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RESEARCH ARTICLE

Diversity and association mapping assessment of an untouched native grapevine genetic resource by iPBS retrotransposon markers

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Abstract Grapes are among the most widely cultivated horticultural crops and have a long history of domestication. They exhibit genetic variation due to natural crossbreeding, bud mutations, and the changing demand for different types of wine and table grapes. Identifying and distinguishing autochthonous grape cultivars is an essential first step in breeding. In this research, an autochthonous grapevine (*Vitis vinifera* L.) population was identified using retrotransposon markers called iPBS (Inter primer binding sites), and genetic relationships with other cultivars from Türkiye and Europe were examined. The association between loci and specific traits was determined using GLM (general linear model) and MLM (mixed linear model) analyses. A total of 136 loci were generated by eight iPBS markers, of which 106 were polymorphic. Genotypes and standard cultivars were clustered into three main groups and seven subclusters by the neighbor-joining method. Structure analysis further classified the genotypes and cultivars into seven populations. Molecular variance analysis revealed that most of the variability occurred among individuals. In the association mapping, 36 loci were correlated with quantitative traits in GLM, while 21 were in MLM. The diversity assessments uncovered significant diversity within the autochthonous grape population, even among individuals with the same name. This diversity retains value for breeding research as it allows identifying distinct genotypes with desirable characteristics. The loci identified through both mapping approaches have the potential to serve as functional markers for selecting genotypes with desired traits.

Keywords *Vitis vinifera* · Quantitative traits · GLM · MLM · Heatmap · Population structure

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Introduction

The agronomic or food significance can be explored through an examination of numerous specific characteristics that exhibit a wide range of variation. Observing divergence in a population ensures

selecting superior types in terms of resistance to biotic or abiotic stressors, suitability to cultivation, and genotypes that have high yield and quality among existing variants (Gonçalves and Martins 2012). The characterization of grape germplasm often involves phenotyping based on various traits such as shoot and leaf characteristics, bunch morphology, berry color, shape, and chemical composition. This approach is widely employed in research aiming to distinguish different grape cultivars and understand their unique characteristics (Leão et al. 2011). Morphological features are sensitive to environmental changes, and ongoing climate change induces elevated temperatures, floods, and drought (Hussain 2015). Hence, morphological characterization is carried out for more than one season or supported by molecular tools to obtain a more reliable distinction of plant individuals (Duminil and Di Michele 2009; Yildiz et al. 2021; Yaman 2022a, 2022b).

Molecular markers are widely used in the discrimination of plants (Başak et al. 2022; Taş et al. 2022). In grape genetic diversity research, some co-dominant molecular markers such as RFLP (Restriction fragment length polymorphism), SSR (Simple-sequence repeats), and SNP (Single nucleotide polymorphism), as well as dominant markers like RAPD (Random amplified polymorphic DNA), AFLP (Amplified fragment length polymorphism), and ISSR (Inter simple sequence repeat) markers were utilized (Tamhankar et al. 2001; Edabi et al., 2019; Rohollahi and Naji 2020). Retrotransposon-based markers have been identified as cost-effective tools with low DNA requirements that offer high reproducibility and polymorphism (Nadeem et al. 2018). iPBS (Inter-primer binding site) was introduced by Kalendar et al. (2010) as a powerful DNA fingerprinting tool that does not require prior sequence information. This technique was performed on various plant species such as safflower (Ali et al. 2019), laurel (Karık et al. 2019; Yilmaz and Ciftci 2021), salep orcids (Palaz et al. 2022), and alfalfa (Eren et al. 2023). Guo et al. (2014) and Milovanov et al. (2019) successfully employed the iPBS technique in the genetic differentiation of grapes.

Grape breeding is time-consuming due to the long juvenile period of the perennial plant. Difficulty in the breeding process of perennial plants urged the researchers to selection that is easy to employ, time-saving, and cost-effective using the existing population (Egorov 2021). Quantitative traits are controlled mainly by multiple genes identified by quantitative trait locus (QTL) analysis in genomic regions. QTL mapping faces difficulty in high plants due to their long juvenile period, high heterozygosity, and genetic divergence between parents (Kaya et al. 2016). Moreover, the cost of propagating a large number of lines also limits QTL research to a narrow recombinant population for mapping (Holland 2007). Both QTL mapping and association mapping analyze the co-inheritance of functional polymorphisms and neighboring DNA variants. The main difference lies in the genetic panels used in the research: QTL mapping requires F_2 populations with known ancestry, while association mapping can be conducted with high resolution on historical and natural recombinants (Zhu et al. 2008).

Grapevine (*Vitis vinifera* L.), the European grape holds a prominent position as one of the most widely propagated and utilized fruit crop species worldwide (Güler 2023). The species has a rich history, spreading from the Mediterranean Basin to Central Asia and becoming deeply intertwined with culture and economies for centuries (Vafee et al., 2017; Dong et al. 2023). Since the berries and by-products of grapes are consumed with pleasure by humans, selection has occurred over the years, shifting from wild species (*V. vinifera* spp. *sylvestris*) to domestic species (*V. vinifera* spp. *sativa*) (Riaz et al. 2018). However, this domestication process and economic concerns have led to a narrow genetic pool due to cultivation primarily focused on a few economically important cultivars and the abandonment of less desirable individuals (Turcotte et al. 2017). The preference for internationally renowned grape cultivars has pushed autochthonous grapes to the brink of extinction, and many of these unique cultivars have been forgotten. In recent decades, recognition of autochthonous grapes has gained momentum due to changes in consumer demand and the need to mitigate the adverse effects of global climate change (Cipriani et al. 2010).

Growers may misname local genotypes by considering only berry shape or color. In our case, the growers in Bolu name genotypes by relying only on berry colors. Plenty of morphologically different grapevines exist with the same local name in the population. To address this issue, our study aimed to assess

the diversity of an autochthonous grapevine population using iPBS retrotransposon molecular markers and to visually illustrate the associations between traits and markers for morphometric features.

Materials and Methods

Research site and plant material

The materials of the study consisted of 36 grapevine genotypes grown in Bolu province, 10 European standard grapevine cultivars, and 30 local cultivars from various sites of Türkiye. The genotypes were determined using the bunch, berry, leaf, and disease tolerance parameters via a survey study conducted in the towns and villages of Göynük at an altitude of 600 m and Seben at an altitude of 700–900 m, where grape cultivation has been made since ancient times (Supp. Table 1) (Güler and Karadeniz 2023). Morphometric evaluations were assessed on 36 genotypes, while the genetic diversity analysis was carried out on the genotypes, standard cultivars, and local cultivars. To extract DNA, pruning waste canes from each genotype/cultivar were collected and transferred to the climate chamber at the Horticulture Department, Faculty of Agriculture, Bolu Abant Izzet Baysal University. These canes were then planted in rooting boxes filled with pure perlite. DNA extraction was performed using young shoots to avoid complications caused by the accumulation of phenolic compounds in the leaves.

DNA isolation

Leaf samples of the genotypes were frozen and homogenized with a mortar and a pestle under liquid nitrogen. A total of 100 mg for each genotype was placed in a 2 ml Eppendorf tube, and DNA extraction was performed by adding the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA). The DNA was diluted to 10 ng/µl with sterile ddH_2O after measuring the DNA concentration using a DS-11 FX series spectrophotometer (Denovix Inc., Wilmington, DE, USA). The prepared DNA was stored at − 20 ℃ throughout the study.

iPBS retrotransposon assay

In this study, 8 iPBS retrotransposons primers designed by Kalendar et al. (2010) were used for the molecular characterization of grapevine genotypes. PCR mix was prepared according to Aydın et al. (2020). PCR amplification was carried out at 95 ℃ for 4 min, followed by 35 cycles 94 ℃ for 30 s, 30 s at the annealing temperature depending on primer (Table 1), and 2 min at 72 ℃ 35 cycles. Finally, 5 min at 72 ℃ was set as the elongation phase. Amplicons were separated in 1.4% agarose gel

Table 1 Some descriptive results for the iPBS markers used in the study

Primer IDs	Primer sequence (5′–3′)	Ta (°C)	GC (%)	TB	PB		PIC	RP
					Number	Ratio (%)		
2095	GCTCGGATACCA	50	58.3	26	21	80.77	0.14	4.58
2395	TCCCCAGCGGAGTCGCCA	50	72.2	10	7	70.00	0.13	1.79
2295	AGAACGGCTCTGATACCA	60	50.0	15	9	60.00	0.20	4.39
2230	TCTAGGCGTCTGATACCA	53	50.0	20	17	85.00	0.24	7.55
2228	CATTGGCTCTTGATACCA	54	44.4	23	20	86.96	0.22	7.45
2232	AGAGAGGCTCGGATACCA	56	55.6	11	10	90.91	0.33	5.16
2415	CATCGTAGGTGGGCGCCA	61	66.7	16	12	75.00	0.28	7.26
2251	GAACAGGCGATGATACCA	53	50.0	15	10	66.67	0.29	7.24
Total				136	106	77.94		
Average/primer				17	13.25		0.23	5.68

Tm Annealing temperature, *GC* Guanine/cytosine ratio, *TB* Total bands, *PB* Polymorphic bands, *PIC* Polymorphism information content, *RP* Resolving power

using 1 × TAE (Tris–acetate-EDTA) buffer at 90 V for 90 min and stained with ethidium bromide for visualisation under ultraviolet (UV-B) light in the G: BOX F3 gel documentation system (Syngene, Synoptics Ltd., Cambridge, UK).

Data analysis and statistical approaches

Morphometric characteristics of Bolu's genotypes were determined based on data collected over two consecutive years, 2019 and 2020 (Güler 2023). In the genetic assays, only strong, clear, and reproducible amplifiable products were used. A binary matrix was then created by scoring the bands observed in the gel as either present (1) or absent (0), which was subsequently employed for further evaluations. The performance of iPBS markers was assessed using various criteria, including polymorphism information content (PIC), resolving power (RP) values, the total number of alleles, and the number of polymorphic alleles (Prevost and Wilkinson 1999; Roldàn-Ruiz et al., 2000). The gene diversity parameters were calculated with PopGene ver. 1.32 (Yeh et al. 2000). Genetic distance (GD) and similarity were calculated according to Nei (1972). The similarity matrix obtained from the data was subjected to analysis using the unweighted pair group method with arithmetic mean (UPGMA) clustering method. This analysis was performed using the "vegan" package in R software (R Core Team 2019). To minimize errors in tree construction and to evaluate all probabilities, probability calculations were made at least 1000 times, and trees were created and compared with each other. MEGA X program was used for the construction of genetic tree. The analysis of population structure was conducted using the binary matrice of the individuals using a Bayesian model-based algorithm by the package program of Structure v.2.3.4 (Pritchard et al. 2000). The algorithm was derived from mixed models of independent values (K) consisting of 1–10 approximate groups of 50.000 iterations of the Markov–Monte Carlo Chain (MCMC) after 100.000 combustion cycles. The algorithm was run using a mixed model of 2–10 putative groups. For the Delta K (ΔK) model and the predicted likelihood values, the web-based tool, STRUCTURE HARVESTER (Earl and VonHoldt 2012), was used to estimate the most probable number of populations. Analysis of molecular variance (AMOVA) was employed to compare the genetic differentiation between studied vines and origins (Bolu, Türkiye, and Europe grapes). The scattering of genotypes and cultivars on the principal coordinate plot was implemented by principal coordinate analysis (PCoA) via GenAlEx v.6.5 (Smouse and Peakall 2012). The associations of the loci determined by iPBS retrotransposon markers with characteristics were evaluated by the generalized linear model (GLM) and mixed linear model (MLM) using the Tassel 5 software (Bradbury et al. 2007).

Results

In the study, PBS2095 exhibited the highest total number of bands, with 26 bands in total, of which 80.77% were polymorphic. The average number of bands per primer was 17, and the average number of polymorphic bands was 13.25. Among the primers, PBS2232 showed the highest polymorphism rate at 90.91%, while PBS2295 had the lowest rate at 60.00%. The polymorphic information content (PIC) values ranged from 0.13 for PBS2395 to 0.33 for PBS2232. The resolving power (RP) of the primers ranged from 1.79 for PBS2395 to 7.55 for PBS2230. Detailed information on the performance of the primers can be found in Table 1.

Among the primers tested, recessive alleles (q) were predominant in five primers, while dominant alleles (p) were predominant in two primers (PBS2251 and PBS2295). The q/p ratio was equal in the PBS2415 primer. The primers PBS2232, PBS2251, and PBS2415 had the highest number of alleles (Na), with very close values of 1.606, 1.600, and 1.604, respectively, while PBS2095 had the lowest Na at 1.218. Shannon's information index (I) and the number of effective alleles (Ne) were consistent with the total number of alleles, with PBS2251, PBS2415, and PBS2232 having the highest values, and PBS2095 having the lowest value for Ne. The values of He (expected heterozygosity) ranged from 0.087 (PBS2095) to 0.246 (PBS2251), while the values of uHe (unbiased expected heterozygosity) ranged from 0.090 to 0.231 for the same primers. When analyzing the diversity values by population, the Na in Turkey's standard cultivars was significantly higher than the Na in European cultivars and Bolu genotypes (1.610, 1.360, and 1.346, respectively). However, the

Table 2 Genetic diversity parameters for the iPBS primers and populations used in the study

Primers	B Freq	p	q	Na	Ne	I	He	uHe
PBS2095	0.292	0.249	0.751	1.218	1.124	0.150	0.087	0.090
PBS2228	0.389	0.306	0.694	1.565	1.266	0.263	0.166	0.170
PBS2230	0.377	0.284	0.716	1.383	1.309	0.286	0.186	0.191
PBS2232	0.404	0.286	0.714	1.606	1.379	0.363	0.235	0.242
PBS2251	0.680	0.557	0.443	1.600	1.440	0.359	0.246	0.253
PBS2295	0.625	0.557	0.443	1.378	1.230	0.210	0.137	0.140
PBS2395	0.419	0.370	0.630	1.233	1.157	0.157	0.098	0.101
PBS2415	0.612	0.499	0.501	1.604	1.383	0.337	0.225	0.231
Populations								
Türkiye	Mean			1.610	1.290	0.276	0.177	0.180
	Std. Er			0.054	0.029	0.022	0.016	0.016
Europe	Mean			1.360	1.271	0.253	0.164	0.172
	Std. Er			0.067	0.030	0.023	0.016	0.017
Bolu	Mean			1.346	1.264	0.247	0.160	0.162
	Std. Er			0.069	0.029	0.023	0.016	0.016
Total	Mean			1.439	1.275	0.258	0.167	0.172
	Std. Er			0.037	0.017	0.013	0.009	0.009

B Freq Band frequency, *p and q* Estimated dominant and recessive allele frequencies, *Na* Number of alleles, *Ne* Number of effective alleles, *I* Shannon's information index, *He* Expected heterozigoty, *uHe* Unbiased expected heterozygosity. *Std. Er.* Standard error

Ne values varied slightly between populations, ranging from 1.264 (Bolu) to 1.290 (Turkey). The I, He, and uHe values were consistent with the Ne values. The overall diversity parameters were calculated as Na: 1.439, Ne: 1.275, I: 0.258, He: 0.167, and uHe: 0.172 (Table 2).

The genotypes were categorized into three main groups based on the UPGMA clustering analysis. The first group (Group A) did not include any genotypes but consisted of Turkey's domestic grape cultivars, as well as well-known grapes used for fresh consumption and the wine industry, such as Syrah, Red Globe, and Muscat of Hamburg. The second group (Group B) had a balanced representation of genotypes and Turkey's cultivars, along with European cultivars, forming a mixed group. This group mainly comprised table grapes, including Michelle Palieri, Alphonse Lavelle, Cardinal, Trakya İlkeren, and the first recognized Etli Beyaz from Bolu. The third group (Group C) further divided into three subgroups, with two subgroups (C1 and C3) consisting entirely of genotypes. Interestingly, the remaining subgroup included renowned wine cultivars such as Merlot and Cabernet Sauvignon (Fig. 1).

The results of the structure analysis, based on the Evanno method, are presented in Supplementary Table 2. The analysis identified the best K value as seven populations, with the highest ΔK value of 22.61. The FST values, representing genetic variances, were calculated as 0.72, 0.29, 0.80, 0.42, 0.67, 0.26, and 0.54 for populations q1 to q7, respectively. Within the populations, the gene diversity (H) ranged from 0.08 in q1 to 0.21 in q6. Populations q1, q3, and q7 exclusively consisted of genotypes, while population q2 included the same cultivars as observed in the UPGMA analysis, except for Merlot and Bozbey, which belonged to subgroup C. The structure analysis provided comparable results to the clustering analysis but offered the advantage of illustrating gene proportions. The visual representation of grapevine gene proportions is depicted in Fig. 2.

In Fig. 3, a significant portion of the genotypes can be observed separated from both the standard cultivars of Turkey and Europe, forming a distinct cluster in the upper left corner of the principal coordinate plane. While genotypes 3 and 4 are grouped together with the standard cultivars, genotypes Bozbey and Su Üzümü also cluster with the standard cultivars. On the other hand, genotype 26 appears relatively distant from the other genotypes and is closely positioned to Michelle Palieri, a well-known standard cultivar. Additionally, genotypes Reçel Üzümü and Sülün Kara, which were registered previously in the 1970s from Bolu, also exhibit proximity to genotype 26.

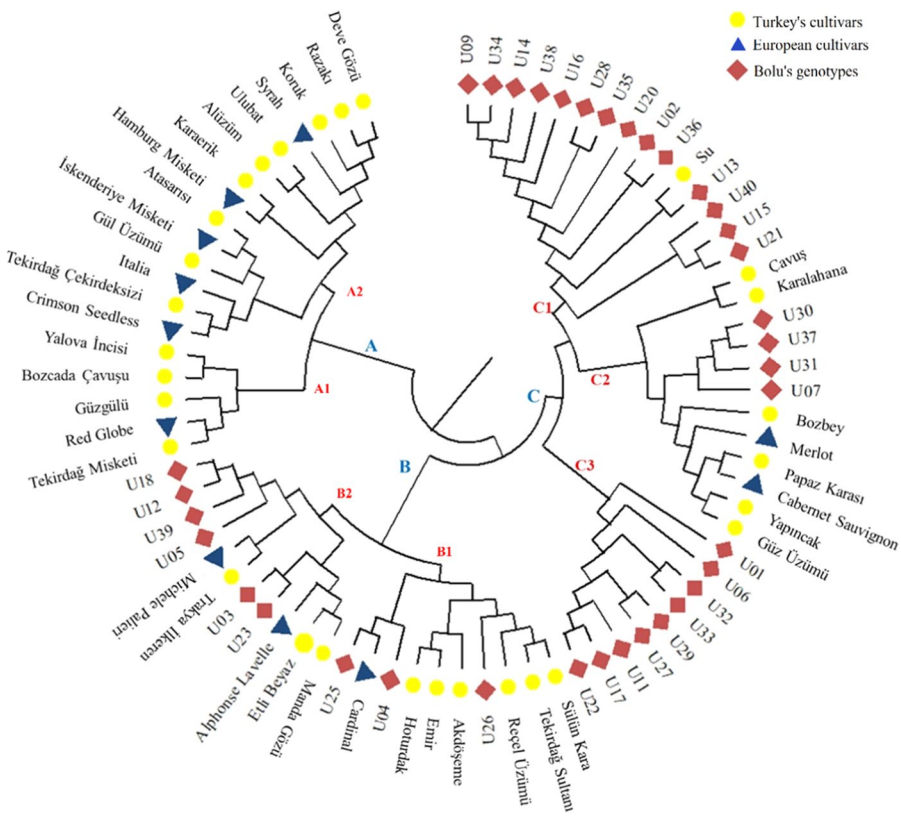

Fig. 1 Genetic tree of grape cultivars and genotypes generated by the neighbor-joining algorithm

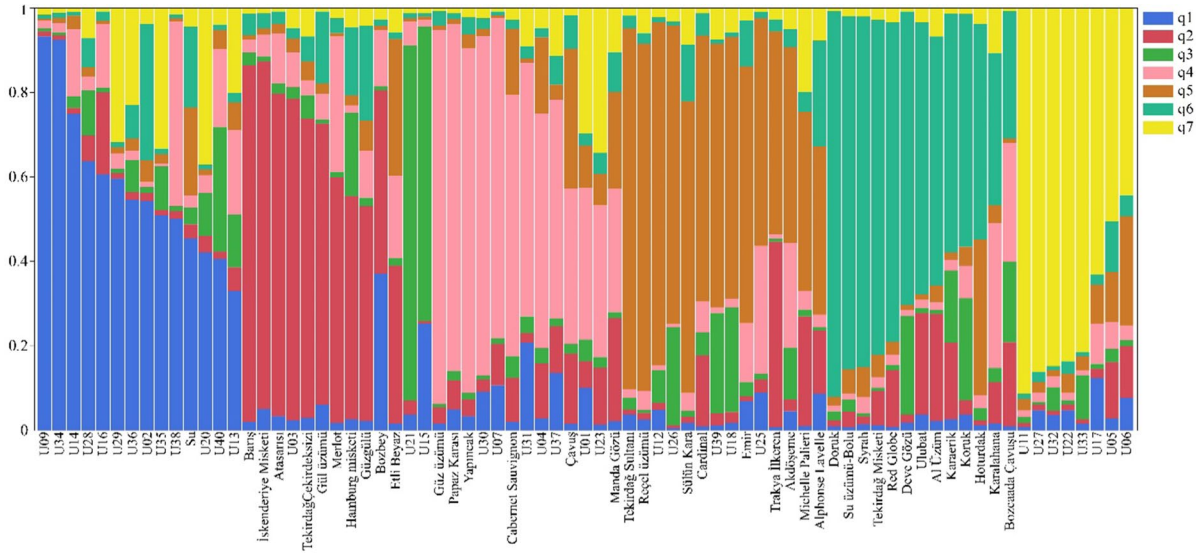

Fig. 2 The distribution and gene proportions of the studied grapevines by STRUCTURE analysis. Numerated individuals by U indicate genotypes of Bolu

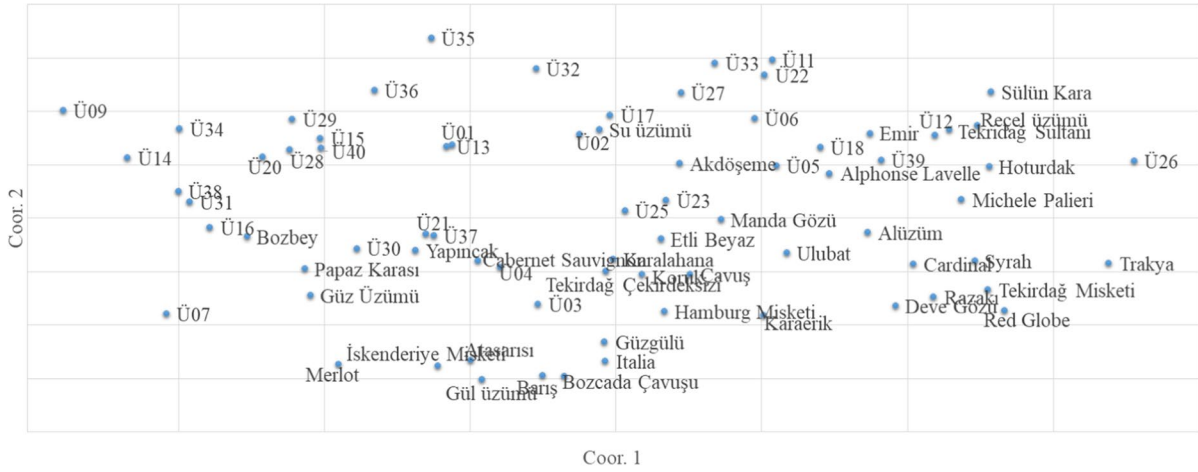

Fig. 3 Distribution of the cultivars and genotypes on principal coordinate plot. The letter "Ü" indicates genotypes of Bolu

Table 3 AMOVA results for eight iPBS primers

Source of variation	Df	SS	MS	Est. Var	Var. (%)
Between populations	2	97.02	48.51	1.49	9%
Within populations	73	1046.34	14.33	14.33	91%
Total	75	1143.37		15.83	100%

Df Degrees of freedom, *SS* Sum of squares, *MS* Mean squares, *Est. Var.* Estimated variance, *Var.* Variance

The genetic variation among the genotypes, Turkey's cultivars, and European cultivars was determined by performing AMOVA. Results demonstrate that the genetic differentiation was significant mainly within the population ($p < 0.001$). Table 3 demonstrates that 9% of the total variation can be attributed to differences between populations, whereas the remaining 91% of the variation occurs within the populations.

Figure 4 illustrates the significant associations ($p < 0.05$) between loci obtained from eight primers and the quantitative traits, according to GLM and MLM. Total soluble solids (TSS) showed a moderate correlation with the 10th locus of the PBS2230 primer in GLM, but no associations were found in MLM. Titratable acidity (TA) was associated with the 19th locus of the PBS2228 primer and the 10th locus of the PBS2230 primer in both GLM and MLM. Sugar-to-acid ratio (SN) had significant but relatively weak correlations with loci from the PBS2230 and PBS2295 primers in MLM, while it was only associated with the PBS2230 locus in GLM. pH exhibited weak correlations with the 14th locus of the PBS2251 primer and the 4th locus of the PBS2395 primer in GLM. In MLM, pH was associated with the 14th locus of the PBS2251 primer, showing a similar correlation value. Additionally, the 20th locus of the PBS2230 primer displayed a relatively strong correlation with pH in GLM. Lightness (L*) was associated with several loci from the PBS2095 primer in GLM, while the 24th locus showed a strong correlation in GLM.

Discussion

Previous research on grapevine genetic resources using SSR markers reported varying mean and polymorphic band numbers. De Michele et al. (2019) reported mean and polymorphic band numbers of 13.70 and 5.06, respectively, while Arnold and Schnitzler (2020) reported 2.14 and 6.69, Zdunić et al. (2020) reported 11.00 and 3.65, Miazzi et al. (2020) reported 11.00 and 5.90, Marsal et al. (2019) reported 12.85 and 10.02, and Cao et al. (2020) reported 12.00 and 3.98. In another study utilizing REMAP, Žulj Mihaljević et al. (2020) reported an average of 3.90 polymorphic bands. Compared to these previous research, the mean and polymorphic band numbers obtained in this study using iPBS retrotransposon markers were higher, indicating that

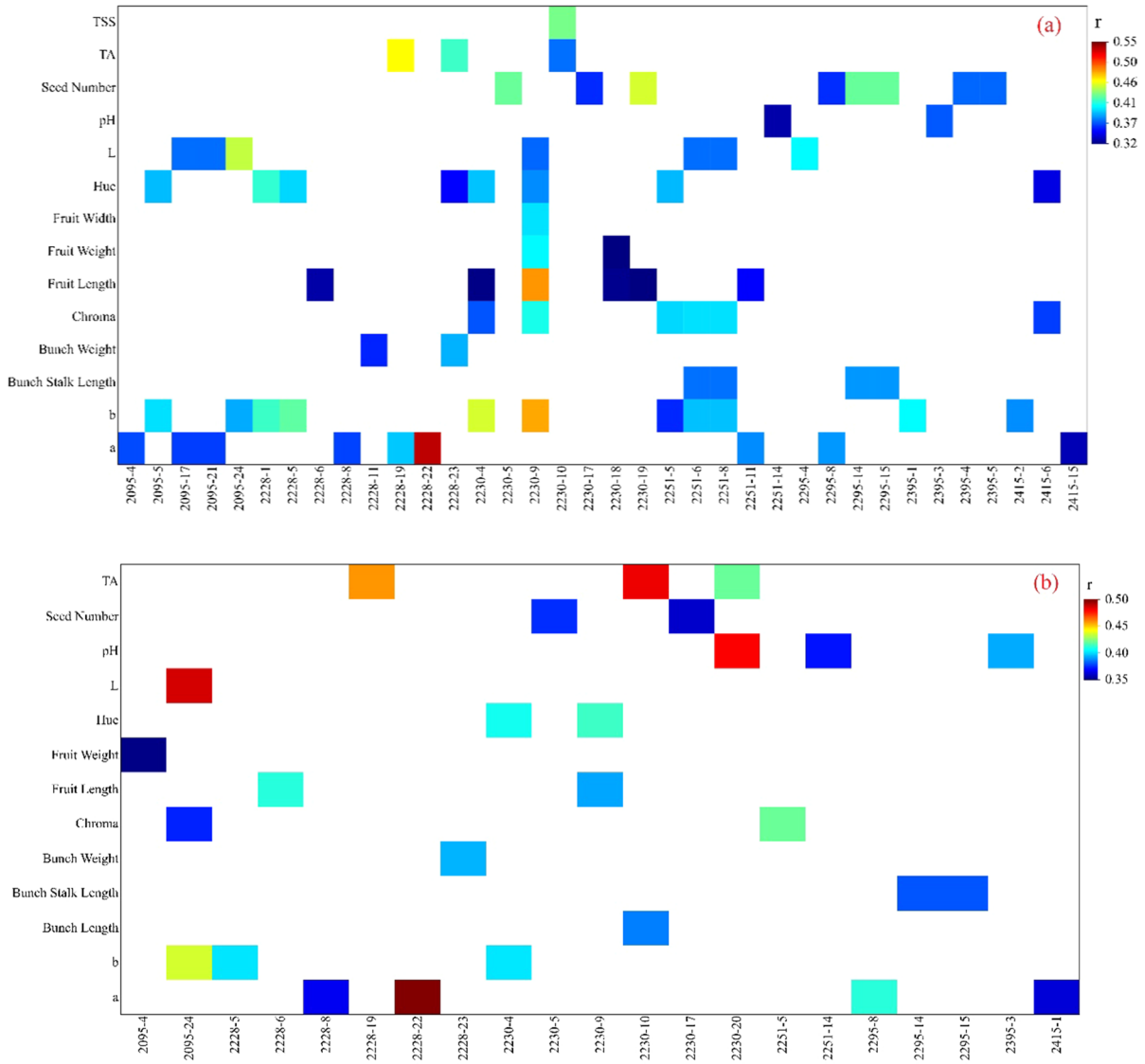

Fig. 4 Trait-loci associations determined by GLM **a** and MLM **b** in autochthonous grapevines of Bolu. The bottom axis represents the primer-locus combinations, while the left axis represents the morphometric traits. Significant associations at the $p \leq 0.01$ level are colored. The color legend, indicating the redundancy of trait-locus associations, is presented in the upper right corner of the figures

iPBS markers were relatively better at distinguishing genetic variation in grapevines.

Genetic polymorphism in a population refers to the differentiation in DNA sequences exceeding 1% (Karki et al. 2015). Polymorphism can result from variations in single nucleotides, known as SNPs, or in repetitive DNA sequences, known as length polymorphism. Previous research has reported polymorphism rates ranging from 26.8 to 85.9% (De Michele et al. 2019), 57.0–100.0% (Papapetrou et al. 2020), 60.8–100.0% (Arnold and Schnitzler 2020), 84.1–96.3% (Yılmaz et al. 2020), and 36.4–75.5% (Zdunić et al. 2020). Notably, a study utilizing iPBS markers reported significantly lower polymorphic band ratios (Milovanov et al. 2019). In our study, the observed polymorphism rates were higher than the majority of previous research, although some overlap exists with certain researchers. It is crucial

to carefully select appropriate markers and primers when assessing polymorphism content, as different primers within the same marker system can yield significantly different polymorphism values. The relatively low variation in polymorphism values observed in our study, despite high polymorphism rates, suggests that the preliminary primer elimination experiment and the chosen primers were successful in achieving a diverse set of polymorphic markers.

In a study by Riaz et al. (2018) using SSR microsatellite markers on *V. vinifera* spp. *sativa* and *sylvestris* accessions, the mean Na was reported as 20.950, Ne as 4.651, and He as 0.678. The researchers also found that sativa had the highest Na and Ne values in the Georgian population, while the Italian population had the lowest values. Another study by De Andrés et al. (2012) utilized 25 microsatellite markers to assess genetic diversity in 192 wild grapevine accessions. The mean Na, Ne, I, and He values were reported as 9.00, 4.22, 1.59, and 0.73, respectively, with slight differences between wild and cultivated forms. In a study by Žulj Mihaljevic et al. (2020) on Croatian grapevines using SSR markers, the mean Na was found to be 9.00, Ne as 3.90, and He as 0.70. The same researchers also noted that Na values ranged from 2.00, Ne values varied between 1.03 and 2.00, and He values ranged from 0.03 to 0.50 in the same population when using SNP markers. These diversity parameters obtained in our study and previous research highlight that the values can vary depending on the marker system employed. Furthermore, the presence of grapevine-specific characterized primers in the SSR marker system suggests that higher values for certain traits can be expected.

During the domestication, significant changes have occurred in the biological properties of grapes, driven by the need to increase sugar content at harvest (Novikova and Naumova 2020). However, the exact mechanisms of genetic change in grapevine gene sources over time are still not fully understood. It remains unclear whether these changes occur gradually through natural crossings or rapidly through processes such as bud mutations, selections, and vegetative propagation (Pelsy et al. 2010). The earliest evidence of wine production dates back approximately 7400 years ago in the Zagros mountains of northern Iran. Additionally, cultivated grape seeds dating back about 8000 years have been discovered in Georgia and Turkey (This et al. 2006). The origin of western European wine grapes is still a subject of debate. One hypothesis suggests that introduced cultivars and local wild populations have undergone reciprocal hybridization as domesticated vines spread westwards, coinciding with the dispersal of wild European grapevines (Magris et al. 2021).

The observed variation between populations in this study, with 9%, aligns with findings from previous studies. Najafi et al. (2006) reported a similar variation of approximately 6% between Iranian and European grape cultivars, indicating that the majority of the variation is found within populations. Ergül et al. (2011) also observed comparable rates of variation, with 92% within populations and 8% between populations. The low genetic variation between populations can be attributed to the high gene flow between regions, as observed in studies on various plant and animal species (Bektaş et al. 2013; Rohollahi and Naji 2020). Grapevines can easily propagate through cuttings, facilitating the exchange of genetic material between regions (McKey et al. 2010). In support of this, Magris et al. (2021) reported gene flow from the Black Sea basin and Balkans, including Bolu, towards primitive European grape cultivars, thereby suggesting that genotypes belonging to the same clade as standard European cultivars could be part of their ancestral tree. Additionally, it should be noted that both the investigated cultivars and the Bolu province genotypes belong to the *V. vinifera* species. Since there is no species distinction, the low variation between populations is an ordinary case.

GLM and MLM are gaining increasing attention in association mapping. MLM, which considers kinship and population structure, is particularly valuable for reducing type 1 errors and spurious correlations (Zhang et al. 2010). In our study, GLM identified a larger number of loci associated with the traits compared to MLM, supporting the notion that GLM can be fruitful in this context. Nevertheless, MLM revealed stronger correlations in some cases, and there were overlapping loci identified by both analyses. Notably, the locus 'PBS2228-22' showed a strong correlation with the trait a*, indicating a potential link to anthocyanin biosynthesis. The TA exhibited associations with the loci 'PBS2228-19' and 'PBS2230-10' in both analyses, suggesting their involvement in acid synthesis pathways. Similarly, L* was strongly associated with the 24th locus of PBS2095 in both approaches, potentially indicating a relation

to cuticular wax. Zhang et al. (2021) reported independent occurrences of cuticular wax and berry skin color, suggesting varietal differences. Since the iPBS retrotransposon markers used in our study are length polymorphic markers (Amiteye 2021), it is possible to generate functional markers by aligning the trait-associated loci with transcribed parts of the genome using gene-targeting approaches and RNA sequence alignments (Poczai et al. 2013). Therefore, it is likely feasible to produce functional markers based on the trait-associated loci identified in this study.

Conclusion

This study aimed to investigate the iPBS retrotransposon markers in various autochthonous grape populations and their association with polymorphic traits related to morphology, color, and physicochemical. The genetic diversity analysis revealed significant differentiation among individuals, indicating their potential value for introducing new cultivars in different usage areas and as suitable parents for hybridization. The diversity assessments using iPBS markers highlighted the importance of examining individuals even if they share the same name. The findings of this study emphasized the genetic differentiation among landraces with the same name, thus suggesting a lack of accuracy in local growers' naming practices. Additionally, association mapping identified trait-associated loci that could be utilized in future research after alignment. Our future research will focus on providing insights into the appropriate cultivation of the investigated genetic resources and further characterizing the trait-associated loci.

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Author contribution EG: Investigation, Methodology, Laboratory research, Data curation, Visualization, Software, Writing–original draft. TK: Supervision. GÖ: Writing–review & editing, Conceptualization. TU: Material supply.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval This article does not contain any research with human or animal subjects and thus does not need ethical approval.

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