

Efficiency of Using ISSR Markers in the Evaluation of Genetic Diversity in Soybean

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Abstract

This study aimed to evaluate the genetic diversity of soybean genotypes using ISSR molecular markers within the scope of soybean breeding studies conducted at the Faculty of Agricultural Sciences and Technology, Sivas University of Science and Technology. A total of 18 soybean (*Glycine max* (L.) Merr.) genotypes, including 13 soybean lines obtained through hybridizations performed in accordance with breeding objectives and 5 commercial cultivars, were analyzed. Using seven ISSR primers, a total of 78 DNA bands were obtained, of which 32 were identified as polymorphic, corresponding to a polymorphism rate of 41.02%. These findings revealed moderate genetic variation among genotypes and confirmed the applicability of ISSR markers for the analysis of soybean genetic resources. According to the UPGMA clustering analysis, the genotypes were divided into two main groups; genotypes S-6 and S-7 exhibited the highest genetic similarity (98.4%), whereas genotypes S-3 and S-13 showed the lowest similarity (79.3%). Principal Component Analysis (PCA) supported the genetic structure obtained from UPGMA and enabled the multidimensional visualization of relationships among the genotypes. The distribution of commercial cultivars in different clusters indicated that these cultivars possess distinct genetic backgrounds. The results demonstrate that ISSR markers are effective, reproducible, and cost-efficient molecular tools for distinguishing genetic similarities and differences. This study provides important scientific data for parent selection in soybean breeding programs, identification of heterotic combinations, and sustainable management of genetic resources.

Keywords: ISSR markers, soybean, genetic diversity, molecular markers

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1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a strategic legume crop widely cultivated worldwide, owing to its high protein and oil content, which makes it an important component of both human nutrition and animal feed (Kumar et al., 2022). In addition, this species is utilized in biodiesel production and various industrial applications and plays a significant role in sustainable agricultural systems by enhancing soil fertility through its ability to fix atmospheric nitrogen (Fetisov et al., 2025). Although soybean production in Türkiye is relatively limited, it is increasingly being considered as an alternative crop due to factors such as climate change, the need for crop diversification, and the reduction of fallow lands, thereby gaining greater importance within the agricultural production system. Modern soybean production faces several challenges, including narrow genetic variation, susceptibility to diseases and pests, and various climatic stress factors (Valliyodan and Ye, 2017). This situation necessitates the effective utilization of existing genetic resources, the accurate identification of genetic diversity, and the development of new varieties with a broad genetic base. Environmental conditions strongly influence traditional phenotypic evaluation methods, complicating objective analysis of genetic diversity. Therefore, the need for rapid, reproducible, and cost effective molecular marker systems that are independent of environmental effects has steadily increased in modern plant breeding programs (Zatybekov et al., 2025; Çilesiz, 2025a). DNA-based molecular markers are considered essential tools in modern plant genetics and breeding studies for identifying genetic resources, determining variation among genotypes, and characterizing breeding materials (Khan, 2026). At the same time environmental factors may influence classical characterization methods based on morphological and physiological traits, but DNA-based markers directly reflect the genetic structure and therefore provide more reliable results independent of environmental influences (Costa et al., 2016; Çilesiz, 2025b). For this reason, molecular markers are widely

used in many research areas, including the determination of genetic diversity, the management of germplasm collections, the elucidation of genetic relationships among genotypes, and the selection of suitable parents. Among molecular marker systems, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers are widely used in genetic diversity studies due to their ease of application and the fact that they do not require prior genomic information. RAPD markers are based on the amplification of genomic regions using short, random primers, whereas ISSR markers offer significant advantages for determining genetic relationships, analyzing population structure, and characterizing breeding materials, owing to their high reproducibility and broad genome coverage (Basak and Imamoglu, 2023; Çilesiz, 2025c). ISSR markers are a powerful tool in plant genetic diversity studies due to their ability to produce high levels of polymorphism by targeting microsatellite regions distributed throughout the genome. However, studies on the use of ISSR markers in soybean breeding materials are limited; therefore, there is a need for research that comprehensively reveals the existing genetic variation. This situation indicates an important research gap for more clearly evaluating the potential of ISSR markers in soybean breeding. With advances in molecular genetics, more advanced marker systems such as Simple Sequence Repeats (SSR), Single Nucleotide Polymorphisms (SNP), and Amplified Fragment Length Polymorphism (AFLP) have also become widely used in the determination of genetic diversity (Ramesh et al., 2020; Çilesiz, 2025d). SSR markers offer significant advantages in genetic mapping and population genetics studies due to their high polymorphism levels and codominant inheritance. SNP markers reveal single nucleotide variations in the genome and enable high-throughput genotyping. AFLP markers, on the other hand, allow the detection of a large number of polymorphic bands through the combined use of restriction enzymes and PCR techniques and have been effectively used in genetic diversity

analyses and cultivar identification studies. These molecular marker systems serve as important scientific tools for evaluating plant genetic resources and improving the efficiency of breeding programs (López Cruz and Díaz Rivera, 2022; Bidyananda, 2024; Çilesiz 2025e). In this study, within the scope of soybean breeding studies conducted at the Faculty of Agricultural Sciences and Technology, Sivas University of Science and Technology, a total of 18 soybean genotypes, including 13 soybean lines obtained through hybridizations carried out in accordance with breeding objectives and 5 commercial cultivars, were evaluated in terms of genetic diversity using ISSR markers. The main objective of the research was to quantify genetic relationships among these genotypes, determine the level of variation between existing cultivars and newly developed lines,

and provide a scientific basis for sustainable soybean breeding programs. The data obtained are expected to contribute significantly to genetic resource management, parent selection, the establishment of core collections, and advanced breeding applications.

2. Material and Methods

In this study, within the scope of soybean breeding programs conducted at the Faculty of Agricultural Sciences and Technology, Sivas University of Science and Technology, a total of 18 soybean genotypes were used as plant material, including 13 soybean (*Glycine max* (L.) Merr.) lines obtained through hybridizations carried out in accordance with breeding objectives and 5 commercial cultivars (Mersoy, ANP 2018, Traksoy, Victoria, and Arisoy) (Table 1).

Table 1. Information on the study plant materials

Genotype code	Name	Species name
G1	Line10	<i>Glycine max</i> (L.) Merr.
G2	Line13	<i>Glycine max</i> (L.) Merr.
G3	Line16	<i>Glycine max</i> (L.) Merr.
G4	Line25	<i>Glycine max</i> (L.) Merr.
G5	Line28	<i>Glycine max</i> (L.) Merr.
G6	Line44	<i>Glycine max</i> (L.) Merr.
G7	Line63	<i>Glycine max</i> (L.) Merr.
G8	Line66	<i>Glycine max</i> (L.) Merr.
G9	Line71	<i>Glycine max</i> (L.) Merr.
G10	Line75	<i>Glycine max</i> (L.) Merr.
G11	Line90	<i>Glycine max</i> (L.) Merr.
G12	Line100	<i>Glycine max</i> (L.) Merr.
G13	Line111	<i>Glycine max</i> (L.) Merr.
G14	Mersoy	Commercial Variety
G15	ANP 2018	Commercial Variety
G16	Traksoy	Commercial Variety
G17	Victoria	Commercial Variety
G18	Arisoy	Commercial Variety

2.1. Genomic DNA isolation

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, SA-88, Bravo, Adasoy, Traksoy, Ataem-7, 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.

2.2. ISSR marker assay

The ISSR 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 20 ISSR primers were initially screened across the samples and 7 primers exhibiting polymorphic patterns were selected for further analysis (Table 2). Among the selected 7 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. ISSR primers used for PCR amplification

ISSR Primers Code	DNA Sequences (5'-3')	T _m °C	PCR Amplification
UBC-811	5'-GAGAGAGAGAGAGAGAC-3'	53	
UBC-810	5'-GAGAGAGAGAGAGAGAT-3'	50	94 °C - 3 min
UBC-826	5'-ACACACACACACACACC-3'	52	94 °C - 1 min
UBC-831	5'-CTCTCTCTCTCTCTT-3'	50	50–55 °C 1 min
UBC-836	5'-AGAGAGAGAGAGAGAGYA-3'	52	72 °C - 1 min
UBC-856	5'-ACACACACACACACACYA-3'	52	72 °C - 10 min
UBC-880	5'-GGAGAGGAGAGGAGA-3'	55	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

In this study, a total of 18 soybean genotypes, including 13 soybean (*Glycine max* (L.) Merr.) lines obtained through crosses carried out for breeding purposes and 5 commercial cultivars (Mersey, ANP 2018, Traksoy, Victoria, Arisoy), within the scope of the soybean breeding program at Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technology, were evaluated for genetic diversity using ISSR markers. A total of 78 bands were obtained using 7 ISSR primers applied for screening purposes, and 32 of these bands were determined to be polymorphic. This indicates a polymorphism rate of 41.02%, indicating that ISSR markers exhibit moderate discrimination power in detecting genetic variation. UPGMA cluster analysis based on ISSR marker data revealed the genetic similarity relationships among the 18 soybean genotypes examined.

Genetic similarity was calculated using the Jaccard similarity coefficient, and similarity values among genotypes ranged from approximately 0.846 to 0.984 (Table 3). These results indicate that, although a generally high level of similarity exists among the genotypes analyzed, a notable degree of genetic variation is also present. The UPGMA dendrogram generated based on the Jaccard similarity

coefficient (Figure 1) divided the genotypes into two main groups. Group A consisted solely of the S-1 and S-3 genotypes, which exhibited distinct genetic differences compared to the others. This suggests that these genotypes are more distantly related and could serve as important materials for genetic diversity studies.

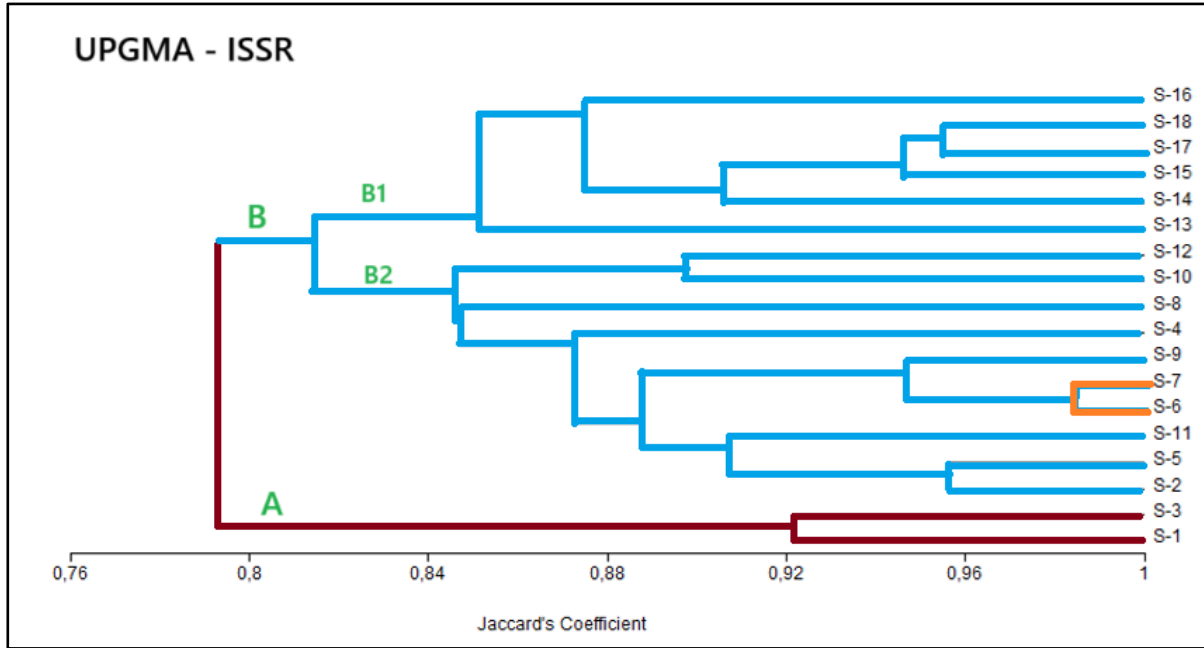


Figure 1. UPGMA dendrogram of genetic similarity between ISSR markers and 18 soybean genotypes

The B group, which constitutes the majority of the dendrogram, includes most of the genotypes and is further divided into two subgroups, B1 and B2. Subgroup B1 comprises the S-13, S-14, S-15, S-16, S-17, and S-18 genotypes, which appear to be relatively closely related to each other. This suggests that these genotypes may share similar genetic backgrounds or have undergone comparable selection processes. The second subset of group B, namely B2, exhibits greater genetic diversity. This subgroup includes S-12, S-10, S-8, S-4, S-9, S-7, S-6, S-11, S-5, and S-2, among which varying levels of genetic similarity are observed. Notably, the S-6 and S-7 genotypes are positioned very closely within the dendrogram, nearly merging at the same node. This indicates a very high level of genetic

similarity between these two genotypes. The highest genetic similarity observed in the dendrogram was 98.4% (0.984) between S-6 and S-7, suggesting that these genotypes may have undergone similar selection processes or originated from the same genetic pool. Similarly, other genotype pairs, such as S-2 and S-5 (95.6%) and S-17 and S-18 (95.5%), also exhibit high levels of genetic similarity (Table 3). A node in the hierarchical clustering process represents a newly formed group that arises when two groups (individuals or previously merged subgroups) are combined at a certain level of similarity. These nodes indicate that previously merged genotypes or groups are further joined with other individuals or nodes at a specific similarity level (Çilesiz, 2025c).

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

Group 1	Group 2	Similarity	Objects in group
S-6	S-7	0.984	2
S-2	S-5	0.956	2
S-17	S-18	0.955	2
Node 1	S-9	0.947	3
S-15	Node 3	0.946	3
S-1	S-3	0.922	2
Node 2	S-11	0.907	3
S-14	Node 5	0.906	4
S-10	S-12	0.897	2
Node 7	Node 4	0.888	6
Node 8	S-16	0.875	5
Node 10	S-4	0.873	7
S-13	Node 11	0.852	6
Node 12	S-8	0.847	8
Node 14	Node 9	0.846	10
Node 15	Node 13	0.814	16
Node 6	Node 16	0.793	18

The polymorphism rate of 41.02% obtained in this study is consistent with the results of previously reported ISSR-based studies on soybean genetic diversity. Arslan et al. (2020), in their study on different soybean genotypes in Türkiye, obtained a high number of polymorphic bands using ISSR markers and observed moderate-to-high levels of genetic variation among the genotypes. Similarly, Aghaei et al. (2012) applied ISSR analyses on Iranian soybean genotypes and reported a polymorphism rate of approximately 45–50%. These studies indicate that ISSR markers are effective and reliable tools for assessing genetic diversity. The slightly lower polymorphism rate observed in the present study compared to some reports in the literature may be attributed to factors such as the number of primers used, the genetic background of the genotypes, and their breeding history. The genetic similarity values calculated based on UPGMA cluster analysis ranged from 0.846 to 0.984, indicating a generally high level of similarity among the genotypes. Sharma et al. (2018), using RAPD markers on different soybean genotypes, reported genetic similarity coefficients ranging from 0.44 to 0.76. Similarly, Ge et al. (2005), found genetic similarity values ranging from 0.36 to 0.91 in a Chinese soybean germplasm collection using ISSR analyses. These findings

suggest that soybean genetic resources generally exhibit limited genetic variation and that similar genetic pools are often utilized in modern breeding programs. According to the dendrogram a high similarity of 98.4% was observed between the S-6 and S-7 genotypes. Likewise, Rayan and Osman (2019) reported genetic similarity values above 0.90 for certain soybean genotype pairs. These high similarities indicate that the respective genotypes may have originated from the same genetic pool or undergone similar selection processes. The separation of genotypes into two main groups in the UPGMA dendrogram, with certain subgroups exhibiting high genetic proximity, is consistent with the findings reported by Sahu et al. (2012) and Tiwari et al. (2019); in these studies, soybean genotypes were also generally divided into two main clusters based on ISSR or SSR analyses, and some genotype pairs showed high genetic similarity. Overall, considering this study together with similar reports in the literature, ISSR markers are effective, reproducible, and reliable tools for assessing soybean genetic diversity. The dendrogram structure and genetic similarity values obtained in the present study indicate that the 18 genotypes analyzed share a common genetic background while also exhibiting sufficient genetic variation to be used in breeding programs.

These findings provide important scientific information to inform the selection of suitable

parent combinations and to broaden the genetic base in soybean breeding programs.

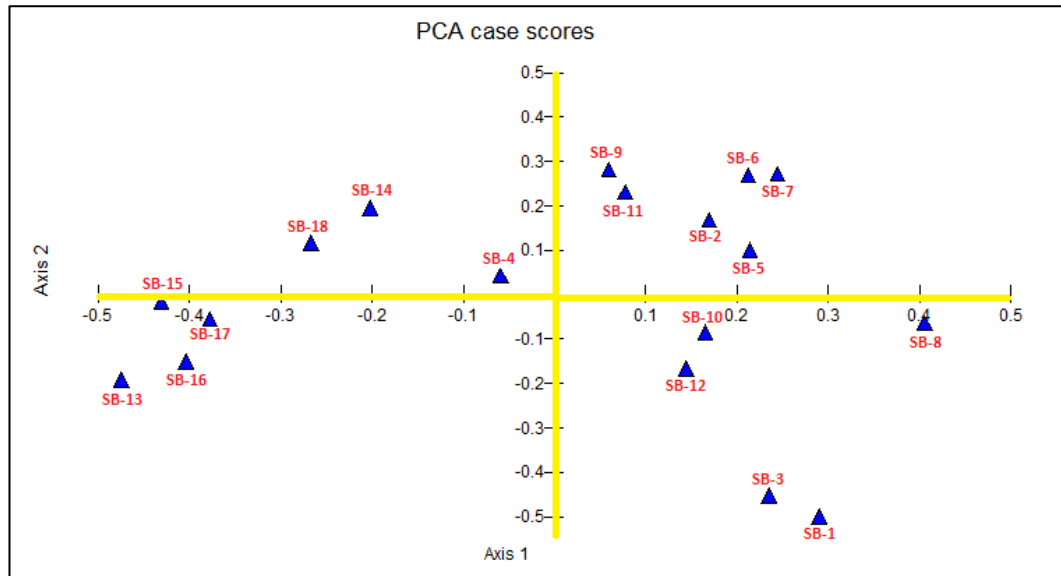


Figure 4. According to ISSR markers principal component analyses of 18 soybean genotypes using MVSP 3.22 software (Kovach Computing Services, Pentraeth, Wales, UK)

Principal Component Analysis based on ISSR markers allowed the visualization of genetic relationships among the 18 soybean genotypes along two principal component axes. The PCA results indicated that the genotypes were positioned in distinct regions of the coordinate plane, forming identifiable clusters. In the PCA plot, Axis 1 and Axis 2 represent the principal components explaining the largest portion of genetic variation. Examining the distribution of genotypes, revealed notable genetic differentiation, particularly along Axis 1. Genotypes located in the positive region of Axis 1 (SB-1, SB-3, SB-6, SB-7, SB-8, and SB-5) were relatively close to one another, suggesting a tendency to form a genetically similar group. Their concentration on the right side of the plot indicates that these individuals share more similar allelic compositions as determined by ISSR markers. In contrast, genotypes located in the negative region of Axis 1 (SB-13, SB-15, SB-16, and SB-17) were positioned further from the other genotypes, forming a separate genetic group. This suggests that these genotypes contain genetic variations distinct from those in the rest of the population. Additionally, some genotypes were positioned in the plot's central region. In particular, SB-2,

SB-4, SB-9, and SB-11 were located near the middle of the PCA coordinate system, suggesting that these genotypes exhibit intermediate characteristics between different genetic groups or possess a more complex genetic structure. Another notable observation in the PCA plot was that certain genotypes were positioned considerably distant from the others. Specifically, SB-1 and SB-3 were distinctly separated along the negative direction of Axis 2, indicating that these genotypes have allelic compositions that are more genetically distinct from the rest. Such genotypes are important in genetic diversity studies and may serve as parent materials in breeding programs (Çilesiz, 2025f). Overall, the PCA results demonstrate a significant level of genetic diversity among the soybean genotypes studied and highlight the effectiveness of ISSR markers in revealing this diversity. The PCA results obtained in this study are consistent with other molecular marker-based genetic diversity studies in soybean. Malik et al. (2013) reported that PCA analyses of soybean genotypes using ISSR markers revealed distinct clusters and clearly separated individuals. Similarly, Wang et al. (2010) emphasized the effectiveness of PCA for visualizing genetic relationships in their

study of molecular diversity. In a study by Ovalıoğlu and Çilesiz (2025) on walnut (*Juglans regia* L.) genotypes using SCoT markers, a high level of genetic polymorphism (77.9%) was detected, and consistency between PCA and UPGMA dendrogram results was observed. This similarity confirms that molecular markers can effectively reveal genetic relationships when combined with PCA and hierarchical clustering analyses. Likewise, Sönmez and Çilesiz (2025) reported that SCoT analysis of grape (*Vitis vinifera* L.) genotypes yielded a dendrogram consistent with PCA results, showing variable similarity among genotypes. This indicates that species-specific genetic background differences can be successfully detected through both PCA and dendrogram analyses, supporting the interpretation of the soybean results in this study. Temrel and Çilesiz (2025), in their SCoT analysis of thyme (*Thymus vulgaris* L.) genotypes also observed high consistency between PCA and dendrogram analyses, highlighting the reliability of both multivariate methods for visualizing genetic differences. In addition, Yücel et al. (2026) reported that PCA analyses of sugar beet genotypes successfully identified both homogeneous groups and distinctive genotypes, in agreement with the cluster structures observed in the dendrogram. This common finding demonstrates that multivariate analyses are complementary tools for visualizing genetic relationships across both vegetable and legume species. Furthermore, Li and Nelson (2001) emphasized the high discriminative power of ISSR markers in assessing soybean genetic diversity, reporting that genotypes formed distinct clusters in PCA analysis. One of the main advantages of PCA in plant genetic diversity studies is its ability to reduce high-dimensional molecular datasets to two- or three-dimensional planes, allowing visual representation of genetic relationships among genotypes. Mohammadi and Prasanna (2003) provided a comprehensive review stating that PCA and similar multivariate methods are widely used in plant genetic diversity studies and yield reliable results. The results obtained in this study are consistent with those reported

in the literature, revealing significant genetic variation among the soybean genotypes analyzed and demonstrating that some genotypes are more genetically distant from others. This offers significant potential to broaden the genetic base, particularly in breeding programs.

4. Conclusion

This study demonstrated that molecular analyses based on ISSR markers are an effective and applicable approach for evaluating genetic diversity among soybean genotypes. Analyses were conducted on a total of 18 genotypes, including 13 lines and 5 commercial cultivars obtained from different ecological regions of Türkiye. Using seven ISSR primers, 78 bands were amplified, of which 32 were polymorphic, resulting in a polymorphism rate of 41.02%. This rate indicates that the soybean materials studied possess notable genetic diversity and that ISSR markers are sufficiently sensitive to detect this variation. When UPGMA cluster analysis and Principal Component Analysis (PCA) were considered together, it was evident that genetic relationships among the genotypes could be evaluated reliably in a multidimensional manner. In particular, the high level of genetic similarity observed between S-6 and S-7 (%98.4) indicates that these genotypes are genetically homogeneous and may be useful in variety verification and pure line development studies. Conversely, the lowest similarity measured between S-3 and S-13 (%79.3) suggests that S-3 is a genetically distant source and may be a candidate for heterotic crossing programs. The placement of commercial cultivars across different subgroups indicates that these varieties have distinct genetic backgrounds and likely originated from different gene pools. This finding highlights that commercial cultivars can serve as complementary parent materials and simultaneously enhance genetic diversity in breeding programs. In conclusion, this study demonstrated that ISSR markers are an effective tool for assessing the diversity of soybean genetic resources, owing to their cost-effectiveness, reproducibility, and

genome-wide analytical capability. The findings provide a strong scientific basis for parent selection, the development of core collections, the identification of heterotic groups, and the sustainable management of genetic resources. These data offer significant contributions toward achieving strategic objectives in soybean breeding programs, including increased productivity, enhanced stress tolerance, and the broadening of 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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