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**ARAŞTIRMA MAKALESİ** **RESEARCH ARTICLE**

# Genetic diversity analysis of sesame (*Sesamum indicum* L.) genotypes in Türkiye using SSR markers

Türkiye'deki susam (*Sesamum indicum* L.) genotiplerinin genetik çeşitliliğinin SSR markörleri ile analizi

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**ABSTRACT**

This study aimed to analyze the genetic diversity of 25 different sesame (*Sesamum indicum* L.) genotypes cultivated in Türkiye, including 16 registered cultivars, 6 local populations, 1 Indian population adapted to the ecological conditions of Konya, and 2 genotypes of Indian origin, using SSR markers. Genetic diversity was assessed using 10 different SSR primers, resulting in a total of 79 polymorphic bands**.** The CUESTSSR02 primer was the most efficient one with high values for Polymorphic Allele Number (PA), Polymorphic Information Content (PIC), Expected Heterozygosity (He), and Marker Index (MI)**.** Genetic relationships between genotypes were analyzed and visualized using a circular phylogenetic tree, PCoA, and a heatmap**.** The findings revealed that the local populations are genetically distinct from both registered cultivars and Indian genotypes**.** Notably, the Akören genotype was identified as genetically isolated from all other genotypes. This study highlights the crucial role of local populations in preserving and enhancing genetic diversity. The integration of Indian genotypes with local populations may contribute to the development of more resilient and productive cultivars. The findings emphasize that the effective use of genetic resources can support sustainable agriculture and improve agricultural performance.

**ÖZET**

Bu çalışma, Türkiye'de yetiştirilen 16 tescilli çeşit, 6 yerel popülasyon, Konya ekolojik koşullarına uyum sağlamış 1 Hint popülasyonu ve Hindistan kökenli 2 genotip olmak üzere toplam 25 farklı susam genotipinin genetik çeşitliliğini SSR (Simple Sequence Repeat) markörleri kullanarak analiz etmeyi amaçlamıştır. Araştırmada, 10 farklı SSR primeri ile yapılan genetik çeşitlilik değerlendirmesi sonucunda toplam 79 polimorfik bant tespit edilmiştir. CUESTSSR02 primeri, yüksek Polimorfik Allel Sayısı (PA), Polimorfik Bilgi İçeriği (PIC), Beklenen Heterozigotluk (He) ve Markör İndeksi (MI) değerleri ile en verimli primer olarak öne çıkmıştır.Genotipler arasındaki genetik ilişkiler, dairesel filogenetik ağaç, Temel Koordinatlar Analizi (TKoA) ve ısı haritası gibi yöntemlerle görselleştirilerek analiz edilmiştir. Elde edilen bulgular, yerel popülasyonların tescilli çeşitlerden ve Hindistan genotiplerinden genetik olarak farklılaştığını ortaya koymuştur. Özellikle Akören genotipi**,** tüm diğer genotiplerden genetik olarak izole bir yapıya sahip bulunmuştur. Çalışma, yerel popülasyonların genetik çeşitliliğin korunması ve artırılmasında önemli bir rol oynadığını göstermektedir. Hindistan genotiplerinin yerel popülasyonlarla birleştirilmesi, daha dayanıklı ve verimli varyetelerin geliştirilmesine katkı sağlayabilir. Sonuçlar, genetik kaynakların etkin kullanımının sürdürülebilir tarımı destekleyerek tarımsal performansı artırabileceğini ortaya koymaktadır.

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## INTRODUCTION

Sesame (*Sesamum indicum* L.) is a globally significant oilseed crop valued for its high-quality edible oil, nutrient-rich seeds, and protein-dense meal. Believed to have originated in Africa, sesame underwent domestication and significant genetic diversification in Asia, particularly in regions such as India and Türkiye (Ashri, 2006) (Uncu et al., 2015). As one of the oldest domesticated crops, it is now widely cultivated across Asia, Africa, and the Americas, playing a crucial role in global vegetable oil production and food security, particularly in developing nations (Meena et al., 2018). With an exceptionally high oil content (46-64%) and a rich nutritional profile, including proteins, antioxidants, and essential micronutrients, sesame is often referred to as the "queen of oilseeds" due to its superior oil quality and stability. Its resilience to drought and adaptability to marginal soils make it an invaluable crop for sustainable agriculture, particularly in arid and semi-arid regions. Beyond its economic significance, sesame has diverse applications in the food industry, pharmaceuticals, and cosmetics, further enhancing its global demand (Yaseen et al., 2021). Recent research highlights its bioactive compounds, such as sesamin and sesamolin, which exhibit antioxidant, anti-inflammatory, and cardioprotective properties, reinforcing its status as a functional food ingredient.

Genetic diversity is fundamental for the continuous improvement of sesame, enabling the development of cultivars with enhanced yield, stress tolerance, and disease resistance. A broad genetic variation within germplasm collections is essential to address the challenges posed by climate change and evolving agricultural demands. Thus, preserving and characterizing this diversity is a strategic priority for future breeding efforts (Dossa et al., 2016).

Türkiye, located at the intersection of Europe and Asia, is recognized as a critical center for sesame cultivation and genetic diversity. The country's diverse agro-ecological conditions have facilitated the development of numerous local landraces and traditional populations, which represent invaluable genetic resources for sesame improvement (Uncu et al., 2015). These genetic resources harbor unique traits that can enhance sesame's resilience and productivity. Additionally, sesame stands out as an indispensable crop for sustainable agriculture due to its ability to thrive in marginal agricultural areas and its resistance to adverse climatic conditions. In Türkiye, where environmental challenges such as drought, salinity, and low-fertility soils are prevalent, sesame's adaptive traits offer a considerable advantage. Its ability to achieve high yields even in water-scarce regions makes sesame a reliable source of income for smallholder farmers, while also contributing significantly to the country's agricultural production systems. These characteristics underscore sesame's strategic importance for Turkish agriculture.

Molecular markers have emerged as indispensable tools in genetic diversity studies due to their precision and reliability. Among these, simple sequence repeat (SSR) markers are particularly valuable because of their high polymorphism rates, reproducibility, and co-dominant inheritance. SSR markers enable detailed analysis of population structure, genetic relationships, and diversity patterns, providing a robust foundation for strategic conservation and utilization of germplasm (Yepuri et al., 2013).

This study aimed to evaluate the genetic diversity of registered sesame cultivars, local populations in Türkiye, and two distinct populations introduced from India using SSR markers. Both Türkiye and India play a critical role in enhancing the genetic diversity of sesame; the unique traits of Türkiye's local populations and the genetic richness of populations from India provide significant contributions to breeding efforts. The findings of this study are expected to offer valuable insights into existing genetic resources and guide strategies to improve sesame's productivity, resilience, and adaptability to future agricultural challenges.

## MATERIALS and METHODS

### *Material*

In this study, the plant material comprised a total of 25 distinct sesame (*S. indicum* L.) genotypes, including 16 registered Turkish sesame cultivars cultivated in Türkiye, five local populations obtained from farmers in various districts of Konya and Antalya, two Indian populations sourced from local farmers in India, and one Indian population adapted to the ecological conditions of Konya. Detailed information on these genotypes is provided in Table 1 and Figure 1.

Table 1. Information on sesame cultivars/populations

*Çizelge 1. Susam çeşitleri/popülasyonları hakkında bilgi*

| Genotype Code | Name of Cultivar/ Population | Institute / Location | Seed Color |
|---|---|---|---|
| Batem Uzun | Batem Uzun | West Mediterranean Agricultural Research Institute (Antalya) | Dark yellow |
| Muganlı 57 | Muganlı 57 | | Yellow |
| Özbek 82 | Özbek 82 | | Dark yellow |
| Baydar 2001 | Baydar 2001 | | Dark yellow |
| Gölmarmara | Gölmarmara | | White |
| Batem Aksu | Batem Aksu | | Brown |
| Kepsut 99 | Kepsut 99 | Aegean Agricultural Research Institute (İzmir) | White |
| Cumhuriyet 99 | Cumhuriyet 99 | | White |
| Osmanlı 99 | Osmanlı 99 | | White |
| Tan 99 | Tan 99 | | White |
| Orhangazi 99 | Orhangazi 99 | | White |
| Sarısu | Sarısu | | Yellow |
| Tanas | Tanas | | Yellow |
| Boydak | Boydak | GAP Agricultural Research Institute (Şanlıurfa) | Dark yellow |
| Arslanbey | Arslanbey | | Black |
| Hatipoğlu | Hatipoğlu | | Brown |
| Küncü | Küncü | Konya- Akören | Yellow |
| Akören | Akören | Konya- Akören | Brown |
| Antalya Sarısı | Antalya Sarısı | Antalya-Manavgat | Yellow |
| YP[1] | Local population[1] | Konya-Doğanhisar | White |
| YP[2] | Local population[2] | Konya-Meram (Kayıhüyük) | Brown |
| YP[3] | Local population[3] | Konya-Meram (Kayıhüyük) | Brown |
| HindistanT | IndiaT* | Konya-Akören * | White |
| Hindistan[1], Hindistan[2] | India[1], India[2] | India | White |

* The cultivar introduced to Türkiye from India.

### *Method*

The seeds of different sesame genotypes were sown in trays containing a peat:perlite (1:1) mixture and germinated under controlled conditions (24±2°C temperature, 65% humidity, 5 LS light intensity, and a 16/8 h photoperiod) in a climate chamber. Fresh leaf samples were collected from germinated plants at the 5–6 leaf stage for DNA isolation. For genotypes that did not germinate in the trays, seeds were germinated on MS (Murashige & Skoog, 1962) medium supplemented with 0.5 mg $L^{-1}$ $GA_3$, and samples were taken from 4-week-old seedlings.

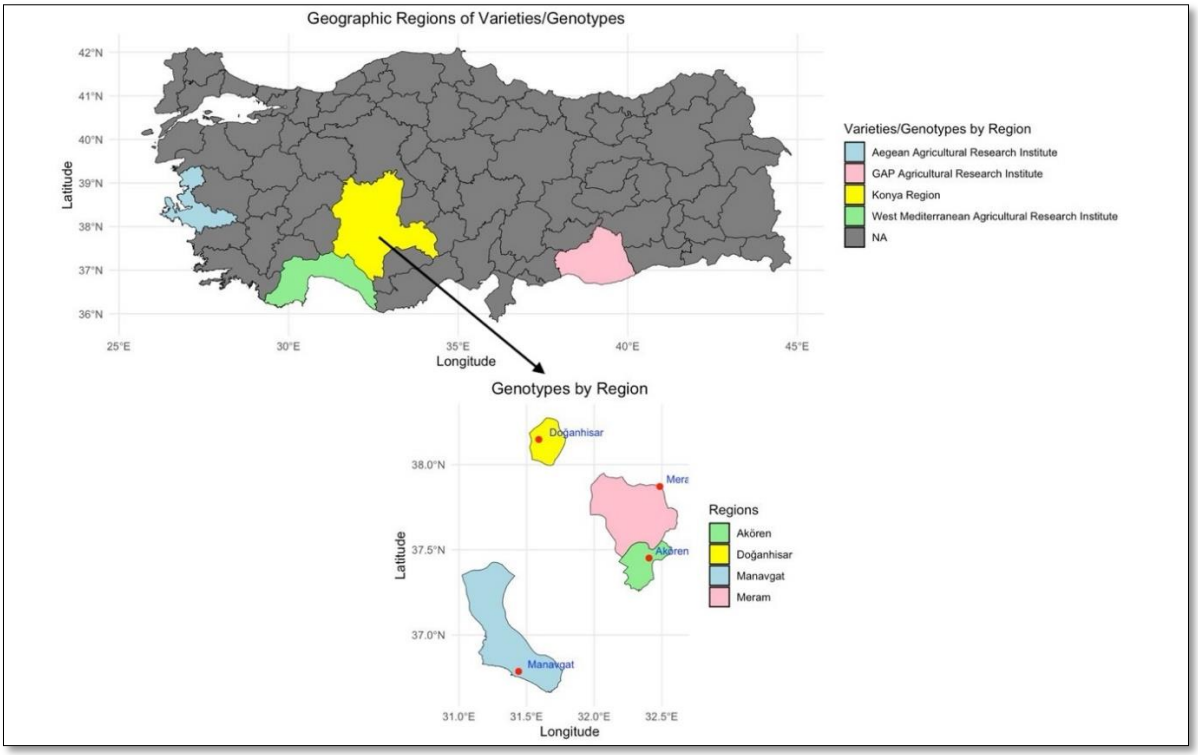


Figure 1. Geographic regions of sesame cultivars/populations belonging to Türkiye
*Şekil 1. Susam çeşitlerinin/popülasyonlarının Türkiye'de ait olduğu coğrafi bölgeler*

***DNA isolation and determination of quality***

Initially, DNA isolation was performed in all genotypes using the CTAB method (Doyle, 1990). However, the high oil content and secondary metabolites in sesame hindered obtaining sufficient quantity and quality of DNA. By modifying the method described by (Porebski et al., 1997), the desired level of DNA isolation was successfully achieved in all genotypes.

According to this method, 0.06 g of young leaf tissue was shock-frozen in liquid nitrogen and ground in a mortar before being transferred into sterile tubes. Lysis buffer (670 µL) and PVP (6.7 mg) were added into the tube and mixed, then the mixture was incubated at 60°C for 1 hour. Subsequently, 800 µL of chloroform:isoamyl alcohol (24:1) was added, and the mixture was centrifuged at 3,000 rpm for 20 minutes. The upper phase (supernatant) was collected, and this step was repeated if necessary. To the supernatant, ½ volume of 5 M NaCl and 2 volumes of cold ethanol (95%) were added, and the solution was precipitated at 4–6°C for 24 hours. Following this step, the samples were centrifuged at 3,000 rpm for 6 minutes, and the DNA pellets were carefully separated and washed with 2,000 µL of 70% ethanol. After, the ethanol was evaporated, and the DNA pellets were dissolved in 300 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and 3 µL of RNase A and Proteinase K were added. The samples were then incubated at 37°C for approximately 1 hour.

For further purification, 150 µL of phenol and chloroform were added to each tube, and after centrifugation, the upper phase was transferred to a new tube. DNA was re-precipitated by adding 1/10 volume of 3 M sodium acetate and 2 volumes of absolute ethanol (99.9%). In the final step, ethanol was carefully removed, and the DNA pellets were dissolved in 100 µL of TE buffer, making them ready for analysis. The obtained DNA samples were quantified and assessed for quality using a NanoDrop ND-1000 spectrophotometer at 260 and 280 nm wavelengths. Stock DNA samples were diluted to a concentration of 20 ng/µL to be used in PCR studies, ensuring equalized DNA concentrations.

***SSR primers and PCR amplification***

In the study, 10 different co-dominant SSR markers, which provide significant polymorphic information content and comprehensively cover all linkage groups in the sesame genome, were utilized (Bhattacharjee et al., 2020; Teklu et al., 2022; Zhang et al., 2012). The annealing temperatures and sequences of the primers are presented in Table 2.

Table 2. SSR primers and sequence information

*Çizelge 2. SSR primerleri ve sekans bilgileri*

| SSR Primers | Primer sequence | Tm (°C) | References |
|---|---|---|---|
| ZMM3261–F | F:5'–CGAAAGCATGAGACGAGTATG–3' | 50.2 | Teklu et al., (2022) |
| ZMM3261–R | R:5'–AACTAGTGCGCAATTCATTCAA–3' | | |
| ZMM5358–F | F:5'–TAGGATGCTTTGAATTGGGC–3' | 51.2 | Teklu et al., (2022) |
| ZMM5358–R | R:5'–AGGAACAAACATACGGCGTC–3' | | |
| ZMM1189–F | F:5'-TATCCAGGGGAAAACCAGAA-3' | 50.5 | Teklu et al.,(2022) |
| ZMM1189–R | R:5'-TTGGATTTTCCTTCTCACGC-3' | | |
| ZMM3312–F | F:5'–GCAAAATCTTCTTTCCTCCG–3' | 45.5 | Teklu et al., (2022) |
| ZMM3312–R | R:5'–GCAGCAAGGGAATTGAATGT–3' | | |
| HS189–F | F:5'–CTCCAACCCCCATAAATCAC–3' | 52.5 | Zhang et al., (2012) |
| HS189–R | R:5'–TGACCCAATAGTGGTGGTCA–3' | | |
| HS94–F | F:5'–CATGTGTTCTCTCCCACCAC–3' | 51.8 | Zhang et al., (2012) |
| HS94–R | R:5'–TCTTGACCATGTTTTCCACC–3' | | |
| HS21–F | F:5'–CGTCCCGTGTTGTCTCTATG–3' | 50.3 | Zhang et al., (2012) |
| HS21–R | R:5'–CAGTGAATTTCTCAACCCGA–3' | | |
| HS233–F | F:5'–CGTCCCGTGTTGTCTCTATG–3' | 52.0 | Zhang et al., (2012) |
| HS233–R | R:5'–GCGGAGAATATGCCGTTATT–3' | | |
| CUESTSSR02-F | F:5'–AAGAAAGCTAAGAAGGCAGAG–3' | 42.5 | Bhattacharjee et al., (2020) |
| CUESTSSR02-R | R:5'–GCTTGATAGAGAAGTTACGACA–3' | | |
| CUSSR29–F | F:5'–CTCACACACGGATAACCTTAG–3' | 50.5 | Bhattacharjee et al. (2020) |
| CUSSR29–R | R:5'–AGGATTTCATGGGTTAAGTTC–3' | | |

PCR amplification reactions were performed using a Techne-512 thermocycler, with a total reaction volume of 20 µL. The reaction mixture contained 2.0 µL of 10X Taq PCR buffer containing $(NH4)_2SO_4$ without $MgCl_2$, 1.70 µL of 25 mM $MgCl_2$, 0.4 µL of 10 mM dNTP, 0.2 µL of 5 U/µL Taq DNA polymerase, 1.0 µL of 10 µM SSR primers (Forward and Reverse), and 40 ng/µL DNA. The PCR amplification temperature and duration were individually optimized based on the base composition of the primers.

The PCR protocol was as follows: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at different temperatures depending on the primers for 1 minute, and extension at 72°C for 2 minutes. A final extension step was performed at 72°C for 5 minutes, followed by cooling at 4°C.

***Detection of amplified DNA fragments***

The PCR products were visualized using 2% agarose gel electrophoresis containing ethidium bromide. Due to the close proximity of the expected band sizes in the amplified PCR products and the difficulty of scoring alleles on agarose gel, fragment visualization was performed using a capillary electrophoresis system.

For this purpose, PCR products were analyzed using the BIOptic® QSEP 100 DNA Analyzer with a high-resolution kit in a capillary system. In capillary electrophoresis, a 20–1000 bp size marker (20 ng/mL) and a 20–1000 bp alignment marker (20 ng/mL) were used for the visualization of PCR products (Figure 2).

***Statistical analysis***

To perform genetic similarity analysis among genotypes, each allele of the SSR marker was numerically converted by coding the presence of a specific fragment as 'present = 1' and its absence as 'absent = 0'.

***Primer efficiency analyses***

The genetic diversity within the population was assessed using heterozygosity (He), which represents the probability that an individual carries two different alleles at a given locus. According to (Nei, 1978), it was calculated using the following formula.

$$He = 1 - \sum (P_i)^2$$

Pi represents the frequency of the i-th allele calculated for each SSR locus. In the formula, "p" denotes the frequency of each allele at a given nSSR locus, while "n" refers to the total number of alleles at that locus.

Polymorphism Information Content (PIC) measures the ability of a genetic marker to detect genetic variation within a population. PIC is a derivative of heterozygosity and provides a more accurate representation of a marker's informativeness in genetic studies (Guo & Elston, 1999). According to Botstein et al. (1980), the PIC value is calculated using the following formula.

$$PIC = 1 - \sum_{i=1}^{n} pi^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2\ pi^2 pj^2$$

Primer efficiency parameters used in this study, EMR (Effective Multiplex Ratio) and MI (Marker Index), were calculated using the following formulas (Chesnokov & Artemyeva, 2015).

$$\text{EMR} = np(\frac{np}{n})$$

MI= $PIC \times EMR$

np: Number of polymorphic bands

n: Total number of bands

***Determination of genetic distance between genotypes***

In this study, the data obtained from PCR amplifications using SSR primers were comprehensively analyzed to assess genetic diversity. Alleles were numerically coded as present (1) / absent (0) based on peak values obtained using the BIOptic® QSEP 100 DNA Analyzer, and these data served as the basis for calculating genetic similarities and differences.

Genetic relatedness analyses were conducted using the R programming language, employing the vegan, ape, and RColorBrewer packages to calculate the Jaccard similarity index (Jaccard, 1908), followed by hierarchical clustering analysis using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) approach. To ensure data integrity, missing values were imputed using the k-Nearest Neighbors (k-NN) method. A circular (fan-type) phylogenetic tree was constructed to visualize genetic relationships, and a heatmap based on the Jaccard similarity matrix was generated to provide a detailed representation of genetic similarities among genotypes (Yi et al., 2021). Furthermore, Principal Coordinate Analysis (PCoA) was performed to gain deeper insights into genetic relatedness, and the results were visualized to confirm the validity of genetic groupings (Cornea-Cipcigan et al., 2023). These analyses facilitated a comprehensive understanding of genetic diversity and phylogenetic relationships.

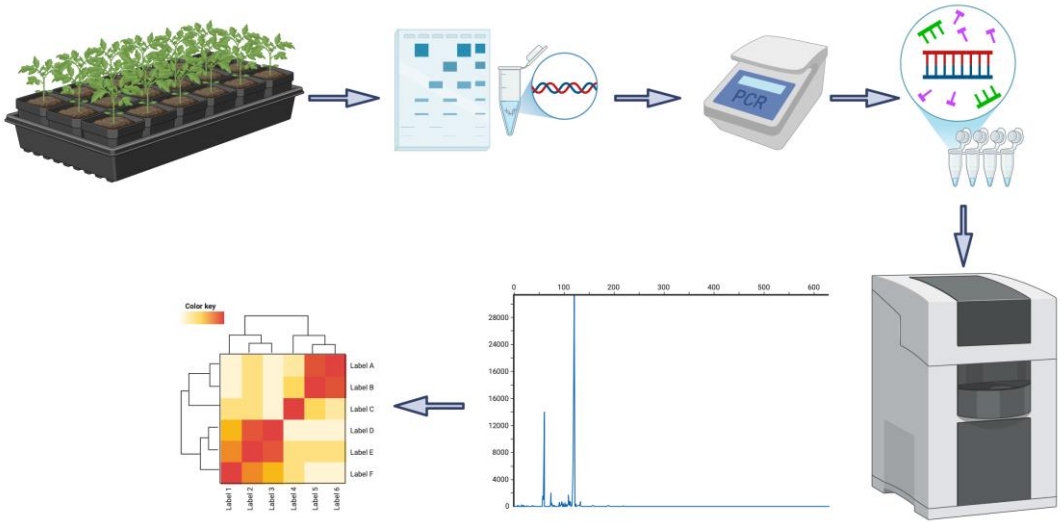


Figure 2. Workflow of DNA isolation, PCR amplification, and bioinformatics analyses in sesame (Created at https://BioRender.com)

*Şekil 2. Susamda DNA izolasyonu, PCR amplifikasyonu ve biyoinformatik analizlerin iş akışı*

**RESULTS and DISCUSSIONS**

***Results of primary efficiency analysis***

This study, which investigated lows-specific SSR primers for genetic diversity, evaluated specific polymorphic characteristics for each primer. SSR markers are powerful tools commonly used to detect genetic differences among organisms, based on repetitive DNA sequences at microsatellite regions (Gupta & Varshney, 2000). Considering this, the parameters such as the Number of Polymorphic Alleles (PA), Effective Multiplex Ratio (EMR), Polymorphic Information Content (PIC), Expected Heterozygosity (He), and Marker Index (MI) are critical for assessing the capacity of primers to detect genetic variation (Botstein et al., 1980).

Heterozygosity (He) generally measures genetic diversity, while PIC advances this assessment by considering the informativeness of genetic markers, making it a preferred metric in linkage analysis and population genetics studies (Elston et al., 2002; Guo & Elston, 1999). The highest number of polymorphic alleles were observed from primers CUSSR29 and CUESTSSR02, with values of 18 and 16, respectively, among the SSR primers analyzed. Number of polymorphic alleles (PA) is an indicator of a primer's capacity to detect variations in genetic diversity, and high PA values indicate that genetic diversity can be studied on a larger scale (Powell et al., 1996). However, the low PIC and He values of 0.51 and 0.55, respectively, for the CUSSR29 primer indicate that the depth of the detected genetic diversity may be limited. The PIC value determines how informative a locus is in terms of allele diversity and distribution; values above 0.5 are generally considered suitable for genetic analyses (Anderson et al., 1993). Despite CUSSR29's limitations, CUESTSSR02 emerges as the most efficient primer, as it not only exhibits a high PA but also high He (0.85) and MI (10.4), making it more suitable for detecting genetic variation.

Expected heterozygosity (He) is a measure of genetic diversity within a population and represents a crucial parameter in genetic structure analyses (Nei, 1978). Primers with lower PA values, such as ZMM3312 and HS233, with PA values of 3 and 4, respectively, exhibit a more limited capacity for detecting genetic diversity. However, the

ZMM3312 primer, with its high PIC and He values of 0.98 and 0.99, respectively, demonstrates the ability to effectively detect genetic variation in specific regions despite its low PA value. The use of such SSR primers may focus more on the analysis of specific target loci rather than overall genetic diversity (Liu et al., 2003).

When evaluating Marker Index (MI) values, the CUESTSSR02 primer achieved the highest level of effectiveness (10.4) compared to other primers. MI is a composite metric used to determine the overall effectiveness of a marker in genetic diversity analysis, and high MI values enhance the suitability of primers for genetic studies (Varshney et al., 2007). In contrast, the ZMM3312 and HS233 primers exhibit more limited effectiveness due to their lower MI values of 2.94 and 3.76, respectively.

This analysis provides valuable insights into the efficiency of SSR primers for genetic diversity studies, aiding in the selection of appropriate primers for Sesamum genotypes. Notably, the CUESTSSR02 primer stands out as the most effective for detecting genetic variation, exhibiting high PA, PIC, He, and MI values. These findings highlight critical factors to consider when selecting primers for genetic diversity and population structure studies, ensuring more accurate and reliable assessments of genetic variation.

Table 3. The analysis of primers' efficiency

*Çizelge 3. Primer etkinlik analizleri*

| SSR Primers | PA | EMR | PIC | He | MI |
|---|---|---|---|---|---|
| ZMM3261 | 6 | 6 | 0.97 | 0.99 | 5.82 |
| ZMM5358 | 4 | 4 | 0.90 | 0.95 | 3.60 |
| ZMM1189 | 12 | 12 | 0.70 | 0.91 | 8.40 |
| ZMM3312 | 3 | 3 | 0.98 | 0.99 | 2.94 |
| HS189 | 4 | 4 | 0.97 | 0.98 | 3.88 |
| HS94 | 5 | 5 | 0.84 | 0.93 | 4.20 |
| HS21 | 7 | 7 | 0.79 | 0.91 | 5.53 |
| HS233 | 4 | 4 | 0.94 | 0.98 | 3.76 |
| CUSSR29 | 18 | 18 | 0.51 | 0.55 | 9.18 |
| CUESTSSR02 | 16 | 16 | 0.65 | 0.85 | 10.4 |

PA: Number of Polymorphic Alleles, EMR: Effective Multiplex Ratio; PIC: Polymorphic Information Content, He: Expected Heterozygosity MI: Marker Index

***Determination of genetic distance between genotypes***

The genetic relationship of 25 different sesame genotypes, including 16 cultivated cultivars in Türkiye, six local populations, two populations of Indian origin, and an Indian population introduced to the ecological conditions of Central Anatolian, was determined using UPGMA cluster analysis based on 10 different SSR markers, as presented in Figure 2. Dendrograms based on the Jaccard coefficient are effective tools for visualizing the distribution of genetic material within populations and for developing strategic plans for the conservation of genetic resources (Powell et al., 1996).

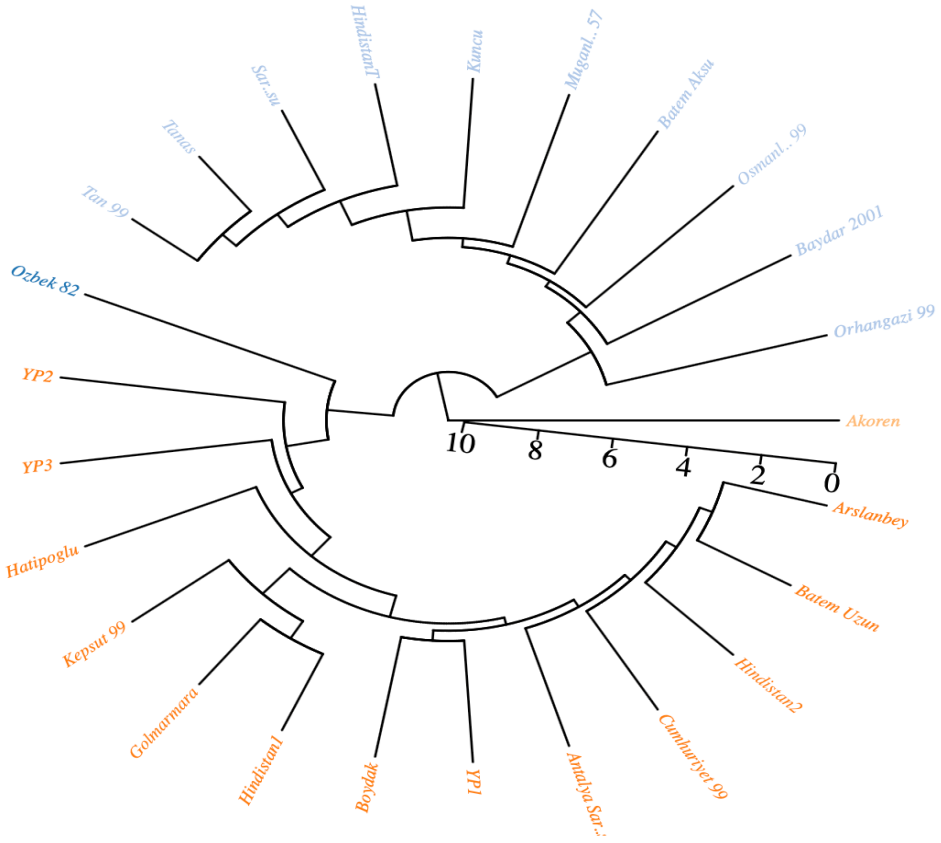


Figure 3. Circular dendrogram showing the genetic relationship between 25 different sesame genotypes
*Şekil 3. 25 farklı susam genotipi arasındaki genetik ilişkiyi gösteren dairesel dendogramı*

According to the constructed dendrogram, the 25 genotypes were divided into three main branches, which were further subdivided into four distinct sub-branches. In the first group, only the Özbek 82 cultivar was present, while the second group included YP[2], YP[3], Hatipoğlu, Kepsut 99, Golmarmara, Hindistan[1], Boydak, YP[1], Antalya Sarısı, Cumhuriyet 99, Hindistan2, Batem Uzun, and Arslanbey. The cultivars obtained from the GAP Agricultural Research Institute were grouped together in the second cluster. In the third group, only the Akören population was present, whereas the fourth group consisted of Orhangazi 99, Baydar 2001, Osmanlı 99, Batem Aksu, Muganlı 57, Küncü, HindistanT, Sarısu, Tanas, and Tan 99. In this study, Tanas and Tan 99 were identified as the most genetically similar genotypes, whereas Özbek 82 and the Akören population exhibited the most distant genetic relationship (Figure 3). These genetic differences were also confirmed by the peak profiles generated by different primers in various genotypes. In particular, the distant positioning of Özbek 82 and the Akören population from other genotypes was supported by the unique peak patterns observed in genetic analyses (Figure 4).

The pollination biology of sesame differs from other plants and it is a species with a high rate of both self- and non-self fertilization (Andrade et al., 2014). Therefore, in the genetic relationships we obtained, plants from distant geographies may be close to each other. Yet, in close geographies, a more distant closeness was observed. This may be due to the pollination biology of the plant. Moreover, especially the fact that the Akören population had a more distant genetic structure than all other genotypes, it could be interpreted that it may have developed in isolation from other cultivars in the geography where it grows. The Indian genotypes (Hindistan1, Hindistan2, and HindistanT) may play a critical role in breeding programs aimed at diversifying genetic resources, expanding genetic variation, and developing new cultivars. Local populations, on the other hand, are valuable for breeding programs

as they exhibit traits associated with regional adaptation processes (Williams & Hoffman, 2009). It can be valuable for breeding programs aimed at preserving specific traits, whereas groups with low similarity ratios are crucial for efforts to enhance genetic diversity. The inclusion of genotypes with low similarity ratios in breeding programs can contribute to an increase in heterozygosity in new cultivars, thereby improving agricultural performance (Powell et al., 1996). These findings align with the literature emphasizing the critical role of genetic diversity in the success of breeding programs. A study by Ashfaq et al. (2024) observed significant genetic diversity among sesame genotypes and highlighted the importance of utilizing this diversity in breeding programs. This underscores the fact that high genetic diversity serves as a strategic resource for developing new cultivars. Similarly, Emon et al. (2023) identified high genetic diversity among sesame genotypes using SSR markers and emphasized the potential of these genotypes for developing high-yielding cultivars.

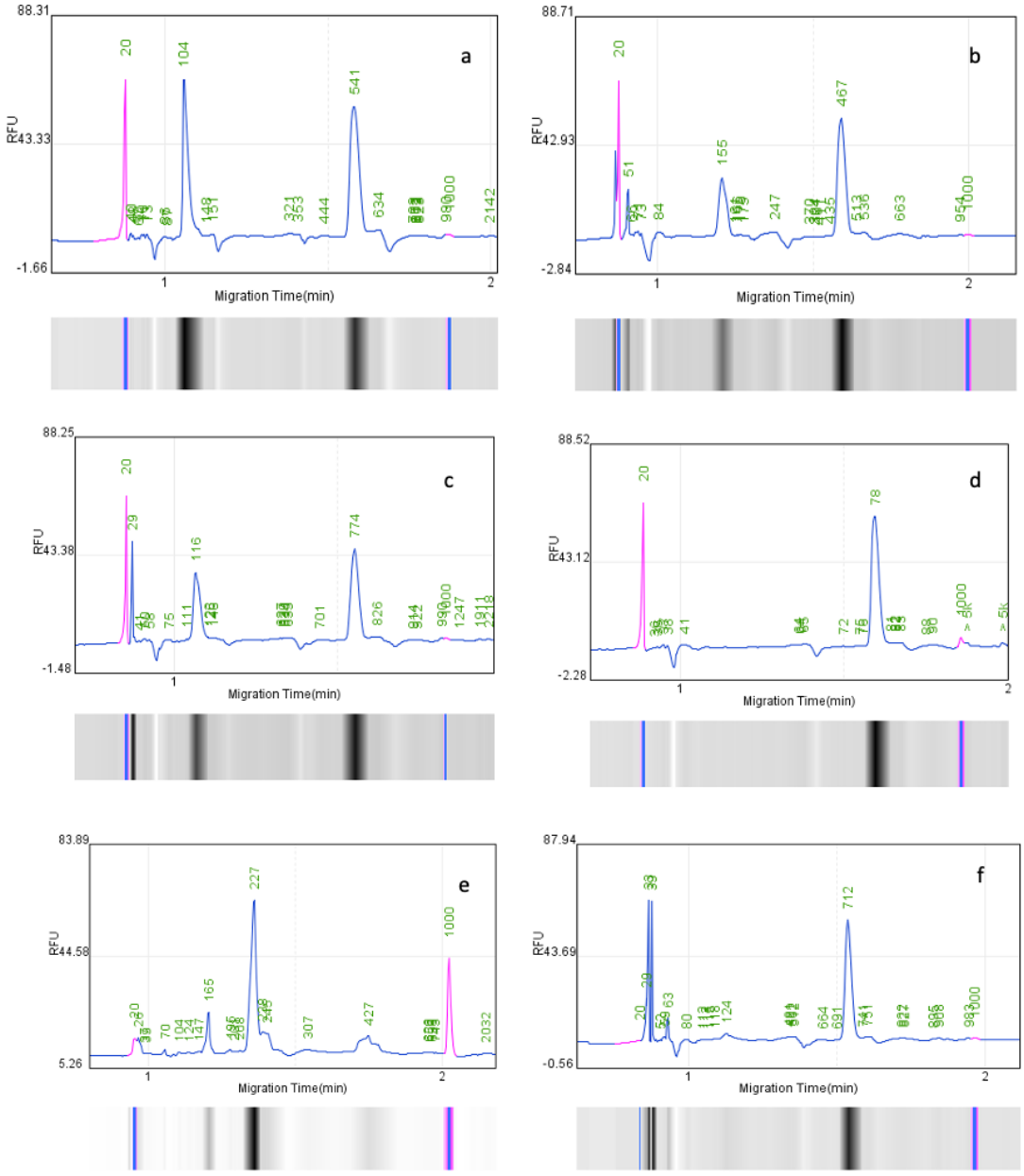


Figure 4. Electrophoresis peak profiles of different primers in various genotypes: a) ZMM5358 b) HS21 c) HS94 d) ZMM3312 e) CUESSTSSR02 f) HS233.

*Şekil 4. Farklı primerlerin farklı genotiplerde elektroforez pik görüntüleri; a) ZMM5358 b) HS21 c) HS94 d) ZMM3312 e) CUESSTSSR02 f) HS233*

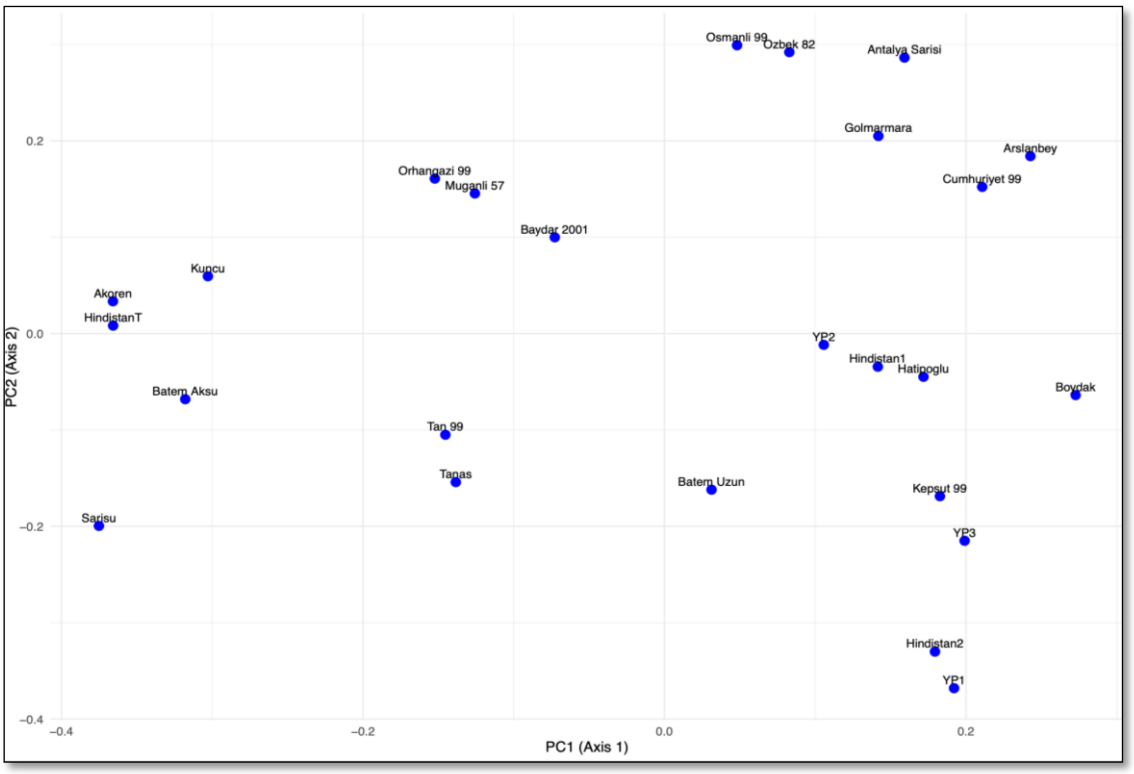


Figure 5. Relationship between 25 sesame genotypes based on principal coordinate analysis (PCoA)
*Şekil 5. Temel koordinat analizi (TKoA) ile 25 susam genotipi arasındaki ilişki*

The PCoA analysis provided a clearer visualization of genetic relationships within a spatial framework (Figure 5). The dendrogram distinctly delineates the boundaries between clusters and categorizes genotypes into well-defined groups. It can be stated that the results we obtained are compatible with the PCoA results.

The heatmap constructed based on Jaccard similarity coefficients (Figure 6) provides numerical support for the dendrogram and PCoA analyses, confirming the pairwise similarity ratios among genotypes in percentage terms. The *Hindistan*$^{1}$ and *Hindistan*$^{2}$ genotypes exhibited 90% genetic similarity, and this result clearly demonstrated that they formed a homogeneous cluster. Among the local populations, the *YP*$^{2}$ and *YP*$^{3}$ genotypes also showed over 95% similarity, indicating a very close genetic relationship. In contrast, the *YP*$^{1}$ genotype shared approximately 80% genetic similarity with *Boydak*, while its similarity to *YP*$^{2}$ and *YP*$^{3}$ remained at around 70–75%, suggesting that *YP*$^{1}$ is genetically closer to *Boydak* but relatively distinct from *YP*$^{2}$ and *YP*$^{3}$.

When examining the genetic similarity of local populations to other genotypes, lower similarity ratios are evident compared to the Indian populations (*Hindistan*$^{1}$ and *Hindistan*$^{2}$). For instance, *YP*$^{2}$ and *YP*$^{3}$ exhibit a genetic similarity of approximately 65–70% with *Hindistan*$^{1}$ and *Hindistan*$^{2}$, confirming that the local populations have genetically diverged from the Indian populations and form a distinct group. Notably, the *Boydak* genotype acts as a bridge population due to its genetic proximity to the Indian populations. Meanwhile, the Akören genotype exhibits less than 30% genetic similarity with all other genotypes, standing out as a distinctly different and isolated population.

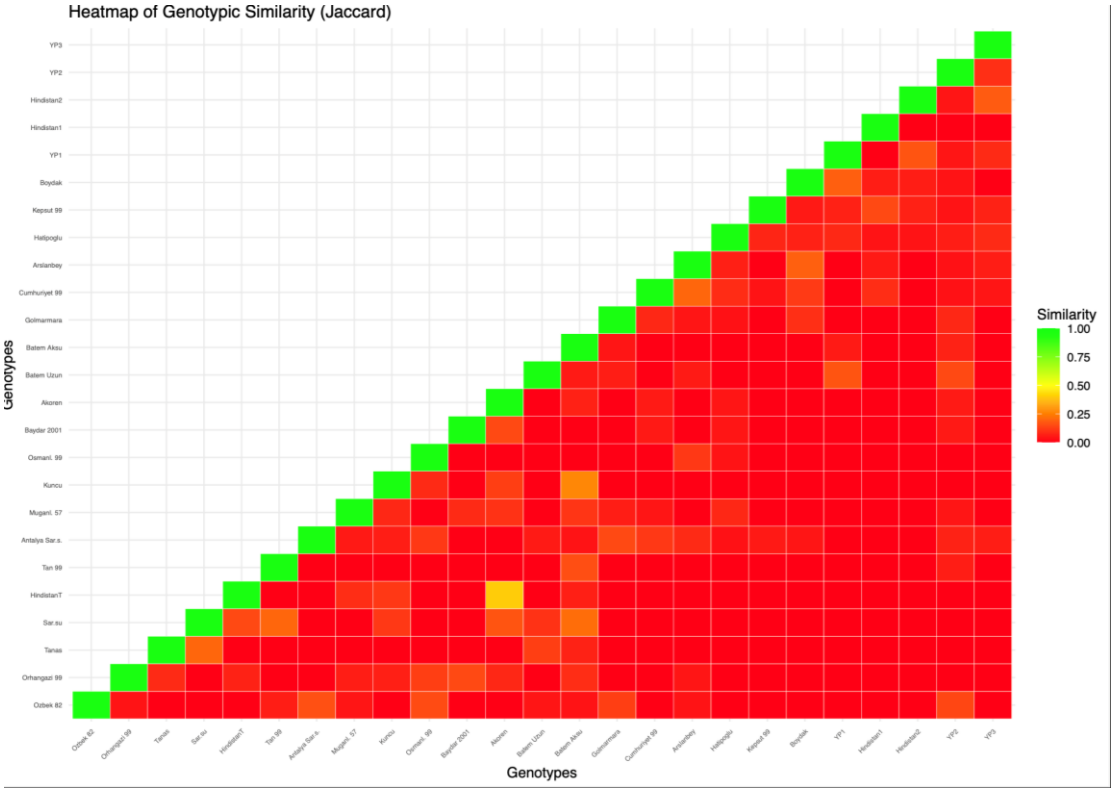


Figure 6. Heat map showing the similarity between 25 different genotypes

*Şekil 6. 25 farklı genotip arasındaki benzerliği gösteren ısı haritası*

This heatmap supported the findings of the PCoA and dendrogram analyses, consistently visualizing the genetic diversity and relationships among genotypes. These findings provided valuable insights for genetic conservation and breeding programs. Furthermore, the heatmap offers a detailed confirmation of the previous dendrogram and PCoA analyses, reinforcing the observed genetic relationships.

Different visualization methods help in understanding various dimensions of genetic data. As observed in the case of Akören, while the dendrogram provided an overall grouping, the PCoA and heatmap highlighted finer differences. This underscores the importance of utilizing multiple methods in genetic analyses, as it allows for a more comprehensive representation of genetic similarities and differences.

***The role of local populations in genetic diversity***

Local populations play a critical role in the conservation of genetic resources and the enhancement of genetic diversity in breeding programs. In this study, local populations collected from various locations in the Central Anatolian, Türkiye, where sesame cultivation is widespread (*Küncü, Akören, Antalya Sarısı, YP*$^{1}$*, YP*$^{2}$*, YP*$^{3}$), emerged as a distinct group in the dendrogram and other analyses, highlighting their significant role in genetic diversity. Compared to Indian populations and registered cultivars, local populations exhibited clear genetic differentiation and formed a separate group. The dendrogram and heatmap analyses revealed that registered cultivars possess a narrower range of genetic variation, whereas local populations exhibited broader genetic diversity. This could be attributed to the long-term adaptation processes of local populations to the environmental conditions of their respective regions, leading to the development of distinct genetic characteristics. Indian populations, particularly in the PCoA and heatmap analyses, formed a genetically homogeneous group while remaining distinctly separate from local populations. This finding suggested that Indian genetic material represents a different genetic background and that its integration with the rich genetic diversity of Turkish local populations could create significant synergies in breeding programs. In this context, associating the genetic characteristics of local and Indian

populations with agricultural performance in future studies could provide valuable insights for more targeted breeding and adaptation efforts. The importance of local populations as genetic resources has been frequently emphasized in the literature. For instance, a study by Anggraeni et al. (2022) using ISSR markers highlighted the critical role of local populations in the conservation of genetic resources. The study emphasized that local populations are a strategic resource in breeding programs due to their potential for adapting to environmental variability and enhancing genetic diversity. Similarly, Asekova et al. (2018) investigated the contribution of local sesame populations to genetic diversity using SSR markers and stated that the conservation of local genotypes is essential for future breeding programs. Additionally, studies by Liang et al. (2021) and Emon et al. (2023) underscored the potential of local populations collected from different ecological regions in developing genotypes resistant to environmental stress factors. These studies highlighted that increasing heterozygosity through hybridization programs involving local populations is a crucial strategy for improving agricultural yield and quality by developing stress-tolerant cultivars. Local populations play a vital role in preserving genetic diversity and ensuring agricultural sustainability. The findings of this study clearly demonstrate the unique genetic characteristics of local populations and their potential contributions to breeding programs.

In this study, the genetic diversity of a total of 25 different sesame (*S. indicum* L.) genotypes was analyzed using 10 species-specific SSR markers. These genotypes included 16 registered Turkish sesame cultivarscultivated in Türkiye, six local populations obtained from farmers in various districts of Konya and Antalya, two Indian populations obtained from local farmers in India, and an Indian population introduced to the ecological conditions of Konya. The results revealed significant genetic differences among the genotypes and highlighted the potential of local populations in preserving and enhancing genetic diversity.

Genetic relationships among the genotypes were thoroughly assessed through complementary analyses such as UPGMA and circular dendrograms, Principal Coordinate Analysis (PCoA), and heat maps. These analyses provided crucial insights into the percentage-based determination and visualization of genetic diversity. The analyses indicated that registered cultivars such as Baydar 2001, Cumhuriyet 99, and Osmanlı 99 are genetically closer to each other, exhibiting narrower genetic variation. Conversely, some registered cultivars, such as Muganlı 57, showed slight genetic differentiation from this group, although still generally clustering within the genetic structure of the registered cultivars. The limited genetic diversity observed among the registered cultivars suggests that these genotypes may have originated from a narrower genetic base.

The local populations demonstrated a distinct genetic structure, differentiating from registered cultivars and Indian genotypes. Particularly, $YP^2$ and $YP^3$ genotypes displayed high genetic similarity, forming a homogeneous group. In contrast, local populations such as Akören exhibited genetically isolated structures, indicating unique genetic characteristics. Indian genotypes formed a genetically close group but showed clear differentiation from both registered cultivars and local populations of Türkiye.

These findings suggest that the broad genetic diversity of local populations provides a strong foundation for improving registered cultivars and developing more resilient and productive cultivars with the contributions of Indian genotypes. The adaptation processes of local populations to different environmental conditions hold strategic importance for conserving genetic diversity and developing cultivars tolerant to environmental stress factors. In the future, examining the relationships between these genotypes and their agronomic performance traits could offer a significant roadmap for sustainable agriculture and breeding programs.

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**STATEMENT OF CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest among them.

**AUTHOR'S CONTRIBUTIONS**

The contribution of the authors is equal.

**STATEMENT OF ETHICS CONSENT**

Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects.

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