn. The information about the probable population number was shown with the highest K of Delta K in the diagram.

3. Results

Molecular definitions were performed using 9 SSR loci on 52 grape varieties, including 50 autochthonous and 2 reference varieties. Allele sizes at all loci were recorded as peak data in the fragment analysis system.

3.1. Genetic diversity and differentiation in the population

Genetic diversity and differentiation indices at the population and locus level were analysed according to the Hardy – Weinberg equilibrium principle (Table 2). Allele – frequency plots were created using the Genalex 6.51b2 programme (Figure 1).

3.2. Genetic relationships among grapevine varieties

When the UPGMA dendrogram was examined, it was observed that the varieties were divided into 2 main clusters. The varieties showing the highest similar rate in cluster 1 were Mor Üzüm (No. 9) and Kabaeldaş (No. 22) with a similarity coefficient of 0.44. Cluster 2 was mainly divided into 3 subgroups. The 1st subgroup of cluster 2 was divided into 2 main branches. While the varieties showing the highest similarity rate were Kuş Üzümü (No. 19) and Çavuş (No. 21) with a similarity coefficient of 0.44 in the branch 1, it was determined as Horoz Üzümü (No. 45) and Karagevrek (No. 47) with a similarity coefficient of 0.5 in branch 2. In the 2nd subgroup of Cluster 2, Merlot (No. 51), Cabernet Sauvignon (No. 52) and Şahmuratlı Üzümü (No. 41) in branch 1; Göğcek (No. 40) and Tatlı Kara (No. 46) took place in branch 2. However, Kaya Üzümü (No. 34) and Ekşi Kara (No. 36) grouped separately in the 3rd subgroup on the dendrogram. These findings indicated that reference varieties had similar alleles with some autochthonous varieties, but the Kaya Üzümü and Ekşi Kara varieties had unique alleles. Consequently, the much branching of the dendrogram, on which the grapevine genotypes were visualized, showed that the sample population had high genetic diversity. The enumerations of the varieties in the Coordinate Graph (Figure 1) and UPGMA Dendrogram (Figure 2) were arranged based on the ranking system presented in Table 1.

3.3. Structure analysis

Structural genetic analysis was performed on 52 grapevine genotypes with 9 SSR primers using Structure and Structure Harvester programmes. As a result of the

Table 2. Number of alleles (n), number of polymorphic alleles (Na), number of effective alleles (Ne), Shannon diversity index (I), polymorphism information content (PIC), observed heterozygosity (Ho), and expected heterozygosity (He) values based on 9 SSR primers used for *V. vinifera* genotypes.

SSRs	**n**	**Na**	**Ne**	**I**	**PIC**	**He**	**Ho**
VVS2	51	20	12.657	2.733	0.916	0.921	0.471
VVMD5	49	24	13.084	2.858	0.919	0.924	0.143
VVMD7	52	27	14.696	2.980	0.928	0.932	0.558
VVMD24	52	20	13.287	2.767	0.920	0.925	0.673
VVMD27	52	25	15.234	2.921	0.931	0.934	0.154
VVMD28	40	36	23.188	3.351	0.955	0.957	0.400
VVMD31	51	22	11.612	2.703	0.908	0.914	0.529
VrZAG62	52	25	13.386	2.885	0.920	0.925	0.942
VrZAG79	52	34	20.880	3.264	0.950	0.952	0.462
Total	451	233	138.025	26.461	8.346	8.384	4.331
Mean	50.11	25.89	15.336	2.940	0.927	0.932	0.481
SE	1.306	1.896	1.328	0.076	-	0.005	0.082

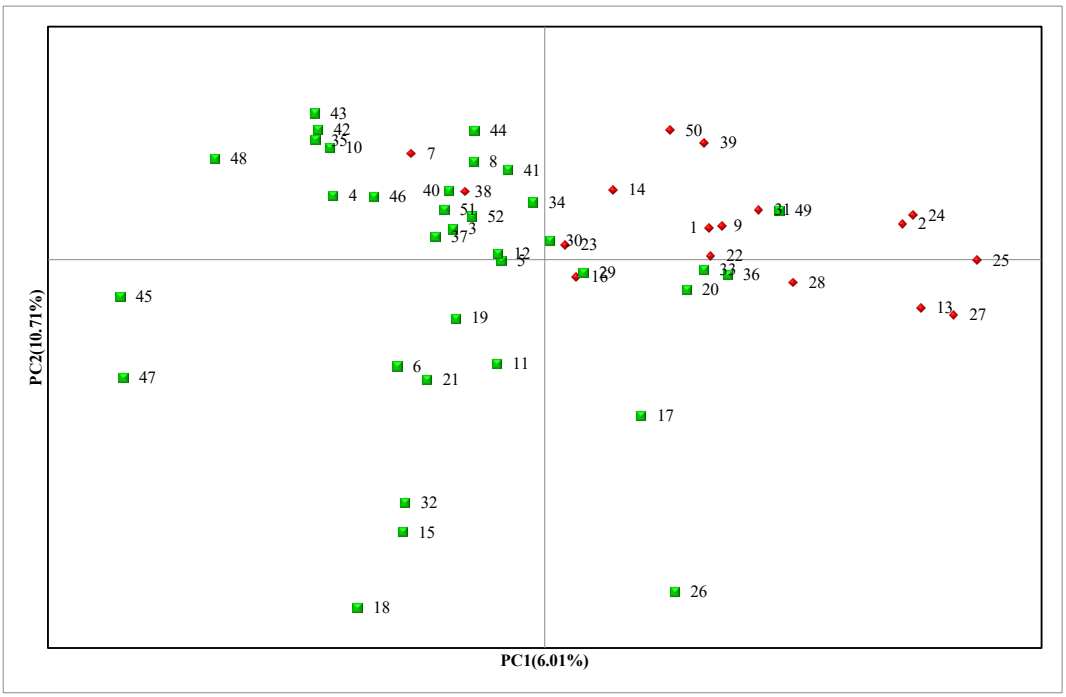

Figure 1. Principal coordinates graph (PCoA) of *V. vinifera* genotypes. In the graph, two main clusters were defined, represented by red (cluster 1) and green (cluster 2) coloured points.

analysis, the highest ΔK value corresponding to the most probable population number was found as 2. Additionally, ΔK = 3, corresponded to the number of subpopulations in the study (Figure 3). According to ΔK = 2, both populations had the admixture of alleles, and no pure line was observed except for Alaca Üzüm (No. 35), Horoz Üzümü (No. 45) and Tatlı Kara (No. 46) genotypes. In the structure analysis, all the genotypes were divided into two main clusters similar to UPGMA tree analysis results (Figure 2). Genetic association dendrograms of the varieties were similar to structural genetic analysis. Furthermore, in ΔK = 3, genotypes were divided into three subpopulations. All three subpopulations had mutual alleles inside and outside of the assigned clusters, with the exception of No.

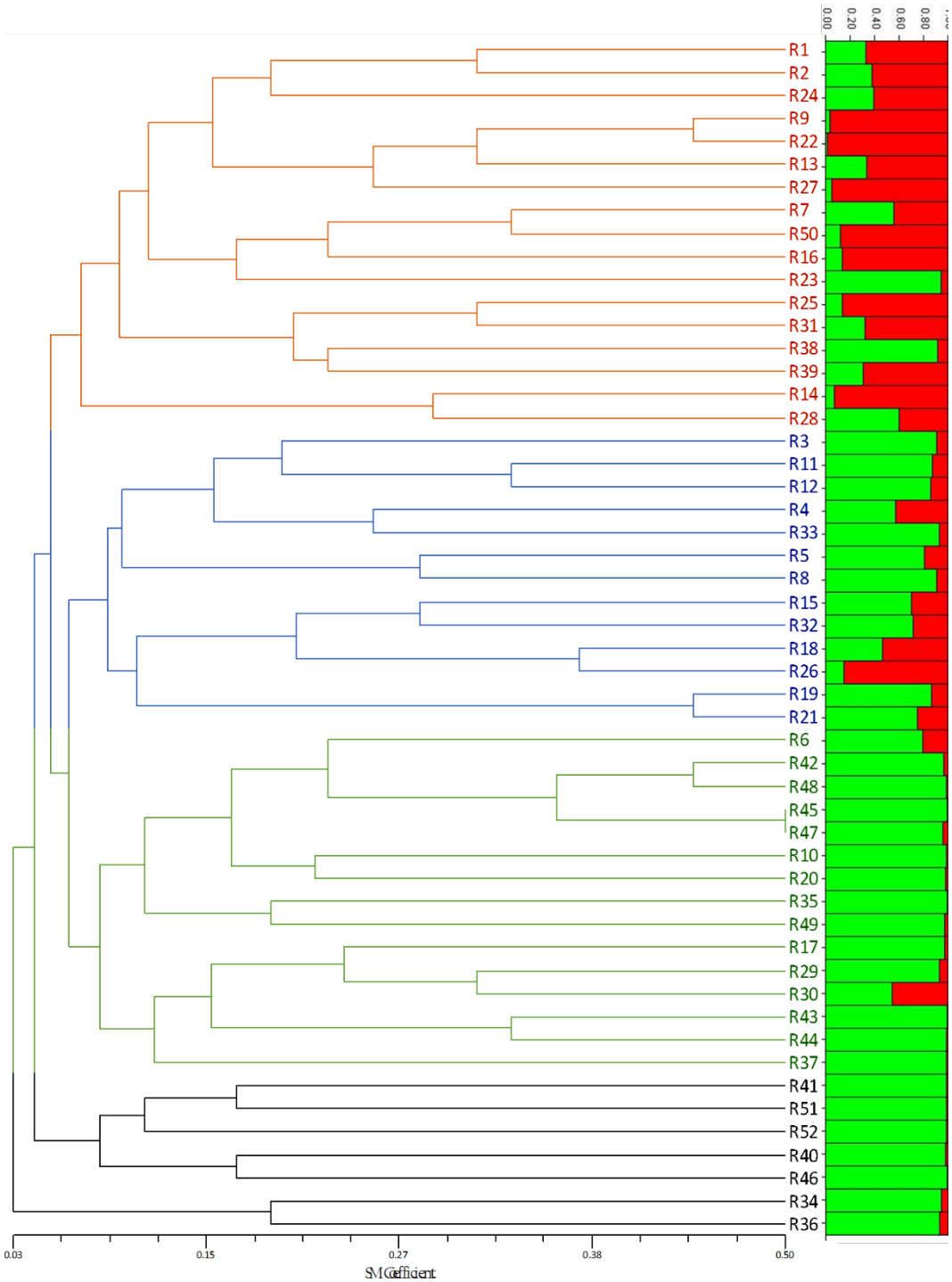

Figure 2. Left: The unweighted pair group method with arithmetic average (UPGMA) clustering pattern. Right: Results of STRUCTURE (ΔK = 2) analysis of 52 *V. vinifera* genotypes. Each bar represented an individual, in which, first and second clusters were presented by red and green, respectively.

25, 39, and 50 in the first subpopulations, No. 10, 35, 45, 46, and 48 in the second subpopulations, No. 19, 21, and 32 in the third subpopulations. In the structure analysis (Figure 4), each individual was represented by a single vertical bar divided into coloured tabs according to their estimated membership in subpopulations 2 to 10. It was the probability of those assigned to any set K on the Y-axis. The black line separates the varieties from each other. In

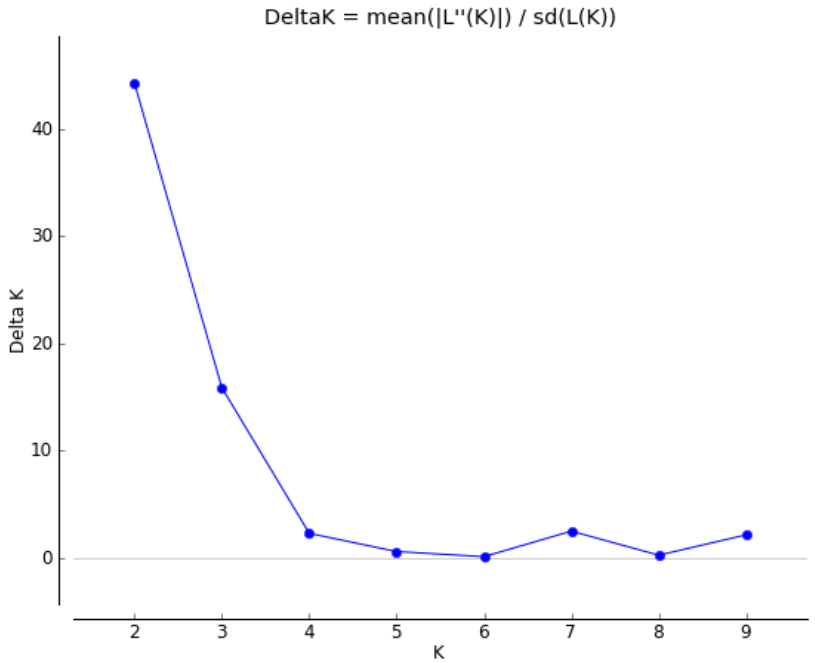

Figure 3. Value of ΔK, that the rate of change in the log probability of data between successive K values, as described by Evanno et al. (2005), estimated for the structure analysis of *V. vinifera* genotypes (ΔK = 2 populations and ΔK = 3 subpopulations).

the Structure analysis (Figure 4), the numbering of the varieties was arranged according to the ranking system given in Table 1.

4. Discussion

4.2. SSR polymorphism

Microsatellites (simple sequence repeats, SSRs) have been the most commonly used genetic marker in population genetics over the past 20 years (Vieira et al., 2016). SSRs, have been preferred due to their codominant structure, abundance in the genome, show high polymorphism, suitability for automation, and reproducibility (Kacem et al., 2017). Additionally, SSRs are widely utilised in grapevine genetic studies for the identification of varieties (Sefc et al., 1999; Martin et al., 2003; Ibañez et al., 2003), parentage analysis (Bowers and Meredith 1997; Bowers et al., 1999a), genome mapping (Doligez et al., 2002; Riaz et al., 2004) and genetic characterisation of germplasm (Lopes et al., 1999; Sefc et al., 1999). In this context, various studies have been carried out to determine the molecular characterization and genetic relatedness of locally distributed autochthonous grapevine genotypes based on SSR markers (Hızarcı, 2010; Karaca - Sanyürek, 2014; Ovayurt, 2017). As a result of our research, 451 alleles (n), 233 of which polymorphic (Na), were obtained. The highest number of alleles was 52 at the VVS2, VVMD24, VVMD27, VrZAG62, and VrZAG79 loci. The lowest number of alleles was determined as 40 at the VVMD28 locus, the average number of alleles 50.11 (± 1.306), and the average number of polymorphic alleles 25.89 (± 1.896). The number of alleles obtained according to the results of population genetics was considerably high. Our results showed that the grapevine population in Yozgat is genetically heterogeneous. Karaca – Sanyürek (2014) obtained 61 alleles because of genetic analysis performed with 6 SSR loci of 54 grape varieties and she found the highest number of alleles as 12 in the VVMD5 locus. In our study, the number of effective alleles (Ne) varied from 11.612 (VVMD31) to 23.188 (VVMD28). The average effective allele numbers as 15.336 (± 1.328) were found to be lower than the mean allele numbers. In our research, expected heterozygosity (He) values were in the range of 0.914–0.957, the observed heterozygosity (Ho) ratios varied between 0.143 and 0.942. The average expected heterozygosity (He) value was calculated as 0.932 (± 0.005) and the average observed heterozygous (Ho) value 0.481 (± 0.082). The expected heterozygosity (He) value at the VVMD28 (0.957) and VrZAG79 (0.952) loci and the observed heterozygosity (Ho) value at the VrZAG62 (0.942) and VVMD24 (0.673) loci were highest. In our study, the expected heterozygosity values were found to be higher than the heterozygosity values observed in 8 loci. Gök Tangolar et al. (2009) determined the average observed heterozygosity as (Ho) 0.743 and expected heterozygosity 0.749. According to the data we obtained from our study, polymorphism information content (PIC)

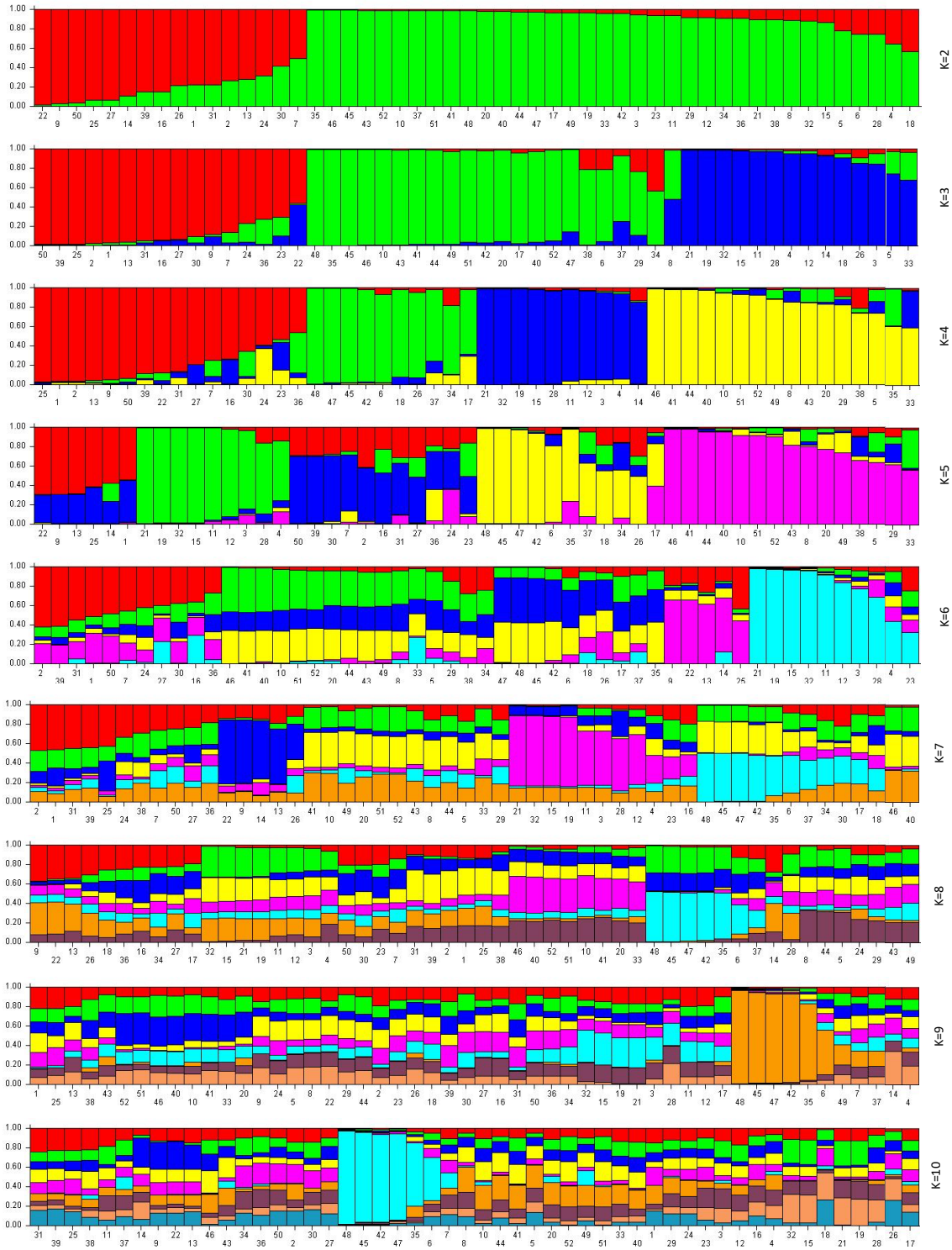

Figure 4. Population structure of 52 *V. vinifera* genotypes estimated from 9 SSRs using structure (ΔK = 2 to 10). Each bar represented an individual, in which, different color represents the estimated membership coefficients.

value varied between the highest 0.955 (VVMD28) and the lowest 0.908 (VVMD31), and the average PIC values of all loci were found to be 0.927. The Shannon diversity index (I) was observed at the highest VVMD28 locus (3.351) and the lowest VVMD31 locus (2.703). Taheri and Ramandi (2020) reported that because of the genetic analysis of 25 local grapevine accessions with 14 SSR markers, the PIC value varied between 0.50 and 0.87, and

the Shannon diversity index varied between 0.79 and 2.13. In our research, allele frequencies ranged between 0.01 and 0.184. When the allele – frequency distribution ratios in the loci were examined, it was observed that it varies between 0.01 and 0.184. 244 with 0.184 allele frequency at VVMD5 locus, 212 with 0.167 allele frequency at VVMD31 locus, and 193 with 0.163 allele frequency at VrZAG62 locus were the most common alleles. Hızarcı (2010) according to the distribution of alleles in loci the highest allele frequencies values determined in the loci VrZAG83 (191), VVMD27 (185), VVMD24 (207) and VVMD7 (246).

4.3. Genetic relationships among grapevine varieties

According to the results of phylogenetic analysis, similarity coefficients among varieties ranged from 0 to 0.50. The varieties showing the highest similarity with 0.50 were Horoz Üzümü (No. 45) and Karagevrek (No. 47). The varieties were divided into two main clusters according to the genetic relationship dendrogram. Cluster 1 revealed less genetic diversity than cluster 2 that showed a wider range of genetic variation, including unique alleles. In the 2nd subgroup of cluster 2, it was determined that the Merlot (No. 51) and Cabernet Sauvignon (No. 52) branched together with some autochthonous varieties (No. 40, 41, and 46). In many studies using Merlot and Cabernet Sauvignon as reference varieties, it was determined that the reference varieties were clustered separately with the autochthonous varieties (Garğin, 2014; Karaca - Sanyürek, 2014; Ovayurt, 2017). However, similar to our findings, Hızarcı et al. (2012), in their study examining the genetic characterisation and relatedness levels of autochthonous grapevine varieties in Northeast Turkey with SSR loci, determined that two reference varieties (Cabernet Sauvignon and Merlot) and two autochthonous varieties (Mandagözü and Beyaz Istanbul) were in the same subgroup. The grouping of European and Turkish autochthonous grapevine populations together indicates that the grapevine from the Yozgat region could have originated from a common genetic background with reference varieties. In addition to these, in the 3rd subgroup of the 2nd cluster, it was determined that Kaya Üzümü (No. 34) and Ekşi Kara (No. 36) varieties were clustered separately on the dendrogram. These high levels of within-group variation observed probably suggest a complex history of the development of grapevine varieties in Yozgat. Our data suggested that these varieties grouped separately might have originated from the Transcaucasia region and introduced through routes like trade or human migration. Similarly, Hızarcı et al. (2012) reported that one of the grapevine varieties they collected from Northeast Turkey clustered separately on the dendogram and that this variety might have been brought to the Çoruh Valley from the island of Cyprus and preserved its genetic structure there. According to the results of the research, 100% similarity synonymous varieties were not found among analysed varieties in the population under study. The Basic Coordinates Analysis (PCoA) showed that according to the codominant genotypic distance method, 31.89% of the cumulative variation for 52 grapevine genotypes was explained in the first three coordinates (Figure 1). However, it was observed that some genotypes spread out of the main clusters. Emanuelli et al. (2013) analysed 2 273 accessions of *Vitis vinifera* spp. *sativa* and their wild relatives (*V. vinifera* ssp. *sylvestris*) using 22 microsatellite loci based on genetic distance matrix. They reported that PCoA was explained in the first and second axes with rates of 38.51% and 21.29%, respectively.

Structural analysis has many applications in population genetic studies and is highly informative for understanding genetic diversity (Eltaher et al., 2018). This analysis is used to obtain a clear insight into the underlying genetic population substructure and is a crucial prerequisite for any analysis of genetic data, such as genome-wide association studies, to eventually reduce false-positive rates (Alhusain and Hafez, 2018). It also provides more information for selecting genetically different varieties for future hybridisation programmes (Olukolu et al., 2012). According to our research results, ΔK criteria proposed by Evanno et al. (2005) reached the maximum value at K=2, which corresponded to the most probable number of populations in the study. The dendrograms of these varieties' relationships were similar to the structural genetic analysis (Figure 2). Similar to our research results, Bakker et al. (2009) analyzed 179 individuals from *B. distachyon, B. hybridum,* and *B. stacei* species with 12 microsatellite loci using structure software and found ΔK=2 indicating two geographic clusters.

5. Conclusion

This article has proven once again that microsatellite analysis is a powerful tool for the characterization of grapevine varieties. Thanks to this study, which has been the first to identify comprehensively the grapevine genetic resources grown in Yozgat province by verifying with molecular techniques, significant variations have been revealed among the varieties. With this research, it has been observed that there was a significant amount of genetic variation in the gene pool of grape varieties grown in Yozgat province. Considering the environmental conditions of the Yozgat, it has been expected that the grapevine germplasm in the region would have economically important adaptive traits that can potentially be incorporated into grapevine breeding programs. The studies performed on germplasm characterisation are essential for effective hybridisation programmes in the future.

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