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RESEARCH ARTICLE

Estimating genetic diversity among selected wild grapevine genotypes from Southern Turkey by simple sequence repeat (SSR) and inter-Primer Binding Site(iPBS) markers

Hatice Ikten · Duygu Sari · Ali Sabir · Hasan Meydan · Nedim Mutlu

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Abstract The examination of germplasm within grapevine accessions derived from wild genotypes holds significant importance within the grapevine breeding program, particularly in the improving of cultivars and rootstocks. Due to the greater genetic variation present in wild genotypes, there is an increased possibility of possessing the desired features. The objective of this study was to determine the genetic diversity and population genetic structure of 64 grapevine genotypes using inter-primer binding site (iPBS) retrotransposon markers and simple sequence repeat (SSR) markers. A total of 236 bands were generated using iPBS markers, of which 162 bands exhibited polymorphism. A comprehensive assessment was conducted on a total of 126 SSR alleles using the SSR markers, revealing that 91 of these exhibited polymorphisms. Despite the similarity in mean values between polymorphic bands generated by iPBS (6.48) and SSR markers (6.5), the iPBS markers exhibited a greater polymorphism information content (PIC: 0.39) in comparison to SSR markers (0.29). The UPGMA analysis classified the genotypes into two primary groups at a similarity index of 0.62 based on combined data. The rootstocks utilized as points of reference are consolidated inside a singular cluster (A), distinct from both the Mediterranean wild population and cultivars. The comparison of genetic variation, represented by FST values, revealed that the maximum differentiation was observed between subpopulations SP3 and SP5. The wild grapevine population grown in the Southeast Mediterranean Region of Turkey exhibited significant differentiation. Both marker systems employed in this study were highly polymorphic and useful for genetic characterization and mapping of grapevine populations.

Keywords Molecular characterization · *Vitis* · Microsatellite · Retrotransposons · Genetic resources

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Introduction

The identification and utilization of natural variability and genotypic divergence have proved essential in enhancing the quality and productivity of agricultural species. Genetic diversity affords breeders the opportunity to discern and choose superior genotypes, which can subsequently be utilized as novel cultivars or included into breeding programs as parental lines. The observable diversity exhibited

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by wild cousins, as well as their capacity to thrive in harsh and challenging conditions, has consistently proven to be of great value to breeders. The wild counterparts of farmed *Vitis vinifera* L. possess significant potential as reservoirs of important genetic resources, which can be utilized for the purpose of breeding novel grapevine cultivars or rootstocks that exhibit desirable traits. In general, cultivated genotypes exhibit a limited range of genetic variability, which poses a challenge for plant breeders seeking to identify and select plants carrying specific desired genes. The transmission of resistance to disease, pests and environmental conditions from wild relatives has been shown in numerous crop species (Qi et al. 2019). The distribution of grape genetic variety in Central Asia (including the Caucasus and China) is comparatively limited when compared to the abundance of naturally growing and cultivated grapevines found in the Mediterranean basin, despite the significant historical significance of Central Asia in the domestication of grapevine (Riaz et al. 2018). According to Arroyo-García et al (2006), researchers suggest that the process of domestication likely originated in the Near-East and subsequently facilitated secondary domestication occurrences throughout the Mediterranean basin. Based on the study carried out by This et al. (2006), archaeological evidence suggests that seeds of cultivated grape cultivars have been discovered in the regions of Georgia and Turkey. Hauptmann (1997) and Pasternak (1998) have documented the discovery of the most ancient seeds of *Vitis vinifera* ssp. *sylvestris*, the wild grape variety, around 8400 years before present. These seeds were unearthed in Turkey, specifically on the valley adjacent to the Euphrates River. Anatolia is situated within the geographical region that is widely recognized as the ancestral territory of winemaking. The plant diversity in the Mediterranean Region is attributed to its geographical, climatic, and historical factors (Valdés Castrillón and Hernández Bermejo 1993). Additionally, the cultivation of grapevines holds significant historical significance in the Mediterranean area. According to FAOSTAT (2023), Turkey has the 5th position globally in terms of grape production area, which spans across 400,000 hectares, accounting for 5.85% of the total. Additionally, Turkey ranks 6th in grape production volume, with a total output of 4.2 million tons, representing 5.32% of the global production.

Genetic erosion, similar to other agricultural crops, leads to a reduction in agrobiodiversity within grapevine cultivation. Grape breeders continue to demonstrate a keen interest in augmenting the genetic variety of grape cultivars and fortifying the population by incorporating novel wild relatives. This endeavor aims to generate resistant cultivars capable of withstanding diseases, pests, and adverse abiotic stress conditions (Li et al. 2021). Despite the significance of wild genotypes in the context of breeding, there has been a scarcity of research undertaken on the characterization of wild genotypes in Turkey. Hence, it is imperative to allocate due attention towards the characterization and protection of wild accessions, as well as the exploitation of local genotypes. Morphological characterizations are commonly employed to evaluate genetic diversity; nevertheless, it is important to acknowledge that they might be subject to environmental influences and the growing cycle of the plant. Whereas, molecular markers offer a means to acquire data of higher accuracy. In this work, the genetic structure of the wild grapevine population under investigation was elucidated by the utilization of simple sequence repeats (SSR) and inter primer binding site (iPBS) markers. The microsatellite marker, commonly known as the simple sequence repeats (SSR), is a highly effective and cost-efficient molecular tool utilized for the measurement of genetic diversity, in comparison to the more advanced genome sequencing technology (Powell et al. 1996). SSR marker system demonstrates a high rate of polymorphism, and is characterized by its repeatability and reliability. SSR markers have been widely utilized in the examination of genetic diversity in several crop species (Zhang et al. 2012; Uzan Eken et al. 2022) as well as in the assessment of grapevine genotypes (Riaz et al. 2018; Sabır et al. 2018; Li et al. 2021; Cretazzo et al. 2022). The utilization of iPBS retrotransposon markers, also known as inter-primer binding site markers, is based on retrotransposons that integrate into novel genomic locations through an RNA intermediate. Retrotransposons classified into two distinct class, namely the long terminal repeat (LTR) retrotransposons and the non-LTR retrotransposons. LTR retrotransposons are qualified by the existence of a primer binding site that conserves tRNA. The development of iPBS markers is reliant on the variety of primer binding sites within

retrotransposon elements, without requiring prior knowledge of a specific sequence (Kalendar et al. 2010; Kalendar and Schulman 2014). iPBS markers have already been used in various plant species (Borna et al. 2016; Coutinho et al. 2018) and *Vitis vinifera* L. (Guo et al. 2014; Milovanov et al. 2019). The aim of this study was molecular characterization of wild grapevine genotypes that are commonly growing in the Southeast Mediterranean area of Turkey, as well as reference cultivars and rootstocks and explore the genetic resources of wild grapevines, identify novel genotypes, and enhance genetic diversity for potential utilization in breeding programs.

Materials and methods

A total of 64 grapevine genotypes were used in this study, of which 54 were collected from mountainous locations of Southeast Mediterranean Region of Turkey in the province of Mersin including Tarsus, Aydıncık, Bozyazı, Gülnar, Anamur and Antalya including Manavgat along with six reference rootstocks (41B, 140Ru, Rupestris du Lot, 5BB, 420A, 1613C) and four international reference cultivars ('Victoria', 'Michael Palieri', 'Alphonse Lavallée' and 'Italia' provided from the grapevine germplasm repository of Selcuk University Agriculture Faculty Horticulture Department, Konya, Turkey. Genotype list was given in Table 1.

DNA was isolated from fresh-frozen leaves using CTAB solution (Doyle and Doyle 1990) and quantified electrophoretically using lambda standard DNA on 0.8% agarose gel.

SSR analysis

SSR markers reported to be highly polymorphic in the previous research were used for analysis. Fourteen polymorphic microsatellite loci were analyzed: VVMD5, VVMD7, VVMD17 (Bowers et al. 1996), VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD31, VVMD32, VVMD34, VVMD36, (Bowers et al. 1999), VrZAG62, and VrZAG79 (Sefc et al. 1999). Five of these markers namely VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 comprise the international standard for grape SSR data for future (This et al. 2004). The PCR amplification reactions performed according to Bowers et al. 1996 and Sefc et al. 1999). Reaction was performed using a thermal cycler (BioRad model My CyclerTM thermal Cycler). PCR amplicons were loaded in 6% polyacrylamide gel (19:1) and visualized in a Li-Cor -IR2 4200 Genetic Analyzer (Li-Cor Biosciences) therefore 5′ end of each forward primer was tagged with either IRDye-700 or -800 fluorescent dye.

iPBS analysis

In this study, 25 iPBS primers (Table 3) were selected on the basis of high polymorphism potential. PCR reaction mix and thermocycler conditions were applied for each primer according to protocol by Kalendar et al. (2010). PCR products were visualized in 2% agarose gel.

Data analysis

All genotypes were evaluated for the presence (1) and absence (0) of the bands. The clearly visible bands were considered for the analysis. The similarity matrices generated using SIMQUAL (Similarity for Qualitative Data) procedure choosing SM (Simple Matching) Coefficient (Sneath and Sokal1973) in NTSYS-pc version 2.0 (Numerical Taxonomy System) (Rohlf 1998) and dendrograms were arranged utilizing the similarity matrices separately for iPBS, SSR and combined data using UPGMA (Unweighted Pair Group Method with Arithmetic Average) and choosing SAHN module (Sneath and Sokal 1973). Genetic identity, genetic distance, expected (He) heterozygosity, number of polymorphic loci (NPL), percentage of polymorphic loci (PPL), number of alleles (Na), effective number of alleles (Ne)Shannon's information index (I) and gene flow (Nm) values were calculated by using POPGENE version 1.32 (Yeh et al. 1997).The ARLEQUIN software (Excoffier et al. 2005) was used to evaluate the genetic variation by the analysis of molecular variance (AMOVA) with 1000 permutations and to calculate the pairwise F_{ST} among subpopulations based on combined data of SSR and iPBS marker patterns. The genetic structure of the population was analyzed using STRUCTURE program ver.2.3 (Pritchard et al. 2000) via Bayesian clustering procedure. Ten independent runs for each K value (2–10) were performed based on admixture model of 100,000 iterations after a burn-in period

Table 1 Geographical location of sampling sites of grapevine genotypes, geographic coordinates and area altitude

No	Code	Origin	GBS	Altitude (m)
1	33BZ01	Bozyazı/Mersin	36 08.316N 032 57.695E	96
2	33BZ02	Bozyazı/Mersin	36 08.274N 032 57.560E	79
3	33BZ03	Bozyazı/Mersin	36 08.337N 032 57.565E	65
4	33BZ04	Bozyazı/Mersin	36 08.345N 032 57.608E	71
5	33BZ05	Bozyazı/Mersin	36 08.375N 032 57.613E	72
6	33BZ06	Bozyazı/Mersin	36 05.336N 032 54.676E	23
7	33BZ07	Bozyazı/Mersin	36 05.375N 032 54.686E	42
8	33BZ08	Bozyazı/Mersin	36 08.001N 033 13.270E	139
9	33BZ09	Bozyazı/Mersin	36 11.715N 033 21.578E	173
10	33BZ10	Bozyazı/Mersin	36 08.109N 033 10.646E	6
11	33AN01	Anamur/Mersin	36 04.58.39N 32 53.56.32E	2
12	33AN02	Anamur/Mersin	36 05.25.66N 32 55.32.05E	63
13	33BZ11	Bozyazı/Mersin	36 05.39.19N 32 56.32.82E	23
14	33BZ12	Bozyazı/Mersin	36 06.09.12N 32 58.10.74E	4
15	33AY01	Aydıncık/Mersin	36 08.00.18N 33 13.33.37E	185
16	33GL01	Gülnar/Mersin	36 16.661N 033 22.837E	639
17	33GL02	Gülnar/Mersin	36 25.300N 033 30.140E	638
18	33GL03	Gülnar/Mersin	36 28.45.11N 33 32.59.03E	118
19	33TA01	Tarsus/Mersin	37 03.27.07N 34 56.2328E	94
20	33BZ13	Bozyazı/Mersin	36 08.033N 033 12.757E	181
21	33AN03	Anamur/Mersin	36 09.793N 032 54.272E	86
22	33AN04	Anamur/Mersin	36 09.810N 032 54.301E	109
23	33AN05	Anamur/Mersin	36 09.690N 032 54.859E	161
24	33AN06	Anamur/Mersin	36 09.349N 032 55.207E	153
25	33AN07	Anamur/Mersin	36 09.342N 032 55.199E	149
26	33BZ14	Bozyazı/Mersin	36 08.957N 032 55.838E	119
27	33BZ15	Bozyazı/Mersin	36 08.519N 032 56.323E	81
28	33BZ16	Bozyazı/Mersin	36 08.242N 032 56.635E	62
29	33BZ17	Bozyazı/Mersin	36 07.486N 032 57.246E	22
30	33BZ18	Bozyazı/Mersin	36 07.338N 032 57.784E	17
31	33AY02	Aydıncık/Mersin	36 08.012N 033 12.288E	156
32	33AY03	Aydıncık/Mersin	36 13.911N 033 22.360E	587
33	33GL04	Gülnar/Mersin	36 25.300N 033 30.140E	180
34	33AN08	Anamur/Mersin	36 11.162N 032 54.883E	310
35	33AN09	Anamur/Mersin	36 11.185N 032 55.008E	280
36	33AN10	Anamur/Mersin	36 11.162N 032 54.883E	191
37	33AN11	Anamur/Mersin	36 11.153N 032 54.542E	196
38	33AN12	Anamur/Mersin	36 10.511N 032 53.761E	64
39	33BZ19	Bozyazı/Mersin	36 08.920N 032 57.212E	230
40	33BZ20	Bozyazı/Mersin	36 08.906N 032 57.247E	213
41	33BZ21	Bozyazı/Mersin	36 08.867N 032 57.288E	218
42	33BZ22	Bozyazı/Mersin	36 08.818N 032 57.325E	190
43	33BZ23	Bozyazı/Mersin	36 08.783N 032 57.418E	158
44	33BZ24	Bozyazı/Mersin	36 08.768N 032 57.435E	161
45	33BZ25	Bozyazı/Mersin	36 08.681N 032 57.473E	150
46	33BZ26	Bozyazı/Mersin	36 08.677N 032 57.523E	131
47	33BZ27	Bozyazı/Mersin	36 08.456N 032 57.700E	118

Table 1 (continued)

No	Code	Origin	GBS	Altitude (m)
48	33BZ29	Bozyazı/Mersin	36 08.396N 032 57.710E	121
49	33BZ30	Bozyazı/Mersin	36 06.229N 032 57.474E	2
50	33BZ31	Bozyazı/Mersin	36 06.228N 032 57.438E	4
51	33BZ32	Bozyazı/Mersin	36 08.388N 032 57.666E	115
52	'Michel Palieri'	Cultivar	Selcuk Uni. Germplasm	–
53	'Alphonse Lavallee'	Cultivar	Selcuk Uni. Germplasm	–
54	'Italia'	Cultivar	Selcuk Uni. Germplasm	–
55	07MA01	Manavgat/Antalya	36 46.190N 031 39.941E	310
56	07MA02	Manavgat/Antalya	36 46.184N 031 39.831E	295
57	07MA03	Manavgat/Antalya	36 11.112N 032 54.993E	368
58	41B	Rootstock	Selcuk Uni. Germplasm	–
59	140 Ru	Rootstock	Selcuk Uni. Germplasm	–
60	R. du Lot	Rootstock	Selcuk Uni. Germplasm	–
61	5BB	Rootstock	Selcuk Uni. Germplasm	–
62	420A	Rootstock	Selcuk Uni. Germplasm	–
63	1613C	Rootstock	Selcuk Uni. Germplasm	–
64	'Victoria'	Cultivar	Selcuk Uni. Germplasm	–

of 10,000 iterations for all runs. The most likely group (K) was investigated based on Evanno method (Evanno et al. 2005) using STRUCTURE Harvester (Earl and von Holdt 2012). Additionally, a two-dimensional (2D) principal component graph developed using PAST software.

Results

The primers utilized in the study consisted of 14 SSR (Simple Sequence Repeat) and 25 iPBS (Inter Primer Binding Site) markers, all of which exhibited multiple alleles and amplified a total of 362 bands, out of which 253 were found to be polymorphic.

SSR analysis

A total of 126 SSR alleles with an average 9 alleles per locus were amplified from the DNA of 64 genotypes. The highest number of alleles (15) was detected in the locus VVMD27 and the lowest (4) in the locus VVMD17. Total number of polymorphic alleles was 91 with an average 6.5 alleles per locus (Table 2). A polyacrylamide gel picture of SSR primer VVMD25 is given in Fig. 1. A dendrogram was generated utilizing the genetic similarity coefficient were classified genotypes into two primary clusters (A and B) based on a similarity index of 0.61. In the context of cluster analysis, it was shown that the genotype 420A (international rootstocks) exhibited a notable separation from other genotypes, namely within group A. All of the remaining genotypes were classified into Cluster B. The six international reference rootstocks, namely 41B, R. du Lot, 140Ru, 5BB, 1613C, and 420A, were isolated from the population and positioned in the lower section of the dendrogram (Fig. 2).

iPBS analysis

A total of 236 bands were amplified with iPBS primers of which 162 were found polymorphic with an average of 6.48 polymorphic bands per primer (Table 3). The agarose gel picture of iPBS primer 2077 is given in Fig. 3. UPGMA analysis separated the genotypes in two clusters (A and B) at the similarity index of 0.53. Cluster A consist of only one genotype 33BZ12 collected from Bozyazı/Mersin. Cluster B comprised the remaining 63 genotypes (Fig. 4).

Combined data analysis

In the analysis of the combined data of SSR and iPBS markers, a total 39 primers produced 253 polymorphic bands out of 362 total bands with average 72.03% polymorphism rate. The analysis of combined

Table 2 Code and sequence of simple sequence repeat (SSR) markers assayed in the characterization of grapevine accessions

No	Primer code	Primer sequence 5′ 3′	NTB	NPB	%Pol	PIC
1	VVMD5	CTAGAGCTACGCCAATCCAA TATACCAAAAATCATATTCCTAAA	10	7	70	0.13
2	VVMD7	AGAGTTGCGGAGAACAGGAT CGAACCTTCACACGCTTGAT	12	4	33.3	0.30
3	VVMD17	TGACTCGCCAAAATCTGACG CACACATATCATCACCACACGG	4	4	100	0.29
4	VVMD21	GGTTGTCTATGGAGTTGATGTTGC GCTTCAGTAAAAAGGGATTGCG	12	8	66.6	0.25
5	VVMD24	GTGGATGATGGAGTAGTCACGC GATTTTAGGTTCATGTTGGTGAAGG	6	5	83.3	0.43
6	VVMD25	TTCCGTTAAAGCAAAAGAAAAAGG TTGGATTTGAAATTTATTGAGGGG	11	11	100	0.22
7	VVMD27	GTACCAGATCTGAATACATCCGTAAGT ACGGGTATAGAGCAAACGGTGT	15	10	66.6	0.24
8	VVMD28	AACAATTCAATGAAAAGAGAGAGAGAGA TCATCAATTTCGTATCTCTATTTGCTG	7	4	57.14	0.40
9	VVMD31	CAGTGGTTTTTCTTAAAGTTTCAAGG CTCTGTGAAAGAGGAAGAGACGC	8	7	87.5	0.27
10	VVMD32	TATGATTTTTTAGGGGGGTGAGG GGAAAGATGGGATGACTCGC	10	9	90	0.35
11	VVMD34	GGTACATCAGTACTTGAAATGGTTGC TTCTCCGTAGAAGCGTAAACAGC	5	5	100	0.39
12	VVMD36	TAAAATAATAATAGGGGGACACGGG GCAACTGTAAAGGTAAGACACAGTCC	9	5	55.5	0.33
13	VrZAG62	GGTGAAATGGGCACCGAACACACGC CCATGTCTCTCCTCAGCTTCTCAGC	7	4	57.14	0.30
14	VrZAG79	AGATTGTGGAGGAGGGAACAAACCG TGCCCCCATTTTCAAACTCCCTTCC	10	8	80	0.23
Total			126	91		
Av			9	6.5	74.79	0.29

NTB, number of total bands

NPB, number of polymorphic bands

%Pol, polymorphism percentage

PIC, polymorphism information content

Av, average

data revealed a range of genetic distances among the 64 wild grapevine genotypes, spanning from 0.52 to 0.95. The UPGMA dendrogram grouped 64 genotypes in two distinct clusters (A and B) at the 0.62 similarity index value. Cluster A separated into two sub-clusters, namely A1 and A2. Sub-cluster A1 comprised 6 international rootstock reference genotypes (420A, 1613C, 5BB, 140Ru, R. du Lot and 41B) (Fig. 5). In the structure analysis based on combined data the Evanno test result represent the highest value for delta K at K = 5 and grapevine genotypes divided into five sub-groups (Fig. 6). The structure analysis allocated 64 grapevine genotypes into 5 subpopulations (SP1, SP2, SP3, SP4, SP5). The largest subpopulation SP1 contained 18 (28.12%) genotypes, 14 of which were collected from Bozyazı and 2 of which were from Manavgat, together with the international cultivars 'Italia' and 'Victoria' (red vertical bars), The SP2, SP3 and SP4 include 3 (4.68%), 4 (6.25%) and 5 (7.81%) genotypes respectively (Fig. 7). The SP5 included 2 international reference rootstocks namely R. du Lot and 41B. Based on the membership component, the genotypes were appointed to a specific subgroup with the likelihood of > 50%. Otherwise

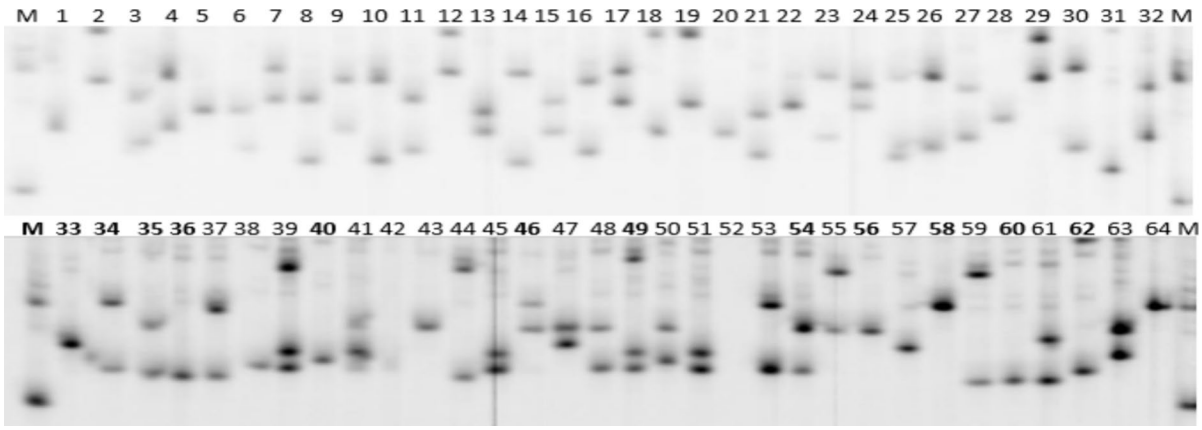


Fig. 1 Segregation of alleles of VVMD25 simple sequence repeat (SSR) marker in 64 grapevine genotypes, separated on 6% polyacrylamide gel

the likelihood of < 50% considered as the admixtures. In this context, 32 genotypes collected from different locations along with the cultivars 'Michel Palieri', 'Alphonse Lavallee', the rootstocks 140 Ru, 5BB and 420A' assigned as admixture (Fig. 7). Overall membership rate of the sample in each cluster is 0.339, 0.240, 0.095, 0.263, 0.062, for SP1, SP2, SP3, SP4 and SP5 respectively. The parameters of population structure and genetic diversity per subpopulations based on combined data of SSR and iPBS marker patterns were shown in Table 4.

According to population genetic analyses, the closest subpopulations were SP1 and SP4 with the lower genetic distance (0.041) and lower pairwise F_{ST} value (0.051). The subpopulations that were genetically most distant from each other were found as SP3 and SP5 with the higher genetic distance (0.28) and higher pairwise F_{ST} value (0.54) (Table 5). The AMOVA analyses showed that the variation among populations was 8.84% of the total variation while the remaining 91.16% was due to the diversity among the individuals within populations, across all the subpopulations studied (Table 6).

Discussion

SSR analysis

The average number of SSR alleles (9) found in the current study was similar to previous research on grapevine such as Boz et al. (2011) found average 8.5 allele for per locus, Fan et al. (2018) reported 8.8, Bibi et al. (2020) 11.54 and Martinez et al. (2006) 9.67.

In agreement with our results (Table 2), previous research reported 8–12 alleles for VVMD5 locus (Bowers et al. 1999; Karataş et al. 2007; Boz et al. 2011; Bibi et al. 2020; Cretazzo et al. 2022), 8–13 alleles for VVDM7 (Bowers et al. 1999; Martinez et al. 2006; Bibi et al. 2020). Karataş et al. (2007) reported average allele numbers as 14.6 in 16 grape cultivars from southeast part of the Turkey and obtained 21 and 18 allele for VrZAG62 and VrZAG79 respectively, higher values reported in our work (Table 2). The observed phenomenon suggests a potentially higher level of genetic variety among grapevine genotypes in the southeastern regions of Gaziantep and Urfa, in comparison to those found in Mersin and Antalya in Turkey. The VVMD24 marker demonstrated the highest PIC value of 0.434, suggesting its potential for enhanced effectiveness in distinguishing between various genotypes of grape genotypes. Genetic distance coefficients between genotypes ranged from 0.61 to 1.0, representing a moderate genetic diversity between genotypes studied and was higher than that reported by Fan et al. (2018) (0.63–0.99) and Martinez et al. (2006) 0.64–1.0). Similar to the report by Carimi et al. (2010), the current study could not identify a significant correlation between the groups and the geographical distribution of cultivars.

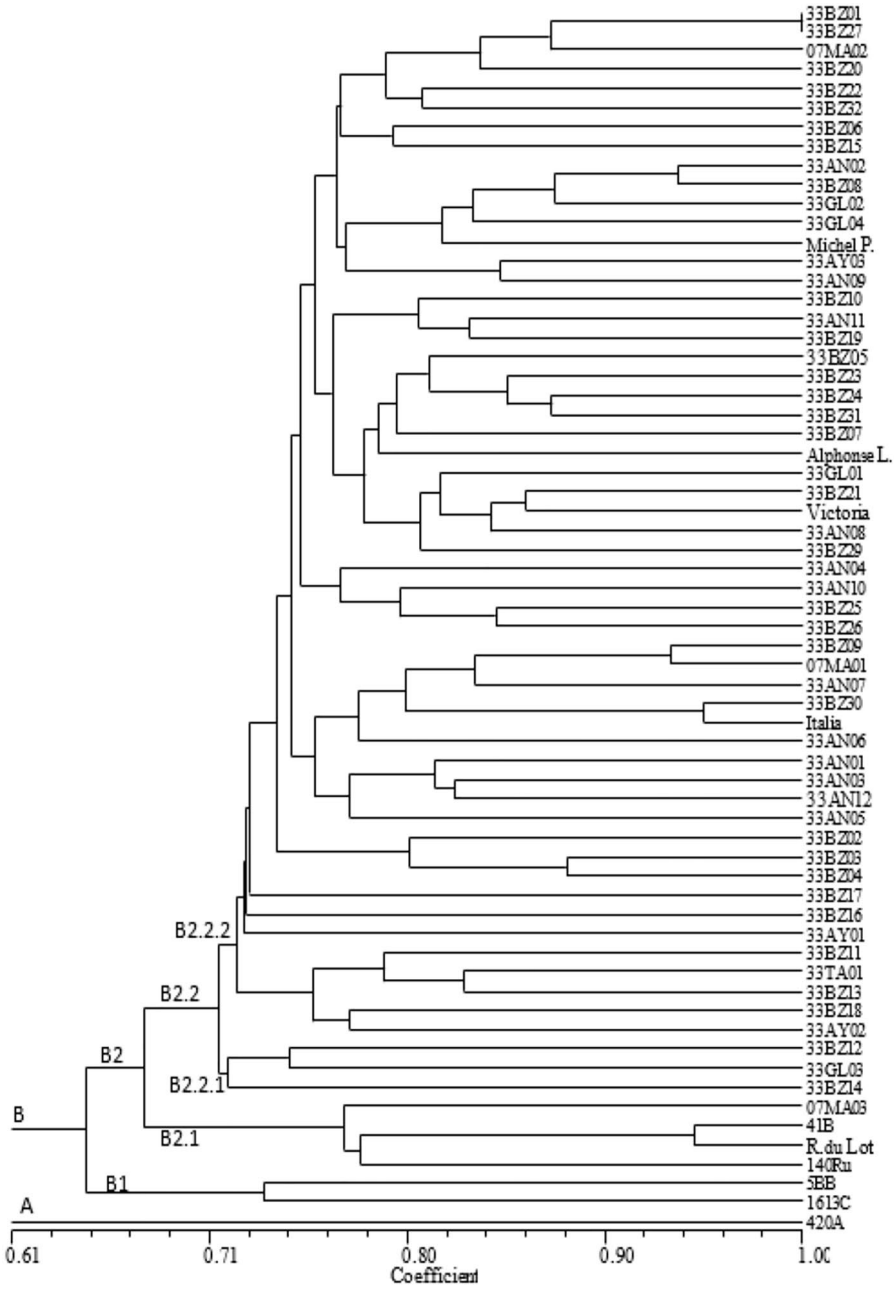


Fig. 2 Dendrogram representing the similarity of simple matching (SM) coefficient among 64 grapevine genotypes, generated by the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on 91 simple sequence repeat (SSR) markers

iPBS analysis

Comparing with the previous research, Guo et al. (2014) reported a total of 99 bands in 35 cultivars with 15 iPBS primers and consistent with our findings, the number of amplified bands per primer ranged from 5 (2217, 2230) to 9 (2251), with an average of 6.6. In the present study, the primer 2382 yielded the greatest polymorphic information content (PIC) value of 0.49, as the primer 2079 exhibited the lowest PIC value of 0.26. The average PIC value across all primers was calculated to be 0.39 (Table 3). The previous work by Guo et al. (2014) revealed comparable values of PIC, ranging from 0.5 when using primer 2232 to 0.30 when using primer 2253. The dendrogram based on the

Table 3 Code and sequence of inter-Primer Binding Site (iPBS) markers assayed in the characterization of grapevine accessions

No	Primer code	Primer sequence 5′ 3′	NTB	NPB	%Pol	PIC
1	2077	ACCTGGCGTGCCA	10	6	60	0.43
2	2079	AGGTGGGCGCCA	8	3	37.5	0.26
3	2080	CAGACGGCGCCA	12	8	66	0.31
4	2085	ATGCCGATACCA	9	6	66.6	0.36
5	2087	GCAATGGAACCA	7	5	71.42	0.30
6	2095	GCTCGGATACCA	15	10	66.6	0.42
7	2221	ACCTAGCTCACGATGCCA	10	6	60	0.44
8	2228	CATTGGCTCTTGATACCA	11	5	45.45	0,32
9	2230	TCTAGGCGTCTGATACCA	12	7	58.33	0.44
10	2231	ACTTGGATGCTGATACCA	5	5	100	0.35
11	2238	ACCTAGCTCATGATGCCA	8	8	100	0.42
12	2239	ACCTAGGCTCGGATGCCA	19	14	73.68	0.45
13	2246	ACTAGGCTCTGTATACCA	7	6	85.71	0.47
14	2255	GCGTGTGCTCTCATACCA	6	5	83.33	0.47
15	2256	GACCTAGCTCTAATACCA	11	8	72.72	0.47
16	2270	ACCTGGCGTGCCA	8	3	37.5	0.38
17	2273	GCTCATCATGCCA	11	6	54.54	0.32
18	2276	ACCTCTGATACCA	12	11	91.6	0.41
19	2298	AGAAGAGCTCTGATACCA	14	11	78.57	0.43
20	2374	CCCAGCAAACCA	7	6	85.71	0.39
21	2381	GTCCATCTTCCA	6	5	83.33	0.37
22	2382	TGTTGGCTTCCA	7	5	71.42	0.49
23	2390	GCAACAACCCCA	8	6	75	0.41
24	2399	AAACTGGCAACGGCGCCA	7	4	57.14	0.37
25	2402	TCTAAGCTCTTGATACCA	6	3	50	0.33
	Total		236	162		
	Average		9.44	6.48	69.28	0.39

NTB, number of total bands
NPB, number of polymorphic bands
%Pol, polymorphism percentage
PIC, polymorphism information content

iPBS-PCR amplification profiles showed that the similarity coefficients among the 64 of genotypes ranged from 0.53 to 0.97, indicating a high genetic diversity among genotypes studied (Fig. 4).

Combined data analysis

The analysis of combined data revealed a range of genetic distances among the 64 wild grapevine genotypes, spanning from 0.52 to 0.95, with an average value of 0.68. These results remarked a substantial level of genetic diversity within the wild grapevine genotypes cultivated in the Southeast Mediterranean Region of Turkey.

The dendrogram derived from the combined SSR–iPBS data in this study exhibited greater consistency with the dendrogram based only on SSR data, as depicted in Figs. 2, 4, 5. Grapevine rootstocks originating from North America were distinctly classified in a different branch, apart from other genotypes. Conversely, reference cultivars have diffused throughout wild grape populations. The observed distribution of genotypes is likely a result of the geographical proximity between reference grape cultivars developed in European countries and their ancestral wild grapes cultivated in Turkey. The principal coordinate analysis, which utilized a combination of iPBS and SSR markers, yielded results that were similar to the cluster analysis, albeit with slight variations (Fig. 8). Compared with the iPBS analysis, the dendrogram generated based on the SSR analysis showed higher consistency with the STRUCTURE analysis. F_{ST} value shows the population differentiation based on genetic structure. A significant variation was estimated within the five subpopulations based on the fixation index (F_{ST}) calculated for each of the

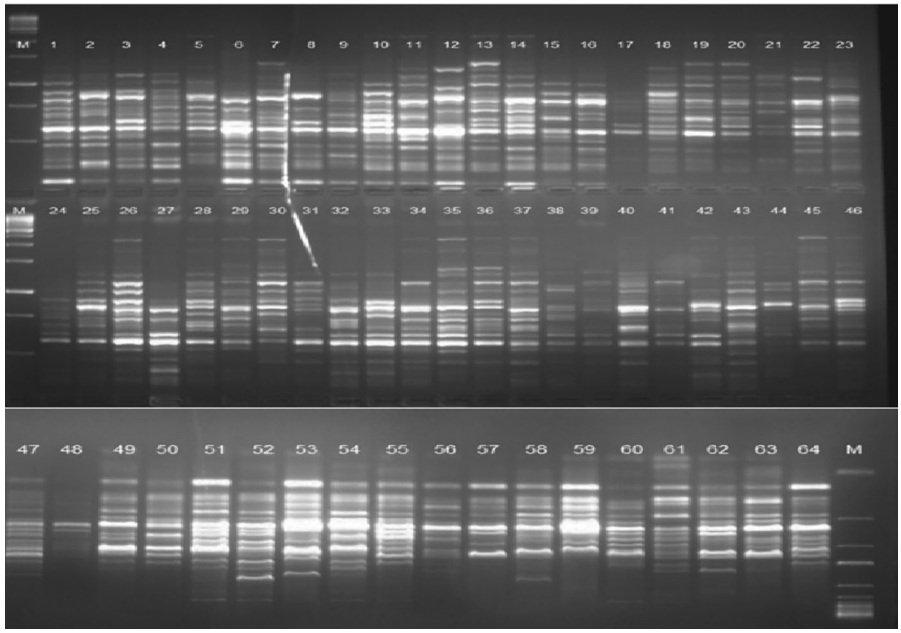


Fig. 3 Agarose gel profile of 2077 inter-Primer Binding Site (iPBS) primers in 64 grapevine genotypes. Lanes 1 to 64 correspond to accessions listed in Table 1, and M is a lane of 100 bp molecular ladder

subpopulations. Mean F_{ST} values were obtained as 0.1642, 0.0571, 0.5141, 0.0193 and 0.8567 for SP1, SP2, SP3, SP4 and SP5, respectively. Estimation the genetic diversity within and among populations is an important consideration in conservation programs for genetic resources (Hilsdorf and Hallerman 2017). Genetic differentiation between the populations was evaluated by pairwise F_{ST} comparisons. Frankham et al. (2002) accepted FST value higher than 0.15 as important in distinguishing populations. In the present study the pairwise F_{ST} values given in Table 5. Wright (1978) suggests that the values of pairwise F_{ST} above 0.25 indicate very great genetic differentiation. According to suggestion of Wright (1978), there are considerable genetic variation between SP1 and SP5, SP2 and SP3, SP2 and SP3, and also SP3 and SP5. Average distances (expected heterozygosity) among the genotypes of the same subpopulation were lowest in SP5 and highest in SP4 and SP2 (Table 4).

The Evanno analysis results strengthen a second maximum peak value for delta K at K = 9 in the plots of L (K) versus delta suggesting a presumably classifying of the grapevine genotypes into 9 sub- groups (Fig. 6) and F_{ST} values (0.2639) between SP5 and SP9; SP1 and SP5 (0.2599), SP5 and SP7 (0.2599) indicating a higher genetic variation between the populations based on the report Frankham et al. (2002). However, the majority of subpopulations did not show a better separation with a low Fst values.

Overall percentage of membership of the sample (Inferred Clusters) in each group from SP1 to SP9 were 0.048, 0.188, 0.138, 0.209, 0.050, 0.194, 0.053, 0.066 and 0.055, respectively. Twenty-eight genotypes did not include into any subpopulation and therefore they classified as admix (Fig. 9). Average distances (expected heterozygosity) between genotypes of the same subgroups were 0.1415, 0.3496, 0.3382, 0.3023, 0.0735, 0.3411, 0.1847, 0.3366, 0.1725 from SP1 to SP9, respectively. These results indicated that SP5 has a low genetic variation and SP2, SP3, SP6 and SP6 show high genetic variation among the genotypes.

Based on structure analysis it is concluded that gene flow may exist among some regions with high number of (28) admixed individuals (43.75%). Furthermore, the UPGMA clustered tree were generally overlap to that from Structure and the PCA results.

In the current study, both SSR and iPBS markers were highly polymorphic and showed an important variation. The polymorphism rate generated by SSR (74.79) is higher than those of iPBS (69.28%) marker. Although number of total bands higher for iPBS than SSR, in both marker systems the average number of polymorphic bands for each primer was similar (SSR = 6.5; iPBS = 6.48). Research

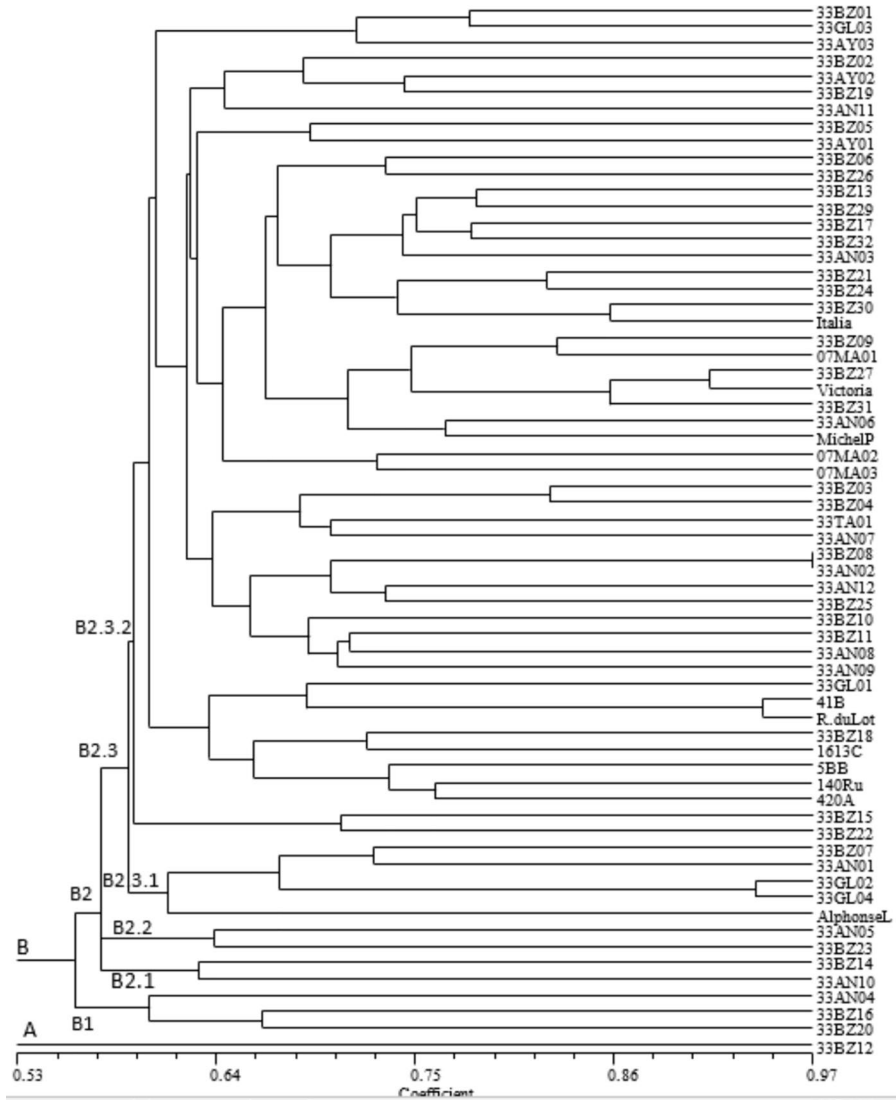


Fig. 4 Dendrogram representing the similarity of simple matching (SM) coefficient among 64 individuals of grapevine genotypes, generated by the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on 162 inter-Primer Binding Site (iPBS) markers

conducted with different plant species indicates that generally SSR demonstrate higher polymorphism per locus than other markers (Solmaz et al. 2016; Aristya et al. 2019). However, researches have also demonstrated that universal markers viz RAPD, ISSR or AFLP, may be evenly or yet more productive than SSR as a marker system (Garcia et al. 2004; Linos et al. 2014).

The selection of an appropriate marker for genetic diversity research presents a complex task, necessitating the utilization of prior information derived from comparative investigations. This study conducted a comparison between two DNA marker systems utilizing wild grapevine plants. The purpose was to get insights into the level of genetic variation that may be anticipated from the marker systems applied. The results indicated that two separate marker systems displayed no significant variation in the genetic criterion obtained. Similar results were acquired using two marker systems, however it should be noted that SSR markers primarily focus on repeated sequences. These markers exhibit co-dominant inheritance and offer essential information that can serve as a foundation for future investigations. However, it is important to acknowledge that iPBS can be considered as a robust and comprehensive multilocus marker system due to its substantial polymorphism and straightforward

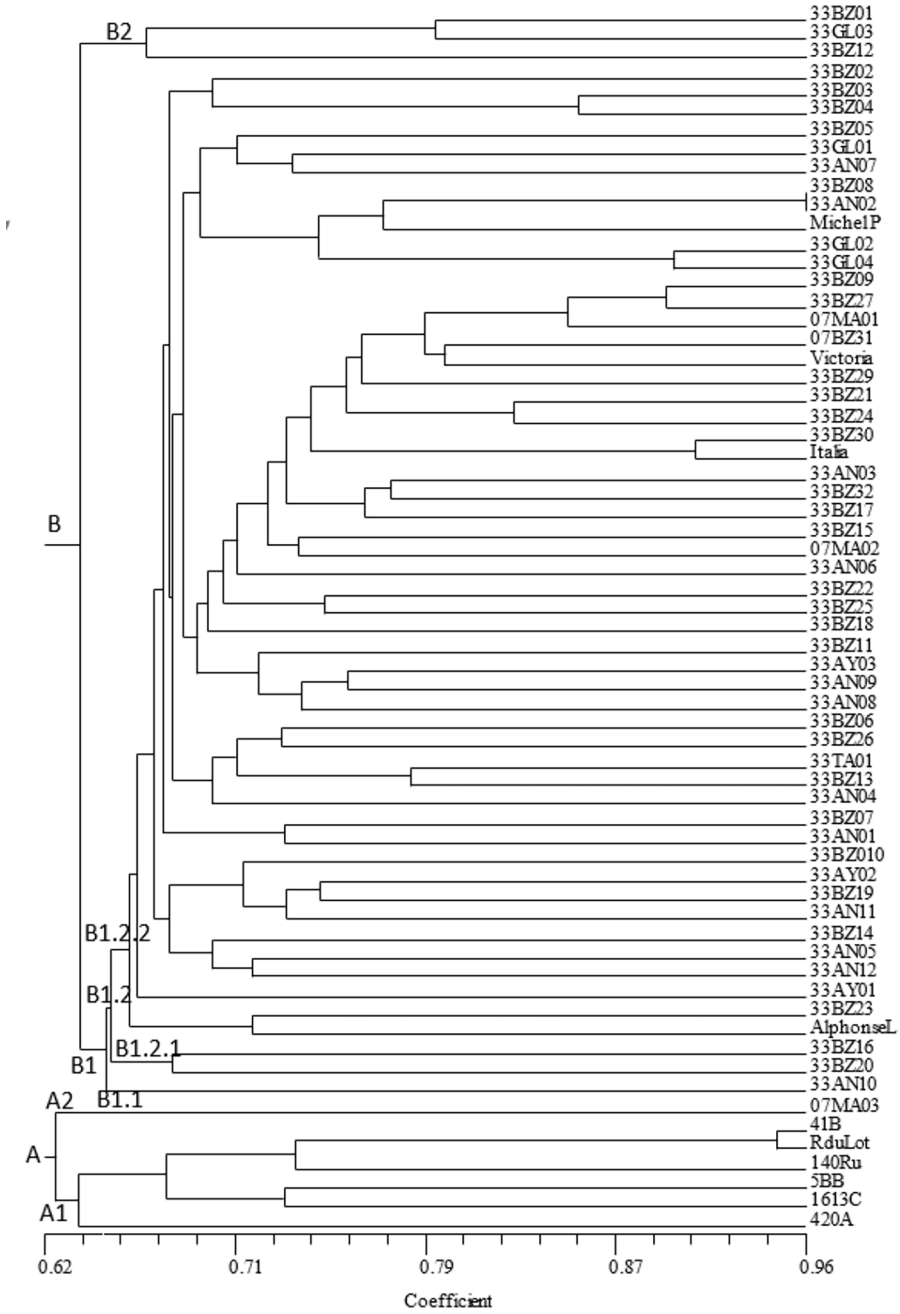


Fig. 5 Dendrogram representing the similarity of simple matching (SM) coefficient among 64 individuals of grapevine genotypes, generated by the UPGMA cluster analysis based on combining 162 inter-Primer Binding Site (iPBS) and 91 simple sequence repeat (SSR) markers

execution. iPBS would also be proven as easy to perform, no need to polyacrylamide gel, absence of fluorescence labeling can be separated in agarose gel electrophoresis and able to identify high levels of polymorphism as SSR.

Conclusion

The wild grapevine population cultivated in the Southeast Mediterranean Region of Turkey exhibited clear differentiation, as evidenced by the genetic distance and population structure values. Both marker

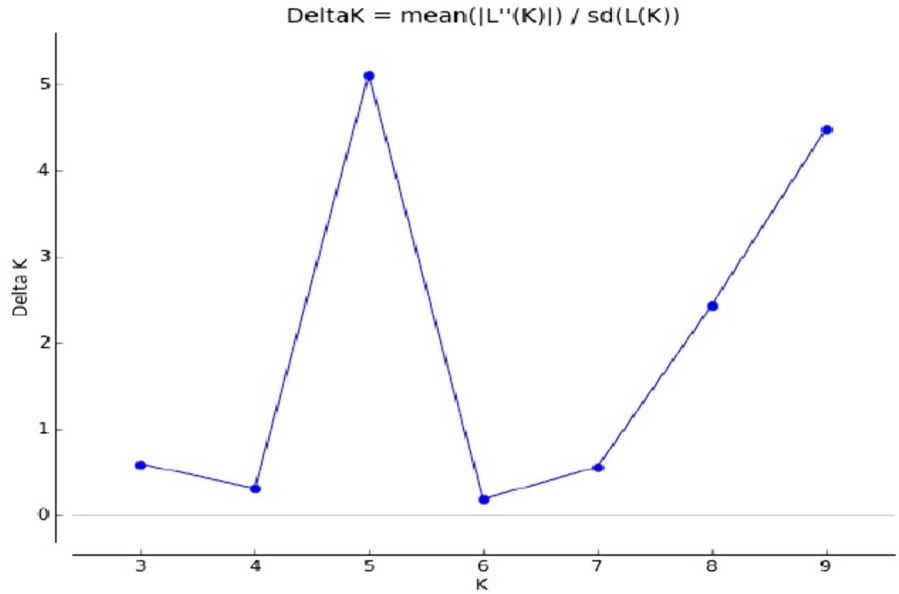


Fig. 6 STRUCTURE assessment of the subgroups for the K values ranging from 2 to 10, by delta K (ΔK) values

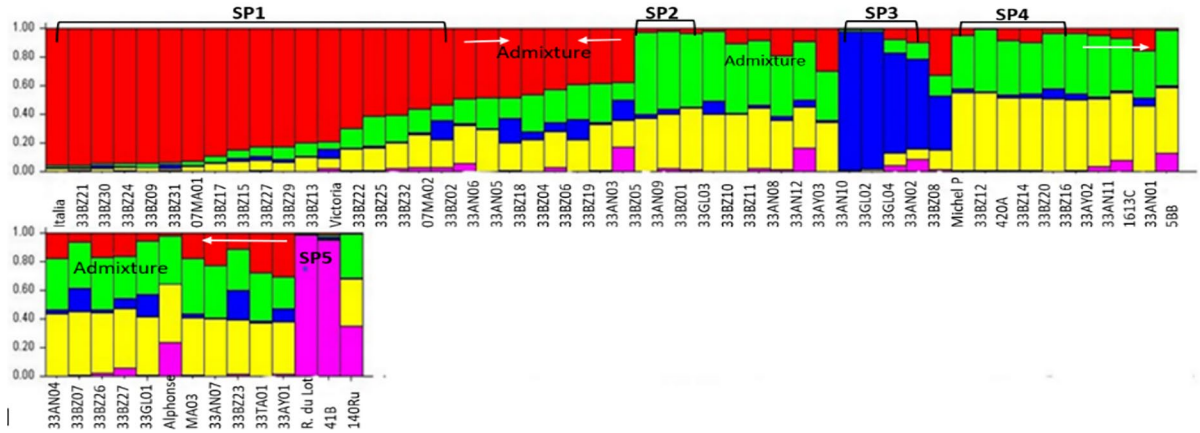


Fig. 7 Genetic structure of the 64 grapevine accessions, produced by STRUCTURE analysis using combined data of inter-Primer Binding Site (iPBS) and simple sequence repeat (SSR) markers based on an admixture model with K = 5. The defined sub-groups (SP) are represented in different colors. SP1, Subpopulation 1; SP2, Subpopulation 2; SP3, Subpopulation 3; SP4, Subpopulation 4; SP5, Subpopulation 5. The genotypes numbers refer to Table 1

Table 4 Number of alleles (Na), effective number of alleles (Ne), expected heterozygosity (He), Shannon's information index (I), number of polymorphic loci (NPL), and percentage of polymorphic loci (PPL) for subpopulations

	Na	Ne	He	I	NPL	PPL (%)
SP1	1.9062 ± 0,2922	1.4768 ± 0.3288	0.2938 ± 0.1665	0.4386 ± 0.2165	174	90.62
SP2	1.7240 ± 0,4482	1.4478 ± 0.3715	0.2766 ± 0.2023	0.3904 ± 0.2684	139	72.40
SP3	1.4010 ± 0,4914	1.3036 ± 0.3911	0.1941 ± 0.2431	0.2458 ± 0.3045	77	40.10
SP4	1.9844 ± 0,1243	1.5985 ± 0.2985	0.3593 ± 0.1375	0.5227 ± 0.1650	189	98.44
SP5	1.2880 ± 0,4540	1.2304 ± 0.3632	0.1536 ± 0.2421	0.1833 ± 0.2890	55	28.65
Average	1.6607 ± 0.3620	1.5620 ± 0.3053	0.3365 ± 0.1377	0.5040 ± 0.1664		

Table 5 Pairwise Fst (above diagonal) and Nei's 0.34 genetic distance (below diagonal) values for subpopulations
- indicates there is no point for comparison of genetic distance ofthe same SP groups

	SP1	SP2	SP3	SP4	SP5
SP1	-	0.0942	0.1859	0.0511	0.3290
SP2	0.0854	-	0.2658	0.0859	0.3577
SP3	0.1341	0.1490	-	0.1458	0.5387
SP4	0.0409	0.0716	0.1209	-	0.2153
SP5	0.2165	0.2455	0.2793	0.1966	-

Table 6 Genetic differentiation of subpopulations by Analyses of Molecular Variance (AMOVA)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among subpopulations	4	202.461	1.16594	8.84
Among individuals within subpopulations	60	1442.985	12.02488	91.16
Total	129	1645.446	13.19082	100

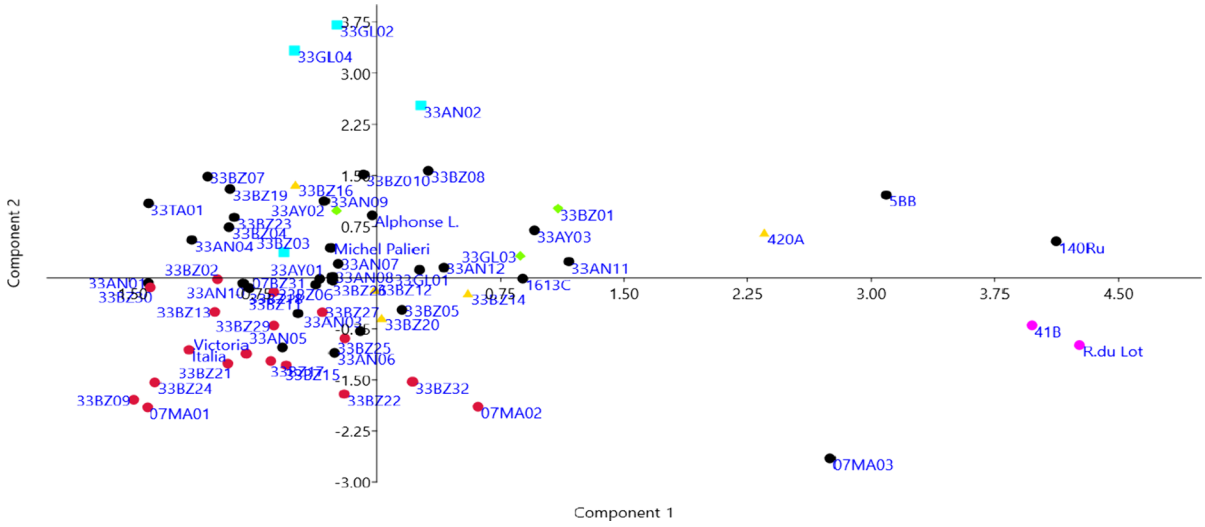


Fig. 8 Principal component analysis (PCA) plot based on combined data of inter-Primer Binding Site (iPBS) and simple sequence repeat (SSR) markers in 64 grapevine genotypes

systems demonstrated significant levels of genetic variation among 64 genotypes of grapevine, with further genetic distinctions observed across different marker technologies. The wild genotypes exhibited a notable degree of genetic variation and were found to be genetically distinct from the reference rootstocks. As a result, these genotypes possess the capacity to serve as genetic resources for breeding purpose.

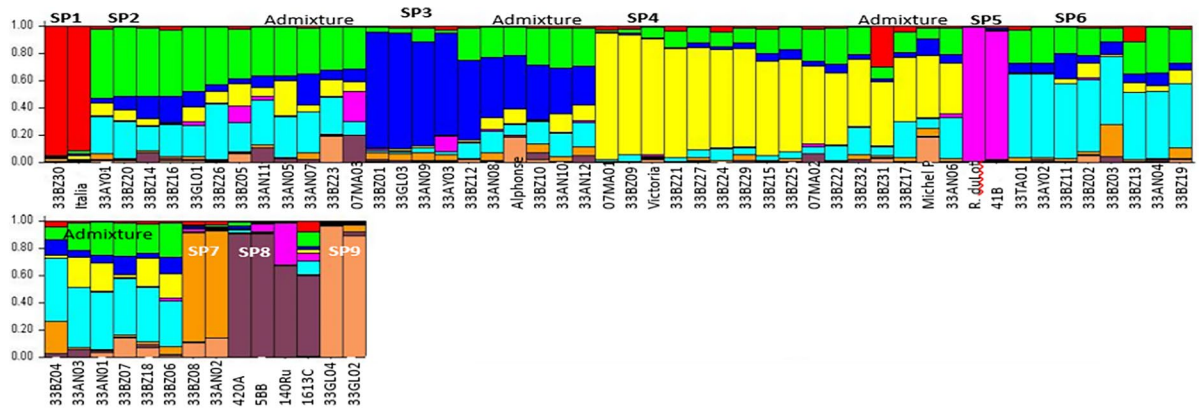


Fig. 9 Genetic structure of the 64 grapevine accessions, produced by STRUCTURE analysis using combined data of inter-Primer Binding Site (iPBS) and simple sequence repeat (SSR) markers based on an admixture model with K = 9. The defined sub-groups (SP) are represented in different colors. The genotypes numbers refer to Table 1

In order to optimize the utilization of wild genetic resources, it is imperative to engage in the mapping of significant features and the incorporation of genotypes that possess genes linked to crucial traits within breeding programs. In future extension of this work an association study may be conducted by correlating the polymorphic markers with important phenotypic features.

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Author contributions Study conception and design: Hİ, DS, AS, NM, collection and providing plant material: AS, NM, data collection: DS, Hİ, analysis of results: Hİ, DS, HM, writing the original draft and visualized the data; Hİ, reviewed and edited the manuscript: Hİ, DS, AS, NM, HM.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

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