

RAPD Based Genetic Diversity in *Glycine max* L.Yeter ÇİLESİZ ^{1*}, Tolga KARAKÖY ¹¹ Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technology, Department of Field Crops, Sivas*Corresponding author: ycilesiz@sivas.edu.tr**Abstract**

This study aims to determine the genetic diversity levels of a total of 18 soybean genotypes consisting of 15 soybean (*Glycine max* (L.) Merr.) lines obtained as a result of hybridizations carried out in accordance with breeding objectives within the scope of soybean breeding studies conducted at the Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, and 3 commercial cultivars (SA-88, Ataem-7, Traksoy), using RAPD markers. In the study, a total of 35 DNA bands were obtained using three different RAPD primers (OPA-5, OPA-7, and OPA-20), and 17 of these bands were identified as polymorphic. This corresponds to a polymorphism rate of 48.5%, indicating that there is a significant level of variation in the genetic structure of the material. The UPGMA dendrogram constructed based on the Jaccard similarity coefficient grouped the genotypes into two main clusters and showed that certain genotypes (S-3, S-4, S-13, S-15, and S-16) were genetically differentiated from the others. The highest genetic similarity value was detected between genotypes S-2 and S-5 (0.933), while the lowest similarity value was observed between genotypes S-16 and S-17 (0.723). These findings indicate that genetically close genotypes can be utilized in studies aimed at developing stable lines and maintaining genetic purity, whereas genetically distant individuals can be used in the formation of heterotic hybrid combinations. The Principal Component Analysis (PCA) enabled the evaluation of genetic relationships in a multidimensional plane and exhibited a high level of consistency with the UPGMA results. The wide distribution of genotypes across the PCA plane indicates that the studied material possesses rich genetic diversity and reveals the presence of subpopulation structures. Consequently, RAPD markers have been shown to be an effective tool for determining genetic diversity in soybean due to their low cost, reproducibility, and the fact that they do not require prior genomic information. The molecular data obtained from this study are considered to provide a scientific basis for parent selection, management of genetic resources, the establishment of core collections, and the development of advanced breeding strategies.

Keywords: RAPD, UPGMA, PCA, soybean

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is an important cultivated plant widely used in both the food industry and the livestock sector due to its high biological value in terms of protein and oil composition (Nyugen et al., 2025). In line with the increasing global population and the growing demand for sustainable agricultural practices, the importance of soybean production is steadily increasing worldwide (Sözen and Karadavut, 2017; Çilesiz, 2025a). In addition, due to its role in biofuel production, its use as an industrial raw material, and its capacity to improve soil nitrogen balance, this species has become one of the strategic components of integrated agricultural systems. Although soybean cultivation in Türkiye is limited, its future potential is gaining increasing importance within the scope of diversification of production systems, utilization of fallow lands, and adaptation strategies against climate change (Sözen et al., 2017; Shaibu et al., 2021; Assfaw et al., 2025). However, the genetic base of currently cultivated soybean genotypes is quite narrow, and this situation negatively affects production sustainability in the face of biotic stress factors, environmental variability, and adaptation difficulties. Therefore, the more effective utilization of existing genetic material, the objective determination of differences among genotypes, and the enhancement of genetic diversity in breeding programs are of great importance (Naflath et al., 2022; Sözen, 2023; Silue et al., 2025). In this context, molecular marker techniques have emerged as an important tool in plant breeding. Traditional morphological characterization methods are susceptible to environmental influences and therefore cannot provide precise results at the genetic level. In contrast, DNA-based molecular markers allow genetic variation to be analyzed more accurately, rapidly, and reproducibly (Rani et al., 2023; Çilesiz, 2025b). One of these methods, RAPD (Random Amplified Polymorphic DNA) markers, is widely used in genetic diversity studies because they do not require prior information about the genetic material, are easy to apply, and reveal a high level of

polymorphism (Potapova et al., 2023). RAPD markers enable the rapid and efficient detection of genetic variation by amplifying different regions of the genome using short and randomly sequenced nucleotide primers. Especially in plant species where genetic mapping studies are still limited or where genomic information has not yet been fully elucidated, the RAPD technique provides significant advantages (Zatybekov et al., 2023). This marker system is frequently preferred in the preliminary evaluation of large germplasm collections due to its low cost, rapid results, and the fact that it does not require prior sequence information. Moreover, RAPD analyses have proven to be effective in many applications such as determining genetic similarity and distance relationships, examining population structure, identifying cultivars, and selecting parental material in breeding programs. Owing to these characteristics, RAPD markers are considered a practical and reliable method, particularly in preliminary genetic diversity analyses. However, molecular marker technologies are not limited only to RAPD, and different marker systems are also widely used in plant genetics studies. For example, ISSR markers target microsatellite regions and can provide a high level of polymorphism, while SSR markers allow a more detailed detection of allelic variation due to their codominant structure (Chiemeké et al., 2024; Çilesiz, 2025c). Advanced marker systems such as AFLP and SNP also contribute to more precise genetic analyses by providing high-resolution genome-wide data. Nevertheless, when factors such as cost, technical infrastructure, and analysis time are considered, RAPD markers remain an important, rapid, and economical alternative, especially in the initial stages of genetic diversity studies. Therefore, different molecular marker systems are considered complementary to each other and make significant contributions to the accurate identification of genetic diversity in plant breeding programs (Chander et al., 2021; Andrijanić et al., 2023; Çilesiz, 2025d). The aim of this study is to determine the genetic similarity and diversity levels of 18 genotypes

using RAPD markers. The findings to be obtained are valuable both for the conservation and management of genetic resources and for contributing to parent selection and population development processes in breeding programs.

2. Material and Methods

2.1. Plant materials

In this study, within the scope of soybean breeding studies carried out at the Faculty of

Agricultural Sciences and Technology of Sivas University of Science and Technology, a total of 18 soybean genotypes were used as material, consisting of 15 soybean (*Glycine max* (L.) Merr.) lines obtained as a result of hybridizations performed in accordance with breeding objectives and 3 commercial cultivars (SA-88, Ataem-7, Traksoy) (Table 1).

Table 1. Information on the study plant materials

Genotype code	Name	Species name
G1	DT	<i>Glycine max</i> (L.) Merr.
G2	Line-3	<i>Glycine max</i> (L.) Merr.
G3	Line-10	<i>Glycine max</i> (L.) Merr.
G4	TK-13	<i>Glycine max</i> (L.) Merr.
G5	Line-42	<i>Glycine max</i> (L.) Merr.
G6	TK-22	<i>Glycine max</i> (L.) Merr.
G7	Line-60	<i>Glycine max</i> (L.) Merr.
G8	TK-30	<i>Glycine max</i> (L.) Merr.
G9	Line-66	<i>Glycine max</i> (L.) Merr.
G10	TK-31	<i>Glycine max</i> (L.) Merr.
G11	Line-83	<i>Glycine max</i> (L.) Merr.
G12	TK-34	<i>Glycine max</i> (L.) Merr.
G13	Line-103	<i>Glycine max</i> (L.) Merr.
G14	Line-118	<i>Glycine max</i> (L.) Merr.
G15	Line-132	<i>Glycine max</i> (L.) Merr.
G16	SA-88	Commercial Variety
G17	Ataem-7	Commercial Variety
G18	Traksoy	Commercial Variety

2.2. Genomic DNA isolation and PCR

Within the scope of the soybean (*Glycine max* L.) breeding program carried out under the ecological conditions of Sivas, registered cultivars such as Arisoy, Blaze, Nova, May 5312, Bravo, Adasoy, Lider, Mersoy, and Viktoria were used as parental material. Considering the morphological, phenological and agronomic characteristics of these cultivars, different hybrid combinations were developed to combine the targeted traits, including earliness, high yield, wide adaptability, and tolerance/resistance to diseases and abiotic stress. DNA extraction from soybean leaf 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^{-1} . 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 dH₂O. 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 18 ISSR primers were initially screened across the samples and 3 primers exhibiting polymorphic patterns

were selected for further analysis (Table 2). Among the selected 3 primers, the number of polymorphic bands was high, and 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	DNA sequences (5'-3')	Tm °C	PCR amplification
OPA-5	5'-AGGGGTCTTG-3'	32	94 °C - 3 min 94 °C - 1 min 32 °C 1 min
OPA-7	5'-GAAACGGGTG-3'	32	72 °C - 1 min 72 °C - 10 min
OPA-20	5'-GTTGCGATCC-3'	32	35 cycles

2.3. Statistical analysis

After the completion of PCR analyses, the resulting banding patterns were evaluated, and each band was scored as “1” if present and “0” if absent. Using this binary data set, genetic similarity among genotypes was calculated using Jaccard's similarity coefficient. A dendrogram illustrating the genetic relationships was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on the calculated similarity coefficients. These analyses were performed using the MVSP 3.22 software (Kovach, 2007). The application of ISSR molecular data to the MVSP 3.22 software enabled the generation of a genetic similarity matrix. In addition, to reveal the genetic differences among the varieties and genotypes in more detail, Principal Component Analysis (PCA) was also conducted using the same software.

3. Results and Discussion

A total of 35 bands were obtained using the three RAPD primers (OPA-5, OPA-7, and OPA-20) evaluated in the study, of which 17 were identified as polymorphic. This result corresponds to a polymorphism level of 48.5%, indicating that RAPD markers have sufficient discriminative power to reveal variation among the genotypes. The obtained band data were evaluated through a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram based on the Jaccard similarity coefficient. The UPGMA analysis showed that the genotypes were grouped into two main clusters. Genotypes coded as S-3, S-4, S-13, S-15, and S-16 were

included in Group A, while the remaining 13 genotypes were clustered in Group B. This separation indicates that the genotypes in Group A possess distinct genetic differences and may potentially carry rare alleles (Figure 1). Pairwise similarity analysis revealed the highest genetic similarity between genotypes S-2 and S-5 (0.933), whereas the lowest similarity was observed between S-16 and S-17 (0.723) (Table 3). This finding suggests that S-2 and S-5 likely share a similar selection history or originated from the same genetic source, while S-16 and S-17 derive from genetically distinct backgrounds. These differences are particularly important for identifying parental combinations with high heterosis potential. Moreover, the node structure in the dendrogram shows the formation of subgroups and indicates varying levels of genetic similarity within these subclusters. This demonstrates that the examined genotypes possess both a wide range of genetic variation and high genetic similarity within certain groups. Specifically, genetically similar individuals (S-6 and S-7) may be utilized in pure line development studies, while genetically distant individuals (S-13 and S-16) can be employed for hybrid combinations. A node, in hierarchical clustering, represents an intermediate merging point in the dendrogram formed when two groups (individuals or previously merged subgroups) are combined at a certain similarity level. These nodes indicate that previously merged genotypes or groups have been further combined with other individuals or nodes at a specific similarity threshold.

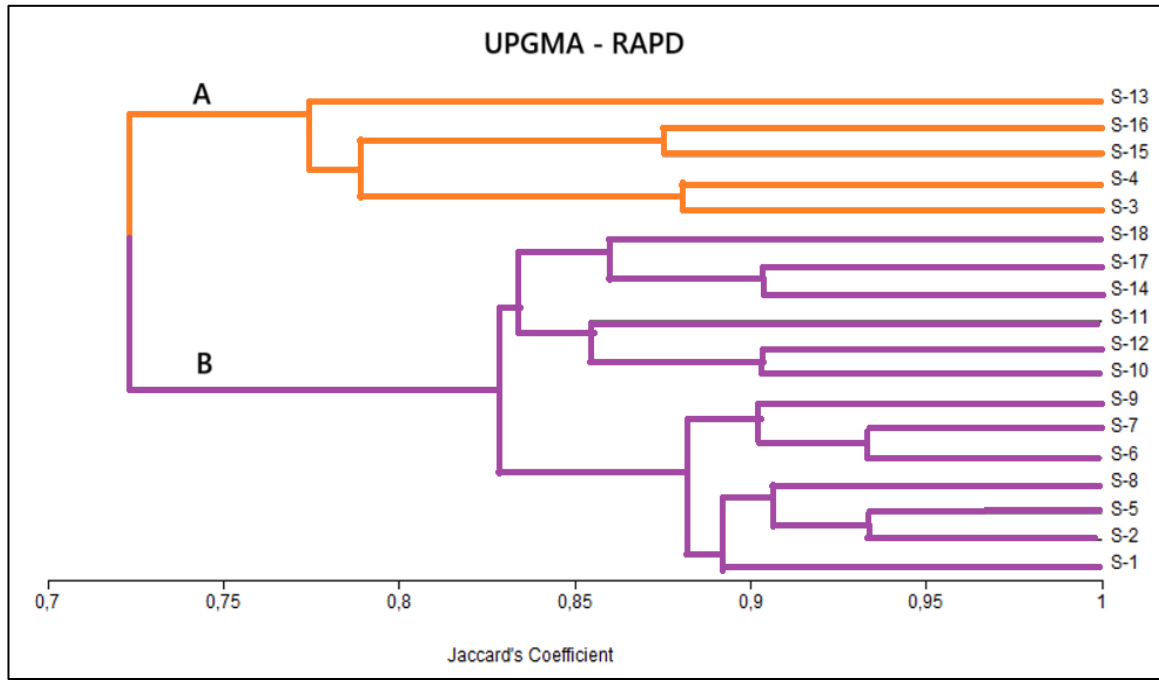


Figure 1. UPGMA dendrogram of genetic similarity between RAPD markers and 18 soybean genotypes

Table 3. Pairwise genetic distance matrix obtained from three RAPD primers

Group 1	Group 2	Similarity	Objects in group
2	5	0.933	2
6	7	0.933	2
Node 1	8	0.906	3
10	12	0.903	2
14	17	0.903	2
Node 2	9	0.902	3
1	Node 3	0.892	4
Node 7	Node 6	0.882	7
3	4	0.880	2
15	16	0.875	2
Node 5	18	0.859	3
Node 4	11	0.855	3
Node 12	Node 11	0.834	6
Node 8	Node 13	0.828	13
Node 9	Node 10	0.789	4
Node 15	13	0.774	5
Node 14	Node 16	0.723	18

In a study conducted by Vivodík et al. (2022) on 28 soybean genotypes, a total of 108 DNA bands were obtained using 13 RAPD primers, and 70 of these were identified as polymorphic. In this study, the average polymorphism rate was reported as 63.68%. The researchers emphasized that RAPD markers are a reliable and effective method for determining genetic diversity among soybean genotypes. These values indicate that the polymorphism rate obtained in the present

study reflects a moderate level of genetic diversity. In the study by Malik et al. (2016), 92 soybean genotypes from different origins were analyzed using RAPD markers, and the polymorphism rate was reported to range from 46.7% to 70.5%. These results show that the 48.5% polymorphism rate obtained in the present study is consistent with the values reported in the literature (Malik et al., 2016). In a more recent study, Mathpal et al. (2024) analyzed 18 soybean germplasm using nine

RAPD primers and obtained a total of 59 bands. The majority of these bands were polymorphic, and the average polymorphism rate was reported as 70.3%. The researchers also stated that in the UPGMA clustering analysis based on RAPD data, the genotypes formed distinct groups and genetic diversity was clearly revealed. These findings support the high discriminative power of RAPD markers in revealing genetic variation. As a result of UPGMA clustering analysis, the genotypes in this study were grouped under two main clusters. Similarly, many studies have reported that dendrogram analyses based on RAPD data separate genotypes into distinct groups. For example, in the study by Vivodík et al. (2022), 28 soybean genotypes were clustered into four main groups. The researchers stated that this clustering was associated with the genotypes' genetic origins and cultivar characteristics. This indicates that molecular markers are effective in revealing kinship relationships among soybean genotypes. In a RAPD study conducted by Wahyudi et al. (2020), mutagenized Grobogan soybean genotypes were analyzed with 20 OPA primers, and polymorphism rates for certain primers ranged from 33.3% to 100%, which further supports the capacity of RAPD markers to reveal genetic differences (Wahyudi et al., 2020). This study demonstrates that RAPD analyses are effective in detecting genetic variation even in different applications. In ISSR and RAPD analyses conducted on walnut genotypes from the Central Anatolia region, a total polymorphism of 77.9% was detected, and a high level of agreement was reported between the PCA results and UPGMA dendrogram structures (Çilesiz, 2025e). Similarly, in ISSR and RAPD analyses conducted on grape genotypes from the same region, genetic relationships among genotypes were revealed with similarity values ranging from 0.22 to 0.87 in the dendrogram, and these results were consistent with PCA findings (Çilesiz, 2025f). These numerical findings demonstrate that molecular markers not only reveal polymorphism rates but also, when supported by multivariate analyses such as PCA and hierarchical clustering, reliably

identify genetic relationships among genotypes. This parallels the 48.5% polymorphism and PCA–UPGMA consistency detected in soybean genotypes in the present study and supports the notion that molecular markers in different species are reliable tools for revealing genetic diversity and genetic relationships through multivariate analyses. The node structures and subclusters observed in dendrogram analysis indicate varying levels of genetic similarity among the examined genotypes. In most studies using RAPD data, genotypes form distinct subgroups, and these groups reflect genetic origin differences. Similarly, in the study by Mathpal et al. (2024), RAPD-based clustering analysis grouped soybean germplasm into different genetic clusters, and this information was reported to be important for parental selection in breeding programs. Overall, the polymorphism rate, genetic similarity coefficients, and UPGMA clustering results obtained in this study are largely consistent with RAPD-based soybean genetic diversity studies reported in the recent literature. In particular, the identification of genetically distant individuals offers significant potential for creating new hybrid combinations and broadening the genetic base. Therefore, RAPD markers can be considered an effective and economical method for evaluating genetic diversity and selecting appropriate parental material in soybean breeding programs. The PCA (Principal Component Analysis) evaluation allowed the genetic differences to be observed more clearly in a multidimensional plane. In the PCA plot, genetically similar genotypes clustered at similar coordinates, whereas genetically distant genotypes were positioned in more separate regions. This indicates a strong consistency between the UPGMA dendrogram and the PCA results. The first few components of the PCA explained a large portion of the total variation, showing that the main genetic differences among the genotypes were concentrated along these axes. The wide dispersion of genotypes in this analysis indicates that the material used in the study is rich in genetic diversity and suggests the presence of subpopulation structures. In

conclusion, this study demonstrated that RAPD markers are a reliable and effective molecular tool for determining genetic diversity among soybean genotypes. The identification of genetically distant and closely related genotypes will provide important contributions both for the determination of breeding strategies and for planning the management of genetic resources. Furthermore, the placement of commercial

cultivars in different genetic clusters indicates that these cultivars can serve as complementary genetic resources in future hybridization programs. In recent studies, high consistency between PCA and UPGMA dendrogram analyses has been reported across various plant species, demonstrating the reliability of combining multivariate methods with molecular markers to reveal genetic relationships.

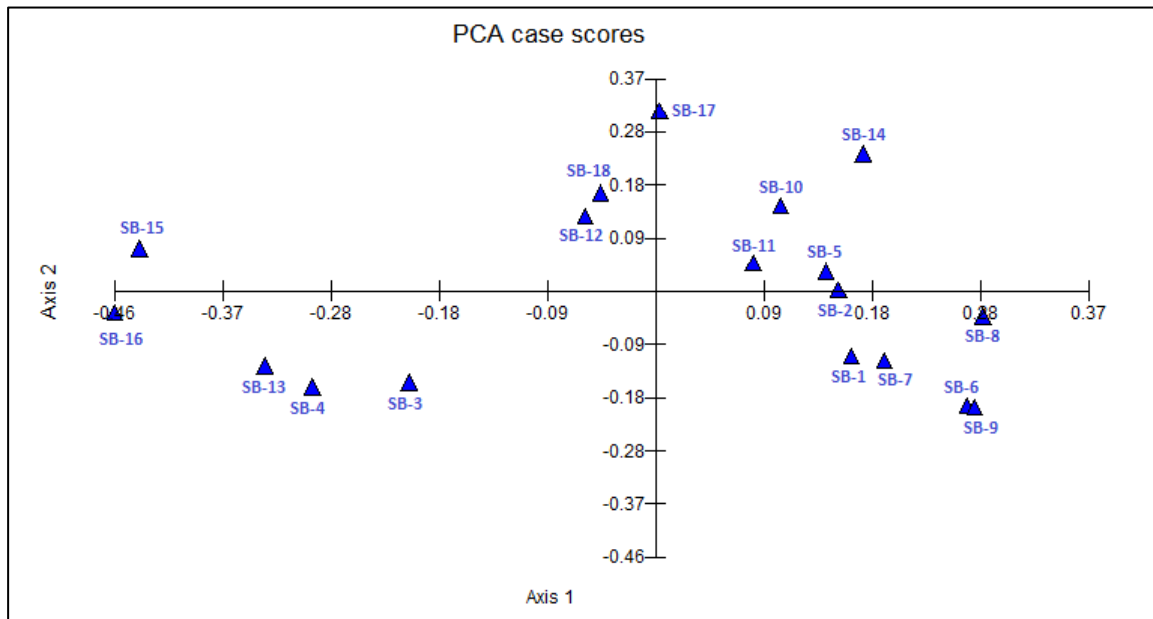


Figure 6. According to RAPD markers principal component analyses of 18 soybean populations using MVSP 3.22 software (Kovach Computing Services, Pentraeth, Wales, UK)

For instance, Ovalıoğlu and Çilesiz (2025) observed a high level of genetic polymorphism (77.9%) among walnut (*Juglans regia* L.) genotypes using SCoT markers, with strong agreement between PCA and dendrogram results. Similarly, Sönmez and Çilesiz (2025) reported that SCoT analysis of grape (*Vitis vinifera* L.) genotypes produced a dendrogram consistent with PCA, reflecting variable similarity among genotypes. Temrel and Çilesiz (2025) also noted high concordance between PCA and dendrogram analyses in their SCoT study of thyme (*Thymus vulgaris* L.) genotypes, further highlighting the robustness of these multivariate approaches. In addition, Yücel et al. (2026) demonstrated that PCA analyses of sugar beet genotypes effectively identified both homogeneous groups and distinctive genotypes, in agreement

with cluster structures observed in the dendrogram. Collectively, these findings indicate that multivariate analyses such as PCA and hierarchical clustering are complementary and reliable tools for visualizing genetic relationships across diverse crop species.

4. Conclusion

This study demonstrated that RAPD markers are an effective and reliable molecular tool for assessing genetic diversity among soybean (*Glycine max* (L.) Merr.) genotypes. In analyses conducted on 18 genotypes, a total of 35 DNA bands were obtained using three different RAPD primers, of which 17 were identified as polymorphic. This 48.5% polymorphism rate indicates that the material under study contains significant genetic

variation. Consequently, RAPD markers have been shown to be an effective method for determining the diversity of soybean genetic resources due to their cost-effectiveness, reproducibility, and broad applicability. The data obtained in this study will provide valuable contributions to parental selection, core collection development, heterotic group identification, and sustainable breeding strategies. In particular, it is considered to establish a solid scientific foundation for studies aimed at improving productivity, enhancing stress tolerance, and broadening the genetic base.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Ethical Committee Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

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