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RESEARCH ARTICLE

Investigation of genetic diversity among autochthonous grape cultivars grown in Türkiye using molecular primers

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Abstract Grape is a type of fruit widely grown globally and in Türkiye thanks to its importance in human nutrition and diversity of usage areas. Türkiye has a long history of grape cultivation, and the genetic diversity of local grapes grown in some regions has still not been revealed. This study showed the genetic diversity between 60 local grape genotypes selected from north Central Anatolia and nine reference grape cultivars;10 inter simple sequence repeats (ISSR) and 7 inter-primer binding site (IPBS) retrotransposon primers were used. The mean values of genetic diversity indices in ISSR and IPBS retrotransposon primers were calculated as expected allelic frequency (p) 0.476/0.195, observed allelic frequency (q) 0.524/0.805, number of effective alleles (Ne) 1.326/1.378, Shannon's information index (I) 0.308/0.367, expected heterozygosity (He) 0.199/0.232 and unbiased expected heterozygosity (uHe) 0.201/0.234, respectively. Unweighted pair group method with arithmetic mean (UPGMA) and model-based STRUCTURE analysis divided the studied germplasm into two groups. However, local genotypes were not grouped per the regions where they grew. As a result of the analysis of molecular variance (AMOVA) analysis, the inter-population variation was determined to be 11%, and the intra-population variation was determined to be 89%. The mean genetic differentiation value (Fst) between populations was 0.303. The results showed that ISSR and IPBS retrotransposon primers effectively explained the genetic diversity.

Keywords *Vitis vinifera* · ISSR · İPBS retrotransposon · Genetic diversity · Population structure

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Introduction

Located at the intersection point of three gene centers from the biodiversity centers in the World (Iran-Turan, Mediterranean, and Europe-Siberia) (Sümbül et al. 2023), Türkiye is the homeland of many fruit species. Among the gene centers of grapes, Türkiye is very suitable for grape cultivation in terms of climate and soil conditions. *Vitis vinifera* is the most grown grape species in the world. This species was

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first cultivated in the Caucasus and Anatolia, and this region is known as the center of diversity of the species (Arroyo-Garcia et al. 2006; Ergül et al. 2006). Grape cultivation in Türkiye dates back to 4000 BC (Selli et al. 2007). In Türkiye, which has an old viticulture culture, grapes are grown for different consumption purposes (table, drying, and wine). For this reason, Türkiye has a wide range of local grape cultivars that are assumed to have emerged due to natural hybridization, mutation, and selection (Aradhya et al. 2003).

In the past, grape cultivation generally consisted of traditionally grown local grape cultivars (Stajner et al. 2014). As a result of the damage caused by phylloxera in the vineyards, local cultivars started to disappear. Commercial cultivars that are superior in yield and quality have started to be grown instead of local cultivars (Alleweldt and Dettweiller 1992). In addition, local cultivars are disappearing due to various biotic (pathogens and pests) and abiotic (temperature, drought lime or salt stress in the soil) causes, especially global climate change. Although the cultivars grown for economic gain are superior in quality and yield, they are usually weak (or susceptible to some pests and diseases) in terms of resistance or tolerance to biotic and abiotic conditions, respectively. However, local cultivars have a genetic structure resistant to pathogens and pests, and tolerance to cold and drought. Breeders can develop new cultivars resistant and tolerant to biotic and abiotic stress factors, respectively, using local cultivars. Therefore, identifying and protecting local cultivars is vital for the future of grape cultivation.

Research on the characterization of grape genetic resources initially started with the ampelographic identification method, which analyses and compares phenological, morphological, and pomological characteristics. However, the features used in the ampelographic identification method are not reliable enough because it is affected by environmental factors and varies according to researchers and the development period of the plant (Lamboy and Alpha 1998; Sefc et al. 1999; Fatahi et al. 2003). Therefore, there is a need to use methods that can accurately identify cultivars and are not affected by environmental factors (Yıldız et al. 2021; Yaman and Uzun 2021).

Breeders prefer molecular primers because they can provide information about the genetic structures of cultivars and are not affected by environmental factors (Khadivi et al. 2019; Pinar et al. 2021). Amplified fragment length polymorphism [AFLP] (Sabır et al. 2010), random amplified polymorphic DNA [RAPD] (Hameed et al. 2020), simple sequence repeats [SSR] (Karaca-Sanyürek 2014; İşçi and Dilli 2015; Khadavi et al. 2019), ISSR (Sabır et al. 2008; Salayeva et al. 2016; Lisek and Lisek 2019), inter-primer binding site [İPBS] (Ziarovska et al. 2022; Güler et al. 2023), sequence-related amplified polymorphisms [SRAP] (Sabır et al. 2018), and single nucleotide polymorphisms [SNP] (Cunha et al. 2020) have been investigated to find genetic diversity among grape cultivars. Inter simple sequence repeats (ISSR) primers are used for the molecular identification of many plants (Pinar et al. 2021; Yildiz et al. 2021, 2023; Yaman 2022a, b; Karakaya et al. 2023). Researchers prefer ISSR primers because they are simple, provide rapid results, have high stability, do not require prior sequence information, and have low cost (Choudhary et al. 2014). Recently, the IPBS retrotransposon primer developed by Kalendar et al. (2010) is frequently used (Guo et al. 2014; Güler 2021; Ziarovska et al. 2022). IPBS retrotransposon primers are highly reproducible, require low amounts of DNA, do not require prior sequence information, and are cost-effective (Nadeem et al. 2018).

Recent advances in molecular primers have created tremendous potential for identifying the genetic diversity of plant germplasm collections (Baran et al. 2023). In molecular research conducted in recent years, diverse types of molecular primers have started to be used together to overcome the limitations of a single molecular primer (Sabır et al. 2009). It has been stated in many investigations that using different primers together will provide more comprehensive results about cultivars (Marakli 2018; Tian et al. 2018; Mao et al. 2018).

The Kelkit Basin is a geographical transition between the Black Sea and Central Anatolia regions and has both a Black Sea and a continental climate. The region is rich in natural resources and biodiversity, and the climate and soil conditions are highly suitable for agricultural production. It is at the intersection of the European-Siberian and Iran-Turanian floristic regions; plants belonging to the Mediterranean floristic region are also seen (Karaer and Kılınç 2001). The Kelkit Basin is located within grid squares A6 and A7 in the grid system created by Davis (Davis, 1965–1988). In a study carried out in the

region, 2800 plants were collected. It was determined that the collected plants consisted of 519 genera and 1316 species and subspecific taxa belonging to 105 families, and 132 were endemic (Karaer and Kılınç 2001). Grape cultivation has been carried out for hundreds of years in the Kelkit Basin. For this reason, the study area is affluent in grape genetic resources.

This study is the first research conducted on local grape genotypes in the region. The study used ISSR and IPBS retrotransposon molecular primer techniques to determine the level of genetic relationship and population structure between local grape genotypes and reference grape cultivars.

SSR marker is a co-dominant marker that has been successfully used in the molecular characterization of various crops, including grapes (Wang et al. 2015; Cao et al. 2020). However, we used two different molecular markers, i.e., iPS-retrotransposons and ISSR. Besides the dominant nature, ISSR markers have been successfully applied for the molecular characterization of various crops (Ekincialp et al. 2019; Ali et al. 2020) and also for the grape as well (Sabır et al. 2008; Argade et al. 2009; Sümbül et al. 2023). Moreover, Retrotransposons contribute to most of the eukaryotic genome and are considered a sound marker system. Kalender et al. (2010) reported iPBS-retrotransposons as a universal marker system successfully utilized for genetic diversity research (Nadeem 2021; Baran et al. 2023; Yeşil et al. 2023). The reproducibility and trust ability of both markers is universally accepted. Therefore, we planned to use both marker systems for molecular characterization.

Materials and methods

Plant material

The study was carried out on local grape cultivars grown in Koyulhisar, Suşehri, the Akıncılar districts of Sivas province, and the Şebinkarahisar district of Giresun province (Fig. 1). In the study, leaf samples of 60 local grape genotypes and nine reference grape cultivars (İzabel, Narince, Kyoho, Alphonse Lavallee, Michele Palieri, Horoz Karası, Muscat Bleu, Philipp, and Glenora) were used as material. Leaf samples of reference grape cultivars were obtained from Selçuk University Faculty of Agriculture Research and Application greenhouses for İzabel, Narince, Kyoho, Alphonse Lavallee, Michele Palieri, and Horoz Karası grape cultivars, and Erciyes University Fruit Research and Application area for Muscat Bleu, Philipp, and Glenora grape cultivars. Leaf samples taken for molecular analyses were brought to the laboratory in a cold chain and stored at -80 °C until the DNA isolation process (Table 1).

Molecular analyses

DNA isolation

Young, healthy, and clean leaves were used in molecular analyses. Leaf samples taken for molecular analyses were brought to the laboratory in a cold chain and stored at -80 °C until the DNA isolation process. The DNA isolation method was the CTAB method Doyle and Doyle (1990) recommended. The DNA samples were dissolved in distilled water, and the prepared DNA samples were stored at -20 °C.

IPBS – retrotransposon analyses

42 different IPBS retrotransposon primers developed by Kalendar et al. (2010) were screened for polymorphism using eight randomly selected grape genotypes. Consequently, only seven of the most polymorphic primers with clear and visible banding patterns were chosen for the genetic diversity assessment of all 60 local grape genotypes and nine reference grape cultivars. The PCR mixture was prepared as 10 µl in total. The PCR mixture consisted of 1 µl 10× PCR Buffer, 1 µl $MgCl_2$, 1 µl dNTPs, 1.5 µl primer, 0.15 µl Tag DNA Polymerase, 2.85 µl distilled water and 2.5 µl template DNA. PCR cycle conditions are as follows: Pre-denaturation at 95 °C for 4 min (1 cycle), denaturation at 95 °C for 15 s (30 cycles), annealing of the primer (depending on the primer) at 49.6 − 54 °C for 1 min (30 cycles), extension phase at 68 °C for 1 min (30 cycles) final extension phase at 72 °C for 5 min (1 cycle).

ISSR Analyses

In total, 20 different ISSR primers were screened for polymorphism using eight randomly picked grape genotypes. Consequently, only ten of the most polymorphic primers with clear and visible banding patterns were chosen for the genetic diversity assessment

Table 1 Passport data of grape cultivars characterised

Genotype Code	Genotype Name	Fruit Skin Color	Origin	Genotype Code	Genotype Name	Origin	Fruit Skin Color
U1	Kokulu	White	Akıncılar	U31	Karadeniz	Suşehri	Colourful
U2	Siyah üzüm 1	Colorful	Akıncılar	U32	Müşkü	Koyulhisar	White
U3	Adıyaman	White	Akıncılar	U33	Beyaz üzüm 2	Koyulhisar	White
U4	Mor üzüm 1	Colorful	Akıncılar	U34	Mor üzüm 3	Koyulhisar	Colourful
U5	Beyaz üzüm 1	White	Akıncılar	U35	Ağ üzüm	Koyulhisar	White
U6	Alyanak	White	Akıncılar	U36	Çavuş 1	Koyulhisar	White
U7	Cemin	Colorful	Akıncılar	U37	Müşküle	Koyulhisar	White
U8	İstanbul	White	Şebinkarahisar	U38	Emcoğlu	Koyulhisar	White
U9	Gazova 1	White	Şebinkarahisar	U39	Dağ üzümü	Koyulhisar	Colourful
U10	Mor üzüm 2	Colorful	Şebinkarahisar	U40	Danagözü	Koyulhisar	White
U11	Uzun üzüm	White	Şebinkarahisar	U41	Çavuş 2	Koyulhisar	White
U12	Parmak üzümü	White	Şebinkarahisar	U42	Çekirdeksiz 1	Koyulhisar	Colourful
U13	Dökülen	White	Şebinkarahisar	U43	Siyah Çekirdeksiz	Koyulhisar	Colourful
U14	Kara üzüm 1	Colorful	Şebinkarahisar	U44	Tokat üzümü	Suşehri	White
U15	Gazova 2	White	Şebinkarahisar	U45	Mor üzüm 4	Suşehri	Colourful
U16	Siyah üzüm 2	Colorful	Şebinkarahisar	U46	Bursa üzümü	Suşehri	White
U17	Sarı üzüm 1	White	Şebinkarahisar	U47	Sarı yanak	Suşehri	White
U18	Gazova 3	White	Şebinkarahisar	U48	Beyaz üzüm 3	Suşehri	White
U19	Siyah Gazova	Colorful	Şebinkarahisar	U49	Güççük	Suşehri	White
U20	Şirelik	White	Şebinkarahisar	U50	Kara Salkım	Suşehri	Colourful
U21	Pembe üzüm 1	Colorful	Şebinkarahisar	U51	Keribar	Suşehri	White
U22	Kara üzüm 2	Colorful	Şebinkarahisar	U52	Ak üzüm	Suşehri	White
U23	Pembe üzüm 2	Colorful	Şebinkarahisar	U53	Davut üzümü	Suşehri	White
U24	Dedem	White	Şebinkarahisar	U54	Işıklar	Suşehri	White
U25	Sık üzüm	White	Şebinkarahisar	U55	İri mor	Suşehri	Colourful
U26	Gevrek	White	Şebinkarahisar	U56	Yeşil üzüm	Suşehri	White
U27	Siyah Gevrek	Colorful	Şebinkarahisar	U57	Geççi	Suşehri	White
U28	Sarı üzüm 2	White	Şebinkarahisar	U58	Mor üzüm 5	Suşehri	Colourful
U29	Keçi Memesi	White	Şebinkarahisar	U59	Uzun Kara	Suşehri	Colourful
U30	Tatlı Kara	Colorful	Şebinkarahisar	U60	Çekirdeksiz 2	Suşehri	White

of all 60 local grape genotypes and nine reference grape cultivars. The PCR mixture was prepared as 20 µl in total. The PCR mixture consisted of 2 µl 10x PCR Buffer, 1.5 µl $MgCl_2$, 1.5 µl dNTPs, 1 µl primer, 0.3 µl Tag DNA Polymerase, 8.7 µl distilled water and 5 µl template DNA. PCR cycle conditions are as follows: Pre-denaturation at 94 °C for 2 min (1 cycle), denaturation at 94 °C for 1 min (45 cycles), annealing of the primer (depending on the primer) at 53 °C for 1 min (45 cycles), extension phase at 72 °C for 2 min (45 cycles) final extension phase at 72 °C for 5 min (1 cycle). The names, sequences, and annealing temperatures of the primers are given in Table 2.

PCR products were loaded onto a 1.7% agarose gel in 1 x TBE (Tris Boric Acid EDTA) buffer solution and run at 110 volts for six hours. While preparing the agarose gel, 25 µl of ethidium bromide was added. A 100 bp DNA ladder (Thermo Fisher Scientific) was used as a standard during each electrophoresis run. After electrophoresis, the gels were placed into the imaging device connected to the computer for visualization. The images on the gel were photographed

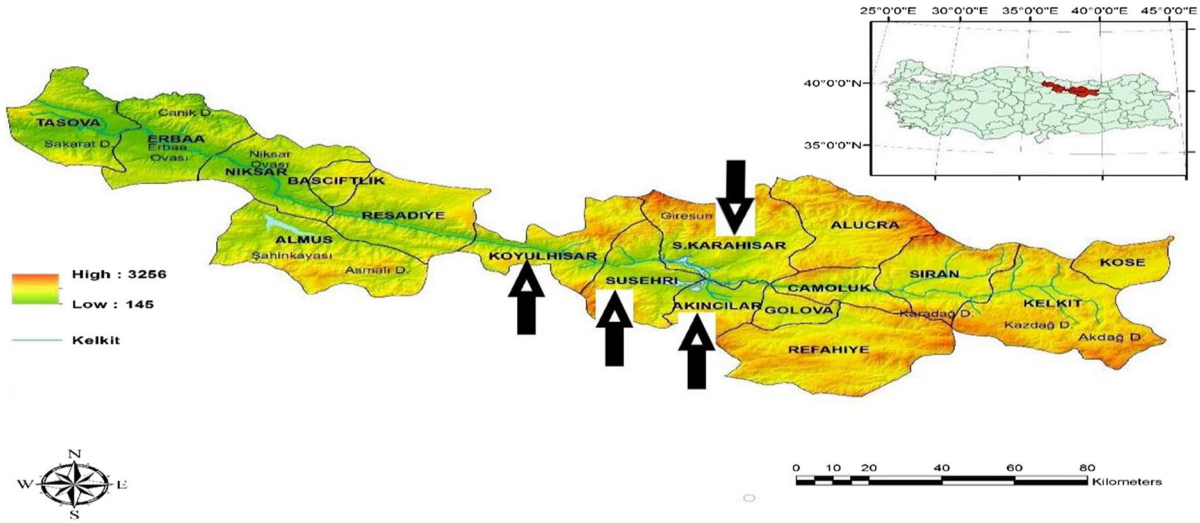

Fig. 1 Geographical location of the study area (Kılıç 2015)

Table 2 Sequence and annealing temperature information of inter-primer binding site (IPBS) retrotransposon and inter simple sequence repeat (ISSR) primers

Primer names	Primer sequence (5”-3”)	Annealing temperature (°C)
ISSR primers		
(AGC)6G	AGCAGCAGCAGCAGCAGCG	53.0
(GACA)4	GACAGACAGACAGACA	53.0
(CT)8TG	CTCTCTCTCTCTCTCTTG	53.0
DBDA(CA)7	DBDACACACACACACACA	53.0
(GA)8YG	GAGAGAGAGAGAGAGAYG	53.0
(AG)8T	AGAGAGAGAGAGAGAGT	53.0
HVH(TCC)7	HVHTCCTCCTCCTCCTCCTCC	53.0
(TAA)8	TAATAATAATAATAATAATAA	53.0
(CA)8R	CACACACACACACACAR	53.0
(CAC)6	CACCACCACCACCACCAC	53.0
İPBS retrotransposon primers		
İPBS 2074	GCTCTGATACCA	49.6
İPBS 2222	ACTTGGATGCCGATACCA	53.0
İPBS 2228	CATTGGCTCTTGATACCA	54.0
İPBS 2251	GAACAGGCGATGATACCA	53.2
İPBS 2383	GCATGGCCTCCA	53.0
İPBS 2391	ATCTGTCAGCCA	52.6
İPBS 2393	TACGGTACGCCA	51.0

under UV light in the imaging device and saved to the computer.

Data analyses

In the gel images of the primers, the values are given as (1) in the presence of bands and (0) in the absence of bands, and binary scoring was done. After

the scoring process, the length of the bands of the primers, the total number of bands, the number of polymorphic bands, polymorphism rates, and polymorphic information content were determined. The polymorphic information content (PIC) of the primers was calculated according to the formula PIC = 2fi (1-fi) suggested by Roldan-Ruiz et al. (2000). According to the formula, fi refers to the frequency of the existing bands of a primary, and (1-fi) refers to the frequency of the absent bands. Expected and observed allelic frequency (p, q), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), and analysis of molecular variance (AMOVA) were determined with GenAlEx 6.5 software (Peakall and Smouse 2006).

In the study, the UPGMA (Unweighted Pair-Group Method with Arithmetic Average) dendrogram was created using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1. Exeter Software, Setauket. N.Y. USA.) software to determine the genetic relationship between local and commercial grapes (Rholf 2000).

STRUCTURE and STRUCTURE Harvester package programs were used to investigate the population structure of the grapes examined in the study. The STRUCTURE program analyses the population structure and calculates the Q probability value by separating the individuals in the population into subpopulations (Pritchard et al. 2000). The number of subpopulations (K) was calculated from 1 to 10. Five repeated analyses were performed for the K value with 10,000 burning cycles and 10,000 repetitions at each step. The resulting file of the STRUCTURE program was analyzed in the STRUCTURE Harvester program (Earl and VonHoldt 2012). As a result of the analysis, the optimum K value and subgroups of the population were determined (Evanno et al. 2005).

Results

In the molecular characterization of grapevine germplasm, 10 ISSR and 7 IPBS retrotransposon primers were used. The length of the bands, number of polymorphic bands, polymorphism rates, and polymorphic information contents of the primers are given in Table 3.

A total of 62 scorable bands were obtained from ISSR primers, and 52 of these bands were detected as polymorphic. The number of bands obtained from the primer varied between 3 (GA)8YG, (TAA)8, (CAC)6) and 13 (DBDA(CA)7). Band lengths of ISSR primers were determined to be between 190 and 2500 bp. The mean number of bands of the primers was 6.20, the mean number of polymorphic bands was 5.20, and the mean polymorphism rate was 83.37%. While the PIC of ISSR primers was between 0.29 ((AG)8T) and 0.49 ((CT)8TG), the mean PIC was determined as 0.40.

Seventy-six scorable bands were obtained from IPBS retrotransposon primers, and 76 were detected as polymorphic. The number of bands obtained from the primer varied between 9 (IPBS 2222, IPBS 2251, IPBS 2383) and 13 (IPBS 2074, IPBS 2228, IPBS 2393). Band lengths of IPBS retrotransposon primers were determined to be between 200 and 3000 bp. The mean number of bands of the primers was 10.86, the mean number of polymorphic bands was 10.86, and the mean polymorphism rate was 100%. While the PIC of IPBS retrotransposon primers was between 0.17 (2228) and 0.50 (2393), the mean PIC was determined as 0.35.

Expected allelic frequency (p), observed allelic frequency (q), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) values of ISSR and IPBS retrotransposon primers are given in Table 4. In the ISSR primers, p and q values respectively ranged from 0.096 ((AG)8T) to 1.000 ((TAA)8, (CA)8R, (CAC)6) and from 0.000 ((TAA)8, (CA)8R, (CAC)6) to 0.904 ((AG)8T). The highest value of Ne = 1.803, I = 0.629, He = 0.439, and uHe = 0.442 were observed for (AGC)6G primer, while the lowest Ne = 1.000, I = 0.000, He = 0.000, and uHe = 0.000 were found for ((TAA)8, (CA)8R and (CAC)6) primers. In the İPBS retrotransposon primers, p and q values respectively ranged from 0.042 (2222) to 0.461 (2391) and from 0.539 (2391) to 0.958 (2222). The highest values of Ne = 1.784, I = 0.615, He = 0.428, and uHe = 0.431 were observed for 2391 primer, while the lowest Ne = 1.093, I = 0.149, He = 0.076, and uHe = 0.077 were found for 2222 primer.

Molecular analyses were evaluated by combining the scoring results obtained from ISSR and IPBS primers. The UPGMA dendrogram was created to

Table 3 Band and polymorphism information of inter simple sequence repeat (ISSR) primers and inter-primer binding site (IPBS) retrotransposon

	Base length (bp)	Number of total bands	Number of polymorphic bands	Polymorphism rate (%)	PIC
ISSR primers					
(AGC)6G	260–2500	11	11	100	0.45
(GACA)4	300–1500	8	8	100	0.45
(CT)8TG	290–1900	7	7	100	0.49
DBDA(CA)7	210–2500	13	13	100	0.32
(GA)8YG	450–900	3	3	100	0.48
(AG)8T	510–1800	5	5	100	0.29
HVH(TCC)7	190–730	5	5	100	0.34
(TAA)8	300–800	3	0	0	-
(CA)8R	300–900	4	0	0	-
(CAC)6	210–700	3	0	0	-
Mean	**190–2500**	**6.20**	**5.20**	**83.87**	**0.40**
Total	**-**	**62**	**52**	**-**	**-**
IPBS retrotransposon primers					
IPBS 2074	200–2700	13	13	100	0.47
IPBS 2222	400–3000	9	9	100	0.15
IPBS 2228	490–3000	13	13	100	0.17
IPBS 2251	230–2400	9	9	100	0.29
IPBS 2383	200–2500	9	9	100	0.43
IPBS 2391	200–2200	10	10	100	0.44
IPBS 2393	250–2600	13	13	100	0.50
Mean	**200–3000**	**10.86**	**10.86**	**100**	**0.35**
Total	**-**	**76**	**76**	**-**	**-**

Table 4 Expected allelic frequency (p), observed allelic frequency (q), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) of inter simple sequence repeats (ISSR) and inter-primer binding site (İPBS) retrotransposon primers in the study

Primer	p	q	Ne	I	He	uHe
ISSR primers						
(AGC)6G	0.446	0.554	1.803	0.629	0.439	0.442
(GACA)4	0.492	0.508	1.567	0.523	0.345	0.348
(CT)8TG	0.262	0.738	1.599	0.524	0.349	0.352
DBDA(CA)7	0.113	0.887	1.251	0.308	0.178	0.180
(GA)8YG	0.230	0.770	1.548	0.504	0.332	0.335
(AG)8T	0.096	0.904	1.221	0.264	0.156	0.157
HVH(TCC)7	0.120	0.880	1.275	0.333	0.195	0.196
(TAA)8	1.000	0.000	1.000	0.000	0.000	0.000
(CA)8R	1.000	0.000	1.000	0.000	0.000	0.000
(CAC)6	1.000	0.000	1.000	0.000	0.000	0.000
IPBS retrotransposon primers						
IPBS 2074	0.213	0.787	1.499	0.480	0.312	0.314
IPBS 2222	0.042	0.958	1.093	0.149	0.076	0.077
IPBS 2228	0.048	0.952	1.102	0.187	0.091	0.091
IPBS 2251	0.102	0.898	1.212	0.244	0.140	0.141
IPBS 2383	0.187	0.813	1.374	0.385	0.240	0.242
IPBS 2391	0.461	0.539	1.784	0.615	0.428	0.431
IPBS 2393	0.313	0.687	1.585	0.509	0.340	0.342

investigate the genetic relationship between grapevine germplasms (Fig. 2). The similarity index of the grapes in the dendrogram formed according to the UPGMA method varied between 0.48 and 0.87. Two different main groups in the dendrogram were formed. While none of the local grape genotypes were found in group A, reference grape cultivars U62, U64, U65, and U68 were found in group A. Group A is divided into two subgroups. The A1 group included U64 and U65 (0.71) grape cultivars, and the A2 group included U62 and U68 (0.72). Group B includes other reference grape cultivars and local grape genotypes. Group B is divided into two subgroups. In the B1 group were included local grape cultivars U4, U6, U11, U14, U16, U17, U20, U24, U27, U28, U34, U43, U44, U47, U59, while the B2 group included other reference grape cultivars and local grape genotypes. U17 and U24 (Şebinkarahisar region) were the most similar genotypes among the genotypes examined, with a similarity ratio of 0.87. Then, genotypes U26 (Şebinkarahisar region) and U37 (Koyulhisar region) had a similarity ratio of 0.86, and genotypes U4 (Akıncılar region) and U34 (Koyulhisar region) had a similarity ratio of 0.85. U14 genotype (Şebinkarahisar region) with a 0.69 similarity ratio and U55 (Suşehri region) genotype with a 0.70 similarity ratio were the most distant genotypes to other genotypes. U61 reference cultivar with a 0.69% similarity rate was the cultivar with the lowest similarity rate in the study. Among the genotypes with a high similarity ratio, genotypes U4 and U34 have colored fruit skin color, while genotypes U17, U24, U26, and U37 have white fruit skin color. According to the information obtained from the dendrogram, it was determined that local grape genotypes did not form similar groups according to the regions where they were grown. In addition, it cannot be said that grapes with colored and white skin color form similar groups. Considering that the genotypes with the closest similarity ratio are taken from different regions, it can be concluded that there is gene flow between regions. This result can be explained by

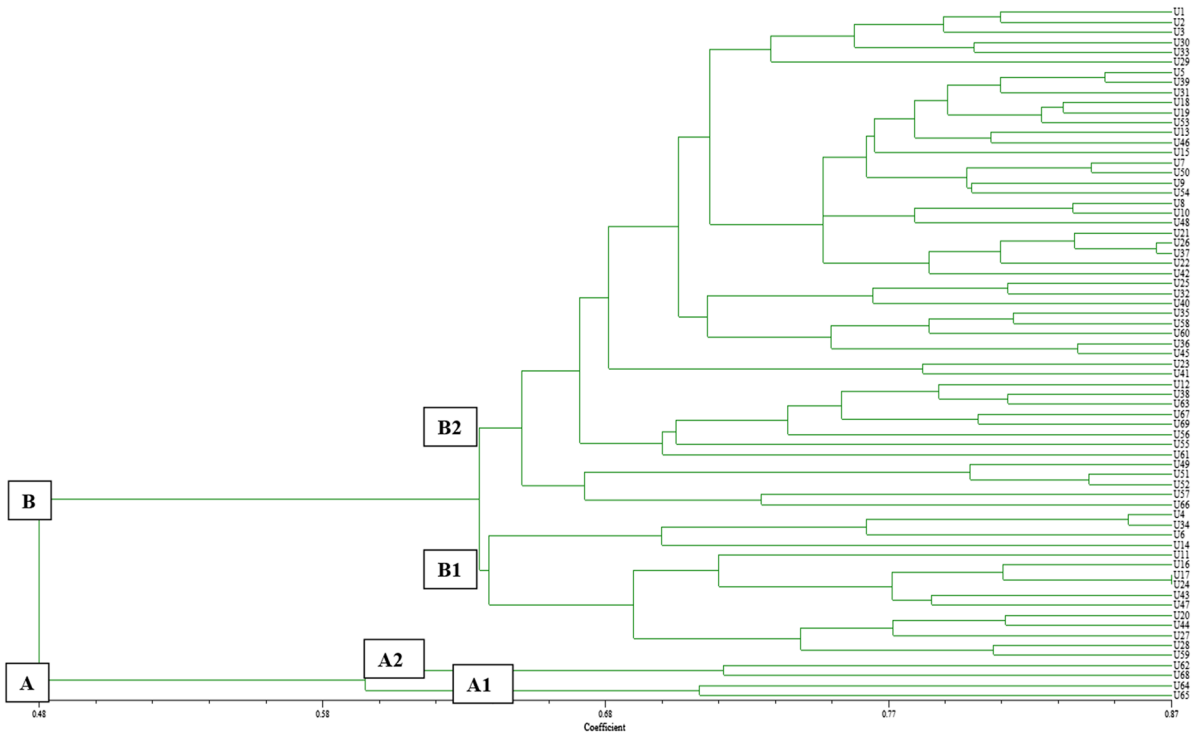

Fig. 2 Unweighted pair group method with arithmetic mean (UPGMA) dendrogram obtained using inter simple sequence repeats (ISSR) and inter-primer binding site (IPBS) retrotransposon primers (Kyoho – U61, Narince – U62, İzabel – U63, Alphonse Lavallee – U64, Michele Palieri – U65, Horoz Karası – U66, Muscat Bleu – U67, Philipp – U68, Glenora – U69)

the producers moving the genotypes from one region to another region.

The population graph of the grape genotypes and cultivars created in the STRUCTURE package program is given in Fig. 3. The model-based STRU CTURE package program divided all the examined genotypes and cultivars into two populations (Fig. 4). Individuals in the population are categorized according to whether they have a membership coefficient $Q \geq 75\%$. Accordingly, 22 grape genotypes and cultivars were determined in the first population, while 20 grape genotypes and cultivars were determined in the second population. Twenty-seven genotypes with a $Q < 75\%$ membership coefficient were considered unclassified. Eighteen local grape genotypes were in the first population, while 16 were in the second population.

The results of AMOVA to reveal the differences between local grape genotypes and reference grape cultivars are given in Table 5. As a result of the analysis, it was determined that 11% of the total variation was among-population and 89% was within-population.

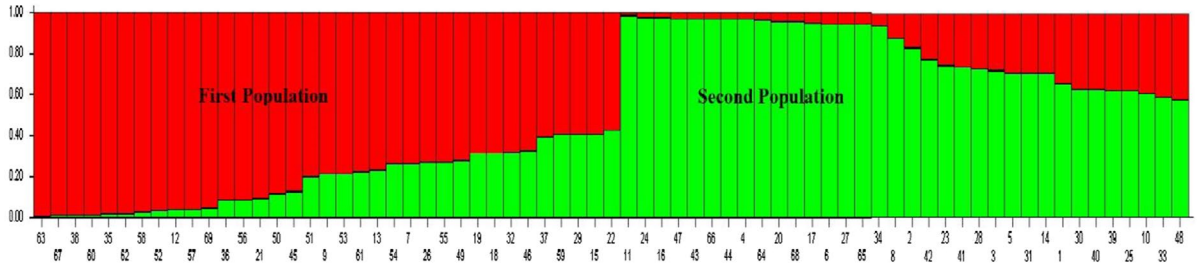

Fig. 3 Population structure of grapes germplasm as determined by the inter simple sequence repeats (ISSR) and inter-primer binding site (iPBS) retrotransposons marker system

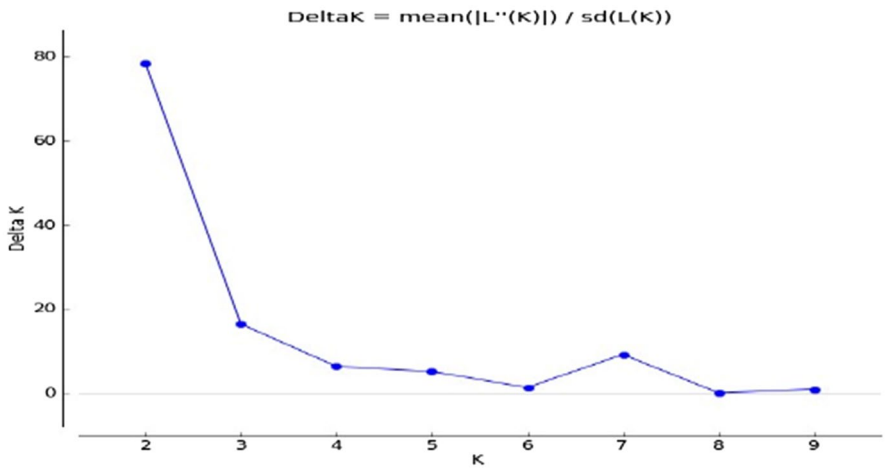

Fig. 4 Delta K (ΔK) the number of populations resulted through the application of inter simple sequence repeats (ISSR) and inter-primer binding site (IPBS) retrotransposons

Table 5 Analysis of molecular variance (AMOVA) revealing genetic diversity within the studied 69 grape accessions

Source of variation	Degrees of freedom	Sums of squares	Mean squares	Estimated variation	Percentage
Among Pops.	4	82,090	20,523	0.961	11
Within Pops.	64	503,939	7,874	7.874	89
Total	68	586,029		8.835	100

The expected heterozygosity, which measures the mean distance between individuals within the same group, was determined as 0.291 in the first subgroup and 0.171 in the second subgroup. The genetic differentiation value (Fst) between subpopulations was calculated as 0.090 in the first population and 0.516 in the second population. The mean genetic differentiation value between populations was 0.303 (Table 6).

Discussion

Identification of the genetic diversity of plant species is vital for breeding them. Therefore, molecular characterization of plants is essential in selecting the right parents in breeding programs. Different molecular markers have been used to reveal genetic diversity in grapevines. (Sabır et al. 2010, 2018; Karaca-Sanyürek 2014; Salayeva et al. 2016; Hamed et al. 2020; Cunha et al. 2020; Pınar et al. 2021; Güler et al. 2023). However, the use of multiple primers for molecular characterization in grapevine breeding is increasing.

When research conducted with ISSR primers in grapevines is examined, it is seen that the mean number of bands varies between 3.26 and 12.28, the mean number of polymorphic bands varies between 2.10 and 9.10, and the mean polymorphism rate varies between 36% and 100% (Sabır et al. 2009; Zeinali et al. 2012; Choudhary et al. 2014; Salayeva et al. 2016; Lisek and Lisek 2019; Basheer-Salimia and Mujahed 2019; Hameed et al. 2020; Sümbül et al. 2023). In research conducted with IPBS retrotransposon primers, the mean number of bands varied between 6.00 and 17.00, the mean number of polymorphic bands varied between 5.7 and 13.25, and the mean polymorphism rate varied between 77.40% and 86.30% (Guo et al. 2014; Güler 2021; Ziarovska et al. 2022). The mean and number of polymorphic bands have shown variability in research conducted on grapevine genetic resources using different molecular primers. In research using SSR, the mean and number of polymorphic bands were 3.8 and 2.7 as reported by Sabır et al. (2018), 8.0 and 7.6 by Dong et al. (2018), 2.14 and 6.69 by Arnold and Schintzler (2020), and 11.0 and 5.90 by Miazzi et al. (2020), respectively. In research with SRAP markers, the mean and number of polymorphic bands were reported as 7.4 and 3.53 by Sabır et al. (2018) and 5.40 and 4.24 by Zhang et al. (2018), respectively. When the results of the study are compared with previous research in terms of mean and number of polymorphic bands, it is seen that ISSR primers have similar values, and IPBS retrotransposon primers have relatively high values.

PIC theoretically takes values between 0 and 1. However, the maximum PIC value for each marker is equal to 0.5 (Doğan and Doğan 2019). In Botstein et al. (1980), PIC value <0.25 is classified as low informative, between 0.25 and 0.5 is classified as reasonably informative, and >0.5 is classified as highly informative. Our study result was in the reasonably informative class for ISSR and IPBS retrotransposon primers regarding PIC values. While the mean PIC value was determined as 0.76 (Sabır et al. 2009), 0.85 (Choudhary et al. 2014), and 0.89 (Lisek and Lisek 2019) in ISSR primers, in IPBS retrotransposon primers, it was determined as 0.44 (Guo et al. 2014), 0.23 (Güler 2021) and 0.38 (Ziarovska et al. 2022). The band lengths obtained from ISSR and IPBS retrotransposon primers within the scope of the study were similar to previous research. While band lengths were determined in the range of 300–2500 bp by Sabır et al. (2009), 100–3000 bp by Zienali et al. (2012), 300–3000 bp by Mwamahonje et al. (2015), 150–900 by Basheer-Salimia and Mujahed (2019) in ISSR primers, in IPBS retrotransposon primers, Milovanov et al. (2019) were determined in the range of 300–6000 bp.

In research on grape cultivars, p, q, Ne, I, He, uHe have shown variability. In a study conducted using ISSR primers, the mean p, q, Ne, I, He, and the values were determined as 0.576, 0.423, 1.321, 0.287, 0.190, and 0.195, respectively (Sümbül et al. 2023). In a study conducted with IPBS retrotransposon primers, the mean p, q, Ne, I, He, and uHe the values were determined as 0.388, 0.611, 1.286, 0.265, 0.172, and 0.177, respectively, by Güler et al. (2023). In research using SSR primers, De Andres et al. (2012) determined Ne = 4.22, I = 1.59, and He = 0.73, Riaz et al.

Table 6 Heterozygosity and fixation index (Fst) value calculated for two sub-populations of grapes

Sub-Population	Expected Heterozygosity	Fst value
1	0.291	0.090
2	0.171	0.516
Mean	0.231	0.303

(2018) determined Ne = 4.651 and He = 0.678, and Zulj Mihaljevic et al. (2020) determined as Ne = 3.90 and He = 0.70.

As a result of the study, although the band and diversity features of ISSR and IPBS retrotransposon primers were generally similar to previous research, they were high values for some features and low values for others. It can be said that these differences are due to the differences in the primers and genetic differences of individuals in the population.

STRUCTURE based analysis was performed better to explain the genetic diversity among the examined individuals. The model-based STRUCTURE package program divided all the examined genotypes and cultivars into two different populations. The mean distance between individuals in the same group was 0.291 in the first group and 0.171 in the second group. A similar study conducted in Bolu province (Türkiye) determined the average distances between individuals to be 0.19 in the first group and 0.24 in the second group (Güler 2021).

The Fst value, which expresses the genetic differentiation value between subpopulations, varies between 0.0 and 1.0. The Fst value of 0.0 is interpreted as no differentiation, and the Fst value of 1.0 is interpreted as complete differentiation (Wright 1951). According to the Fst values obtained in the study, the differentiation in the second population (0.516) is higher than in the first population (0.090). According to these results, it can be stated that genetic diversity is high among the grapes examined. The mean Fst value (0.303) obtained in our study was relatively higher than in previous research. The mean Fst value was reported as 0.05 by Najafi et al. (2006) in their study investigating the genetic diversity of Iranian and European grapes, as 0.05 by Ergül et al. (2011) in their study investigating wild grapevines in Anatolia, as 0.23 by Riaz et al. (2018) in their study on wild grapevines, as 0.25 by Riaz et al. (2019) in their study investigating the genetic diversity of grapevine rootstocks, as 0.15 by Yılmaz et al. (2020) in their study on the genetic characterization of grapevines collected from Central Anatolia, and as 0.26 by Güler (2021) in their study in the Bolu province of Türkiye.

AMOVA was conducted to reveal the differences between local grape genotypes and reference grape cultivars. As a result of the analysis, it was determined that 11% of the total variation was inter-population and 89% was intra-population Najafi et al. (2006) reported inter-population variation of 6% and intra-population variation of 94% in Iranian and European grape cultivars, Ergül et al. (2011) reported inter-population variation to be 8% and intra-population variation to be 92% in wild grapevines of Anatolia, and Güler (2021) reported inter-population variation to be 9% and intra-population variation to be 91% in grape cultivars in Bolu province of Türkiye. The high results of intra-population variation indicate that gene flow within the population is high. The high intra-population variation may be due to the ease with which producers can propagate grape cultivars by cuttings and transport to desired regions. This situation is supported by reports of gene flow from the Black Sea basin toward European grape cultivars (Magris et al. 2021).

Conclusion

The current study elucidated genetic variation and population structure between local grape genotypes and reference grape cultivars using ISSR and IPBS retrotransposon primers. This study is the first research on the region's genetic diversity of local grape genotypes. It revealed genetic diversity indices, UPGMA method dendrogram, model-based STRUCTURE, AMOVA, and PIC values. As a result of the analyses, UPGMA dendrogram and STRUCTURE analyses divided genotypes and cultivars into two main groups. However, regional differentiation was not observed among the local grape genotypes. AMOVA analysis results showed a higher genetic variation intra-population compared to inter-populations. As a result of the study, it was concluded that ISSR and IPBS retrotransposon primers can be used successfully to identify grape cultivars. In addition, using two different primers together is more effective in revealing plant genetic diversity. As a result, it is thought that the examined local grape genotypes will shed light on future research in grapevine breeding.

Author contributions AS, EY and AS conceptualized and established the methodology. AS and MAY performed molecular characterization. AS, EY and AS performed statistical analysis. AS and MAY wrote the manuscript.

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Data availability All data needed to conduct this study is provided in the manuscript.

Declarations

Conflict of interest The authors confirm that this article's content has no conflict of interest.

Consent to publish The authors read the manuscript and showed their willingness to publish this study.

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