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THE USE OF MINISATELLITE MARKERS FOR DNA FINGERPRINTING IN *Nigella sativa* L. (BLACK CUMIN)

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Abstract: In this study, 10 genotypes, including diverse Nigella accessions collected from different regions of Turkey, were evaluated for genetic diversity using minisatellite (URP/DAMD) markers. Genomic DNA was isolated using the modified CTAB method, and PCR amplifications were performed with a total of 20 minisatellite primers. The obtained band profiles were scored in 1/0 format; band number (N), Polymorphism Information Content (PIC), Nei–Li similarity coefficient, UPGMA dendrogram, and PCoA analyses were applied. All primers used produced amplification, with band numbers ranging from 4 to 8. PIC values were determined to be in the range of 0.080–0.253, and the average PIC value was calculated as 0.173. These results indicate that minisatellite markers provide a moderate level of polymorphism in the differentiation of *Nigella* genotypes. Nei–Li similarity coefficients revealed high similarity values (0.92–0.97) among N. sativa populations; however, N. damascena was determined to be genetically distinct from N. sativa (0.39–0.43). UPGMA dendrogram and PCoA results supported the Nei–Li similarity analysis; N. sativa populations clustered together, while N. damascena formed a distinct genetic group on its own. This confirms that minisatellite markers are effective in determining both intraspecific and interspecific genetic differentiation. Furthermore, N. sativa populations were found to be highly similar to each other, suggesting that the gene pool may be relatively narrow. Overall, minisatellite markers are reliable tools for the genetic evaluation of *Nigella* species, and limited genetic variation was detected in the material studied. This study is one of the first examples of the use of minisatellite markers in *Nigella* species, providing important information for genetic resource management and breeding programs.

Keywords: *Nigella sativa*, Minisatellite markers (URP/DAMD), Genetic diversity, UPGMA/PCoA

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1. Introduction

The Nigella (Ranunculaceae) genus includes species cultivated across wide geographical areas due to their medicinal and aromatic properties (Alberts et al., 2024). In particular, Nigella (black cumin) is considered an important species today, both economically and pharmacologically, due to the use of its seeds as a spice and in traditional medicine (Gürel and Polat, 2025). Black cumin has a wide distribution, primarily in the Middle East, Southern Europe, and North Africa. Turkey is considered one of the important centers of genetic diversity for this species (Gürel and Polat, 2025). N. damascena L. is known as an ornamental plant and is morphologically similar to N. Sativa (Jabbour et al., 2015; Dönmez, 2021). This close taxonomic relationship makes it important to distinguish between species and determine genetic diversity at the population level. Due to the similar morphological characteristics of the species, classical taxonomic distinctions are often insufficient; therefore, molecular markers are used as more reliable tools for establishing genetic relationships (Dönmez, 2021).

The sustainable use of plant genetic resources and the success of breeding programs depend on the accurate determination of genetic diversity among populations (Govindaraj et al., 2015; Salgotra and Chauhan, 2023; Eren et al., 2023). DNA-based molecular markers have long been widely used to assess both intraspecific and interspecific variation (Eren et al., 2023; Bidyananda et al., 2024; Eren, 2025). In recent years, advances in marker systems and PCR-based fingerprinting approaches have further strengthened the efficiency of genetic diversity analyses. Among these markers, minisatellite-based DAMD (Directed Amplification of Minisatellite DNA) and URP (Universal Rice Primers) markers are preferred due to their high level of polymorphism, reproducibility, short laboratory process, and cost-effectiveness (Karaca and Ince, 2008; Ince and Karaca, 2012; Rezaee Danesh et al., 2022; Saghir et al., 2022).

Among DNA-based marker systems, SSRs are co-dominant and provide allele-specific information with high resolution for population genetic analyses (Gupta et al., 1999), whereas ISSR markers generate dominant

multilocus banding patterns and are widely used for rapid diversity screening (Zietkiewicz et al., 1994). Minisatellite-based fingerprinting systems such as DAMD (Directed Amplification of Minisatellite DNA) and URP (Universal Rice Primers) similarly produce multilocus profiles but are particularly valued for their high reproducibility, broad genome coverage, and cost-effective laboratory workflow (Heath et al., 1993; Kang et al., 2002; Karaca and İnce, 2008; İnce and Karaca, 2012). Therefore, URP/DAMD markers were preferred in the present study because they provide robust DNA fingerprints suitable for distinguishing closely related Nigella genotypes and for evaluating both intraspecific diversity and interspecific differentiation.

Mini-satellite markers target repetitive sequences in the genome, enabling them to reveal genetic differences with high resolution and are used to create DNA fingerprints in many species (Jeffreys et al., 1985; Alexandrov and Romanov, 2024). This method provides reliable results in areas such as determining the genetic similarities and differences of populations collected from different locations, correctly classifying breeding material, and revealing phylogenetic relationships (Debenham, 2002; Latif et al., 2011; Limborska et al., 2012).

Although various morphological, physiological, and chemical characterization studies on Nigella sativa and N. damascena exist in the literature, DNA fingerprinting studies using minisatellite markers appear to be limited (Orooji et al., 2022). In particular, DAMD/URP-based minisatellite analyses have rarely been performed on Nigella species, making it essential to evaluate genetic diversity using these markers (Mehri et al., 2022).

In this context, the main objective of the study is to determine the genetic diversity of N. sativa populations collected from Turkey, the registered 'Çameli' variety, and the N. damascena species using minisatellite markers. To this end, 20 different minisatellite primers were tested; the Nei–Li similarity coefficient was calculated based on band profiles, genetic relationships between populations were revealed using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering analysis, and these relationships were graphically supported by PCoA (Principal Coordinate Analysis) analysis. The findings will contribute to the conservation of Nigella genetic resources, the understanding of phylogenetic relationships, and breeding programs.

2. Materials and Methods

2.1. Plant Material, Conditions, and DNA Isolation

The study utilized a total of nine Nigella sativa genotypes (eight local landraces and one registered commercial variety) and one Nigella damascena genotype. Seven local N. sativa varieties are traditionally cultivated by farmers in different provinces of Türkiye (Amasya, Burdur, Eskişehir, Konya, Tokat, Şanlıurfa, and Samsun) as village-type local varieties. In addition, a local Syrian N. sativa variety was obtained from Syrian-origin material cultivated by local farmers. The registered N. sativa variety and N. damascena genotype were obtained from commercial seed sources and are currently used in cultivation.

Ten seeds from each genotype were grown under controlled conditions (50% relative humidity, 25–30 °C) under a 16 h light/8 h dark photoperiod (Celik and Aydın, 2023). After germination, the seeds were collected in bulk when they had 5-6 leaves and homogenized with liquid nitrogen in 2.0 mL Eppendorf tubes. DNA isolation was performed according to Aydin et al. (2018) with modifications.

DNA quality and quantity were determined using a NanoDrop spectrophotometer (Maestrogen, MN-913) and 1% (w/v) agarose gel electrophoresis. DNA purity was evaluated based on A260/280 ratios, and only samples with ratios between 1.8 and 2.0 were used for downstream PCR analyses.

2.2. Touch-Down DAMD-PCR

The Touch-down polymerase chain reaction (PCR) method was used to amplify minisatellite DNA regions. PCR reactions were adjusted to a volume of 25 µL and performed in a solution containing 85 nanograms of total genomic. PCR amplification was performed in a reaction mixture containing DNA template, 2.4 µM primer, 0.28 mM of each dNTP, 2.5 mM $MgCl_2$, 1 U Taq DNA polymerase, and 2.5 µL of Taq Buffer (Thermo Fisher Scientific, USA; Cat. No. EP0402). The Td-DAMD-PCR conditions were as follows: initial denaturation at 94°C for 3 min; 10 touchdown cycles of 94 °C for 30 s, annealing starting at 55°C and decreasing by 0.5 °C per cycle for 30 s, and extension at 72 °C for 2 min; followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 2 min; with a final extension at 72 °C for 10 min (İnce and Karaca, 2012). The touchdown annealing range (55–50 °C) was selected to improve amplification specificity and reduce non-specific products, as previously described for DAMD-PCR protocols (İnce and Karaca, 2012). The final extension step was included to ensure complete polymerization and fill-in of the amplified products (İnce and Karaca, 2012).

2.3. Agarose Gel Electrophoresis of PCR Products

PCR products were mixed with 6× loading dye and separated on a 2% agarose gel. RedSafe (according to the instructions) was added to the prepared agarose gel, and the agarose gel was run in 1x TBE (Tris-Borate-EDTA) solution at 5 V/cm for 2-6 hours. Images were recorded every 2 hours during electrophoresis, and the gel was visualized when the bands were clearly visible for more detailed analysis.

2.4. Scoring and Diversity Analysis

After PCR, bands in the gel images were scored as present (1) or absent (0) for each minisatellite primer. Polymorphic Information Content (PIC) was calculated for each marker according to Smith et al. (1997) based on the pattern it formed among varieties.

The presence/absence matrix was used for all similarity-based analyses, and genetic relationships were inferred

using the Jaccard similarity coefficient. Accordingly, Principal Coordinate Analysis (PCoA) was performed based on the Jaccard similarity matrix using the Multi-Variate Statistical Package (MVSP 3.13O, Kovach Computing Services, Pentraeth, UK). A Distant Neighbor dendrogram was generated using Jaccard genetic similarity indices in MVSP (Kovach, 2007). In addition, the heat map was constructed based on the Jaccard similarity matrix to visualize genetic relationships among genotypes. The reliability of the dendrogram was evaluated using parsimony analysis in PAUP version 4.0 with 10,000 bootstrap replicates (Elmasulu and Karaca, 2020). In addition, Bayesian inference was performed using MrBayes to further support the robustness of the clustering pattern. Bootstrap and Bayesian support values were used to assess the robustness of the clustering topology (validation), rather than serving as direct evidence for biological interpretation.

3. Results

3.1. Minisatellite primer performance

All 20 minisatellite primers used in the study amplified and were evaluated (Figure 1). The total number of bands differed among primers; the lowest number of bands was 4, and the highest number of bands was 8. The primers producing the most bands were 33.6 and URP38F (8 bands), while the primers producing the fewest bands were HBV-5, PVII-C, and URP-13R (4 bands) (Table 1).

The highest PIC was obtained with the URP-1F (0.253) primer. This primer was followed by the URP-4R (0.236) and URP-13R (0.226) primers. PIC values generally ranged from 0.080 to 0.253, indicating low to moderate polymorphism among the tested minisatellite primers. Nevertheless, the markers provided sufficient resolution to detect genetic variation among the Nigella genotypes included in this study.

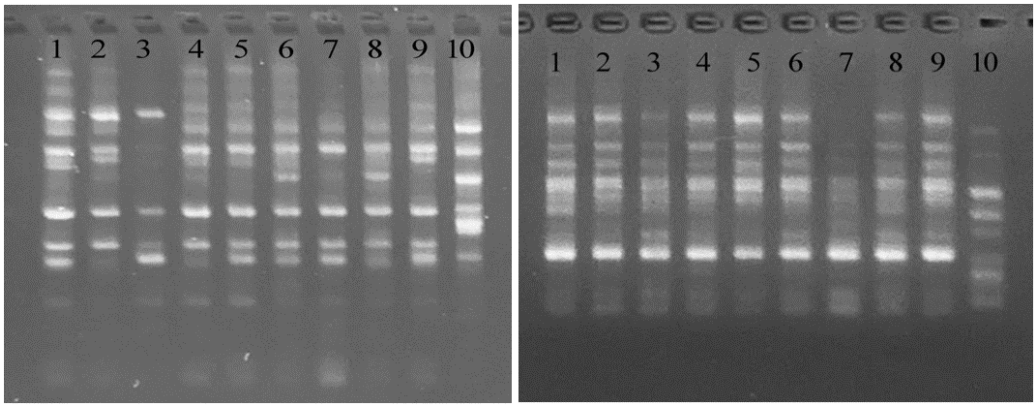

Figure 1. Gel image of the 14C2 and URP38F primers.

Table 1. Minisatellite primers used in the study and their amplification performance

No	Primer*	5→3' Sequence	N_amplicons	PIC
1	14C2	GGCAGGATTGAAGC	6	0.205
2	HBV-3	GGTGAAGCACAGGTG	5	0.227
3	HBV-5	GGTGTAGAGAGGGGT	4	0.185
4	PVII-8	ATGCACACACACAGG	6	0.141
5	PVII-C	CCTGTGTGTGTGCAT	4	0.165
6	YNZ-22	CTCTGGGTGTGGTGC	7	0.151
7	33.6	GGAGGTGGGCA	8	0.135
8	6.2(-)	CCCTCCTCCTCCTTC	6	0.173
9	6.2(+)	AGGAGGAGGGGAAGG	5	0.169
10	URP-1F	ATCCAAGGTCCGAGACAACC	5	0.253
11	URP-2F	GTGTGCGATCAGTTGCTGGG	6	0.135
12	URP-4R	AGGACTCGATAACAGGCTCC	6	0.236
13	URP-6R	GGCAAGCTGGTGGGAGGTAC	7	0.080
14	URP-9F	ATGTGTGCGATCAGTTGCTG	5	0.160
15	URP-13R	TACATCGCAAGTGACACAGG	4	0.226
16	URP-17R	AATGTGGGCAAGCTGGTGGT	7	0.111
17	URP25-F	GATGTGTTCTTGGAGCCTGT	7	0.209
18	URP-30F	GGACAAGAAGAGGATGTGGA	5	0.150
19	URP-32F	TACACGTCTCGATCTACAGG	7	0.156
20	URP38F	AAGAGGCATTCTACCACCAC	8	0.147
Means			5.9	0.170

*Detailed information about the sources of primers was puplished in Ince et al. (2009), (N_amplicons: total number of amplified bands).

The average PIC value among the primers used in the study was 0.170, indicating that the minisatellite markers exhibited low information content (PIC<0.25) for discriminating Nigella populations. Nevertheless, the marker set provided useful and reproducible polymorphic banding patterns that enabled the detection of genetic variation among the studied genotypes. A general association was observed between the number of amplified bands and PIC values, and most primers producing a high number of bands showed low to moderate PIC values. Similar PIC ranges and marker informativeness levels have also been reported in dominant marker–based fingerprinting studies, indicating that such marker systems can still provide informative polymorphic profiles depending on the germplasm and sampling scale (Botstein et al., 1980; Rezaee Danesh et al., 2022).

3.2. Nei–Li Similarity Matrix and Genetic Relationships Between Populations

The Nei–Li similarity coefficient values calculated among Nigella populations ranged from 0.39 to 0.97. A high level of similarity was observed among Nigella sativa populations; intraspecific similarity values generally ranged from 0.92 to 0.97. The highest similarity value was determined between the Eskişehir and Çameli populations (S=0.97), followed by the Konya–Tokat, Amasya–Çameli, Amasya–Konya, and Samsun–Tokat population pairs (S≈0.95–0.96). The high intraspecific similarity values suggest that the N. sativa populations used in the study are genetically very close to each other and that the gene pool may be narrow. In contrast, low levels of differentiation were also detected between populations. Similarity values between the Nigella damascena sample and N. sativa populations were found to be significantly lower. The inter-species similarity coefficient ranged from 0.39 to 0.43, with the lowest value recorded for the Amasya–Nigella damascena pair (S=0.39). This indicates that N. damascena is genetically distinct from N. sativa.

The findings show that the Nei–Li similarity coefficient is effective in distinguishing between Nigella species and that the minisatellite markers used provide sufficient polymorphism to reveal genetic variation. Furthermore, the high similarity (0.94–0.97) of the registered Çameli variety with other N. sativa populations indicates its close genetic relationship with local populations. These results are supported by the lower triangular similarity heat map presented in Figure 2, confirming that N. sativa populations show high similarity to each other, while N. damascena is clearly separated.

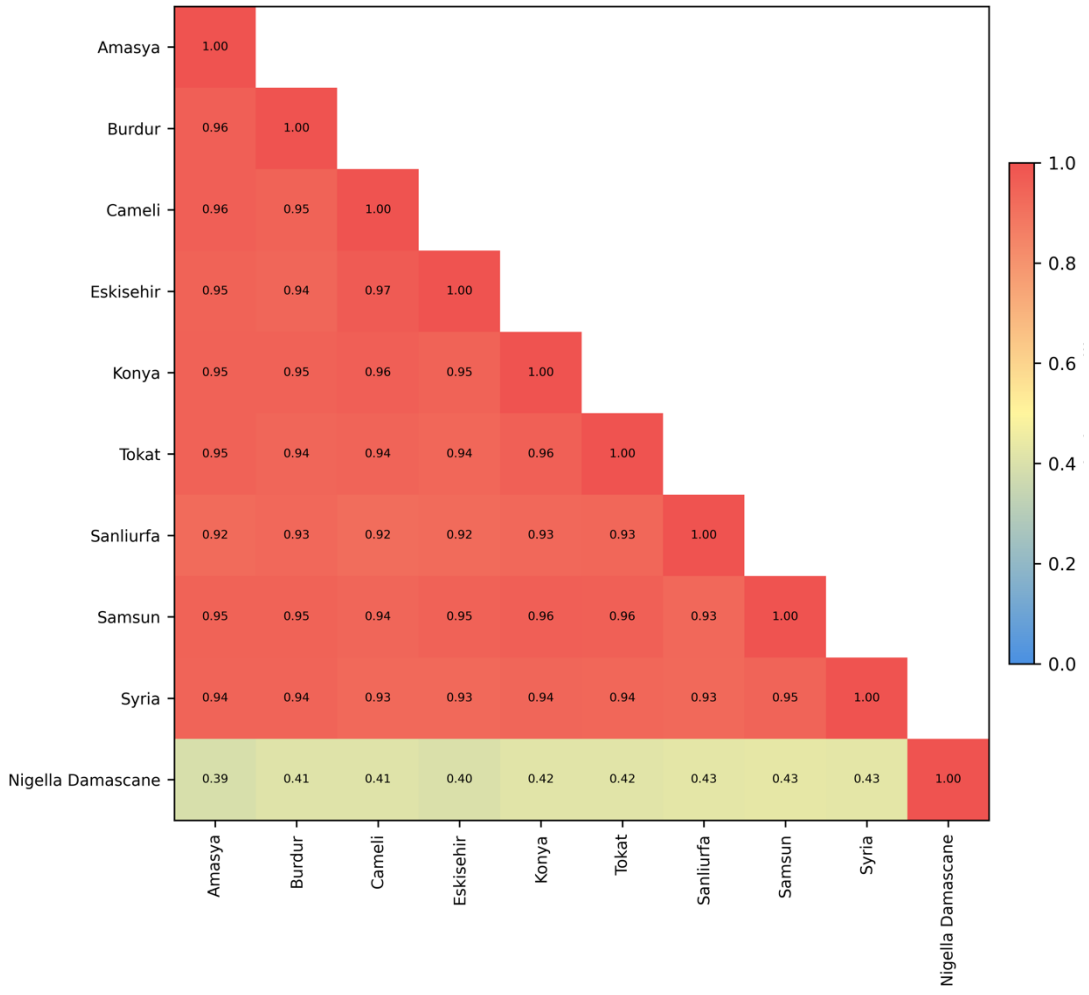

Figure 2. Heat map showing the genetic relationships of Nigella samples based on the Nei–Li similarity coefficient obtained from minisatellite markers.

3.3. Genetic Relationships Based on UPGMA According to Minisatellite Data

The dendrogram created according to the UPGMA method clearly reveals the genetic relationships of the Nigella populations studied (Figure 3). The dendrogram structure shows that Nigella sativa populations are genetically very close to each other; in contrast, Nigella damascena is distinctly separated. The N. damascena sample formed an independent branch in the dendrogram and did not share a cluster with N. sativa accessions. This supports the Nei–Li similarity analysis results, indicating that this species is genetically distinct from N. sativa.

Although the N. sativa genotypes formed a single main cluster, they exhibited clear sub-structuring. The closest relationships were observed between Amasya and Burdur, Çameli and Eskişehir, Konya and Tokat, and Samsun and Syria. Şanlıurfa was placed as a separate branch within the N. sativa cluster, joining the other N. sativa subgroups at a higher level. In contrast, Nigella damascena was clearly separated from all N. sativa genotypes, indicating a markedly lower similarity.

This pattern of differentiation demonstrates that minisatellite markers have sufficient resolution to reveal genetic differences between Nigella populations. Furthermore, there is a partial correlation between geographical origin and genetic similarity, but in some cases, this relationship is not explanatory. This situation points to the possible effects of seed/plant material exchange and common selection processes among populations.

When the results are evaluated together, the formation of a separate branch for N. damascena in the UPGMA dendrogram clearly demonstrates the inter-species distinction; the minimal genetic differentiation within N. sativa indicates that the genotypes studied may have a narrow gene pool.

3.4. Genetic Differentiation of Nigella Populations Using Principal Coordinate Analysis (PCoA)

The PCoA scatter plot indicates a clear first-axis differentiation among Nigella samples (Figure 4). Along PCo1, the Nigella damascena sample strongly separates to the left (negative direction), while all *N. sativa* populations cluster on the right side (positive direction). This indicates that the primary axis captures the genetic differentiation between species and that *N. damascena* is clearly distinct from *N. sativa* populations.

The PCo2 axis reflects the relative variation within N. sativa. The Sanlıurfa and Syria samples are positioned at more negative values on PCo2, diverging downward from the group, while Burdur and Tokat are located in the middle section of the axis, Amasya is in the center, and Konya–Samsun are quite close to each other. The fact that the registered Çameli variety and the Eskişehir sample are located in the upper region (positive PCo2) suggests that these two genotypes indicate a subgroup that is co-located compared to other populations. In general, the tight distribution of N. sativa populations on the right side indicates a high level of intraspecific genetic relatedness, while the vertical spread in PCo2 shows that there is limited but detectable differentiation between populations.

This pattern obtained from the PCoA result is fully consistent with the Nei–Li similarity heat map and UPGMA dendrogram: N. damascena is confirmed to be separated at the species level, while N. sativa populations show a high degree of similarity with each other.

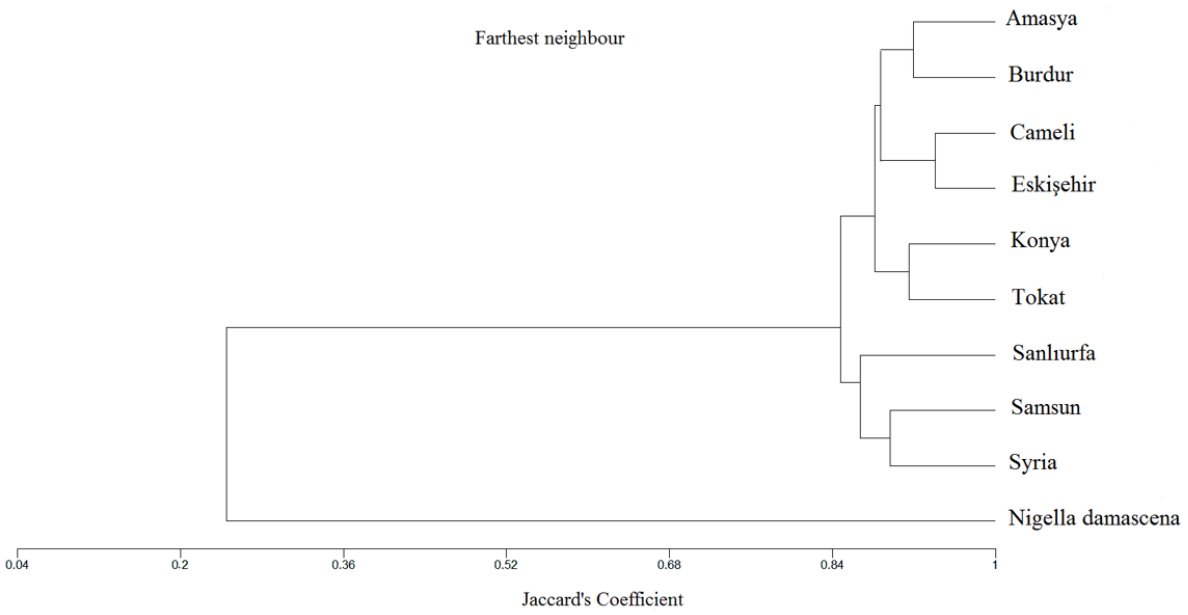

Figure 3. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram showing genetic relationships among Nigella genotypes based on the Jaccard similarity coefficient. The x-axis represents Jaccard's similarity coefficient (0–1), where higher values indicate greater genetic similarity.

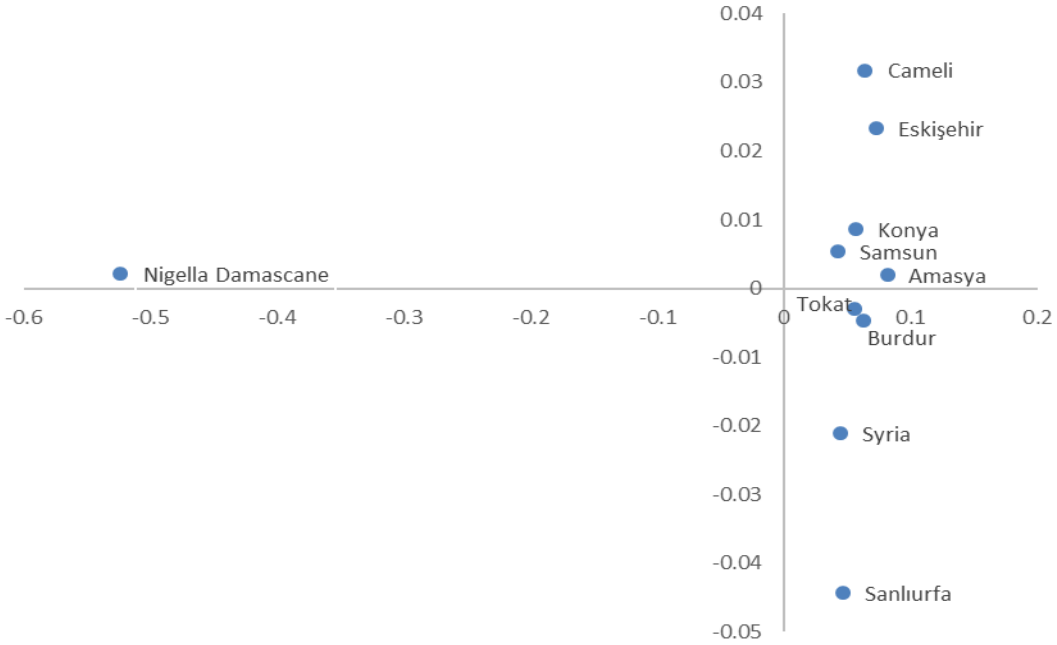

Figure 4. Principal Coordinate Analysis (PCoA) plot showing genetic relationships among Nigella genotypes.

4. Discussion

Overall, the minisatellite-based URP and DAMD marker systems provided reproducible banding patterns and enabled the detection of genetic variation among the studied Nigella genotypes. These findings contribute to the molecular characterization of Nigella genetic resources and may support germplasm conservation strategies and future breeding programs. Further studies including larger sample sizes and complementary marker systems may provide a more comprehensive understanding of genetic diversity in Nigella.

The number of amplified bands (N) and PIC values obtained in the initial evaluation suggest that the minisatellite markers provided informative polymorphic profiles for the Nigella material studied. Although most PIC values were low (PIC<0.25), a few primers showed comparatively higher PIC values (e.g., URP-1F=0.253; URP-4R=0.236), indicating that these primers were more informative within this dataset. In studies using minisatellite or URP markers in the literature, PIC values are generally reported as 0.2 and above, indicating a similar level (Mostafavi et al., 2021; Ibrahimi et al., 2023). On the other hand, low PIC values have been observed in some primers (e.g., URP-6R=0.080); this indicates that this primer offers limited variation in the studied populations and should be reevaluated during the selection phase.

Minisatellite-based marker systems such as DAMD and URP provide a rapid, cost-effective, and reproducible PCR-fingerprinting approach for genetic diversity assessments, particularly useful for preliminary screening of germplasm collections. Compared with co-dominant SSR markers, which allow allele-specific genotyping and provide higher resolution for population structure, heterozygosity, and allelic richness, DAMD/URP markers are dominant and therefore yield less detailed allelic information. Nevertheless, DAMD-PCR has been shown to produce reliable and informative multilocus profiles and can efficiently resolve genetic relationships in plant genetic resources (Karaca and İnce, 2008; Seyedimoradi and Talebi, 2014). Similar to ISSR markers, which also generate dominant multilocus banding patterns, DAMD/URP approaches may reveal clustering trends among accessions; however, the level of polymorphism detected can differ depending on primer motifs, genome composition, and sampling scale (Pakseresht et al., 2013). Therefore, integrating minisatellite-based fingerprinting with SSRs (or high-resolution SNP approaches) would provide a more comprehensive framework for Nigella diversity analysis and improve the effectiveness of conservation and breeding strategies.

Nei–Li similarity analyses revealed high similarity values, indicating intraspecific genetic proximity (Govindaraj et al., 2015). The fact that N. sativa populations generally showed high (>0.90) similarity values suggests that these populations have a genetically close structure. These data indicate that minisatellite markers gave a strong signal of proximity among the genotypes studied. Similarly, another study reported high similarity coefficients with minisatellite markers in Cynodon species (Karaca and İnce, 2008). However, such a high degree of genetic proximity may indicate relatively low genetic diversity among populations and a potentially limited gene pool for breeding material (Ellegren, 2004; Govindaraj et al., 2015).

In contrast, the clear differentiation of the N. damascena sample from the N. sativa group (Nei–Li=~0.39–0.43) and its placement as a separate branch in the UPGMA dendrogram indicate a distinct genetic difference between the two species (Orooji et al., 2022). This result

points to the genetic group difference between species, even though they are morphologically similar, and demonstrates that minisatellite markers are also effective in determining taxonomic differences (Karaca and İnce, 2008; Karaca et al., 2015). This aspect of the study is unique as one of the first examples of minisatellite-based genetic analysis in Nigella species. Recent SSR-based studies in Nigella sativa also support the growing use of molecular markers for genetic characterization, although minisatellite-based approaches remain scarce (Celik and Aydin, 2023).

When UPGMA clustering analysis and PCoA scatterplot are evaluated together, the clustering of N. sativa populations and the placement of N. damascena in an independent group provide a consistent genetic relationship map (Pakseresht et al., 2013). The genotype positions in PCoA are consistent with the similarity heat map and dendrogram results. Such multidimensional analyses provide important insights for genetic resource management and breeding programs using molecular data, as suggested in the literature (Guo et al., 2014).

When comparing geographic origin with genetic relationships, although some populations (e.g., Amasya–Çameli and Konya–Samsun) clustered closely, no clear overall correspondence between geographic distribution and genetic structure was observed. This situation suggests that factors such as gene flow, seed exchange, and human influence are decisive on genetic structure; a similar situation is frequently reported in minisatellite studies (He et al., 2014; Mostafavi et al., 2021).

A limitation of the present study is that interspecific comparisons involved a single N. damascena genotype. Therefore, the observed differentiation between N. sativa and N. damascena should be interpreted cautiously and may not fully represent the overall species-wide divergence. Future studies including a broader representation of N. damascena accessions from different geographic origins will be necessary to confirm and refine the patterns of interspecific differentiation. Likewise, the relatively low genetic variation observed among N. sativa samples reflects the specific set of populations analyzed in this study and should not be generalized to all Turkish N. sativa germplasm.

In conclusion, this study is the first to analyze genetic diversity in Nigella species using minisatellite markers; the findings indicate that these markers are suitable for revealing genetic relationships and diversity. However, the relatively low genetic diversity should be carefully considered in terms of conserving local populations and developing breeding material. Future studies should include different genetic resources, use more markers, and examine geographical variation on a larger scale.

5. Conclusion

This study assessed the genetic diversity of Turkish *Nigella sativa* populations together with the registered 'Çameli' variety and *Nigella damascena* using minisatellite (URP/DAMD) markers. Overall, the marker set generated reproducible fingerprints and revealed limited genetic variation among the *N. sativa* populations analyzed in this study, suggesting a relatively narrow genetic base within the sampled material. In contrast, *N. damascena* was clearly differentiated from *N. sativa*; however, this inference is based on a single *N. damascena* genotype and should therefore be interpreted cautiously.

These findings provide useful baseline information for germplasm characterization and may support conservation strategies and breeding efforts aimed at broadening the genetic diversity of black cumin in Turkey. Future studies incorporating larger collections—particularly including multiple *N. damascena* accessions from diverse origins—and complementary marker systems (e.g., SSRs and SNPs) are recommended to obtain a more comprehensive view of genetic diversity and population structure in Nigella.

Author Contributions

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

	B.E.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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