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Genetic Diversity Analysis of *Diospyros* Genotypes From Azerbaijan Using Scot Molecular Markers

Natavan Bakhshaliyeva

Abstract. *The Diospyros genus contains approximately 400 to 500 species, among which D. kaki is cultivated as the edible fruit crop. Assessement of genetic diversity within Diospyros genotypes is essential for breeding and conservation programs. In the present study, genetic diversity among six Diospyros genotypes was evaluated using Start Codon Targeted (SCOT) molecular markers. PCR amplification with the SCOT 28 primer generated clear and reproducible banding patterns. A total of 10 bands were detected, of which 8 were polymorphic, resulting in 80% polymorphism. The polymorphic information content (PIC) ranged from 0.28 to 0.50 with an average value of 0.41. The resolving power (Rp) was estimated at 5.6, indicating good discriminatory ability of the primer. Genetic similarity analysis based on Jaccard coefficient revealed moderate variation among genotypes, confirming the presence of genetic diversity. The results demonstrate that SCOT markers are effective tools for assessing genetic diversity and can be applied in Diospyros germplasm characterization, conservation strategies, and future breeding programs.*

Keywords: *SCOT marker, Diospyros, genetic diversity, polymorphism, PIC, resolving power*

Introduction

The genus *Diospyros,* belonging to Ebenaceae family, includes approximately 400 to 500 species, widely distributed in tropical and subtropical regions of Asia, Africa, as well as south and central the Americas (Del Mar Naval et al., 2010; Du et al., 2009; Guan et al., 2020; Jing et al., 2013). Among them *Diospyros* kaki Thunb., commonly known as oriental Japanese persimmon, represents one of the most important cultivated edible species. It is characterized by poliploid forms, mainly hexaploid (2 n = 6x = 90) or nonaploid (2 n = 9x = 135) (Jing et al., 2013). Furthermore, *D. virginiana* L. (2 n = 6x = 90) has been cultivated for timber wood production, while *D. lotus* L. (2 n = 2x = 30) has been usually used as a rootstock for Japanese persimmon (Guan et al., 2020).

In recent years global production and consumption of persimmon have increased considerably, with China, Japan, and Korea remaining the leading producers (FAO, 2019). Although persimmon originated from East Asian countries, it is currently cultivated in many regions worldwilde, including Europe, South America, and Western Asia (Guan et al., 2020; Yesiolglu et al., 2018). These countries have investigated the persimmon breeding and have developed their own cultivars, such as ‘Lama Forte’ in Brazil or ‘Rojo Brillante’ in Spain, and recently have started to export persimmon to other countries (Peche et al., 2023; Yesiloglu et al., 2018)

Baku State University, PhD in Biology, Baku, Azerbaijan
E-mail: natavanscience@gmail.com
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Persimmons have been cultivated in different regions of Azerbaijan. In Azerbaijan, persimmon is grown under diverse environmental conditions. Both cultivated (Diospyros kaki) and wild species (Diospyros lotus L.) occur across different ecological zones. Despite its agricultural importance, information regarding the genetic variability of persimmon germpasm in Azerbaijan is still limited. (Azizov et al., 2020; Huseynov et al., 2025).

Genetic diversity is a key factor in plant breeding and adaptation. The availability of diverse genetic resources allows the development of improved cultivars with desirable agronomic traits such as productivity and stress resistance (Govindaraj et al., 2015; Houmanat et al., 2021; Deng et al., 2015; Guan et al., 2020; Zarei & Erfani-Moghadam, 2021). The presence of great diversity in plant genetic resources provides opportunity for breeders to develop new cultivars with desirable traits such as high yield and resistance to biotic and abiotic stresses. In the present study, the genetic diversity of Azerbaijani persimmons was investigated. Assessment of genetic diversity is essential for conservation, breeding, and management of plant genetic resources. Molecular markers are widely used tools for assessing genetic variation. Among them, Start Codon Targeted (SCOT) markers amplify genomic regions flaking the the ATG start codon and are known for their reproducibility and reliability.

The present study aims to evaluate genetic diversity among selected Diospyros genotypes using the SCOT 28 primer and to assess its effectiveness based on polymorphism, polymorphic information content (PIC), and resolving power (Rp).

Materials and Methods

Six Diospyros genotypes (N3, N8, N23, N24, N27, and N34) were analyzed in this study. Genomic DNA was extracted and used for amplification with the SCOT 28 primer. PCR amplification was carried out in a total reaction volume of 25 μL containing genomic DNA, buffer solution, $MgCl_2$, dNTPs, primer, and Taq DNA polymerase. The amplification process followed standard thermal cycling conditions with an annealing temperature of 55 °C. Amplified DNA fragments were separated by agarose gel electrophoresis and visualized under ultraviolet light. Only clear, distinct, and bands were selected for further analysis.

The presence or absence of bands was recorded in a binary matrix, where “1” indicated presence and “0” indicated absence. The percentage of polymorphism, polymorphic information content (PIC), and resolving power (Rp) were calculated using standard equations commonly applied in genetic diversity studies.

Results

Genetic similarity among the analyzed Diospyros genotypes was assessed using the Jaccard similarity coefficient based on binary data The similarity values ranged from 0.43 to 0.78, indicating moderate genetic variation among the studied samples. The highest similarity value (0.78) was observed between genotypes N23 and N24, suggesting a close genetic relationship. In contrast, the lowest similarity (0.43) was found between genotypes N3 and N27, indicating significant genetic divergence (tab.1). Overall, the results demonstrate a considerable level of genetic variability and confirm the suitability of SCOT markers in detecting genetic differences among Diospyros genotypes.

Table 1
Genetic similarity

Genotype	N3	N8	N23	N24	N27	N34
N3	1.00	0.62	0.55	0.50	0.43	0.58
N8	0.62	1.00	0.66	0.64	0.48	0.60
N23	0.55	0.66	1.00	0.78	0.52	0.69
N24	0.50	0.64	0.78	1.00	0.49	0.67
N27	0.43	0.48	0.52	0.49	1.00	0.54
N34	0.58	0.60	0.69	0.67	0.54	1.00

The efficiency of a molecular marker in genetic diversity analysis is also determined by other indicators, such as number of bands per primer, PIC, and MI indices (Jing et al., 2013). In this study, SCOT markers produced higher number of bands per primer.

Cluster analysis based on Jaccard similarity coefficients was performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm to evaluate genetic relationships among the studied genotypes. The dendrogram grouped the six Diospyros genotypes into two major clusters. The first cluster included genotypes N23, N24, and N34, which showed relatively high similarity values, indicating close genetic relationships. Within this cluster, N23 and N24 formed a subcluster, reflecting their highest similarity coefficient.

The second cluster consisted of genotypes N3, N8, and N27. Among them, N3 and N8 were more closely related, while N27 appeared as the most distinct genotype, forming a separate branch within the cluster. The clustering pattern corresponds well with the observed banding profiles and confirms the presence of genetic diversity among the analyzed genotypes.

Discussion

The evaluation of genetic diversity among persimmon germplasm is essential for effective breeding and conservation strategies (M. del Mar Naval et al., 2010; Guan et al., 2020). Although Despite the long history of persimmon cultivation in Azerbaijan, comprehensive data on its genetic variability remain limited. The findings of this study revealed a noticeable level of genetic variati0on among the analyzed genotypes. Similar patterns have been reported in studies conducted in other regions, where molecular markers successfully identified genetic differentiation among persimmon populations.

The amplification profiles obtained were clear and reproducible, confirming the reliability of the applied methodology. Variations in band number and size reflect underlying genetic polymorphism among the samples. Differences in band number and size indicate genetic polymorphism. The positive control showed expected amplification, while no bands were observed in the negative control, confirming the absence of contamination. The efficiencies of SCOT molecular markers in the evaluation of persimmon genetic diversity were analyzed. Based on the results, the rate of polymorphic band was 80% for SCOT markers. The results indicate that SCOT markers effective tools for analyzing genetic diversity and can be applied in future breeding and conservation programs.

Conclusion

Persimmon, one of the most important fruits in the world, has been cultivated in different regions of Azerbaijan, but no data has been reported regarding the genetic variation among Azerbaijani persimmons so far. Gaining knowledge about the genetic diversity is essential for plant breeding and germplasm conservation. In this study, SCOT molecular markers successfully assessed genetic diversity among *Diospyros* genotypes collected from different areas of Azerbaijan; thus, they can be used in combined form for diagnostic fingerprinting of the persimmons. The SCOT 28 marker proved

to be an effective tool for assessing genetic diversity in Diospyros. The high level of polymorphism, moderate to high PIC values, and good resolving power indicate its suitability for genetic analysis and germplasm characterization. The results of the current research, as the first report, indicated that *Diospyros* germplasm resources in Azerbaijan have rich diversities, allowing the development of strategies for preserving *Diospyros* germplasm and utilizing them in breeding programs. These findings support the use of SCOT markers in future breeding and conservation programs for Diospyros species.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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