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RESEARCH ARTICLE

Genetic diversity and population structure of some blackberry genotypes collected from different parts of Türkiye using inter simple sequence repeat (ISSR) markers

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Abstract Molecular marker breeding is a powerful tool for screening targeted genetic material, evaluating genetic differences and targeting a crop improvement potential. Hence, DNAs of leaf samples belonging to 87 *Rubus* genotypes from different regions of Türkiye were isolated with the application marking system. Polymerase chain reaction (PCR) products were obtained with 16 inter simple sequence repeat (ISSR) primers and band lengths ranging from 150 bp [$(AGC)_6G$, $(AG)_7YC$, $VHVG(TG)_7$] to 1500 bp [$HVH(CA)_7$ T] were detected. A total of 293 bands were obtained in terms of genetic diversity and 292 were shown to be polymorphic and 99.75% polymorphism rate. When the obtained results were evaluated, it was confirmed that there was a significant genetic diversity in the genetic material. In the molecularly presented dendrogram, the similarity level between genetic materials can be determined between 0.68 and 1, the average similarity coefficient was determined as 0.84 and two main groups (A and B) were established. Almost all of the genotypes are genetically separated from each other.

Rubus cultivars and genotypes were divided into five populations using a model-based structure algorithm. Based on the STRUCTURE analysis, individuals in the second, fourth, and fifth subpopulations were identified as pure cultivars and are expected to contribute to hybridization research. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis and principal component analysis (PCA) also supported these five different populations and confirmed the reliability of the STRUCTURE analysis. According to the obtained data, the low number of populations was due to the high gene flow rate between the regions where the samples were taken.

The study is the first in this field with blackberry materials representing different regions of Türkiye. The genetic diversity observed in the *Rubus* genetic material reveals that this material is a valuable genetic resource for comprehensive breeding programs. In addition, these cultivars and genotypes are considered as promising candidates for breeding aimed at increasing blackberry production.

Aydın Uzun and Hasan Pınar have contributed equally to this work.

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Introduction

Blackberry belongs to the genus *Rubus* of the *Rosaceae* family, consisting of twelve subspecies, taxonomically included in the subgenera *Idaebatus* and *Eabatus* (Surya et al. 2018). It is a plant with biennial shoots and perennial roots that grows in the form of a bush and has a botanically compound fruit (Ağaoğlu and Gerçekoğlu 2013). Blackberry production in Türkiye was approximately 4.03 thousand tons in 2024 (TUIK 2024).

Research on the blackberry fruit started in the mid-eighteenth century, in the 1930s, with the improvement of thornless species, and enclosure gardens were built (Demirsoy et al. 2006; Bayrak 2024). The wild forms of blackberry fruit, which spread over a very wide area around the world, are known to be of North American origin and its homeland is stated as the temperate regions in the northwestern part of Asia, North Africa, Central, Western, and Southern Europe (Ağaoğlu 1986; Moore and Clark 1993). Climate characteristics of Türkiye create a great product growing potential and diversity that very few countries in the world have, and our country is located within the natural distribution areas of the blackberry plant (Altunbaş 2019). Although its cultivation is not widespread in Türkiye, the first research was reported to have been carried out in the 1960s at the Yalova Atatürk Horticultural Central Research Institute (Ağaoğlu 1986; Yıldız 2011).

It is a valuable fruit species that has become widespread in many areas, with its cultivation increasing in Türkiye. Its fruits have health-enhancing and healing properties due to their high nutritional value, unique taste and aroma, and beneficial effects on health. Its fruits are preferred not only for human consumption but also for their medicinal properties. They are used fresh and in various industrial sectors, including baked goods, jellies, desserts, ice cream, tea, jam, juice, cakes, canned products, beverages, marmalade and chewing gum (Aines and Byers 2003; Zia-Ul-Haq et al. 2014; Cosme et al. 2022; Martins et al. 2023).

There are genetically rich blackberry populations that are widely found in different ecological regions of Türkiye and have been growing naturally for many years. Although it is known that morphological and biochemical characteristics are affected by environmental conditions in blackberry cultivation, it is important that the genetic material is original and the genetic relationship of these materials is revealed (Iqbal 2019). Therefore, different methods must be used to correctly identify genotypes (Ahmad et al. 2017; Güney et al. 2019).

Türkiye has a very special position in terms of fruit genetic resources due to different geographical and ecological conditions (Pınar et al. 2019; Geçer et al. 2020). For this reason, many molecular marker techniques have been developed and DNA-based markers provide more successful and reliable results in the management of genetic resources in defining genotypes and distinguishing phenotypically similar ones. Among these, simple sequence repeats (ISSRs), developed by Zietkiewicz et al. (1994), are considered a preferred marker system due to their PCR (Polymerase Chain Reaction) basis and high polymorphism and productivity (Nadeem et al. 2018). This marker system is widely used in research to determine phylogenetic relationships between different blackberry species, determine genetic diversity, reveal parents, and create genetic maps (Hormaza et al. 2002; Zhebentyayeva et al. 2003; Barkley et al. 2006; Hayashi et al. 2008; Pedryc et al. 2009; Yılmaz et al. 2012; Bakır et al. 2015; Gürcan et al. 2015; Corrado et al. 2021; Ehliz et al. 2021; Sheikh et al. 2021; Wang et al. 2023). In this respect, it is known that the use of molecular markers is very important in terms of speed and convenience (Roose 1988; Uzun et al. 2017). In addition, structure method used to determine individuals into assumed groups and find the most appropriate number of groups by trial and error in line with the ISSR molecular genetic data obtained from individuals belonging to populations, and requires markers that show low correlation.

There are widespread blackberry genotypes in nature in Türkiye. To date, no genetic diversity study has been encountered that includes blackberries from different regions. In order to properly evaluate the existing genetic pool, the diversity within the populations must be revealed. On the other hand, determining the relationship levels of natural populations and standard cultivars is also important. In this study, a

total of 87 blackberry genotypes were used to determine the genetic differences between some blackberry genotypes collected from different regions of Türkiye and some standard blackberry cultivars. After DNA isolation from the plants, molecular research and population structures were conducted with 16 ISSR primers to reveal the genetic diversity between blackberry genotypes and cultivars. The results obtained provide important information for the organization of subsequent research. This is the first comprehensive study on this subject.

The aim of this study is to reveal the genetic diversity between local blackberry genotypes naturally collected from different ecological regions of Türkiye and commercially used standard cultivars using ISSR (Inter-Simple Sequence Repeat) molecular markers. Within this scope, genetic similarities and differences among the genotypes were determined and population structures were analyzed. Thus, it was aimed to evaluate the naturally grown blackberries in Türkiye as a genetic resource, to reveal their potential use in breeding programs, and to establish a scientific basis for the conservation of this genetic material. For this purpose, a comprehensive molecular analysis was carried out using the materials and methods detailed below.

Material and methods

Plant material and DNA isolation

In this study, 67 blackberry genotypes and 20 blackberry cultivars [Jumbo, Chester (*SIUS 47* × *Thornfree*), Bursa I, Bursa II, Bursa III, Bursa IV, Brazos *(Lawton* × *Nessberry),* Early Fat, Boysenberry *(Rubus* × *loganobaccus*), Wineberry (*Rubus phoenicolasius*), Tayberry (*Rubus fruticosus* × *ida*eus), Loganberry (*Rubus ursinus* × *Rubus idaeus*), Dirksen thornless (*Rubus fruticosus*), Thornles Evergreen (*Rubus ulmifolius*), Ness (*Rubus caucasicus*), and Cacastra bestra (*Cacak Thornless*)] collected from different regions of Türkiye were used (Table 1, Fig. 1). The collected cultivars and genotypes were propagated by cuttings and transferred to a plot in Kayseri province. Young leaf tissues of the plants in this plot, free from diseases and pests, were used for DNA isolation. Laboratory research was carried out in the Molecular Genetics Laboratory of the Department of Horticulture, Faculty of Agriculture, Erciyes University. DNA isolation was carried out according to the CTAB protocol defined by Doyle and Doyle (1990). The isolated DNA qualities were determined in a spectrophotometer (BioSpec-Nano) device (Coşkun 2019a).

ISSR analysis

For PCR research, 20 ISSR primers were tested and 16 that gave clear bands were used for all samples (Table 2).

PCR components and PCR cycles were arranged according to the method reported by Uzun et al. (2009) and Pınar et al. (2017). PCR products were run on 2% agarose gel in a tank containing 1 X TBE buffer (108 g Trizma, 55 g Boric acid, 20 mL EDTA) under 110–120 V electric current for 3–3.5 hours and gel images were recorded under UV light in a gel imaging unit connected to the computer. A 100 bp standard DNA Ladder (Biorun, Nantes, France) was used to evaluate the size of ISSR fragments.

Data analysis

Genetic similarity values between genotypes were analyzed using NTSYS-pc ver. 2.11X computer package program. The numbers were given for the presence of bands (1), absence of bands (0), and absence of amplification (9) and converted to a text file. The matrix was created and the genetic similarity coefficient was calculated according to the SM (Simple Matching) method and a dendrogram showing genetic relationships between 87 samples was obtained in the SAHN module with the UPGMA method (Fig. 3). Using the ultrametric distance matrix obtained from the pedigree and similarity matrix, a cophenetic correlation number 'r' was calculated by performing the goodness of fit test called the Mantel test (Mantel 1967) and showing how well the pedigree represents the similarity data.

In addition, the distribution of blackberry genotypes and cultivars on a two-dimensional plane is presented in Fig. 4 by performing principal component analysis (PCA) with the data obtained as a result of statistical analysis with the NTSYS-pc 2.11X computer package program using ISSR data

Table 1 Places and names of the main blackberry genotypes brought from different regions of our country and abroad as plant material in the study

Genotype no	Place	Genotype no	Place
1	Kayseri-Tavlusun	45	Mersin- Alata Research Institute
2	Kayseri-Tavlusun	46	Bursa-Karacabey
3	Kayseri-Akkışla-Kesdoğan	47	Zonguldak
4	Kayseri-Akkışla-Akbayır	48	Adana-Yüregir
5	Kayseri-Yahyalı- Agriculture Fair	49	Bartın- Centre
6	Kayseri-Yahyalı	50	Rize-Küçükçayır
7	Kayseri- Erciyes University-Campus	51	İstanbul-Sarıyer
8	Kayseri-Cırgalan	52	İstanbul-Çatalca
9	Kayseri- Erciyes University-Campus	53	İzmir-Narlıdere
10	Malatya- Apricot Research Institute	54	İzmir-Çeşme
11	Malatya-Merkez- Yeşilyurt District	55	Nevşehir
12	Kastamonu-Küre	56	Erzurum
13	Brazos	57	Kahramanmaraş
14	Chester	58	Afyonkarahisar
15	Bursa II	59	Balıkesir-Bandırma-Susurluk
16	Jumbo	60	Balıkesir-Bandırma
17	Jumbo	61	Tokat
18	Early Fat	62	Kıbrıs
19	Boysenberry	63	Aydın
20	Bursa III	64	Kırşehir
21	Thornless Evergreen	65	Erzincan
22	Bursa I	66	Elazığ
23	Bursa-Kestel	67	Chester
24	Jumbo	68	Sivas-Sızır
25	Chester	69	Sivas
26	Bursa IV	70	Kütahya
27	Osmaniye-Kadirli- Kumbet Village	71	Hatay
28	Trabzon-Akçaabat	72	Isparta
29	Trabzon-Vakfıkebir	73	Cacastra Bestra-Rusya
30	Hollanda-Vlaardingen	74	Sakarya
31	Ness	75	Niğde
32	Dirksen Thornless	76	Samsun-Atakum
33	Kayseri-Tavlusun	77	Samsun-Çarşamba
34	Mersin-Erdemli-Akbıyık Street	78	Samsun-Çarşamba
35	Mersin-Erdemli-Arpaçbahşiş Neighborhood	79	Ordu
36	Mersin-Erdemli	80	Yalova
37	Mersin-Mezitli	81	Kocaeli
38	Mersin-Mezitli	82	Çanakkale
39	Muğla- Centre	83	Çanakkale
40	Batman	84	Amasya-Merzifon
41	Wineberry	85	Antalya-Serik
42	Tayberry	86	Antalya-Alanya
43	Loganberry	87	Kayseri- Haymana Vineyards
44	Mersin- Alata Research Institute		



Fig. 1 Map showing the provinces (in purple) where blackberry genotypes were collected

Table 2 Inter simple sequence repeat (ISSR) primers and base sequences used in polymerase chain reaction (PCR) research

Primary no	Primary name	Base index (5' > > 3'
1	$(AGC)_6G$	AGCAGCAGCAGCAGCAGCG
2	$(CAC)_3GC$	CACCACCACGC
3	$HVH(TCC)_7$	HVHTCCTCCTCCTCCTCCTCCTCC
4	$BDB(CA)_7C$	BDBCACACACACACACACAC
5	$(CA)_6AC$	CACACACACACAAC
6	$VHVG(TG)_7$	VHVGTGTGTGTGTGTGTG
7	$(GACA)_4$	GACAGACAGACAGACA
8	$(TCC)_5RY$	TCCTCCTCCTCCTCCRY
9	$(GT)_8YA$	GTGTGTGTGTGTGTGTYA
10	$(CT)_8TG$	CTCTCTCTCTCTCTCTG
11	$(AG)_7YC$	AGAGAGAGAGAGAGYC
12	$DBD(ACA)_7$	DBDACAACAACAACAACAACAACA
13	$(GA)_8YG$	GAGAGAGAGAGAGAGAYG
14	$(CA)_8R$	CACACACACACACACAR
15	$HVH(CA)_7$ T	HVHCACACACACACACAT
16	$(GAA)_6$	GAAGAAGAAGAAGAAGAA

based on SM (Simple Matching) similarity coefficients. The graphical representation of the genetic structure analysis obtained using the Structure program (Version 2.3.2) (Pritchard et al. 2009) to determine the population structure of 87 blackberry genotypes is also given in Fig. 6.

Results and discussion

Genetic diversity

In this study, blackberry genotypes and cultivars collected from various regions of Türkiye were analyzed using the ISSR-PCR method to determine genetic diversity. A total of 16 different ISSR primers were used for this purpose. The obtained gel images were scored as "presence (1)" and "absence (0)" and

converted into a digital format. For each primer, the total band count (TBC), polymorphic band count (PBC), and corresponding polymorphism rates were calculated.

To further evaluate the level of polymorphism, the Polymorphic Information Content (PIC) values, reflecting the informativeness of the molecular markers, were also calculated. The calculations were performed using the formula proposed by Smith et al. (1997) as $PIC = 1 - \Sigma fi^2$, and the average information content for each primer was determined. These calculations were used to assess the capacity of each ISSR primer to distinguish genetic variation.

Additionally, to better assess the discriminatory power of the markers, the resolving power (RP) values were calculated based on the formula proposed by Prevost and Wilkinson (1999) [$I_b = 1 - (2 \times |0.5 - p|)$]. The obtained RP values demonstrated that the ISSR primers used had a high capacity to reflect genetic diversity accurately.

As a result of the analyses, it was determined that the band lengths of the primers varied between 150 and 1500 bp. The shortest bands (150 bp) were obtained from the primers $(AGC)_6G$, $(AG)_7YC$, and $VHVG(TG)_7$, while the longest band (1500 bp) was obtained from the $HVH(CA)_7$ T primer. A total of 293 bands were obtained, of which 292 were polymorphic, and the overall polymorphism rate was found to be 99.75%. The primer that produced the highest number of bands (33) was $(AGC)_6G$, while the primers that produced the fewest bands (12) were $(GAA)_6$ and $(CA)_8R$.

When comparing polymorphism rates, the lowest polymorphism rate of 96% was observed for the $(AGC)_6G$ primer, while the other 15 primers exhibited a polymorphism rate of 100%.

In conclusion, this study not only evaluated the band profiles and polymorphism rates, but also provided a detailed scientific assessment of the ability of the ISSR markers to reflect genetic diversity, considering advanced molecular analysis parameters such as PIC and RP (Table 3).

Gel images of the $(AGC)_6G$ primer, which yielded a total of 33 bands, 32 of which were polymorphic and 1 was monomorphic, with a polymorphism rate (PR) of 96% and band lengths ranging from 150/1400 as a result of amplification, are given in Fig. 2.

In previous research on blackberries, it has been observed that the total band number per marker and

Table 3 Band properties of inter simple sequence repeat (ISSR) primers used in the study

ISSR Primary Name	Band Length (base pairs) (bp)	Total Band Count (TBC)	Polymorphic Band Count (PBC) (PBS)	Polymorphism Rate (%) (%)
$(AGC)_6G$	150/1400	33	32	96
$(GT)_8YA$	200/1300	16	16	100
$(AG)_7YC$	150/1100	19	19	100
$BDB(CA)_7C$	200/1000	17	17	100
$(CT)_8TG$	220/1100	17	17	100
$VHVG(TG)_7$	150/1200	24	24	100
$(GACA)_4$	220/1100	18	18	100
$(CAC)_3GC$	250/1100	15	15	100
$HVH(TCC)_7$	210/1200	22	22	100
$(GA)_8YG$	160/1200	26	26	100
$(CA)_6AC$	200/1300	17	17	100
$HVH(CA)_7$ T	400/1500	13	13	100
$DBD(ACA)_7$	250/1000	15	15	100
$(GAA)_6$	200/1000	12	12	100
$(TCC)_5RY$	200/1100	17	17	100
$(CA)_8R$	250/1000	12	12	100
Total		293	292	
Average		18.31	18.25	99.75

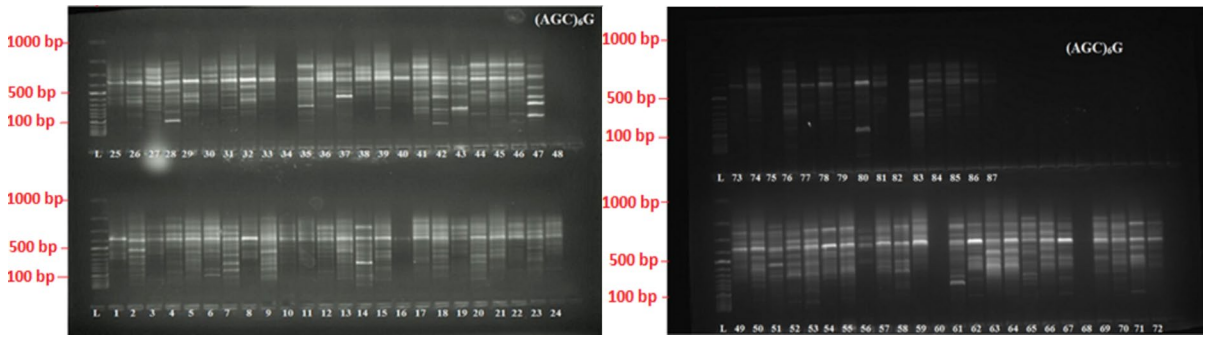


Fig. 2 (AGC)6G primer with amplification result inter simple sequence repeat (ISSR PCR products 1st and 2nd gel images (L: 100 bp DNA ladder)

the polymorphism rate are generally higher. This difference can be explained by factors such as the genetic background used and the diversity of ISSR primers. Below are the findings from various investigations on this topic.

In research on blackberries, the total number of bands per marker and the polymorphism rate were generally higher. Hunková et al. (2023) found that five out of six ISSR primers produced strong, clear, and reproducible band patterns for the detection of potential polymorphism in blackberry genotypes. They reported that the number of fragments for each primer ranged from seven to twelve bands per primer and the band lengths ranged from 250 to 2000 bp.

Abdi et al. (2021) reported that 344 out of an average of 34.5 bands in 45 blackberry genotypes from the regions south of the Caspian Sea showed high polymorphism, while Innis et al. (2011) reported that 4 primers were polymorphic in *R. phoenicolasius* species, and at least 3 out of 13 bands out of 4 primers were polymorphic in *R. argutus* species. According to the results of the researchers, we can state that the reason for this difference in our study can be attributed to the genetic background and ISSR primers used.

Marulanda et al. (2012) reported that in the Andean blackberry species *R. glaucus*, a large group with 95% similarity in SSR markers and 44 *Rubus* genotypes belonged to *R. glaucus* genotypes, and thus, the grouping of genotypes was based on genetic distances but not on the region of origin. The similarity between the *R. glaucus* species was also quite high, some of them showing 100% similarity.

Agar et al. (2011) reported that a total of 223 AFLP fragments with a polymorphism rate of 53% were produced in nine wild blackberry (*Rubus caucasicus* L.) and Chester cultivars, while Barut et al. (2009) obtained a similarity level of 65% among 10 blackberry cultivars using AFLP markers. Stafne and Clark (2003) determined a similarity level of 65% among 13 blackberry genotypes in their Maximum Potential Similarity (MPS) among blackberry genotypes, while Karakoç (2011) reported that a total of 85 bands were formed with 15 primers among 55 blackberry genotypes collected from eight different areas in the Black Sea Region, and 87 of these bands showed polymorphism. It is seen that the similarity levels between blackberry genotypes in our study are similar to the values obtained with the different marker systems explained above.

The polymorphism rates and band numbers obtained in the research mentioned above show significant differences depending on the markers used and genetic diversity. In our study, it has been observed that the similarity levels among blackberry genotypes are in accordance with the results obtained from different marker systems in previous research. These findings provide an important contribution to understanding genetic diversity and further exploring the population structure.

Various investigations conducted have provided important findings regarding the genetic diversity of *Rubus* species from different geographical regions and species. These literature works highlight the effects of molecular marker systems and environmental factors on genetic diversity and population

structures. Below are some key results obtained from these investigations.

Debnath (2015) evaluated forty-eight wild *Rubus chamaemorus* L. species from four Canadian provinces in terms of genetic diversity and found that nine primers produced 138 polymorphic bands. However, in our study, they differed due to both genetic and environmental factors.

Garrido et al. (2020) evaluated the genetic diversity of 106 individuals in Andean blackberry cultivars from the main production areas in the Ecuador region and found that RAPD primers did not show any differentiation among individuals identified as *R. glaucus*, but an ISSR primer was useful in selecting 29 individuals for analysis with AFLP markers and in finding polymorphisms. They reported that 152 of them showed polymorphism in *R. glaucus* by obtaining 203 bands with AFLP markers. AFLP markers clearly distinguished *R. glaucus* from the screened wild *Rubus* species and also revealed an unexpected genetic structure among *R. glaucus* cultivars. This genetic differentiation and the detection of mixed genotypes suggest a possible introgression of wild *Rubus* species into *R. glaucus*.

Ryu et al. (2014) found that 18 bands were produced among 55 plants, and eight of them were polymorphic bands representing 44.4% and 1.6% on average per primer pair. However, they reported the highest percentage of polymorphic bands as 66.7% and the lowest as 33.3%. They also calculated the genetic diversity value for each primer to range from 0.095 to 0.234, with an average of 0.178.

The genetic diversity and polymorphism rates obtained in the above-mentioned literature show differences depending on the marker systems used and environmental factors. These investigations have made significant contributions to understanding the genetic diversity and genetic structure among blackberry species. In our study, similar marker systems were used to evaluate genetic diversity and polymorphism rates, and the obtained results, in line with the findings in the literature, clearly highlight the impact of environmental and genetic factors.

The cophenetic correlation coefficient, which shows the correlation between the similarity indices and the dendrogram, was found to be $r = 0.7$. It has been stated that if this coefficient is greater than 0.9, there is a very good relationship between the similarity indices and the dendrogram obtained, if it is between 0.8 and 0.9, there is a good relationship, if it is between 0.7 and 0.8, there is a weak relationship, and if this value is less than 0.7, there is a very weak relationship (Mohammadi and Prasanna 2003). From this point of view, it is seen that there is a weak correlation between the similarity indices and the dendrogram obtained. In this regard, the results obtained from other research help us better understand the relationship between genetic diversity and environmental factors.

Sedighi and Rahimmalek (2015) reported that there was a good fit between the dendrogram clusters and cophonetic matrices by calculating the highest cophonetic correlation coefficient $r = 0.802$ as a result of the evaluation of genetic diversity of *R. hyrcanus* and *R. discolor* species in Iran. We can state that the relatively lower cophonetic correlation coefficient obtained in our study is probably because the genotypes used belong to different regions of Türkiye, and environmental factors are effective.

Leišová-Svobodová et al. (2023) determined 162 different genotypes among 184 individuals in the species *Rubus chamaemorus* L. and calculated the correlation index value between genetic and geographical distances as $r = 0.44$ and stated that gene flow may exist even over long distances. They reported that *Rubus chamaemorus* populations differed in regions, but some individuals within the population shared common alleles even between regions.

In light of these literature findings, it can be concluded that both geographical origin and environmental conditions play a significant role in genetic diversity analyses, and that the correlation values obtained are meaningfully influenced by these factors. In this context, the difference between the cophenetic correlation coefficient obtained in our study and the values reported in the literature indicates that the blackberry genotypes were collected from different ecological regions of Türkiye, and that the influence of environmental factors on genetic structure should be considered as important elements in the evaluation of genetic diversity.

In the study, the similarity levels of genotypes in the family tree obtained using the SM (Simple Matching) method were determined to be between 0.68 and 1.00, and the average similarity coefficient was determined as 0.84. The materials used were divided into 2 main clusters (A and B) at a similarity level of 0.69,

and the second main cluster consisted of three subgroups, B1, B2, and B3.

When we examine the first main cluster in Fig. 2, it can be observed that it predominantly consists of genotypes collected from the Mediterranean region, including 10 individuals (27, 34, 35, 36, 37, 38, 39, 44, 45, 48), followed by 3 from the Black Sea region (28, 29, 47), 1 from the Southeastern Anatolia region (40), 1 from the Marmara region (46), and 1 from the Central Anatolia region (33) (Table 1). Additionally, this main cluster includes one genotype obtained from the Netherlands (30), as well as the cultivars Chester, Bursa IV, Tayberry (*Rubus fruticosus*×*idaeus*), Wineberry (*Rubus phoenicolasius*), Dirksen Thornless (*Rubus fruticosus*), Ness (*Rubus caucasicus*), and

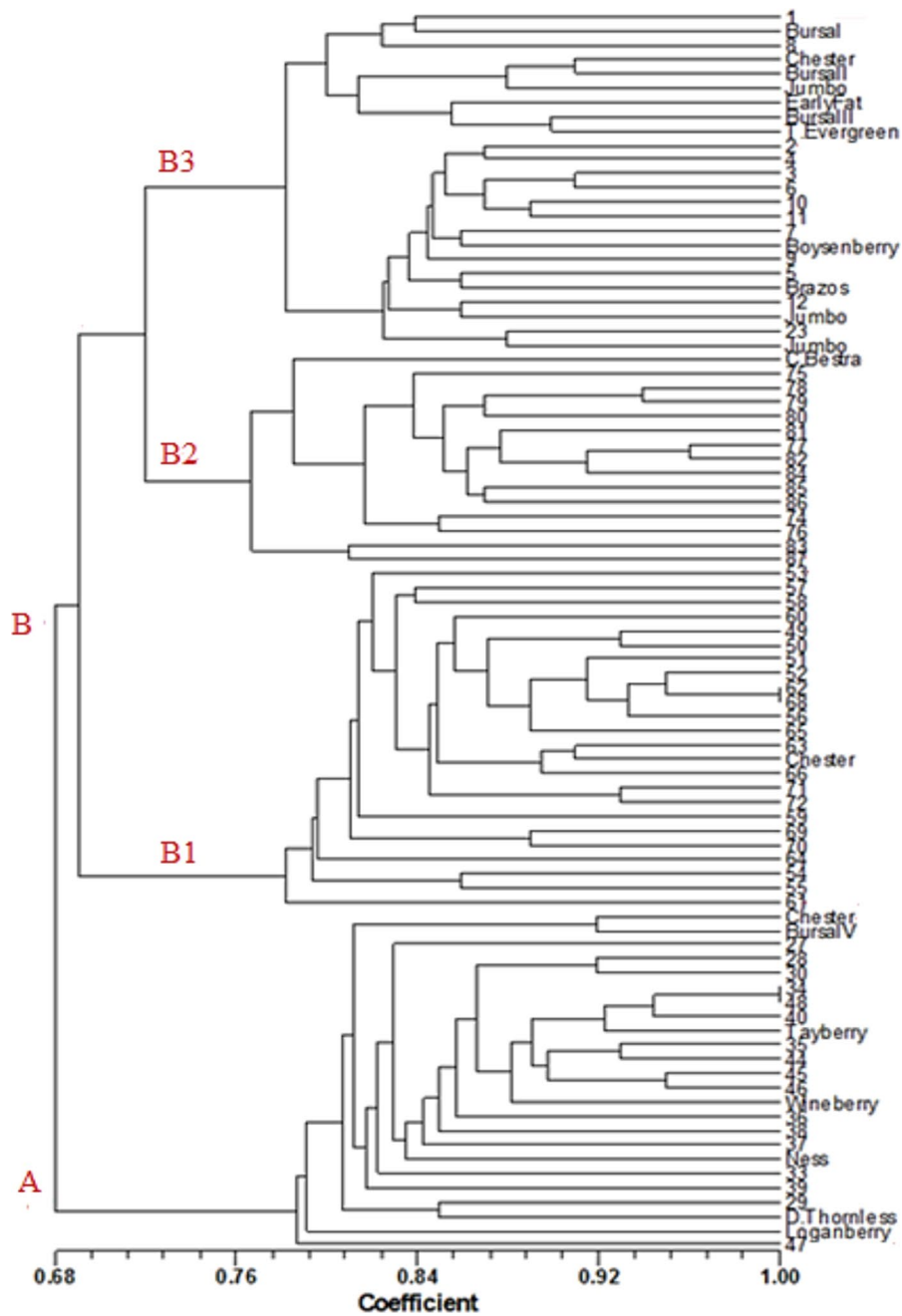


Fig. 3 Dendrogram created in the SAHN module according to the similarity indices obtained from the inter simple sequence repeat (ISSR) data of blackberry genotypes

Loganberry (*Rubus ursinus*×*Rubus idaeus*) (Fig. 3, Table 1).

The second main cluster (B) consisted of individuals from the Black Sea (49, 50, 61), Marmara (51, 52, 59, 60), Central Anatolia (55, 64, 68, 69), Eastern Anatolia (56, 65, 66), Aegean (53, 54, 58, 63, 70), and Mediterranean Regions (57, 71, 72) in the first subgroup (B1) and consisted of a total of 24 individuals, including genotype number 62 obtained from Cyprus and the Chester (*SIUS 47*×*Thornfree*) variety (Fig. 3, Table 1).

The second main cluster (B) second subgroup (B2) contains genotypes 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, and 87, totaling 14 individuals (Fig. 2). If we examine these individuals regionally, we can state that 5 individuals (74, 80, 81, 82, 83) were obtained from the Marmara Region, 4 individuals (76, 77, 78, 79) from the Black Sea Region, 2 individuals (85, 86) from the Mediterranean Region and 2 individuals (75, 87) from the Central Anatolia Region (Table 1).

In the dendrogram, genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9 (Central Anatolia Region), 10, 11 (Eastern Anatolia Region), 23 (Marmara Region), 12 (Black Sea Region), and the cultivars Brazos (*Lawton*×*Nessberry*), Boysenberry (*Rubus*×*loganobaccus*), Thornless Evergreen (*Rubus ulmifolius*), Chester (*SIUS 47*×*Thornfree*), Early Fat, Jumbo, Bursa I, Bursa II, and Bursa III are in the second main cluster (B) and the third subgroup (B3) (Fig. 3, Table 1).

When the dendrogram is examined, it is seen that there are 24 individuals in the first main cluster (A), the second main cluster first subgroup (B1) and the third subgroup (B3), and 15 individuals in the second subgroup (B2). According to the obtained dendrogram, it was determined that the genotypes numbered 34 and 48 from the Mediterranean Region in the first main cluster (A), 62 in the second main cluster (B) first subgroup (B1), and 68 obtained from the Central Anatolia region are the closest genotypes to each other with a similarity ratio of 1.00. In addition, at the similarity ratio point of 0.78, we can say that the genotypes numbered 47 in the first main cluster (A), 61 in the first subgroup (B1) of the second main cluster (B) obtained from the Black Sea Region and the Cacastra bestra (*Cacak Thornless*) variety in the second subgroup (B2) of the second main cluster (B) are the most distant genotypes (Fig. 3, Table 1).

Standard cultivars are generally located in the first main cluster (A) and the third subgroup of the second main cluster (B3). In the first main cluster (A), the cultivars include Chester (*SIUS 47*×*Thornfree*), Bursa IV, Tayberry (*Rubus fruticosus* x *idaeus*), Wineberry (*Rubus phoenicolasius*), Ness (*Rubus caucasicus*), Dirksen Thornless (*Rubus fruticosus*), and Loganberry (*Rubus ursinus* x *Rubus idaeus*); In the third subgroup (B3) of the second main cluster (B), the cultivars include Jumbo, Brazos (*Lawton* x *Nessberry*), Boysenberry (*Rubus* x *loganobaccus*), Thornless Evergreen (*Rubus ulmifolius*), Bursa I, Bursa II, Bursa III, Early Fat, and Chester (*SIUS 47*×*Thornfree*), as shown in Fig. 2. In addition, it was determined that the majority of the individuals in the first main cluster (A) consisted of genotypes obtained from Mersin (34, 35, 36, 37, 38, 44, 45), while the individuals in the first and third subgroups of the second main cluster consisted of genotypes obtained from coastal regions (49, 50, 51, 52, 53, 54, 57, 58, 59, 60, 61, 63, 70, 71, 72). When we evaluate the pedigree obtained as a result of the analysis as a whole, it is seen that the genotypes morphologically show the thorny feature. We can say that the leaf shape of Genotype Number 23 in the third subgroup (B3) of the second main cluster (B) obtained from the Marmara region is different and can be a decisive criterion for subsequent research (Fig. 3, Table 1).

According to the dendrogram showing genetic similarities, the following conclusions were reached (Fig. 3, Table 1):

Genotypes 35 and 44 from the Mediterranean Region were in the first main cluster (A) with a similarity rate of 0. Genotypes 49 (Black Sea Region) and 50 (Black Sea Region) and genotypes 71 (Mediterranean Region) and 72 (Mediterranean Region) belonging to genotypes 0.9264 were placed in the first subgroup (B1) of the second main cluster (B). Genotypes 63 (Aegean Region) and the variety Chester (*SIUS 47*×*Thornfree*) were in the first subgroup (B1) of the second main cluster (B) with 0. 9072. Cultivars Chester (*SIUS 47*×*Thornfree*) and Bursa II were located in the third subgroup (B3) of the second main cluster (B), and genotypes 3 and 6 from Central Anatolia Region; 0. 888, genotypes 65 (Eastern Anatolia Region), 69 (Central Anatolia Region) and 70 (Aegean Region) were in the second main cluster, first subgroup (B1). Genotypes 10 and 11 from the Eastern Anatolia Region were grouped in the second

main cluster (B) and third subgroup (B3). Finally, the Jumbo variety is in Subgroup B3 and showed the highest similarity with Genotype 23 from the Marmara Region. The Wineberry variety (*Rubus phoenicolasius*) was placed in Cluster A.

The dendrogram results obtained in our study revealed that the genotypes formed distinct groups based on their geographical origins and morphological characteristics. The similarity ratios used in the evaluation demonstrated that genetic diversity is influenced by regional differences. In light of these findings, making a comparative assessment with previous research is important to support the accuracy and validity of the data obtained.

In previous research on blackberries, Sedighi and Rahimmalek (2015) reported that three main clusters were formed in the dendrogram in the Iranian population, while Marulanda et al. (2012) reported that the similarity ratio varied between 1.00 and 0.49 in the *R. glaucus* Andean blackberry species. As a result of the researchers' investigations, the grouping of genotypes and the similarity ratios depended on genetic distances but not on the regions of origin, but our study showed that the main clusters were grouped according to their geographical origins and that especially the genotypes found in the Mersin province experienced less gene flow compared to other regions of Türkiye.

Innis et al. (2011) used *R. argutus* and *R. phoenicolasius* species and found the variation between settlements to be 1.538 for *R. argutus* and 0.418 for *R. phoenicolasius* species, and the variation within settlements was 0.370 for *R. argutus* and 0.075 for *R. phoenicolasius* species.

Barut et al. (2009) stated that in order to determine the genetic similarities between blackberry genotypes, 2 clusters were formed with 1.00 and 0.35 similarity levels, and only Arapoha variety was included in the first cluster. As a result of our study, it was concluded that Chester variety was also similar to the investigations of the researchers and independent and wild blackberry genotypes were also clearly clustered. Hence, we can state that in both research, genotypes experienced less gene flow according to geographical regions. It was also determined that it was genetically more distant from other wild blackberry genotypes.

Stafne and Clark (2003) investigated the Maximum Potential Similarity (MPS) among blackberry genotypes and found a similarity level between 1.00 and 0.45 among the 13 blackberry genotypes they used in their study.

Based on the dendrogram created from molecular analysis results, Karakoç (2011) determined that there are two main clusters. The smaller of these clusters differs from the other genotypes, particularly in terms of morphology. It is separated from the others by its soft, thin spines with a feathery structure. The larger main cluster is divided into two geographical groups: the Black Sea coastline and the inland areas.

Debnath (2015) reported that there was significant genetic diversity among the wild collection, with 48 wild genotypes falling into six main clusters and the remaining one genotype being an outlier. Furthermore, in four of the clusters, genotypes tended to form subclusters consistent with a principal coordinate (PCO) analysis and geographical distribution explained 8% of the total variation.

Leišová-Svobodová et al. (2023) reported that three main clusters were formed as a result of cluster analysis, two of which included genotypes from the Krkonose Mountains and the Norwegian population, while the other cluster consisted of genotypes from both the Norwegian and Spitsbergen populations. They also stated that according to the results of Principal coordinate analysis (PCoA), the distance between the samples showed the differentiation between the populations.

Ryu et al. (2014) reported that according to the cluster analysis results they obtained among 55 plants, they were classified into five clusters, and the genetic similarity level was 82.3%.

The findings of this study, when compared with previous research conducted on blackberry species, reveal that the genetic diversity and clustering patterns of genotypes can vary depending on both intraspecific variation and geographical origin. While the literature generally suggests that genetic similarity is primarily related to genetic distances rather than geographical origin, our study shows that genotypes, particularly those collected from specific regions such as Mersin, tend to form distinct clusters. This indicates that geographic isolation and limited gene flow may significantly influence genetic structure. Therefore, it supports the idea that geographical origin should be considered an important factor in assessing the genetic diversity of blackberry genotypes.

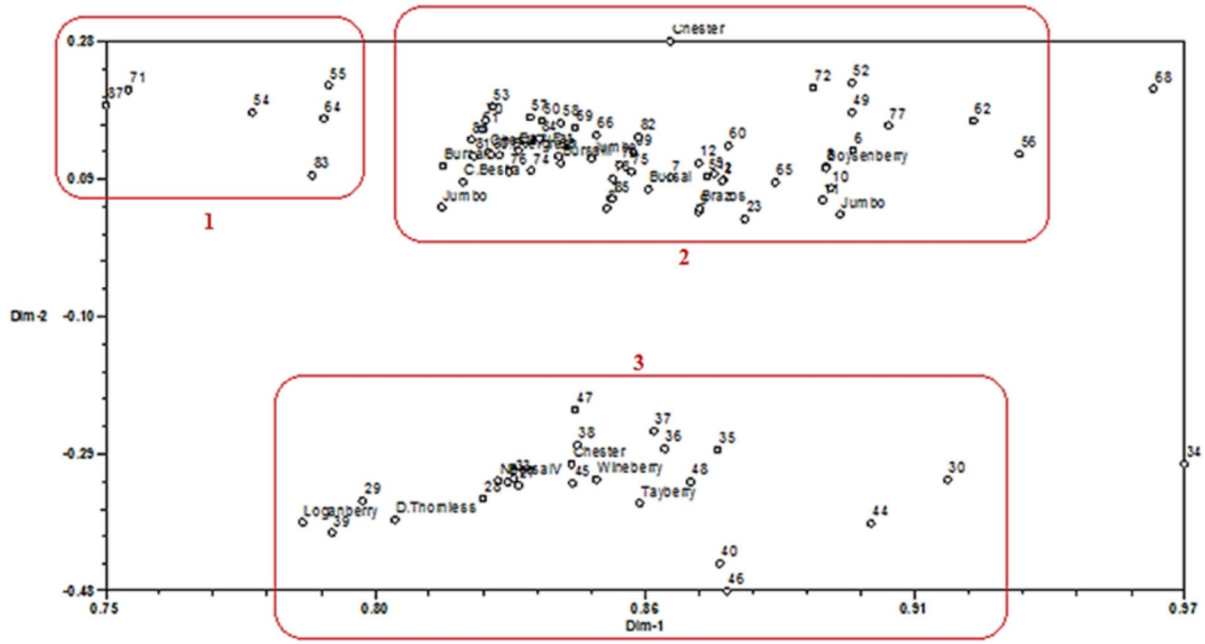


Fig. 4 Two-dimensional principal component analysis (PCA) for all blackberry cultivars and hybrid plants

The distribution of blackberry genotypes on a two-dimensional plane was determined by performing principal component analysis (PCA) with the data obtained as a result of statistical analysis performed with the NTSYS-pc 2.11X computer package program (Fig. 4). In the two-dimensional distribution graph obtained, the similarities and differences within the blackberry genotypes were clearly revealed. When 3 main groups and 2 outgroups were formed and compared with the pedigree, it was seen that the similarities and differences between the genotypes were expressed on a plane parallel to the results obtained in the pedigree. While the individuals in the second main group on the plane formed clusters closer to each other than the individuals in the first and third main groups, the genetic variations were low. Due to the high level of similarity between the individuals in the second group, the clustering was squeezed into a very narrow area. The individuals in the first main group showed a slightly less dense clustering, and their genetic variations were high. It is seen that the second and third groups mostly consist of cultivars (Fig. 4).

The distribution obtained from the PCA (Principal Component Analysis) allowed for the visual evaluation of genetic relationships among the genotypes and clearly revealed that the levels of genetic variation differed between groups. These analytical findings demonstrate that PCA is an effective tool in understanding the genetic structure of blackberry genotypes, and similar approaches have also been used in previous research, yielding significant results in identifying genetic groupings.

Sedighi and Rahimmalek (2015) emphasized that genotypes in Iran were gathered under two main groups, and in the principal component analysis (PCA) *R. hyrcanus* was an important wild blackberry species distributed around the Caspian Sea. Agar et al. (2011) also found that independent and wild blackberry genotypes of the Chester variety were clearly clustered according to AFLP markers.

These findings demonstrate that the PCA method is a powerful tool for determining the genetic relationships of blackberry genotypes, and that different investigations have successfully used this approach to better understand the genetic diversity and distribution of blackberries. These investigations shed light on how genetic diversity is shaped by geographical factors and inter-species relationships, making significant contributions to the development of future blackberry breeding and conservation strategies.

Population structure

To determine the population structure, the software STRUCTURE (version 2.3.2) was used to analyze 87 blackberry genotypes (Pritchard et al. 2009). The analysis was based on a model assuming that the population could be divided into 1 to 10 subgroups (K=1–10), with the assumption that allele frequencies were correlated (Falush et al. 2007). For each K value, a run was performed with a burn-in period of 100,000 iterations followed by 100,000 Markov Chain Monte Carlo (MCMC) repetitions, and this procedure was repeated 10 times (Falush et al. 2007). The ΔK values for each population were calculated using the web-based Structure Harvester software (Earl and VonHoldt, 2012). The optimal K value was determined based on these results, and the number of groups into which the populations should be classified was decided accordingly. The obtained K and ΔK values were used to determine the most likely number of genetic clusters based on the probability-based results of the data (Evanno et al. 2005). The Estimated Ln Probability of Data [LnP(D)] reached its maximum at K = 5, and this value was considered the most appropriate number of populations. It was observed that the same number of groups was obtained through UPGMA clustering analysis (Fig. 5). Based on the obtained data, the low number of populations can be attributed to a high rate of gene flow among the regions from which the samples were collected.

This analysis is an effective method used to understand the population structure of blackberry genotypes, and the results provide valuable insights into how genetic diversity and gene flow are shaped by regional differences. Additionally, it has helped us better understand how the genetic structure varies at the regional level and how each population forms its genetic makeup. Previous research on *Rubus* species found that Zhou et al. (2024) identified 4 subpopulations in 132 wild *R. chingii* individuals. Han et al. (2024) reported that black raspberry (*Rubus coreanus* Miq.), which has a wide distribution area among the naturally occurring *Rubus* species in Korea, consists of three genetic clusters. Veljković et al. (2019), including Loganberry and Tayberry blackberry cultivars, identified 3 subpopulations among 135 *Rubus idaeus* individuals from 7 populations, while Lee et al. (2016) similarly examined the population structure of 69 *Rubus* species and reported the number of subpopulations as K=6. Sedlák et al. (2022), using Bayesian analysis, identified 8 subpopulations among all the samples analyzed.

The findings obtained provide an opportunity to better understand the genetic diversity and evolutionary processes of different regional populations, while also revealing how the differences in the genetic structures and gene flow of blackberry species are influenced by regional conditions.

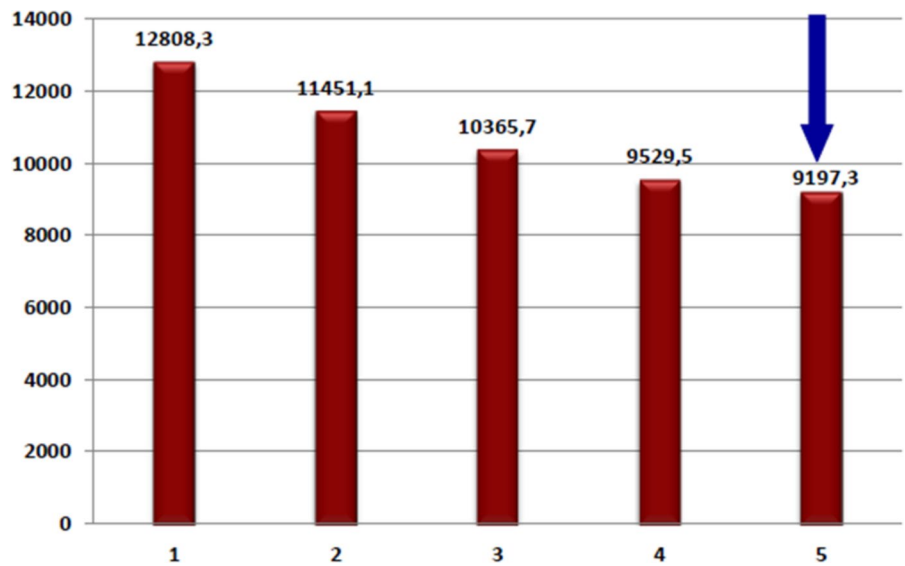


Fig. 5 Number of subpopulations of blackberry genotypes

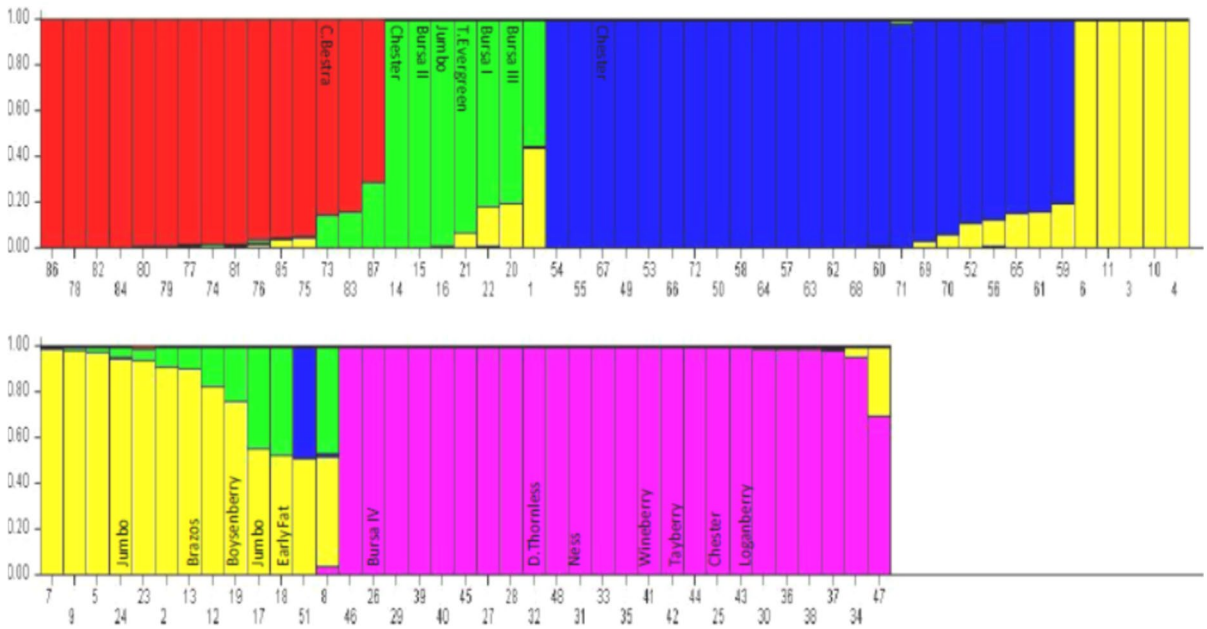


Fig. 6 Graph of grouping of 87 blackberry accessions obtained with Structure version 2.3.2 software indicating membership coefficiencies and level of admixture to each subpopulation

It was observed that the results of the structure analysis were consistent with the groupings obtained in the Dice dendrogram presented in Fig. 3. In the structure analysis, a grouping consistent with the geographical distribution of the genotypes was obtained, which was also found to be in agreement with the dendrogram results. The groupings in the dendrogram in Fig. 3 and those in the population graph in Fig. 6, including cultivars and genotypes, show a high degree of similarity. According to the findings, the low number of populations is thought to be due to the high rate of gene flow between the regions where the samples were collected. This high gene flow can be explained by the widespread transport and use of propagation materials (e.g., seedlings, cuttings) between different regions. The use of the same or similar genetic material in different geographical areas in agricultural practices reduces local variation and increases genetic similarity, thereby decreasing genetic differentiation among populations. This has led to the grouping of genetically similar individuals despite geographical distances in genetic structure analyses.

In the study, the genetic structure of blackberry genotypes was analyzed using the STRUCTURE software, and the individuals were divided into five subpopulations ranging from K=1 to K=5. Individuals with a Q value ≥ 0.80 were classified as pure lines, while those with a Q value ≤ 0.80 were considered as admixed or hybrid individuals (Coşkun 2019b).

According to Fig. 6, in the first and second bar plots, the K values obtained according to the marker system and primer number using the Structure program defined by Pritchard et al. (2009) are shown in red, 1st subpopulation (K = 1) in green, 3rd subpopulation (K = 3) in blue, 4th subpopulation (K = 4) in yellow, and 5th subpopulation (K = 5) in pink. It is seen that there are 15 genotypes in the first subpopulation (K = 1), 7 in the second subpopulation (K = 2), 23 in the third subpopulation (K = 3), 18 in the fourth subpopulation (K = 4), and 24 in the fifth subpopulation (K = 5) (Fig. 6).

There are 15 genotypes in the red group, and their Q value is above 0.80. Genotypes numbered 86, 78, 82, 84, 80, 79, 77, 74, 81, and 76 in this group showed an almost homogeneous structure. In addition, genotypes numbered 85, 75, 73, 83, and 87 were included in the red group, and it was seen that they contained substructure in the other two groups. On the other hand, it was determined that these genotypes showed almost more than half of the yellow and

green group resolution (Fig. 6). It is seen that the red group in the bar plot graph consists of genotypes in the second small subgroup (B2) of the second largest cluster (B) in the Dice dendrogram (Fig. 3). This group generally consists of genotypes belonging to coastal regions (Table 1, Fig. 1).

This study is the first comprehensive research examining genetic diversity and population structure of blackberry genotypes collected from different regions of Türkiye using ISSR markers. No previous genetic structure analysis has been conducted on blackberry genotypes in Türkiye, and in this study, the relationships between genetic structures and populations were examined in detail. The findings were compared with previous research, providing valuable insights into genetic diversity and population structure. Hence, this study makes a significant contribution to the literature as the first comprehensive genetic structure analysis of blackberry genotypes conducted in Türkiye. The results are also consistent with earlier research conducted on *Rubus* species. For instance, in a study by Zhou et al. (2024), 132 wild *Rubus* chingii individuals were divided into four subpopulations, and it was reported that the differences in genetic structure were largely influenced by lineage, geographical origin, and distribution areas. This finding closely aligns with the coastal versus inland genetic differentiation observed in our study. Similarly, in research conducted by Han et al. (2024) in South Korea, a Bayesian clustering analysis of natural *Rubus coreanus* populations revealed the presence of three genetic clusters, which showed a clear geographic pattern. Han and colleagues also noted that regions with high genetic diversity (such as Chiak, Hamyang, and Jeju) could serve as priority sources for breeding programs. In this context, our study also concludes that the pure genotypes with $Q \geq 0.80$ may be considered valuable genetic resources for use in hybridization and breeding efforts.

There are 7 genotypes (14, 15, 16, 21, 22, 20, 1) in the green group, and six of them have a Q value above 0.80 and a homogeneous structure. In addition, genotypes 20, 21, 22, and 1 are included in the green group, and it is seen that they also contain substructure in the other group. On the other hand, it was determined that these genotypes show almost more than half of the yellow group resolution (Fig. 6). The green group in the bar plot graphic is mostly composed of cultivars, showing compatibility with the genotypes in the third small subgroup (B3) of the second main cluster (B) in the dendrogram. It was determined that all genotypes in this green group in particular showed the thornless feature (Fig. 6, Fig. 3).

Similarly, research conducted in different geographical regions has also demonstrated that the genetic diversity of *Rubus* species forms distinct subpopulations based on species-level and geographic distribution. For example, Veljković et al. (2019) reported that in their genetic structure analysis of 135 *Rubus idaeus* individuals from seven different populations in the Central Balkans, cultivars and wild genotypes were found to cluster into genetically distinct subpopulations. Likewise, in a Bayesian clustering analysis conducted by Lee et al. (2016) on 69 different *Rubus* species, individuals were assigned to subpopulations ranging from $K=1$ to $K=6$. Among these, $K=2$, $K=3$, $K=4$, and $K=6$ were found to include only a single species: *R. crataegifolius var. subcuneatus, R. crataegifolius, R. fruticosus*, and *R. coreanus*, respectively. In addition, it was emphasized that $K=1$ included five *R. idaeus* and one *R. fruticosus* individual, while $K=5$ included three *R. parvifolius* and one *R. crataegifolius var. subcuneatus* individual. These findings highlight that both genetic structure and species origin play a determining role in the genetic differentiation among *Rubus* species.

In the study, individuals with a Q value ≥ 0.80 were considered "pure lines," and it was determined that the majority of genotypes in the blue and pink groups possessed a genetically homogeneous structure suitable for use in hybridization research. Sixteen genotypes within the blue group were classified as pure individuals, originating from both coastal regions (Black Sea, Marmara, Aegean, Mediterranean) and continental climate regions (Central Anatolia and Eastern Anatolia). Additionally, genotype number 62 from Cyprus and the 'Chester' cultivar were also included in this group. Similarly, in the pink group, 23 out of 24 individuals were classified as pure and thus carry hybridization potential. Although the yellow group had Q values above 0.80, it included genetically admixed individuals, mostly consisting of genotypes collected from the Kayseri region. The clustering patterns of these groups in the dendrogram were also found to be consistent with the results from the bar plot graph (Fig. 6).

The findings of our study are consistent with those reported by Sedlák et al. (2022), in which genetic

clustering analysis was conducted on *Rubus* species originating from Northern and Southern Norway as well as the Czech Republic. In that study, the K2, K4, and K8 clusters corresponded to populations from Northern Norway, while K1, K3, K6, and K7 included individuals from Southern Norway. The K5 cluster represented genotypes from the Czech Republic. It was also reported that some clusters were shared between the two countries. These results indicate that *Rubus* genotypes from different geographical regions exhibit distinct genetic structures, highlighting the significant influence of geographic origin on genetic diversity. Similarly, in our study, the blackberry genotypes collected from various regions of Türkiye showed a tendency to cluster based on geographic distribution, demonstrating region-based genetic differentiation.

The strong concordance between the resulting bar plot and the Dice dendrogram supports the reliability of the marker system and methodology used in this study. In particular, the correspondence of the red group with the B2 subcluster in the dendrogram indicates that the observed genetic differentiation is also reflected at the phylogenetic level. The distribution of genotypes among groups is directly related to their geographical origin. For example, the red group includes individuals from coastal regions such as the Marmara and Aegean, while the yellow group consists of genotypes from inland regions like Kayseri. The fact that all individuals in the green group exhibit a thornless morphological trait demonstrates a direct relationship between genetic structure and phenotypic characteristics (Fig.6, Fig. 3).

In conclusion, this study represents one of the first comprehensive genetic structure analyses conducted on blackberry genotypes. It not only aligns methodologically and in terms of results with international research on the genus *Rubus*, but also provides valuable comparative data on regional diversity, genetic purity, and population structure.

Although no previous genetic structure analysis has been conducted on blackberry genotypes, this study represents the first of its kind and will serve as a valuable reference for future research in this field. Therefore, our study and future research will guide us in testing which taxonomic classifications individuals from different populations can be assigned to.

Conclusion

Nowadays, in order to increase agricultural production and stabilize it at a high level, the collection, storage, and use of genetic resources are gaining more importance. Our plant genetic resources are subject to genetic erosion due to environmental and other pressures and are facing the danger of extinction. In particular, the protection of diversity in plant genetic resources belonging to cultivated species is extremely important for the sustainability of plant production and molecular research should be carried out in order to avoid further delays in the protection of plant genetic resources for the future.

In our study conducted with a total of 87 blackberry materials, it was determined that the samples were grouped into 2 main groups according to the dendrogram obtained. Almost all of the materials used were separated from each other. This situation shows that there is a significant diversity between the genotypes growing in nature and the standard cultivars. The variations in natural populations were found to be important in particular. These variations in nature can be explained by the reproduction of plants by seeds and the dispersal of seeds to different areas by animals. This interaction between plants in the process has provided the emergence of a rich diversity. In future research, it is necessary to reveal the fruit and plant characteristics of these genotypes and to determine their responses to diseases, pests, and abiotic stress conditions. Thus, the populations can be examined in every aspect and better evaluated.

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Data availability No datasets were generated or analysed during the current study.

Declarations

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

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