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Utilization of Rapd Markers for Determining Genetic Diversity in Sugar Beet

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Abstract
This study aimed to evaluate the effectiveness of RAPD (Random Amplified Polymorphic DNA) markers in determining the genetic diversity among 18 sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) genotypes. Of the 11 RAPD primers initially tested, 6 primers showing the highest level of polymorphism were selected and analyses were performed across all genotypes. A total of 49 bands were obtained, 40 of which were identified as polymorphic, corresponding to a polymorphism level of 81.6%. As a result of the RAPD analyses applied, it was determined that there were significant levels of genetic differences among the genotypes. To visualize genetic relationships, UPGMA cluster analysis based on the Jaccard similarity coefficient and Principal Component Analysis (PCA) were performed. Diversity parameters such as the effective number of alleles, gene diversity, and Shannon's diversity index as well as Nei's genetic distance were calculated using POPGENE version 1.32 software. In terms of PIC, the highest values were found in OPA-18 (0.30) and OPA-2 / OPA-5 (0.29), while the lowest PIC value was observed in OPA-3 (0.20). The pairwise genetic distance matrix was obtained using MVSP 3.22 software, while the Q-matrix bar plot was generated with STRUCTURE software. The results of the analyses showed that genetically similar genotypes clustered together, whereas distinct genotypes were separated. In particular, the differentiation of SB-2 as the most genetically distant individual revealed that this genotype has the potential to increase genetic variation in breeding programs. In contrast, SB-10 and SB-12 genotypes exhibited high genetic similarity. The fact that commercial cultivars were distributed in different clusters demonstrated that these materials have different genetic origins and may share common alleles with gene bank accessions. The findings obtained revealed that RAPD markers are a reliable method for the characterization of sugar beet genetic resources and provide important information for parent selection and sustainable breeding strategies.

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1. Introduction

Sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) is an industrial crop widely cultivated around the world and serves as one of the primary sources of sucrose (Panella, 2010; Susar and Şatana, 2024). It grows efficiently under temperate climatic conditions and plays a strategic role not only in sugar production but also in the generation of animal feed, bioethanol, and various industrial by-products (Şahin, 2024). Türkiye is among the leading producers of sugar beet in Europe, and the crop occupies a central place in the country's agricultural economy and rural development (Holtgräwe et al., 2014; Stevanato et al., 2019). However, modern sugar beet cultivation is increasingly challenged by a range of biotic and abiotic stress factors, including reduced genetic diversity, pressure from pathogens and pests, climate change, soil salinity and water scarcity (Schneider et al., 2007). The narrow genetic base of breeding materials used in current programs limits adaptive potential and may hinder future yield improvements (Broccanello et al., 2015). Therefore, accurate assessment and conservation of existing genetic resources are crucial for the development of sustainable breeding strategies and for the effective management of plant genetic resources (Richards et al., 2004). Conventional phenotypic characterization methods are often insufficient to accurately evaluate genetic diversity (Ouchar et al., 2023) due to their susceptibility to environmental variation and their limited discriminatory power (Shimira et al., 2021). To overcome these limitations, molecular marker technologies have become essential tools for providing reliable and detailed insights into plant genetic diversity (Sayed et al., 2024; Çilesiz et al., 2025). These markers, which operate independently of environmental influences, enable accurate assessment of genetic similarity, variation, and population structure at the DNA level (Abbasi et al., 2014; Sevindik et al., 2023; Tan and Arabacı, 2024).

To assess genetic diversity, RAPD markers were employed. RAPD markers are widely used in plant genetic studies due to their advantages such as low cost, minimal DNA requirement, and no need for prior sequence information (Temrel and Çilesiz, 2025; Sönmez and Çilesiz, 2025). However, as a dominant marker system, RAPD has certain limitations, including the inability to distinguish between heterozygous and homozygous individuals and relatively lower reproducibility compared to sequence-specific markers (Sevindik et al., 2024; Küçük et al., 2024). When compared with other marker systems, such as Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) markers, RAPD offers the advantage of rapid and cost-effective screening across a large number of genotypes without prior genomic knowledge (Ovalıoğlu and Çilesiz, 2025). ISSR markers, on the other hand, generally exhibit higher reproducibility and polymorphism levels due to longer primer lengths and higher annealing stringency, making them more robust for distinguishing closely related genotypes (Smulders et al., 2010; Çetin et al., 2025). Similarly, SCoT markers target the short conserved region flanking the ATG start codon in plant genes, which often results in high reproducibility and a strong association with functional genomic regions, providing insights not only into genetic diversity but also into potential trait-linked variation (Gülay et al., 2023; Çelik et al., 2024). In contrast, Simple Sequence Repeat (SSR) markers, which are co-dominant, allow for the detection of both alleles in heterozygous individuals and offer high reproducibility and cross-laboratory comparability; however, they require prior sequence information and can be more costly and time-consuming to develop (Baloch et al., 2022; Karaköy et al., 2024). Considering the trade-offs among these systems, RAPD remains a practical choice for preliminary diversity assessment, especially when resources are limited or rapid screening is needed. In the present study, a total of 18 sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) genotypes obtained from the USDA Agricultural Research Service (USDA) genebank were used. In this study, the selection of RAPD markers aimed to provide a broad and efficient evaluation of genetic

variation among sugar beet genotypes, serving as a foundation for identifying diverse parental lines and structuring future analyses with more specific or co-dominant markers. The main objective of this research is to investigate the genetic diversity and similarity levels among the 18 sugar beet genotypes, to gain insights into their genetic structure, and to provide a scientific basis for parent selection, genetic resource management, core collection development, and future breeding programs. The findings are expected to contribute to the improvement of existing varieties and the development of new, high-performing cultivars with enhanced stress tolerance and yield potential.

2. Material and Methods

2.1. Plant materials

In the study, 18 beet genotypes obtained from the USDA Agricultural Research Service (USDA) genebank were used as materials (Table 1).

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

Genotype code	Plant name	Registration number	Species name
SB-1	PI 590673	SP73514 01	*B. vulgaris* subsp. *vulgaris*
SB-2	PI 612765	A77 51	*B. vulgaris* subsp. *vulgaris*
SB-3	PI 590670	SP70682 01	*B. vulgaris* subsp. *vulgaris*
SB-4	PI 590837	FC 607	*B. vulgaris* subsp. *vulgaris*
SB-5	PI 590842	L53 CMS	*B. vulgaris* subsp. *vulgaris*
SB-6	NSL 28026	GARDENERS MODEL	*B. vulgaris* subsp. *vulgaris*
SB-7	NSL 31344	GIANT YELLOW ECKENDORF	*B. vulgaris* subsp. *vulgaris*
SB-8	NSL 43404	PARMA GLOBE	*B. vulgaris* subsp. *vulgaris*
SB-9	NSL 93280	A76 39	*B. vulgaris* subsp. *vulgaris*
SB-10	NSL 141985	JANASZ	*B. vulgaris* subsp. *vulgaris*
SB-11	NSL 176304	YUGO 2	*B. vulgaris* subsp. *vulgaris*
SB-12	NSL 195503	EL40 BREEDING LINE 24 and 31	*B. vulgaris* subsp. *vulgaris*
SB-13	PI 117117	No. 299	*B. vulgaris* subsp. *vulgaris*
SB-14	PI 120707	No. 3264	*B. vulgaris* subsp. *vulgaris*
SB-15	PI 140350	No. 5926	*B. vulgaris* subsp. *vulgaris*
SB-16	PI 140362	No. 7249	*B. vulgaris* subsp. *vulgaris*
SB-17	PI 140357	No. 6820	*B. vulgaris* subsp. *vulgaris*
SB-18	PI 144675	No. 8148	*B. vulgaris* subsp. *vulgaris*

2.2. Genomic DNA isolation

DNA extraction from the leaves of sugar beet samples was performed using the CTAB protocol as described by Doyle and Doyle (1990). 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.

2.3. RAPD marker assay

The RAPD primers selected for PCR amplification and the corresponding PCR cycling conditions are provided in 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. For electrophoresis of the PCR products, a 2% agarose gel prepared in Tris-borate-EDTA buffer was used. DNA bands were visualized under UV light. A total of 11 RAPD primers were initially screened across the samples and 6 primers exhibiting polymorphic patterns were selected for further analysis (Table 2). In the selected 6 primers, the number of polymorphic bands was high and the band intensity was at the desired level and clear. The other primers did not exhibit these characteristics.

Table 2. RAPD primers used for PCR amplification

RAPD primers code	Sequence (5'-3')	Tm˚C	PCR amplification
OPA-2	TGCCGAGCTG	32	94 ˚C - 3 min
OPA-3	AGTCAGCCAC	34	94 ˚C - 1 min
OPA-5	AGGGGTCTTG	34	32-34 ˚C 1 min
OPA-7	GAAACGGGTG	32	72 ˚C - 1 min
OPA-18	AGGTGACCGT	32	72 ˚C - 10 min
OPA-20	GTTGCGATCC	34	35 cycles

2.4. Statistical analysis

The bands obtained from the PCR analysis were scored as 1 for presence and 0 for absence. 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):

$$PIC = 2fi\ (1\text{-}fi) \quad (1)$$

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

In this study, RAPD markers were used to reveal the genetic relationships among 18 sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) genotypes. Of the 11 OPA primers initially screened, 6 with high levels of polymorphism were selected and analyzed across all genotypes. These 6 primers produced a total of 49 DNA bands, 40 of which were identified as polymorphic. The obtained polymorphism rate of 81.6% clearly demonstrates that the RAPD markers used are an effective tool for revealing genetic diversity in sugar beet. Although RAPD markers cannot distinguish between heterozygous and homozygous individuals due to their dominant nature, they are frequently preferred in the preliminary evaluation of genetic resources because of their low DNA requirements, rapid processing time, and cost-effectiveness. This situation indicates that the analyzed group of genotypes has a broad genetic base and carries richness in terms of potential breeding material. Table 3 demonstrates that RAPD markers effectively revealed genetic diversity among sugar beet genotypes. The highest polymorphism rates were observed in primers OPA-5 and OPA-7 (100%), indicating that all bands were polymorphic and provided maximum variation among the genotypes. The lowest polymorphism rate was detected in primer OPA-20 (63%), suggesting a relatively weak discriminatory power in terms of genetic diversity. In particular, primers OPA-2 (91%) and OPA-3 (78%) also exhibited high polymorphism rates, further supporting their efficiency in detecting genetic variation. The highest effective number of alleles (ne) was recorded in OPA-2 (1.53) and OPA-3 (1.50), reflecting a relatively broad allelic diversity. For genetic diversity (h) values, OPA-2 (0.30) and OPA-18 (0.28) stood out as the most informative primers. Regarding Shannon's diversity index (I), the highest values were observed in OPA-3 (0.45) and OPA-2 / OPA-18 (0.43), indicating a strong level of

information richness. In terms of Polymorphic Information Content, the highest values were found in OPA-18 (0.30) and OPA-2 / OPA-5 (0.29), while the lowest PIC value was observed in OPA-3 (0.20), suggesting weaker discriminatory ability (Table 3).

Table 3. 6 primers used for RAPD assay, number of total and polymorphic bands and diversity parameters

RAPD primers code	Number of bands		Diversity parameter				
	Polymorphic bands	Total bands	P%	ne	h	I	PIC
OPA-2	10	11	91	1.53	0.30	0.43	0.29
OPA-3	7	9	78	1.50	0.29	0.45	0.20
OPA-5	5	5	100	1.29	0.22	0.37	0.29
OPA-7	6	6	100	1.38	0.22	0.33	0.22
OPA-18	7	10	70	1.49	0.28	0.43	0.30
OPA-20	5	8	63	1.34	0.20	0.31	0.22

There are many studies in the literature conducted using SCoT markers. The average polymorphism rate of SCoT markers was determined as 69.74% by Sönmez and Çilesiz (2025), 67.3% by Hromadová et al. (2022), 83.43% by Temrel and Çilesiz (2025), 93.99% by Nosair (2016), and 49.11% by Rayan and Osman (2019). Sönmez and Çilesiz (2025), obtained a total of 119 bands from 12 SCoT primers in their study on grapes. Among these, 83 were polymorphic bands, 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 found between genotypes G37 and G39. Temrel and Çilesiz (2025) obtained a total of 169 bands from 14 SCoT primers in thyme. Among these, 141 were polymorphic bands, 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 genetic similarity (0.326) was observed between samples G12 and G17, while the highest similarity (0.854) was found between G8 and G10. Peng et al. (2024), in their study on sugar beet, showed that the polymorphism information content (PIC) ranged from 0.419 to 0.773 (mean = 0.610). In addition, the mean number of effective alleles detected by SSR and InDel markers was 3.054 and 2.298, respectively. Meanwhile, the PIC values ranged from 0.130 to 0.602 (mean = 0.462). Nagl et al. (2011), using 8 RAPD primers, obtained a total of 44 bands, with an average number of bands per primer of 6.13. The number of polymorphic loci was 44, which constituted 75.86% of all scored loci. Nei's genetic diversity index was found to be 0.252. Ghasemi et al. (2014), in their study, determined the genetic diversity of thirty sugar beet genotypes using RAPD markers. The polymorphism of all primers was found to be within similarity limits at 82.33%. The cophenetic correlation coefficient for the similarity matrix and the obtained curve was calculated as r = 0.75. With a similarity coefficient of 0.91, Genotypes 4 and 18 showed the highest similarity, while with a similarity coefficient of 0.63, Genotypes 21 and 15 showed the lowest similarity. Among the primers used, primer OPB-18 produced 12 bands (the highest number of bands), while primer OPA-09 produced 5 bands (the lowest number of bands). Çilesiz (2025), assessed the genetic diversity of 18 walnut genotypes using ISSR and RAPD markers. Polymorphic bands were scored to calculate Jaccard similarity coefficients. Genetic relationships were analyzed using the UPGMA dendrogram and Principal Component Analysis (PCA). A total of 72 bands (54 polymorphic; 75.0%) were generated with nine ISSR primers, and 53 bands (42 polymorphic; 79.2%) were generated with five RAPD primers. Both marker systems indicated moderate genetic diversity within the genotypes. In the dendrogram constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm based on the Jaccard similarity coefficient, the genotypes were divided into two main groups (Figure 1).

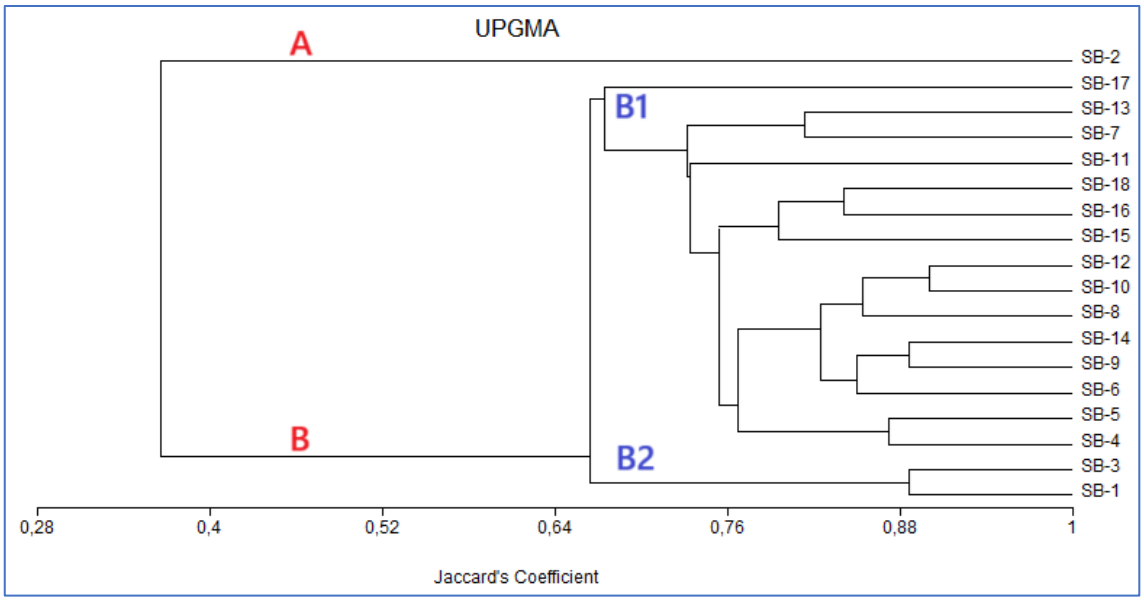

Figure 1. UPGMA Dendrogram of Genetic Similarity Between RAPD Markers and 18 Sugar Beet Genotypes

Group A consists only of the SB-2 genotype, indicating that this genotype shows significant genetic differentiation from all other genotypes. This suggests that SB-2 carries rare or low-frequency alleles and demonstrates that it could be an important parental candidate in breeding programs aimed at increasing genetic variation through hybridization (Figure 1). The other genotypes, however, were clustered under Group B, which was further divided into subgroups B1 and B2. Subgroup B1 contained 15 genotypes (SB-4, SB-5, SB-6, SB-7, SB-8, SB-9, SB-10, SB-11, SB-12, SB-13, SB-14, SB-15, SB-16, SB-17, and SB-18), and the high genetic similarity among these genotypes suggests that they may share a common genetic origin or have been subjected to similar selection pressures. Group B2, on the other hand, included 2 genotypes, SB-1 and SB-3. In particular, the similarity rate of 0.900 observed between SB-10 and SB-12 revealed that these two genotypes have a very close genetic structure. Similarly, high levels of similarity were also observed between SB-1 and SB-3 (0.886), SB-4 and SB-5 (0.872), and SB-9 and SB-14 (0.886) (Table 4). The lowest similarity rate was found between SB-2 and SB-14 (0.366), indicating the maximum genetic distance between these two genotypes. Such genetically distant genotypes can be preferred in the creation of heterotic hybrid combinations, allowing the maximization of heterosis (hybrid vigor) in F_1 hybrids. Especially in species such as sugar beet, where heterozygosity is important, the identification of such distant genetic relationships forms the basis of breeding strategies. Ghasemi et al. (2014) stated that the cluster analysis confirmed the correlations obtained from the Jaccard similarity coefficient as well as the profiles obtained in the genetic differentiation of the examined varieties. According to the obtained results and the resulting dendrogram, they were divided into 13 groups. The results of the cluster analysis performed using the Jaccard similarity coefficient revealed the genetic diversity among the genotypes and emphasized the effectiveness of selection in sugar beet genotypes (Ghasemi et al., 2014).

Table 4. Pairwise genetic distance matrix obtained from six RAPD primers

Group 1	Group 2	Similarity	Subject in group
SB-10	SB-12	0.900	2
SB-9	SB-14	0.886	2
SB-1	SB-3	0.886	2
SB-4	SB-5	0.872	2
SB-8	Node 1	0.854	3
SB-6	Node 2	0.850	3
SB-16	SB-18	0.841	2
Node 6	Node 5	0.825	6
SB-7	SB-13	0.814	2
SB-15	Node 7	0.796	3
Node 4	Node 8	0.768	8
Node 11	Node 10	0.755	11
Node 12	SB-11	0.734	12
Node 13	Node 9	0.732	14
Node 14	SB-17	0.675	15
Node 3	Node 15	0.664	17
Node 16	SB-2	0.366	18

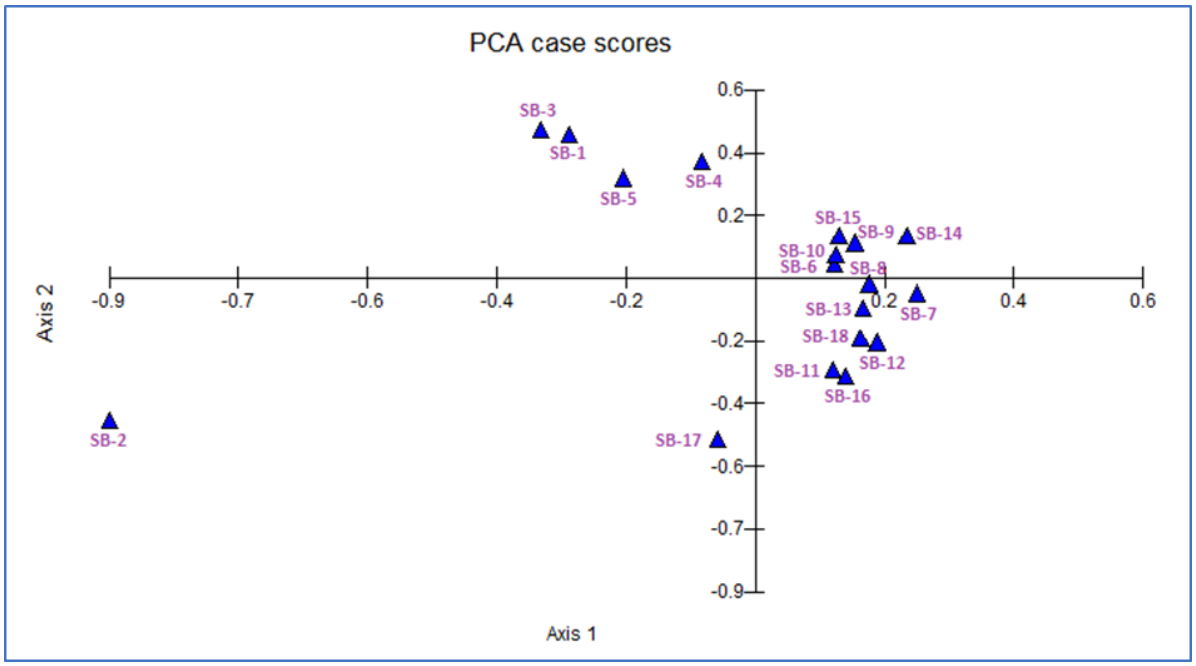

Figure 2. According to RAPD Markers Pricipal Component Analyses of 18 Sugar Beet Genotypes Using MVSP 3.22 Software (Kovach Computing Services, Pentraeth, Wales, UK)

In order to provide a more detailed visualization of genetic relationships, Principal Component Analysis (PCA) was applied based on RAPD data. In the two-dimensional plot obtained from the PCA, it was observed that genetically similar genotypes were positioned close to each other, while different genotypes were located farther apart. For example, SB-10 and SB-12, which were the most similar genotypes in the UPGMA analysis, were also positioned close to each other in the PCA plot, whereas genetically distant genotypes such as SB-2 and SB-14 were located at quite distant positions along the PCA axes. This indicates a high correlation between UPGMA and PCA analyses and confirms that the RAPD markers used accurately reflect the genetic structure. The fact that the first three components in the PCA explained a significant portion of the total variation shows that genetic diversity can largely be represented by these axes. The scattered distribution of the genotypes across the PCA plane reveals that the material possesses high genetic heterogeneity and

originates from different sources. This reflects the effects of both natural selection and human-mediated selection. In this context, the obtained findings serve as an important reference for the establishment of core collections in sugar beet, the identification of adaptive traits, and the planning of modern breeding approaches such as marker-assisted selection (MAS) and genomic selection (GS). In particular, genetically distant individuals such as SB-2 are considered to have the potential to carry resistance genes against biotic and abiotic stresses, and the use of such genotypes is recommended in advanced variety development studies.

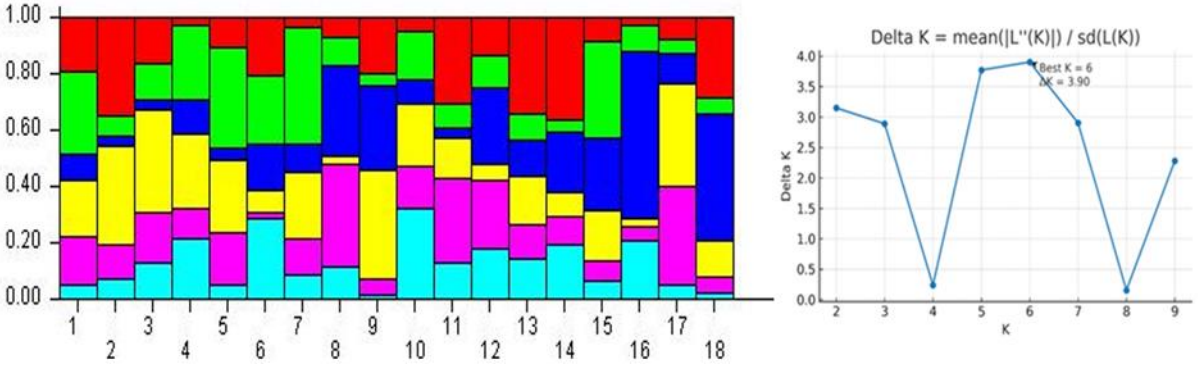

Figure 3. Q-matrix bar plot showing the population structure of 18 sugar beet genotypes (K = 6) based on STRUCTURE software

The results shown in Figure 3 were obtained as a result of the STRUCTURE analysis. According to the ΔK graph generated using the Evanno et al. (2005) method, the sugar beet samples were divided into six populations (K=6). In other words, the maximum ΔK value was obtained at K=6, and it was concluded that the structure of the samples was best explained by six subpopulations. According to the Q-matrix bar plot, all genotypes exhibit multiple colors, indicating genetic contributions from different populations. This indicates the presence of both pure lines and mixed individuals among the genotypes. While colors are almost equally distributed in some genotypes, in others, a single dominant color indicates stronger affiliation to a particular population. The ΔK analysis showed the highest value at K=6, confirming that genetic diversity is best explained by dividing it into six gene pools. Consequently, sugar beet genotypes have a complex population structure. Peng et al. (2024), in their study on sugar beet, reported that population structure analysis revealed the optimal K value to be three populations (K=3). Genetic distances among 129 genetic sources ranged from 0.099 to 0.466 (mean = 0.283), and cluster analysis results revealed that the genetic materials were divided into three main groups.

4. Conclusion

This study revealed that RAPD markers are an effective, reliable, and cost-efficient molecular tool for determining genetic diversity among sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) genotypes. As a result of applying 6 RAPD primers to a total of 18 genotypes, a polymorphism rate of 81.6% was obtained, demonstrating that the studied material contains a significant level of allelic diversity. This diversity is of critical importance for long-term plant breeding and the conservation of genetic resources, especially today, when the narrowing of the genetic base poses a major threat to modern breeding programs. In the study, UPGMA cluster analysis and Principal Component Analysis (PCA) were used together, and the genetic relationships among the genotypes were evaluated in a multidimensional manner. A high degree of consistency was observed between the two analytical methods, with genetically similar genotypes grouped into clusters, while highly divergent genotypes were clearly separated both in the dendrogram and in the PCA plane. This confirms that the

applied methodology allows for the accurate determination of genetic distances and population structure. The identification of genetically distant genotypes such as SB-2 and SB-14 offers important opportunities for the evaluation of heterotic combinations in future breeding studies. In contrast, genotype pairs such as SB-10 and SB-12, which showed high genetic similarity, can be considered as candidates for use in phenotypic stability, varietal purity, and registration processes. In conclusion, this study comprehensively revealed the existing genetic variation in sugar beet and demonstrated that RAPD markers can be used as a highly reproducible, genome-wide, and low-cost tool in germplasm characterization. The genetic information obtained provides direct contributions to decision-making in breeding processes such as parent selection, population design, core collection development, and conservation strategies. These findings form a scientific basis for the development of more efficient, environmentally stress-tolerant, and sustainable production-adapted varieties in future sugar beet breeding programs.

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