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DETERMINATION OF GENETIC DIVERSITY IN SUGAR BEET USING ISSR MARKERS

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Abstract: This study aimed to assess the genetic diversity and population structure of 18 sugar beet (*Beta vulgaris* L.) genotypes using ISSR molecular markers. Initially, 18 ISSR primers were screened, of which six were selected based on their polymorphism and amplification efficiency. A total of 41 bands were scored, 29 of which were polymorphic, resulting in a polymorphism rate of 75.83%. Genetic similarity coefficients calculated using Jaccard's index were used to generate a UPGMA dendrogram, revealing both closely related and highly divergent genotypes. Principal Component Analysis (PCA) confirmed these results, indicating a complex genetic structure with potential heterotic pools. genotypes SB-7 and SB-17, which showed the highest similarity (0.806) in the UPGMA dendrogram, were also positioned in close proximity on the PCA plot. In contrast, genotypes SB-2 and SB-15, which demonstrated the lowest genetic similarity (0.461), occupied distant locations along the principal axes, reinforcing their genetic divergence. STRUCTURE software was employed to analyze population structure, showing that some genotypes exhibited high genetic purity while others displayed admixture. The findings underscore the effectiveness of ISSR markers in evaluating genetic variation and provide valuable insights for future sugar beet breeding programs aimed at improving adaptability, productivity, and genetic resource conservation.

Keywords: *Beta vulgaris*, ISSR markers, Genetic diversity, UPGMA, Structure analysis

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1. Introduction

Sugar beet (*Beta vulgaris* L.) is a strategically important agricultural crop and one of the primary industrial sources of sugar cultivated worldwide (Tan and Arabacı, 2024; Turgut and Koca, 2024). This species, which thrives particularly in temperate climate zones, holds not only economic but also strategic value due to its utilization in sugar production, animal feed, biofuel (especially bioethanol) generation, and a variety of industrial by-products (Srivastava et al., 2007; Sen and Alikamanoglu, 2012; Keykhosravi et al., 2017). Türkiye ranks among the leading sugar beet producers in Europe, and this crop constitutes a significant component of the country's agricultural production system (Ouchar et al., 2023; Susar and Şatana, 2024; Şahin, 2024). However, modern sugar beet cultivation is increasingly challenged by several critical factors, including reduced genetic diversity (genetic bottlenecks), pressure from various phytopathogens and insect pests, climate change, soil salinization, water scarcity, and uniform cultivation practices (Sadık et al., 2025). Therefore, the conservation of existing genetic resources, the identification of inter varietal genetic variation, and the broadening of the genetic base are essential for ensuring sustainable production systems and developing modern plant breeding strategies (Çilesiz, 2025a). Traditional phenotypic characterization methods are often insufficient for reliably evaluating genetic variation due to their high susceptibility to environmental influences and limited discriminatory power (Çilesiz, 2025b). To overcome these limitations, molecular marker technologies have become increasingly prevalent in recent years for the precise, objective, and detailed analysis of plant genetic resources (El-Mouhamady et al., 2021; Sevindik et al., 2023; Çilesiz, 2025c). Molecular markers allow the detection of polymorphisms directly at the DNA level, independent of environmental factors, thereby enabling the accurate assessment of parameters such as genetic similarity, phylogenetic relationships, population structure, variation dynamics, and levels of genetic erosion (Karaköy et al., 2024; Ovalıoğlu and Çilesiz, 2025; Çetin et al., 2025).

In this context, dominant molecular markers such as ISSR (Inter Simple Sequence Repeat) and RAPD (Random Amplified Polymorphic DNA) have been widely used for evaluating genetic diversity in sugar beet and many other plant species (Shimira et al., 2021; Baloch et al., 2022; Çilesiz et al., 2025). ISSR markers bind to microsatellite regions between repeating sequences and generate high levels of polymorphism. Due to their ability to cover a broad range of the genome and their high reproducibility, ISSR markers are particularly suitable for genetic mapping, intra-species variation analysis, and phylogenetic studies (Küçük et al., 2024; Sayed et al., 2024). Since ISSR markers exhibit dominant inheritance, they cannot distinguish between heterozygous and homozygous individuals. This limitation prevents the full revelation of allelic diversity and the true genetic structure (Sevindik et al., 2024). In contrast, co-dominant systems such as SSR or SNP provide more detailed genetic information due to their ability to differentiate both allelic forms, and they are particularly preferred in population genetics, linkage mapping, and genomic selection studies. Therefore, while ISSR markers stand out as a rapid and cost-effective screening method, more balanced and reliable results can be achieved when they are used together with, or as a complement to, systems such as SSR and SNP (Çilesiz, 2025d). The main objective of the research is to quantitatively evaluate the levels of genetic similarity and variation among these genotypes. Furthermore, the study aims to assess the current status of the genetic base and to contribute to genetic resource management, parental selection strategies, core collection development, and future oriented sustainable breeding programs. The findings are expected to serve as a valuable reference for both the improvement of existing cultivars and the development of new, superior varieties.

2. Materials and Methods

2.1. Plant Materials

In the study, 13 sugar beet genotypes obtained from the USDA Agricultural Research Service (USDA) genebank and 5 commercial beet varieties (Serenada, Varias, Evelina, Jaguar, Balaban) were used as materials (Table 1).

Table 1. Details of sugar beet populations used in the study

Genotype No	Genotype Code	Registration Number	Species Name
G1	Serenada	Commercial Variety	
G2	Varias	Commercial Variety	
G3	Evelina	Commercial Variety	
G4	Jaguar	Commercial Variety	
G5	Balaban	Commercial Variety	
G6	NSL 28024	Extra Early	*B. vulgaris subsp. vulgaris*
G7	PI 179180	CICLA	*B. vulgaris subsp. vulgaris*
G8	NSL 95223	A77-52	*B. vulgaris subsp. vulgaris*
G9	NSL 6320	Winter Keeper	*B. vulgaris subsp. vulgaris*
G10	NSL 6624	Half Sugar Rose	*B. vulgaris subsp. vulgaris*
G11	PI 608798	A77-50	*B. vulgaris subsp. vulgaris*
G12	PI 590679	C01	*B. vulgaris subsp. vulgaris*
G13	PI 610306	A79-55	*B. vulgaris subsp. vulgaris*
G14	PI 610300	A78-31	*B. vulgaris subsp. vulgaris*
G15	PI 590732	MELANGE U	*B. vulgaris subsp. vulgaris*
G16	PI 610278	71/3-1-3-L13 A75-32	*B. vulgaris subsp. vulgaris*
G17	PI 590709	1503	*B. vulgaris subsp. vulgaris*
G18	PI 171576	No. 7154	*B. vulgaris subsp. vulgaris*

2.2. Genomic DNA Isolation

All 18 sugar beet samples were grown in a greenhouse and 100 mg of fresh leaves were collected for DNA isolation. After freezing the leaves in liquid nitrogen, they were ground into fine powder in a porcelain mortar. DNA extraction from the leaves of sugar beet samples was performed using the CTAB protocol as described by Doyle and Doyle (1990) with slight modifications. The concentrations of stock DNA were measured using a MaestroNano Pro spectrophotometer (MN913A, MaestroGen) and the DNA samples were subsequently diluted to a final concentration of 5 ng/µL. DNA quality was assessed solely by spectrophotometry.

2.3. ISSR Marker Assay

To determine the genetic relationships among sugar beet samples, 18 ISSR primers were initially screened in 5 samples and 6 primers exhibiting polymorphism were selected and applied to all samples. Six ISSR primers were used (Table 2). The PCR reaction mixture consisted of 4 µL of DNA (20 ng), 1 µL of primer, 10 µL of PCR master mix (Eco Tech, Cat No: ET5) and 10 µL of dH_2O. In total, 20 ng of template DNA was used for 25 µL PCR reaction. PCR conditions started with initial denaturation for 3 min, following 35 cycles of denaturation at 94°C for 1 minute, annealing at 50-52°C for 1 min and polymerisation at 72°C for 1 min; last, final polymerisation was performed at 72°C for 10 min. For electrophoresis of the PCR products, a 2% agarose gel prepared in Tris-borate-EDTA buffer was used. The gel was stained using ethidium bromide, and visualised with UV Imager (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Table 2. Six primers used for ISSR assay, their sequence, GC content, Tm value, number of total and polymorphic bands, and diversity parameters

ISSR Markers	Sequence (5'-3')	GC%	Tm°C	Number of Bands		Diversity parameter				
				Polymorphic Bands	Total Bands	P%	ne	h	I	PIC
UBC-807	AGAGAGAGAGAGAGAGT	47	50	4	4	100	1.27	0.15	0.22	0.15
UBC-810	GAGAGAGAGAGAGAGAT	47	50	4	5	80	1.67	0.35	0.50	0.35
UBC-831	CTCTCTCTCTCTCTCTT	47	50	6	6	100	1.73	0.40	0.59	0.40
UBC-834	AGAGAGAGAGAGAGAYT	41	52	6	11	55	1.59	0.32	0.47	0.32
UBC-836	AGAGAGAGAGAGAGAGYA	44	52	4	7	57	1.75	0.42	0.61	0.42
UBC-840	GAGAGAGAGAGAGAGAAT	44	52	5	8	63	1.35	0.20	0.30	0.20

P%= polymorphism percentage.

2.4. Statistical Analysis

The scoring process was conducted using a binary approach, where a value of 1 indicated the presence of a band, and a value of 0 indicated its absence. The investigations focused exclusively on bandsthat were bright, clear, and wellseparated (Yıldız et al., 2021). Diversity parameters such as effective number of alleles, gene diversity, and Shannon diversity index as well as Nei's genetic distance were calculated using POPGENE ver. 1.32 software (https://sites.ualberta.ca/~fyeh/popgene.html) (Yeh et al., 2000). The mean polymorphism information contents (PIC) of each SCoT primer were deduced using the following formula (equation 1) (Roldan-Ruiz et al., 2000):

$$PIC = 2fi\ (1-fi) \quad (1)$$

where PIC isthe polymorphic information content, fi represents the frequency of presence of the band and 1 – fi shows the absence of the band.

A dendrogram based on Jaccard's similarity coefficients was generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in MVSP 3.22 software (Kovach, 2007). Application of MVSP 3.22 to the ISSR molecular data enabled the construction of a genetic similarity matrix. Pairwise genetic distance matrix was generated using MVSP 3.22 software, and the bar plot graph was created using STRUCTURE software (https://web.stanford.edu/group/pritchardlab/structure.html). In addition, Principal Component Analysis (PCA) was performed using MVSP 3.22 to assess the genetic relationships among sugar beet populations.

3. Results and Discussion

To determine the genetic relationships among sugar beet samples, 18 ISSR primers were initially screened in 10 samples and 6 primers (that produced strong PCR results) exhibiting polymorphism were selected and applied to all samples (Table 2). Using these primers, a total of 41 scorable bands were generated. Among these bands 29 were polymorphic with an average of 4.8 bands per primer. The number of bands produced by each primer ranged from 4 (UBC-807) to 11 (UBC-834), with an overall average of 6.8 bands per primer (Table 2). The use of six ISSR primers resulted in a significant level of polymorphism across 18 samples. The average polymorphism rate observed among these samples was calculated as 75.83%. Among the primers, UBC-836 and UBC-807 exhibited the highest (1.75) and lowest (1.27) effective number of alleles, respectively, with a mean value of 1.56. Gene diversity observed using ISSR primers ranged from 0.15 (UBC-807) to 0.42 (UBC-836), with an average of 0.31. Shannon's diversity index values varied from 0.22 for UBC-807 to 0.61 for UBC-836, with a mean of 0.45. The PIC (Polymorphic Information Content) values ranged from 0.15 for primer UBC-807 to 0.42 for primer UBC-836, with an average PIC value of 0.31 (Table 2). This result reflects the high efficiency of the selected ISSR primers in detecting DNA-level variations across diverse sugar beet genotypes. Such a high polymorphism percentage indicates that ISSR markers successfully amplified multiple genomic loci and were able to differentiate between the genotypes effectively. The use of ISSR markers known for their reproducibility and genome-wide coverage enabled the identification of both intra- and intergroup variability, which is critical in crop improvement programs. In the study conducted by Izzatullayeva et al. (2014), in which they performed a comparative analysis of genetic variation in 42 sugar beet genotypes using RAPD and ISSR markers, a total of 24 polymorphic primers (12 RAPD and 12 ISSR) were used. The RAPD primers generated 204 amplification products, while the ISSR primers produced 178 fragments; of these, 190 and 173 were found to be polymorphic, respectively. The average polymorphism level obtained with the ISSR markers (97.2%) was found to be higher than that of the RAPD primers (93%). Based on the high genetic diversity indices (0.86 for RAPD and 0.91 for ISSR), it was determined that these methods were equally effective in identifying genetic variation in sugar beet genotypes. Temrel and Çilesiz (2025), screened a total of 36 SCoT markers in thyme and selected 14 markers showing the highest polymorphism for analysis. A total of 169 bands were obtained from 14 SCoT primers. Of these, 141 were found to be polymorphic, resulting in a polymorphism rate of 83.43%. According to the UPGMA dendrogram and the correlation matrix generated from the SCoT molecular data, the lowest similarity (0.326) was observed between samples G12 and G17, while the highest similarity (0.854) was detected between G8 and G10. In another study, four elite diploid sugar beet populations (IISR Comp-1, LKC-11, LS-6, and Ramonskaya-06) were analyzed for genetic diversity using isozymes and molecular markers (RAPD and ISSR). RAPD and ISSR markers showed lower genetic similarity values compared to isozymes, indicating their higher ability to detect polymorphism. Genetic diversity among eight different sugar beet varieties was evaluated using ISSR markers and agronomic traits obtained over two growing seasons. The generation of 169 amplified bands by 12 ISSR primers, of which 122 were polymorphic, resulted in a polymorphism rate of 72.18%, indicating high genetic diversity among the eight sugar beet varieties (El-Mouhamady et al., 2021).

Table 3. Pairwise genetic distance matrix obtained from six ISSR primers

Group 1	Group 2	Similarity	Objects in group
SB-7	SB-17	0.806	2
SB-1	SB-9	0.800	2
SB-13	SB-14	0.800	2
SB-8	SB-18	0.788	2
SB-10	SB-12	0.788	2
SB-4	SB-6	0.774	2
Node 2	SB-5	0.761	2
SB-11	SB-15	0.750	3
Node 8	Node 3	0.726	2
Node 4	Node 5	0.723	4
Node 1	SB-16	0.706	4
Node 7	Node 10	0.702	3
Node 12	Node 11	0.684	7
SB-3	Node 6	0.678	10
Node 13	Node 9	0.665	3
Node 15	Node 14	0.645	14
Node 16	SB-2	0.461	17

Sönmez and Çilesiz (2025), in their study on grapes, obtained a total of 119 bands from 12 SCoT primers. Of these, 83 were found to be polymorphic, resulting in a polymorphism rate of 69.74%. According to the UPGMA dendrogram and the correlation matrix generated from the SCoT molecular data, the lowest genetic similarity (0.382) was observed between genotypes G6 and G38, while the highest similarity (0.786) was observed between genotypes G37 and G39. In the study conducted by Sun et al. (2024) on sugar beet, the total number of alleles (Na) was 298, of which 182.426 (approximately 61.2%) were identified as effective alleles (Ne). The average genetic diversity index was calculated as 0.836. The polymorphic information content (PIC) ranged from 0.639 to 0.907, with a mean of 0.819, indicating a high level of polymorphism. The sugar beet varieties were grouped into six clusters based on UPGMA cluster analysis. The population structure analysis revealed that the optimal K value was 6, which was consistent with the clustering results, indicating that the test materials could be divided into six groups.

To assess the genetic relationships and diversity patterns among the 18 sugar beet genotypes, a cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard's similarity coefficients. The resulting dendrogram (Figure 1) revealed a wide range of genetic similarity values, ranging from 0.461 (SB-2 and SB-15) to 0.806 (SB-7 and SB-17), indicating the presence of both closely related and highly divergent genotypes within the evaluated population (Table 3).

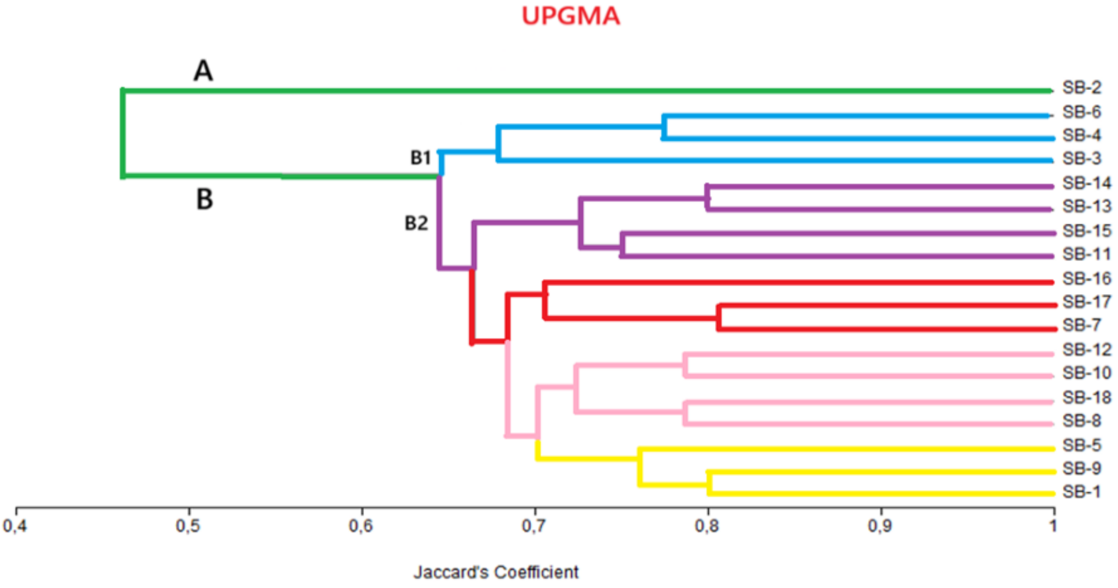

Figure 1. UPGMA dendrogram of genetic similarity between ISSR markers and 18 sugar beet genotypes.

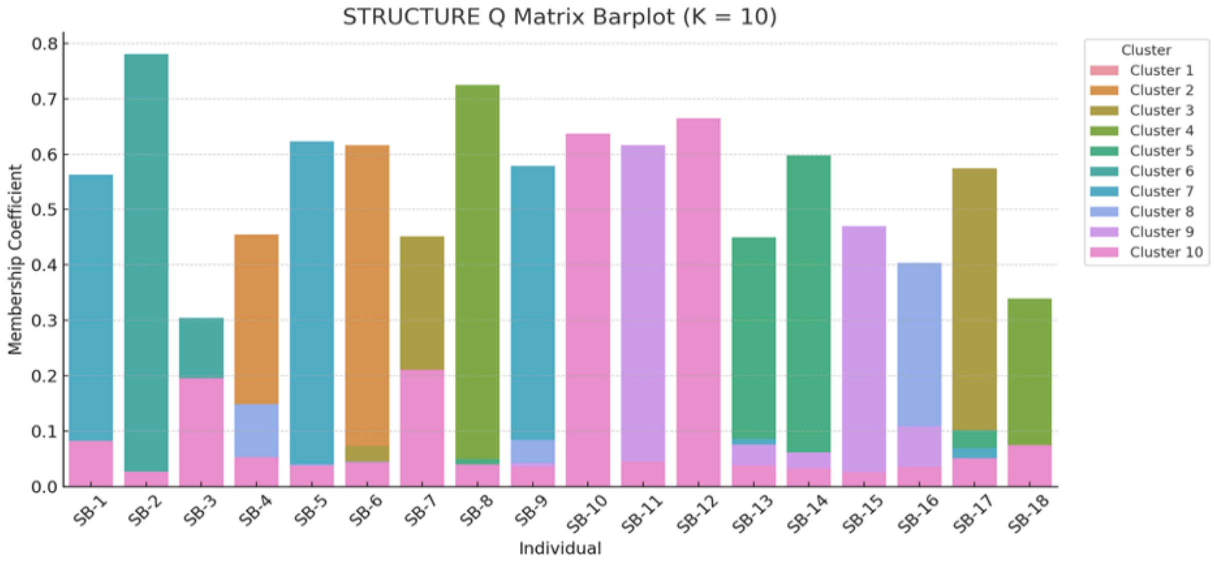

Figure 2. Bar plot of STRUCTURE Q matrix showing the membership coefficients of 18 sugar beet genotypes across 10 inferred genetic clusters (K = 10).

This broad variation in pairwise genetic similarity highlights the rich genetic heterogeneity of the germplasm set, which is essential for effective cultivar improvement and long-term conservation strategies. The UPGMA dendrogram clustered the genotypes into multiple distinct groups, each representing varying levels of genetic affinity. Cluster I, for instance, comprised genotypes with the highest similarity values such as SB-7 and SB-17, SB-1 and SB-9, and SB-13 and SB-14 all of which shared similarity indices at or above 0.800. The tight clustering of these genotypes suggests a relatively narrow genetic base within these groups, potentially reflecting shared ancestry, similar selection histories, or limited genetic drift. Conversely, genotype SB-2 exhibited the lowest similarity (0.461) when compared to SB-15 and was placed in a terminal solitary branch of the dendrogram, indicating its unique genetic composition. This level of divergence implies that SB-2 harbors rare or underrepresented alleles that distinguish it from the rest of the genotypes. As such, SB-2 may serve as a valuable outcrossing parent in breeding programs aimed at enhancing heterozygosity and broadening the genetic base through hybridization with more genetically distant lines. Interestingly, the five commercial sugar beet cultivars (Serenada, Varias, Evelina, Jaguar, and Balaban) were dispersed across different clusters rather than forming a unified group. This observation suggests that these cultivars, despite their commercial status, possess diverse genetic backgrounds and may share considerable allelic overlap with gene bank accessions. This dispersion may be attributed to historical introgression events, hybrid breeding, or shared ancestral lines between cultivated and exotic germplasm. Such interrelatedness underlines the significance of germplasm exchange and international breeding collaborations, which have likely contributed to the observed allelic complexity. Furthermore, the observed clustering pattern reinforces the utility of ISSR markers in distinguishing among closely related and genetically distant genotypes. The resolution power of ISSR-based UPGMA analysis enables not only the identification of redundant accessions but also the detection of genetically unique individuals that are critical for core collection development, marker-assisted selection, and adaptive trait discovery. In summary, the UPGMA dendrogram constructed from ISSR data offers a clear and detailed visualization of the genetic structure within the studied sugar beet panel. The clustering results provide valuable insight into the levels of genetic similarity and divergence, which can guide strategic decisions in genotype selection, parental choice for crossing, and the design of effective breeding programs targeting genetic gain and adaptability.

The STRUCTURE Q matrix barplot illustrates the membership coefficients of 18 sugar beet genotypes assigned to K = 10 different genetic clusters. The plot visually represents each individual's proportional contribution to these ten genetic pools using color-coded bars (Figure 2).

This graphical representation is commonly utilized in genetic structure analyses to visualize population stratification. SB-2 appears to belong predominantly to Cluster 6 (turquoise) with approximately 78% membership, while SB-11 and SB-12 show over 70% membership in Cluster 10 (pink), and SB-5 is approximately 63% assigned to Cluster 6. These individuals are highly likely to possess a pure or distinct genetic structure, indicating genetically homogeneous profiles. In contrast, individuals such as SB-6, SB-7, SB-16, SB-17, and SB-18 demonstrate substantial contributions from multiple clusters. For instance, SB-6 has components from Cluster 2 (orange), Cluster 4 (green), and to a lesser extent, Cluster 9 (pink). SB-7 is assigned to Cluster 4 (~45%) and Cluster 10 (~22%). This pattern indicates that these genotypes exhibit an admixed genetic structure, likely resulting from historical hybridization or artificial selection events. The clusters are not uniformly distributed across all individuals. Some clusters (e.g., Cluster 7, Cluster 8) are represented only at low proportions across genotypes, suggesting that certain clusters are genetically dominant, while others are marginal or rare. Individuals with ≥70% assignment to a single cluster can be considered core members of that genetic group. Those with lower assignment probabilities are admixed and may be valuable in breeding programs aimed at increasing heterozygosity. For instance, hybridizing SB-2 (Cluster 6) and SB-11/SB-12 (Cluster 10) with admixed individuals such as SB-6, SB-7, or SB-16 could enhance genetic variation. In order to further elucidate the genetic relationships among the studied sugar beet genotypes and to reduce the dimensional complexity of the ISSR marker data, Principal Component Analysis (PCA) was conducted using the MVSP 3.22 software (Kovach Computing Services, UK) (Figure 3).

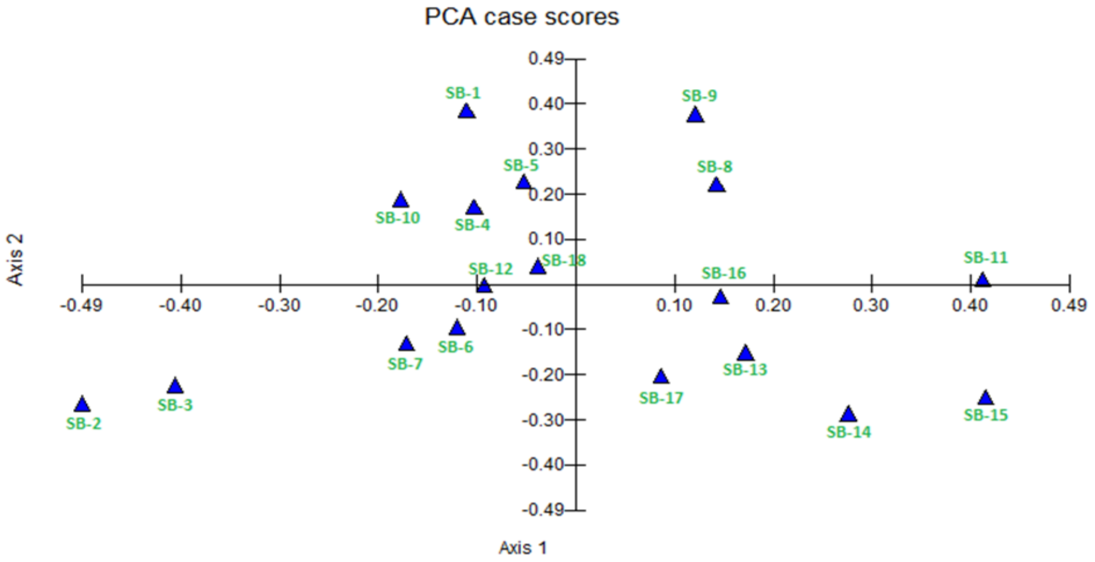

Figure 3. According to ISSR markers pricipal component analyses of 18 sugar beat genotypes using MVSP 3.22 software (Kovach computing services, Pentraeth, Wales, UK).

PCA is widely recognized as an effective multivariate statistical method for simplifying high-dimensional genetic datasets while retaining most of the original variation. In this study, the PCA results provided a complementary perspective to the UPGMA cluster analysis and validated the overall genetic structure of the sugar beet germplasm. The distribution of genotypes on the PCA biplot (Figure 3) exhibited a clear separation among genetically distinct individuals, whereas genetically similar genotypes were closely clustered. For instance, genotypes SB-7 and SB-17, which showed the highest similarity (0.806) in the UPGMA dendrogram, were also positioned in close proximity on the PCA plot. In contrast, genotypes SB-2 and SB-15, which demonstrated the lowest genetic similarity (0.461), occupied distant locations along the principal axes, reinforcing their genetic divergence. This concordance between the PCA and cluster analysis supports the reliability and consistency of the ISSR marker system used in this study.

The first three principal components (PCs) explained a substantial portion of the total genetic variance, indicating that the major axes captured the most significant sources of variation within the population. This multivariate dispersion implies that the studied sugar beet genotypes possess a broad and complex genetic background. Such a pattern is indicative of considerable intra- and inter population diversity, which may have originated from the integration of diverse genetic materials, including gene bank accessions and commercial cultivars. Moreover, the spread of genotypes across the PCA plot suggests the presence of genetically distinct subpopulations, which could represent ecotype differentiation or historical selection pressures. The detection of these subgroups is particularly useful for the identification of potential heterotic pools, enabling more informed decisions in parental selection and hybrid development. Additionally, such structure can inform genome-wide association studies (GWAS) by accounting for population stratification, thereby reducing the risk of false-positive associations (Ghazy et al., 2022). In conclusion, PCA analysis not only confirmed the clustering patterns revealed by UPGMA but also provided deeper insights into the genetic structure and variability of sugar beet genotypes. The spatial arrangement of individuals on the PCA plot underscores the high discriminatory power of ISSR markers and demonstrates their effectiveness in dissecting the genetic complexity of diverse germplasm collections. The molecular diversity revealed by ISSR markers in this study has profound implications for sugar beet breeding and genetic resource management. The identification of both genetically similar and genetically divergent genotype pairs among the evaluated material presents strategic opportunities for developing targeted breeding schemes with enhanced genetic efficiency.

Genotypes exhibiting high genetic similarity, such as SB-7 and SB-17, represent lines with close genetic backgrounds. These genotypes are valuable for use in phenotypic stability, uniformity assessments and genetic fidelity studies, particularly in variety registration trials. Their high similarity also suggests that they could serve as recurrent parental lines for maintaining specific agronomic traits in elite breeding populations, such as sugar yield, disease resistance, or phenological uniformity. In contrast, genetically distant genotype pairs, notably SB-2 and SB-15, which exhibited the lowest similarity index (0.461), are ideal candidates for heterotic hybrid combinations. Crossing such divergent lines has the potential to produce F1 hybrids with enhanced vigor (heterosis), improved biomass accumulation, and broader tolerance to biotic and abiotic stresses. This approach is particularly advantageous in modern sugar beet breeding, where yield plateauing and stress sensitivity have emerged as key challenges under increasingly variable environmental conditions. The significant genetic divergence observed among the USDA gene bank accessions indicates that these materials represent a reservoir of underutilized allelic variation. These accessions likely harbor novel or rare alleles associated with adaptive traits such as drought resistance, salinity tolerance, disease resistance (e.g., Rhizoctonia root rot or Cercospora leaf spot), enhanced root architecture, or higher sucrose accumulation. The strategic incorporation of these gene bank materials into commercial breeding pipelines can lead to the development of broadly adapted cultivars with improved yield stability and environmental resilience. Moreover, the dispersed clustering of commercial cultivars within the UPGMA dendrogram rather than forming a single clade suggests that commercial breeding programs may have historically drawn from a genetically diverse foundation. However, the limited number of elite parental lines currently in use in industrial breeding has likely resulted in a constricted genetic base over time. This genetic bottleneck increases vulnerability to pest/pathogen outbreaks and climate-induced stress, and limits breeders' ability to respond to new challenges. Therefore, the substantial variation identified in this study provides a valuable genetic framework for rational parental selection, core germplasm development, and pre-breeding efforts (Zhao et al., 2025). These findings advocate for the integration of diverse gene bank accessions into elite backgrounds, using marker assisted selection (MAS) or genomic selection (GS) approaches to accelerate genetic gain. Ultimately, leveraging the full spectrum of molecular diversity documented here will be essential to develop next-generation sugar beet cultivars with improved productivity, adaptability, and sustainability (Ferweez and Bashandy, 2021; Wu et al., 2024).

4. Conclusion

In summary, the present study demonstrated that ISSR marker-based molecular analysis is a robust and informative approach for evaluating the genetic diversity of sugar beet genotypes. The application of six ISSR primers across 18 genotypes resulted in a high polymorphism rate (75,83%), indicating the presence of substantial allelic variation within the examined germplasm. Such diversity is critical for sustaining long-term crop improvement and genetic conservation efforts, particularly in the context of modern breeding programs where narrowing genetic bases pose serious challenges. The combined use of UPGMA clustering and Principal Component Analysis (PCA) allowed for an in-depth exploration of genetic relationships among genotypes. The congruency between the two methods validated the observed groupings, with genotypes showing strong genetic similarities forming tightly linked clusters, and highly divergent genotypes clearly separated both in the dendrogram and on the PCA plot. This multi-faceted analytical approach ensured both numerical and visual confirmation of genetic distances and population structure. Importantly, the identification of genetically distant genotypessuch as SB-2 and SB-15 underscores the potential to exploit heterotic combinations in future hybrid development, while genetically similar pairs such as SB-7 and SB-17 could be useful in establishing phenotypic stability and uniformity in varietal selection. Moreover, the wide dispersion of gene bank accessions alongside commercial cultivars highlights the potential value of exotic and underutilized germplasm as a reservoir of novel alleles for biotic and abiotic stress tolerance, sugar yield, and adaptability. The findings of this study contribute significantly to the current understanding of genetic variation in sugar beet and demonstrate the applicability of ISSR markers as cost-effective, reproducible, and genome-wide tools for germplasm evaluation. The genetic insights gained here not only support breeding decisions such as parental selection and population design, but also serve as a baseline for conservation strategies targeting the preservation of genetic resources under future climatic and agricultural challenges. Therefore, the outcomes of this research provide a solid scientific foundation for future sugar beet improvement programs, ensuring sustainable production, enhanced resilience, and effective genetic resource management.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	Y.Ç.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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