Genetic Diversity and Population Structure of *Berberis crataegina* DC. in Türkiye Revealed by ISSR and SCoT Markers

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Berberis species are valuable wild plants widely distributed in Türkiye and other regions. They are known for their nutritional, medicinal, and ecological importance. Despite their significance, molecular studies on *Berberis* are limited, particularly regarding the use of SCoT markers. In the present study, the genetic diversity of selected *Berberis* populations was investigated using both ISSR and SCoT molecular markers. A total of 51 scorable bands were obtained with ISSR markers, 41 of which were polymorphic, resulting in a polymorphism rate of 80.4%. SCoT markers produced an average of 6.2 bands per primer, with 5.3 polymorphic bands and a polymorphism of 86.4%, indicating a higher sensitivity in detecting genetic variation. Genetic similarity values ranged from 0.39 to 0.84 for ISSR and 0.32 to 0.89 for SCoT markers. These results were compared with previous studies on *Berberis* and other plant species, confirming the robustness of the applied markers. Notably, this study represents the first application of SCoT primers in *Berberis*, demonstrating their effectiveness in resolving genetic relationships and detecting polymorphism. The findings provide a valuable molecular basis for conservation strategies, germplasm management and future breeding programs, while also contributing to a better understanding of genetic structure and diversity within the genus. Overall, the combined use of ISSR and SCoT markers offers a reliable approach for molecular characterization and supports the sustainable utilization of *Berberis* genetic resources.

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INTRODUCTION

Berberis crataegina DC. (Berberidaceae), commonly known as barberry, is an important berry-producing species that grows naturally in the mountainous and forested regions of Anatolia. The species has a wide geographical distribution across America, Asia, Europe, and Africa, and is also reported in the mountainous regions of Pakistan (Khan *et al*. 2016). In Türkiye, it is widely distributed in Eastern Anatolia, Central Anatolia, and the Black Sea regions, where it typically develops as a highly branched woody shrub reaching 1 to 3 m in height (Güner *et al*. 2012; Ekim *et al*. 2000). Adapted to cold climates, *B. crataegina* thrives at elevations up to 2000 m, particularly in pine-dominated forest ecosystems (Kındersley and Brickell 2008), and demonstrates high tolerance to drought, low temperatures, and nutrient-poor soils.

Morphologically, the species is characterized by lignified stems, reddish-brown thorny branches bearing stiff spines, and simple, serrated leaves (Davis 1965; Arslan 2021). The leaves exhibit a glossy dark green upper surface and a paler underside, turning yellow to red in autumn (Yıldırım 2000). Flowering occurs between May and June, producing 4 to 10 yellow, nectar-rich flowers arranged in short racemes, which provide an important resource for pollinators (Zhaleh *et al*. 2025). The fruits, which ripen from late summer to early autumn, are fleshy, ellipsoidal, and dark red to purplish-black in color, typically containing a single seed. Due to their sour taste, the fruits are consumed by both humans and birds, contributing to biotic seed dispersal (Kargıoğlu *et al*. 2010).

In addition to its ecological importance, *B. crataegina* has attracted increasing attention due to its nutritional and phytochemical properties. With the growing interest in healthy diets and natural food sources, wild fruits have gained importance as functional foods and nutraceuticals due to their antioxidant, antimicrobial, and anti-inflammatory properties (Ercan 2024). Compared to cultivated fruits, wild species are generally richer in bioactive compounds (Yıldız *et al*. 2010; Milivojevic *et al.* 2012). In traditional medicine, *B. crataegina* has been used for the treatment of diarrhea, fever, liver disorders, and infectious diseases. Its pharmacological importance is largely attributed to its rich phytochemical composition, particularly the presence of berberine, along with other alkaloids, flavonoids, and phenolic compounds (Ekim *et al.* 2000; Eroğlu *et al.* 2020; Zhaleh *et al.* 2025). Berberine, an isoquinoline alkaloid, is well known for its antibacterial, antifungal, antiparasitic, and anti-inflammatory properties (Sevindik *et al.* 2020).

Beyond its nutritional and medicinal value, *B. crataegina* also draws attention as an important source of lignocellulosic biomass. As a woody shrub contributing to the understory biomass in forest ecosystems, it constitutes a part of the natural lignocellulosic material pool. Lignocellulosic biomass is considered a significant renewable and carbon-neutral resource that enables the production of high value-added products and is widely utilized, particularly in biofuel and biochemical production (Quevedo-Amador *et al.* 2024). In addition, by-products generated during fruit processing, such as peels, seeds, and pomace, contain substantial amounts of biomass but are often underutilized. It has been reported that such fruit and vegetable wastes can be used in the production of biogas, bioethanol, biodiesel, and other biofuels, thereby contributing to sustainable energy conversion (Adamu *et al.* 2023). Moreover, fruit pomace and similar by-products are highlighted as having significant potential for the development of bioactive compounds, biomaterials, and functional products, playing a key role in the circular economy approach (Venkidasamy *et al.* 2024). In this context, when *B. crataegina* is evaluated both in terms of woody biomass production and fruit-processing by-products, it emerges as a versatile and sustainable biological resource for biofuel production, biomaterial development, and biorefinery applications (Ufitikirezi *et al.* 2024).

In this context, determining the genetic diversity and population structure of *B. crataegina* is important for the conservation of natural populations and the identification of genotypes with desirable traits such as stress tolerance, adaptability, and biomass production potential. Genetic diversity plays a crucial role in the long-term survival and evolutionary potential of plant species, particularly under changing environmental conditions (Frankham *et al.* 2002; Allendorf *et al.* 2012). In addition, the assessment of population genetic structure is fundamental for identifying adaptive variation that can be utilized in breeding and conservation programs (Hoban *et al.* 2020; Amiteye 2021).

Genetic variation within and among populations can significantly influence ecological fitness, environmental adaptability, and resource use efficiency, thereby shaping the evolutionary success of species in natural ecosystems (Sevindik *et al.* 2023; Jahangir

and Nasernakhaei 2025). These variations are also closely associated with phenotypic performance traits, such as growth, stress tolerance, and biomass accumulation, highlighting their importance in both ecological and applied plant sciences (Igwe *et al.* 2022; Alan *et al.* 2025). Therefore, molecular characterization studies are considered indispensable tools for understanding genetic resources and supporting sustainable utilization strategies in plant species.

Molecular marker systems have become indispensable tools for assessing genetic diversity, population structure, and phylogenetic relationships in plants. Among these, Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) markers are widely used due to their high reproducibility, cost-effectiveness, and ability to detect polymorphism without prior genomic information (Pakseresht *et al.* 2013; Amiteye 2021; Igwe *et al.* 2022; Jahangir and Nasernakhaei 2025). ISSR markers target microsatellite regions and are effective in revealing genome-wide variation (Çelik *et al.* 2024), whereas SCoT markers amplify gene-targeted regions flanking the ATG start codon, providing insights into functional genetic diversity (Gupta *et al.* 2019). The combined use of these marker systems enables a more comprehensive and reliable evaluation of genetic variation (Sevindik *et al.* 2023; Alan *et al.* 2025).

Therefore, the aim of this study was to determine the genetic diversity of *B. crataegina* populations naturally distributed in Kayseri (Türkiye) using ISSR and SCoT markers. The findings are expected to contribute to the conservation of genetic resources, the development of sustainable utilization strategies, and the evaluation of the species' ecological and biomass potential.

EXPERIMENTAL

Materials

The collected plant specimens were processed as herbarium material and preserved in the Ondokuz Mayıs University (OMU) Herbarium (Samsun, Türkiye). The specimens were placed in the registration queue and assigned accession numbers upon completion of cataloging. The species identification was performed by Dr. Alper Durmaz (Artvin Çoruh University, Ali Nihat Gökyiğit Botanical Garden Application and Research Center, 08000, Artvin, Türkiye).

In this study, leaf samples of *B. crataegina* were collected from 9 different populations located in various districts of Kayseri province, Türkiye. The sampling points were determined according to the methodology described in previous studies (Demir 2024; Demir and Başayiğit 2024; Demir *et al*. 2024; Dursun *et al*. 2025) and are presented in Fig. 1 and Table 1. To ensure replication and representativeness, three individual plants were sampled from each location, and all analyses were conducted using these biological replicates. Genomic DNA (gDNA) was extracted using the GeneMark Plant Genomic DNA Kit (Catalog No: DP022) and the obtained gDNA samples were stored at –20 °C until further analysis. Polymerase chain reaction (PCR) amplifications were performed using a Thermocycler Gradient system. Selected ISSR and SCoT primers (Tables 2 and Table 3) were employed for PCR and reactions were carried out using a PCR 5X Master Mix (Catalog Nos: RP02-II-400, RP02-II-2000) according to the protocol described in Tables 1 and 2. Amplified products were separated by electrophoresis on 1.5% agarose gels prepared in Tris-borate-EDTA (TBE) buffer and DNA bands were visualized under ultraviolet (UV) light (Fig. 2).

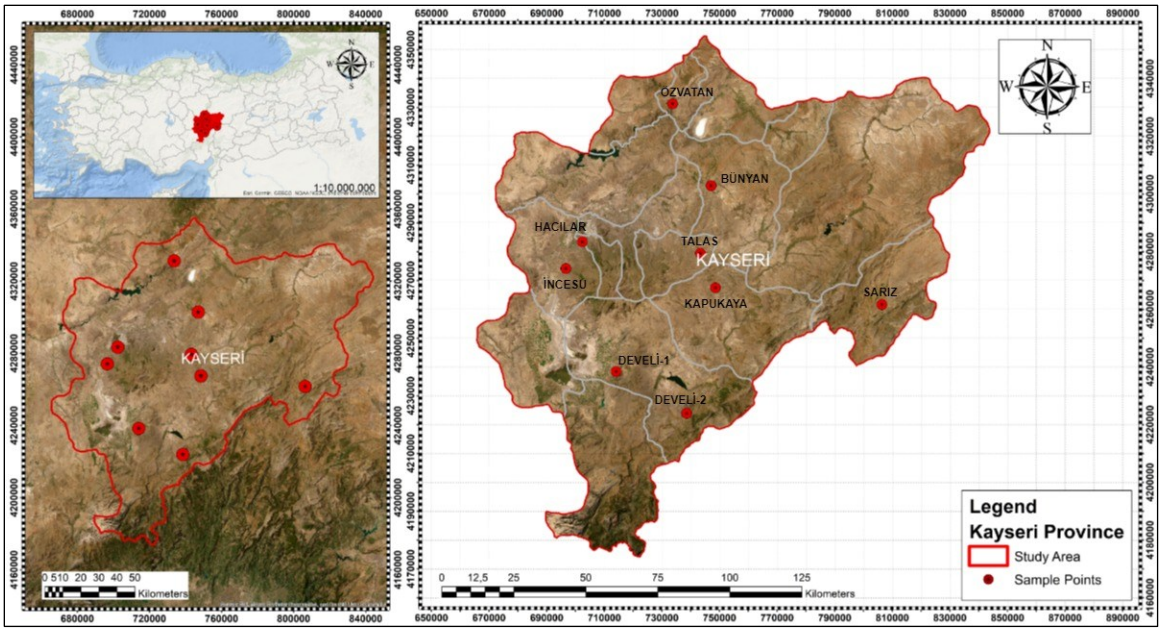

Fig. 1. Location of the Kayseri province

Table 1. Sampling Locations and Coordinates of *B. crataegina*

Population	Location	Latitude (N)	Longtitude (E)
1	BÜNYAN	38.9420	35.8506
2	Develi-1	38.2660	35.4487
3	Develi-2	38.2187	35.7214
4	Hacılar	38.6764	35.3271
5	İncesu	38.5942	35.2583
6	Özvatan	39.0994	35.7030
7	Sarız	38.4505	36.5112
8	Talas	38.6301	35.7955
9	Kapukaya	38.5210	35.8517

Table 2. ISSR Primers and PCR Components Used for PCR Amplification

ISSR primers	DNA Sequences (5'–3')	Tm C	PCR components	PCR amplification (35 cycles except final extension step)
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50 C	1 µL Genomic DNA, 1 µL primers, 5 µL master mix (PCR buffer, 2 mM $MgCl_2$, 250 µM dNTP, 0.75 U Taq DNA polymerase) and 18 µL dH_2O. (Total 25 µL)	94 °C/1 min 94 °C/1 min 47 to 53 °C/1 min 72 °C/1 min 72 °C/10 min (Final extension: 1 cycle)
UBC-856	5'-ACACACACACACACACYA-3'	52 °C		
UBC-855	5'-ACACACACACACACACYT-3'	52 °C		
UBC-873	5'-GACAGACAGACAGACA-3'	48 °C		
UBC-840	5'-GAGAGAGAGAGAGAGAAT-3'	52 °C		
UBC-811	5'-GAGAGAGAGAGAGAGAC-3'	53 °C		
UBC-826	5'-ACACACACACACACACC-3'	52 °C		
UBC-831	5'-CTCTCTCTCTCTCTCTT-3	50 °C		
UBC-892	5'-TAGATCTGATATCTGAAT-3'	52 °C		
UBC-830	5'-TGTGTGTGTGTGTGTGG-3'	52 °C		

Table 3. SCoT Primers and PCR Components Used for PCR Amplification

SCoT primers	DNA sequences (5'–3')	T_m °C	PCR components	PCR amplification (35 cycles)
Primer 1	CAACAATGGCTACCACCA	54 °C	1 µL genomic DNA 1 µL primer, 5 µL master mix (PCR buffer, 2 Mm $MgCl_2$, dNTP, 0.75 U Taq DNA polymerase) & 18 µL dH_2O	95 °C/1 min 95 °C/30 s 54 to 56 °C/30 s 68 °C/1 min 68 °C/5 min
Primer 2	CAACAATGGCTACCACCC	56 °C		
Primer 4	CAACAATGGCTACCACCT	54 °C		
Primer 8	CAACAATGGCTACCACGT	54 °C		
Primer 9	CAACAATGGCTACCAGCA	54 °C		
Primer 11	AAGCAATGGCTACCACCA	54°C		

Following PCR amplification, the presence or absence of bands was scored in a binary format, where "1" indicated the presence of a band and "0" indicated its absence. Genetic similarity among populations was assessed using Jaccard's similarity coefficients and a dendrogram was generated by applying the unweighted pair group method with arithmetic mean (UPGMA) algorithm in the NTSYSpc 2.1 (Exeter Software, Setauket, NY, USA). Based on ISSR and SCoT marker data, a genetic similarity matrix was constructed. Furthermore, principal component analysis (PCA) was performed using MVSP 3.22 software (Kovach Computing Services, Pentraeth, Wales, UK) to evaluate the genetic variation among *B. crataegina* populations and their spatial relationships were illustrated accordingly.

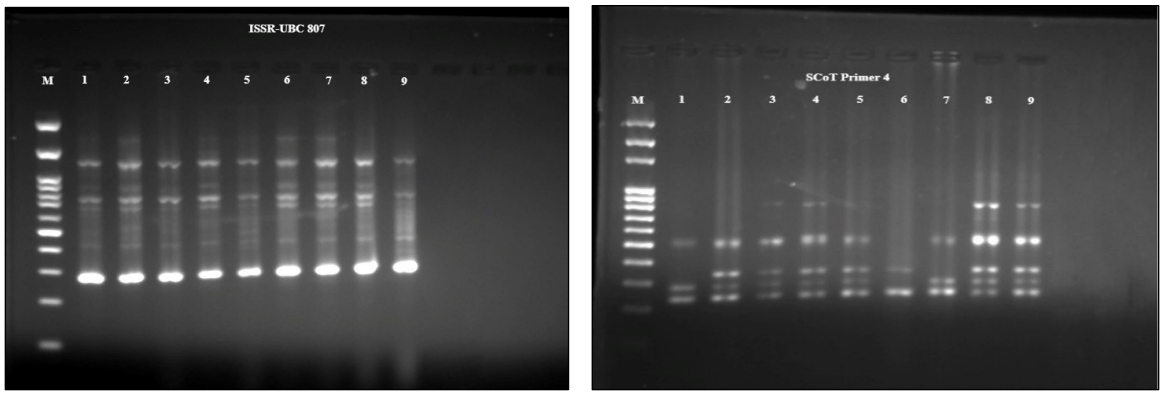

Fig. 2. Gel images of ISSR UBC-807 and SCoT Primer 4 (M: Marker; 1: Bünyan; 2: Develi-1; 3: Develi-2; 4: Hacılar; 5: İncesu; 6: Özvatan; 7: Sarız; 8: Talas; and 9: Kapukaya)

RESULTS AND DISCUSSION

The dendrogram generated using 10 ISSR markers revealed the genetic relationships among the *B. crataegina accessions*. As a result of the study, a total of 51 scorable bands were obtained. It was determined that 10 of the bands were monomorphic and 41 were polymorphic, giving a polymorphism of 80.39%. The UPGMA clustering based on Jaccard's similarity coefficients grouped the accessions into two major clusters (Fig. 3).

The first major cluster comprised Bünyan, Talas, Özvatan, and İncesu, which showed relatively high similarity coefficients, indicating close genetic relationships among

these accessions. Within this group, Bünyan and Talas exhibited the highest genetic similarity, followed by Özvatan, while İncesu was slightly more distant but still clustered within the same branch. The complete similarity observed between the Bünyan and Talas populations may be attributed to gene flow occurring between these populations as a result of their geographical proximity. In addition, it should not be overlooked that the ISSR markers used in this study may not have generated sufficient polymorphism to reveal potential genetic variation between these populations.

The second major cluster was divided into two subgroups. One subgroup contained Develi-1 and Develi-2, which clustered very closely, reflecting a high degree of genetic similarity, possibly due to their geographical proximity or shared genetic background. This subgroup was further related to Kapukaya. The other subgroup consisted of Hacılar, which was separated from all other accessions, suggesting that it possesses distinct genetic characteristics.

Sarız was placed between the two major groups but remained on an independent branch, indicating a moderate level of genetic divergence.

Overall, the clustering pattern suggests that geographic origin may partially explain the genetic similarities observed, particularly in the case of Develi-1 and Develi-2, as well as Bünyan and Talas. However, the distinct separation of Hacılar highlights the presence of unique genetic variation within the population. The results demonstrate that ISSR markers are effective in revealing the genetic diversity and relationships among *B. crataegina* accessions.

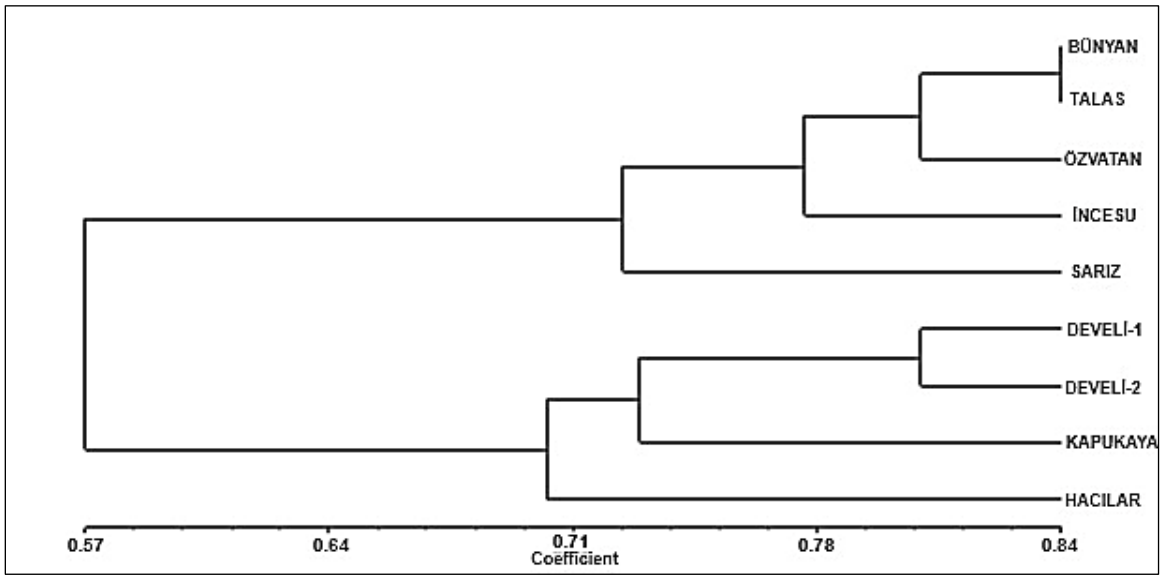

Fig. 3. UPGMA dendrogram showing genetic relationships among *B. crataegina* accessions based on ISSR markers

The PCA based on ISSR data provided further insights into the genetic relationships among *B. crataegina* accessions. The first two principal components explained a substantial proportion of the total genetic variation, effectively separating the accessions into distinct groups that were largely consistent with the UPGMA dendrogram results (Fig. 4).

In the PCA plot, Sarız was clearly separated along the positive axis of Component 2, confirming its intermediate and distinct position observed in the dendrogram. Similarly, Develi-1 and Develi-2 clustered closely together on the right side of the PCA space, again

supporting their close genetic relationship revealed in the dendrogram. Kapukaya was positioned near this cluster, consistent with its grouping in the dendrogram.

Bünyan, Talas, Özvatan, and İncesu were located on the left side of the PCA plot, forming a compact group, which supports their clustering within the same branch in the dendrogram. In contrast, Hacılar appeared separated in the lower right quadrant, reflecting the strong genetic divergence also indicated by its independent branch in the dendrogram.

Overall, the PCA and dendrogram analyses were largely congruent, both indicating that *B. crataegina* accessions share certain regional genetic similarities while also possessing distinct variations. These complementary approaches highlight the utility of ISSR markers in resolving genetic structure and diversity within natural populations of *B. crataegina*.

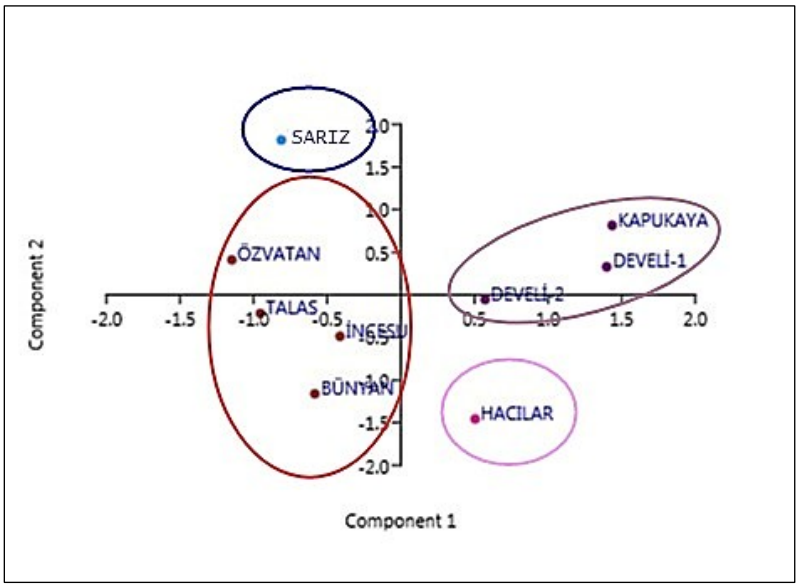

Fig. 4. PCA plot showing the genetic relationships among *B. crataegina* accessions based on ISSR markers

The genetic similarity matrix derived from ISSR markers quantitatively revealed the degree of similarity and divergence among *B. crataegina* genotypes (Table 4). The matrix values ranged from 0.39 to 0.84, indicating a moderate to high level of genetic variation within the studied population.

The highest genetic similarity was observed between Bünyan and Talas (0.84). This finding is consistent with their clustering under the same main branch in the dendrogram and their compact grouping in the PCA plot. Similarly, Develi-1 and Develi-2 exhibited a high degree of similarity (0.80), which is also supported by their close clustering in the dendrogram and their proximity in the PCA analysis.

In contrast, the lowest similarity was detected between Özvatan and Kapukaya (0.39). This divergence corresponds to the separation of Kapukaya from Özvatan and other northern genotypes in the PCA analysis, as well as its placement in a distinct subgroup in the dendrogram. Moreover, the Hacılar genotype showed moderate levels of divergence from most other genotypes (ranging between 0.53 and 0.78), which is consistent with its isolated position in both the PCA plot and the dendrogram.

The Sarız genotype exhibited moderate similarities with the other genotypes (ranging from 0.45 to 0.75), which explains its intermediate position in the PCA analysis and its placement on an independent branch in the dendrogram.

Overall, the genetic similarity matrix results are largely congruent with both PCA and dendrogram analyses. These findings demonstrate that ISSR markers are reliable tools for assessing genetic diversity in *B. crataegina* and support the overall conclusions of the study.

Table 4. Genetic Similarity Matrix among *B. crataegina* Genotypes Based on ISSR Markers

	Bünyan	Develi-1	Develi-2	Hacilar	İncesu	Özvatan	Sariz	Talas	Kapukaya
Bünyan	1.00								
Develi-1	0.59	1.00							
Develi-2	0.75	0.80	1.00						
Hacilar	0.78	0.73	0.69	1.00					
İncesu	0.82	0.57	0.69	0.69	1.00				
Özvatan	0.76	0.43	0.55	0.59	0.71	1.00			
Sariz	0.71	0.49	0.61	0.53	0.69	0.75	1.00		
Talas	0.84	0.47	0.67	0.67	0.78	0.84	0.75	1.00	
Kapukaya	0.47	0.80	0.65	0.69	0.57	0.39	0.45	0.47	1.00

The dendrogram constructed using SCoT markers revealed the genetic relationships among the *B. crataegina* accessions. As a result of the study, a total of 37 scorable bands were obtained. It was determined that 5 of the bands were monomorphic and 32 were polymorphic, giving a polymorphism of 86.40%. Based on Jaccard's similarity coefficients, the accessions were grouped into two major clusters (Fig. 5).

In the first main cluster, Bünyan and Talas exhibited the highest level of genetic similarity, forming a very close sub-cluster. This pair was further associated with Kapukaya, indicating a relatively close genetic relationship. İncesu and Sarız were also placed within the same cluster, suggesting moderate levels of relatedness with the aforementioned accessions.

The second major cluster included Develi-1 and Develi-2, which showed high similarity and grouped together tightly, consistent with their likely geographic and genetic relatedness. Hacılar was positioned near this subgroup, though on a separate branch, reflecting a certain degree of genetic divergence. Özvatan was clearly separated from the other accessions, forming the most distinct branch of the dendrogram and indicating unique genetic characteristics.

Overall, the clustering pattern derived from SCoT markers highlighted both close genetic relationships (*e.g.*, Bünyan–Talas, Develi-1–Develi-2) and strong divergences (*e.g.*, Özvatan). These results demonstrate the effectiveness of SCoT markers in revealing genetic diversity and population structure in *B. crataegina* and complement the findings obtained from ISSR marker analysis.

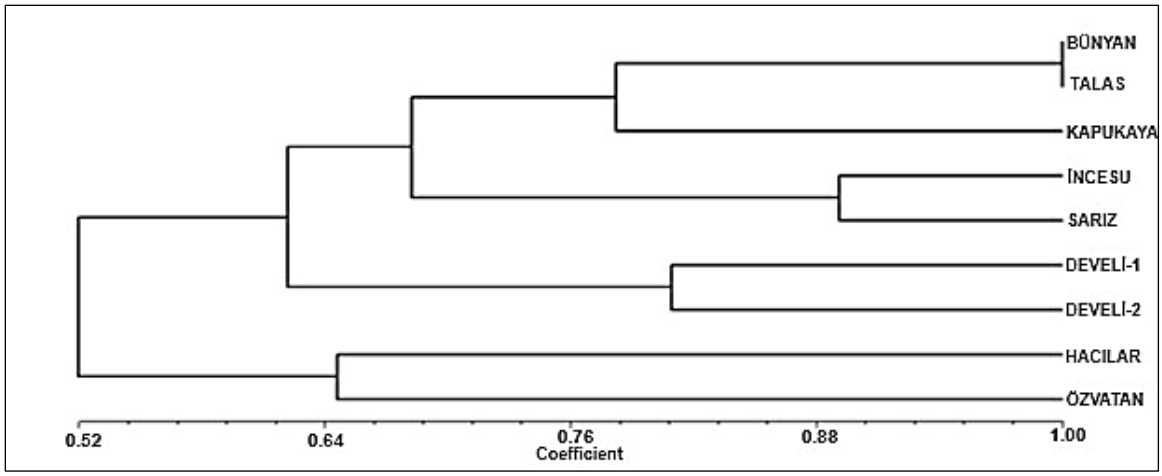

Fig. 5. UPGMA dendrogram showing genetic relationships among *B. crataegina* accessions based on SCoT markers

The PCA based on SCoT markers provided a clear visualization of the genetic relationships among *Berberis crataegina* genotypes. The first two principal components effectively distinguished the accessions into distinct groups (Fig. 6).

Bünyan and Talas were positioned very close to each other in the PCA space, with Kapukaya clustering near this pair, indicating high genetic similarity among these three accessions. Develi-1 and Develi-2 were located in the lower right quadrant and clustered closely together, confirming their strong genetic relatedness.

In contrast, İncesu was placed in the upper central region, clearly separated from the other groups, suggesting that it possesses a unique genetic profile. Sarız appeared in the lower central area in a relatively isolated position, reflecting a distinct genetic structure. Özvatan, located near the center of the plot, occupied an intermediate position, indicating moderate genetic similarity with multiple accessions. Hacılar was placed on the far left side, separated from all other accessions, thus representing the most genetically divergent sample.

Overall, the PCA results demonstrate that SCoT markers were effective in distinguishing both close genetic relationships (*e.g.*, Bünyan–Talas, Develi-1–Develi-2) and marked divergences (*e.g.*, Hacılar) within *B. crataegina*.

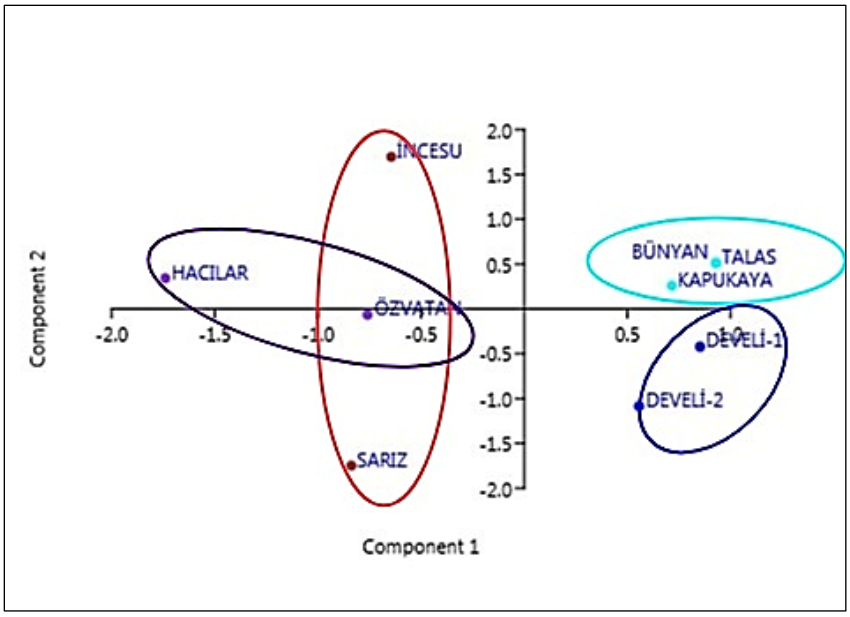

Fig. 6. PCA plot showing the genetic relationships among *B. crataegina* accessions based on SCoT markers

The genetic similarity matrix generated from SCoT markers quantitatively revealed the levels of genetic similarity among *Berberis crataegina* genotypes. The similarity values ranged from 0.32 to 1.00, indicating the presence of considerable genetic variation within the population (Table 5).

The highest similarity was detected between Bünyan and Talas (1.00), showing that these accessions share an almost identical genetic background. Other highly similar pairs included İncesu–Sarız (0.89) and Develi-1–Develi-2 (0.81), reflecting their close genetic affinity.

In contrast, the lowest similarity values were observed between Hacılar and Bünyan/Talas/Kapukaya (0.32). This result highlights the distinct genetic nature of Hacılar and supports its separation from the other accessions. Özvatan showed moderate similarity values with most genotypes (0.49 to 0.65), indicating that it is neither highly divergent nor strongly clustered, but instead occupies an intermediate genetic position. Kapukaya also exhibited relatively high similarity with Bünyan and Talas (0.78), suggesting that it shares a closer relationship with this cluster.

Overall, the SCoT-based genetic similarity matrix revealed both close genetic relationships (*e.g.*, Bünyan–Talas, İncesu–Sarız, Develi-1–Develi-2) and distinct divergences (*e.g.*, Hacılar), thereby confirming the presence of substantial genetic diversity within *B. crataegina*.

The UPGMA dendrogram, PCA, and genetic similarity matrix based on SCoT markers together revealed a highly consistent genetic structure among *Berberis crataegina* accessions. Bünyan and Talas showed complete similarity (1.00), while Develi-1–Develi-2 (0.81) and İncesu–Sarız (0.89) also exhibited strong relatedness, each forming tight clusters across all analyses. Kapukaya grouped with Bünyan and Talas (0.78), confirming its close affiliation. In contrast, Hacılar appeared as the most divergent genotype (similarities as low as 0.32), forming a distinct branch and an isolated PCA position. Özvatan displayed moderate similarity (0.49 to 0.65), occupying an intermediate placement. Overall, these findings demonstrate both highly related clusters and clearly distinct genotypes, underscoring the substantial genetic diversity within *B. crataegina*.

Table 5. Genetic Similarity Matrix among *B. crataegina* Genotypes Based on SCoT Markers

	Bünyan	Develi-1	Develi-2	Hacilar	İncesu	Özvatan	Sariz	Talas	Kapukaya
Bünyan	1.00								
Develi-1	0.59	1.00							
Develi-2	0.68	0.81	1.00						
Hacilar	0.32	0.57	0.54	1.00					
İncesu	0.70	0.57	0.65	0.51	1.00				
Özvatan	0.62	0.49	0.62	0.65	0.59	1.00			
Sariz	0.70	0.57	0.70	0.51	0.89	0.70	1.00		
Talas	1.00	0.59	0.68	0.32	0.70	0.62	0.70	1.00	
Kapukaya	0.78	0.65	0.57	0.32	0.65	0.57	0.65	0.78	1.00

When previous studies on *B. crataegina* are examined, it is evident that ISSR markers have been successfully used to reveal genetic diversity and to reliably determine the relationships among genotypes (Safamanes *et al*. 2017; Yaman *et al*. 2024). However, no studies employing SCoT primers on this species have been reported in the literature. Although genetic diversity in this species has previously been investigated using SSR and ISSR (Safamanes *et al*. 2017), ISSR and RAPD (Aslan *et al*. 2024) primers in combination, the present study represents the first attempt to assess genetic variation based on both ISSR and SCoT primers. The findings of this study are generally consistent with previous research in terms of ISSR marker banding patterns and genetic diversity features. However, some parameters exhibited higher values, which can be attributed to differences in the markers used and the genetic variance among genotypes (Yaman *et al*. 2024). ISSR markers, which successfully revealed polymorphism in *B. crataegina*, are recommended as a reliable tool for future molecular studies on *Berberis* species (Pinar *et al*. 2021). Furthermore, the combined use of ISSR and SCoT markers in this study allowed for a more comprehensive assessment of the genetic diversity of *B. crataegina*, representing the first report in the literature to evaluate these two marker systems together, thus highlighting the originality of this research.

In the current study, a total of 51 scorable bands were obtained with ISSR markers, of which 41 were found to be polymorphic, resulting in a polymorphism of 80.39%. This value is remarkably high compared to previous studies conducted with ISSR markers in *Berberis* species. For example, Pinar *et al*. (2021) analyzed 32 *Berberis* species using 20 ISSR primers and reported a total of 150 scorable bands, 111 of which were polymorphic, corresponding to a polymorphism of 74%. This value is lower than the 80.39% polymorphism obtained in the current study. Such differences may be attributed to the diversity of the primer sets used, the variation in the genetic material included, or the degree of genetic diversity within the studied populations (Pinar *et al*. 2021).

Similarly, Safamanesh *et al*. (2017) documented an average of 9.80 bands per ISSR primer, 8.60 polymorphic bands, and a polymorphism of 79.98% utilising 10 ISSR and 5 SSR markers (Safamanes *et al*. 2017). Despite the fact that the mean number of bands per ISSR marker was lower in the present study (5.1 bands per primer, with 4.1 polymorphic bands), the higher polymorphism (80.39%) suggests that both primer efficiency and the genetic material examined played a role in revealing this high variation. Furthermore, the results obtained from SCoT markers complemented the ISSR findings, with an average of 6.2 bands per primer, 5.3 polymorphic bands and a polymorphism of 86.40%. This finding

suggests that SCoT markers exhibit superior sensitivity in detecting genetic diversity when compared to ISSR markers, thus underscoring their potential as a valuable tool in genetic characterisation studies.

With regard to genetic similarity, values ranged between 0.39 and 1.00 with ISSR markers and between 0.32 and 1.00 with SCoT markers (Côté and Leduc 2007). These findings are consistent with the results of Côté and Leduc (2007), who used ISSR markers on *B. thunbergii* and reported similarity values ranging from 0.30 to 1.00 among 41 different species. Likewise, Lubell *et al*. (2008) used AFLP markers in *B. thunbergii*, *B. julianae*, *B. Koreana*, and *B. vulgaris* var. *atropurpurea*, reporting genetic similarity values between 0.37 and 1.00, which overlaps with the ranges obtained in the current study using ISSR and SCoT markers (Lubel *et al*. 2008). This demonstrates that both ISSR and SCoT markers are reliable tools for revealing broad spectra of genetic variation within *Berberis* species.

Furthermore, Heidary *et al*. (2009) conducted an AFLP study on 33 different *Berberis* ecotypes and two ornamental species and found that *Mahonia aquifolium* formed a separate group from *Berberis*, while the seedless *Berberis* cultivar clustered within the *B. integerrima* population. At 17% similarity, *B. thunbergii* was reported to be distinctly separated from other *Berberis* species such as *B. gagnepaini*, *B. Vulgaris*, and *B. integerrima* (Heidary *et al*. 2009). These results parallel the current study's findings of wide similarity ranges obtained with ISSR and SCoT markers, highlighting strong interspecific genetic differentiation within the genus *Berberis*.

A review of the literature revealed that no studies have yet been conducted using SCoT primers in *Berberis* species. However, SCoT primers have been widely applied in genetic diversity studies of other plant species and cultivars (Bhattacharyya *et al*. 2013; Al-Qurainy *et al*. 2015; Igwe *et al*. 2022; Rai 2023; Altaf *et al*. 2025). Vivodik *et al*. (2017) analyzed 20 maize genotypes from different countries using 5 SCoT primers, obtaining a total of 29 bands, 77.90% of which were polymorphic (Vivodik *et al*. 2017). In comparison, the current study yielded an average of 6.2 bands, 5.3 polymorphic bands, and a polymorphism of 86.40% with SCoT markers. These results suggest that the SCoT markers used in the current study provided a higher polymorphism rate in *Berberis* species and are in line with the findings of Vivodik *et al*. (2017) in maize (Vivodik *et al*. 2017).

Similarly, Hromadová *et al*. (2023) evaluated the effectiveness of 10 RAPD and 10 SCoT markers across 33 common bean genotypes and reported that both marker systems produced high levels of polymorphism, but SCoT markers were more effective, with higher MI (7.474) and DDI (2.265) values compared to RAPD (Hromadová *et al*. 2023). Dendrogram and PCoA analyses also confirmed sufficient separation among bean genotypes. In agreement, the current study demonstrated that SCoT markers revealed a higher polymorphism (86.40%) and a broader genetic similarity range (0.32 to 1.00) compared to ISSR markers, further supporting the conclusion that SCoT markers are highly effective tools for detecting genetic diversity in *Berberis* species, as has been shown in other plant taxa.

CONCLUSIONS

In conclusion, the present investigation provides comprehensive insights into the genetic diversity and relationships among *B. crataegina* species through the application of Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) markers.

1. The relatively high levels of polymorphism detected by ISSR markers confirmed their usefulness as reliable molecular tools in diversity studies. However, the higher polymorphism rate and broader genetic similarity values obtained with SCoT markers clearly emphasized their greater efficiency and discriminatory power in detecting genomic variability.
2. Importantly, this study represents the first application of SCoT primers in *Berberis* species, thereby establishing a novel molecular framework for future research in this genus.
3. The findings underline the complementary value of combining different marker systems, which not only enhances the resolution of genetic characterization but also provides a stronger basis for conservation strategies, germplasm management, and breeding programs.
4. Overall, the molecular evidence generated in this study contributes considerably to understanding the genetic structure of *Berberis* and sets the stage for more targeted utilization of its genetic resources in both applied and fundamental research.

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