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SCoT marker-based evaluation of genetic diversity in selected walnut (*Juglans regia* L.) accessions

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Abstract

This study investigates the genetic diversity among different walnut (*Juglans regia* L.) genotypes using the SCoT (Start Codon Targeted) marker system. A total of 136 bands were obtained from 15 markers, of which 106 were polymorphic, with an average of 9.07 bands per primer. The SCoT markers yielded a polymorphism rate of 77.9%. In order to interpret the genetic distance among *Juglans regia* L. genotypes, a UPGMA dendrogram was constructed using MVSP 3.22 software. According to the resulting dendrogram, the lowest similarity (0.593) was observed between samples W6 and W26, while the highest similarity (0.970) was observed between W1 and W4, followed by 0.962 between W4 and W5. Principal Component Analysis (PCA) was also conducted using MVSP 3.22. The PCA results revealed a homogeneous distribution and wide variation. The UPGMA dendrogram and PCA analysis were consistent with each other. The SCoT analyses conducted on walnut genotypes provide highly valuable information for assessing the level of genetic diversity, understanding population structure and selecting superior individuals. Such studies contribute to the conservation of genetic resources and support the development of new cultivars with high yield, disease resistance and strong adaptation to climate change in future breeding programs. Based on this study, SCoT analyses demonstrate that the Sivas province constitutes an important genetic diversity reservoir and that walnut genotypes possess rich genetic variation.

Keywords: *Juglans regia* L., SCoT marker, Genetic diversity

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INTRODUCTION

Walnut (*Juglans regia* L.), a member of the *Juglandaceae* family, is a significant fruit species distinguished by its broad ecological adaptability and high economic value (Martínez et al., 2010). Believed to have originated in Central Asia, this species is now widely cultivated across various geographical regions around the world. Particularly notable for its wide variation in morphological and agronomic traits such as fruit shape, size, shell thickness and yield, walnut exhibits a high degree of genetic diversity. This diversity is of great importance for local adaptation, disease tolerance and the development of high-quality products (Wani et al., 2016; Hayes et al., 2016). Walnuts are consumed both fresh and dried, as well as in processed forms (e.g., walnut oil, walnut paste), making them a valuable commodity in both domestic markets and international trade (Delaviz et al., 2017; Bernard et al., 2018). In recent years, global trends toward healthy diets have led to a significant increase in walnut production and consumption. Nutritional analysis of walnuts reveals that they are a rich source of high-quality vegetable oils (up to 60%), proteins (15–20%), dietary fiber, vitamins (particularly vitamin E and B-group vitamins) and minerals (such as potassium, magnesium and phosphorus). Furthermore, walnuts contain considerable amounts of antioxidant compounds (including flavonoids, polyphenols and tocopherols), which contribute to reducing oxidative stress and preventing chronic diseases. Owing to these properties, walnuts are considered protective against cardiovascular diseases, diabetes and certain types of cancer (Vischi et al., 2017; Güney et al., 2021; Shah et al., 2023).

Globally, walnut cultivation represents a major agricultural sector and Türkiye holds a significant position in terms of both production volume and richness of genetic resources (Yıldız et al., 2021). Local walnut varieties that have evolved through natural selection and farmer selection over centuries in various regions of Türkiye possess high adaptive capacity against biotic and abiotic stress factors, owing to their genetic diversity. These local varieties serve as valuable genetic resources for modern breeding programs due to their internal quality traits and resilience (Yıldız et al., 2021). The conservation of local genotypes is crucial for sustainable walnut cultivation and the preservation of biological diversity. In addition, these genotypes offer a competitive edge in both local and international markets due to their unique flavor and aroma profiles (Tabasi et al., 2020; Yıldız et al., 2021; Xie et al., 2025). Preserving the genetic diversity of local walnut (*Juglans regia* L.) populations is essential not only for the sustainability of agricultural biodiversity but also for transmitting genetic resources to future generations and enhancing agricultural resilience against environmental challenges such as climate change. In this context, molecular characterization of local walnut genotypes plays a critical role in both conservation efforts and the identification of genetic material for breeding. Today, molecular marker technologies are widely used for the evaluation of plant genetic resources. Among these, the SCoT (Start Codon Targeted) markers have gained prominence in recent years, as they target coding regions, offering high reproducibility and polymorphism rates, thus serving as an effective tool for analyzing genetic diversity (El-Sheikh et al., 2020; Mahammadi et al., 2021; Zhang et al., 2024). SCoT markers are designed based on the universal ATG start codons found in plant genomes and provide information directly associated with functional regions of the genetic structure. Compared to random markers such as ISSR and RAPD, SCoT markers offer greater specificity and reliability and they are advantageous in reflecting genetic differences in phylogenetic relationships among varieties (Collard ve Mackill, 2009). SCoT analyses conducted on walnut genotypes provide valuable insights into genetic diversity, population structure and the identification of superior individuals. Such studies contribute to the conservation of genetic resources and support the development of new cultivars with high yield potential, disease resistance and enhanced adaptability to climate change (Pan et al., 2024; Zarinkolah et al., 2024). In this study, the genetic diversity of *Juglans regia* L. germplasm, locally grown for many years in the Divriği district of Sivas Province, was investigated using SCoT marker groups. This research aims to reveal the genetic structure of local walnut varieties in the region and to contribute to their conservation and use in future breeding programs.

MATERIALS AND METHODS

Plant Materials

Fresh leaves from 32 different walnut (*Juglans regia* L.) samples, belonging to germplasm that has long been established and adapted to the natural flora of the Divriği district in Sivas, were collected. Information regarding the walnut samples is provided in Table 1.

Genomic DNA Isolation and PCR

DNA extraction from the leaves of walnut 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. The SCoT 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 dH_2O. 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 36 SCoT primers were initially screened across the samples and 15 primers exhibiting polymorphic patterns were selected for further analysis (Table 2).

Statistical Analysis

The bands obtained from the PCR analysis were scored as 1 for presence and 0 for absence. A dendrogram based on Jaccard's similarity coefficients was generated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in MVSP 3.22 software (Kovach, 2007). Application of MVSP 3.22 to the SCoT molecular data enabled the construction of a genetic similarity matrix. In addition, Principal Component Analysis (PCA) was performed using MVSP 3.22 to assess the genetic relationships among walnut populations.

RESULTS AND DISCUSSION

To determine the genetic relationships among walnut samples, 36 SCoT primers were initially screened in 10 samples and 15 primers exhibiting polymorphism were selected and applied to all samples.

A total of 136 bands were obtained from the 15 markers, of which 106 were polymorphic, with an average of 9.07 bands per primer. The SCoT markers yielded a polymorphism rate of 77.9%. In the UPGMA dendrogram constructed based on SCoT data, 32 samples were clustered according to their genetic distance matrix (Figure 1). According to the dendrogram, the lowest similarity (0.593) was observed between samples W6 and W26, while the highest similarity was observed between W1 and W4 (0.970), followed by W4 and W5 (0.962) (Figure 1, Table 3).

Table 1. Details of walnut populations used in the study

Genotype Code	Taxonomy	Province	Province	Longitude	Latitude	Altitude (m)
W1	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W2	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W3	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W4	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W5	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W6	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W7	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W8	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W9	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W10	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W11	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W12	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W13	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W14	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W15	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W16	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W17	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W18	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W19	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W20	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W21	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W22	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W23	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W24	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W25	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W26	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W27	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W28	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W29	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W30	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W31	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150
W32	*Juglans regia* L.	Sivas	Divriği	39°22'39.7"N	38°06'53.1"E	1150

Table 2. SCoT primers used for PCR amplification

SCoT Primers Code	DNA Sequences (5'-3')	Tm ºC	PCR Amplification
SCoT-6	CAACAATGGCTACCACGC	56	94 ºC - 3 min 94 ºC - 1 min 48–55 ºC 1 min 72 ºC - 1 min 72 ºC - 8 min 35 cycles
SCoT-14	ACGACATGGCGACCACGC	61	
SCoT-15	ACGACATGGCGACCGCGA	61	
SCoT-16	ACCATGGCTACCACCGAC	58	
SCoT-17	ACCATGGCTACCACCGAG	58	
SCoT-18	ACCATGGCTACCACCGCC	61	
SCoT-19	ACCATGGCTACCACCGGC	61	
SCoT-21	ACGACATGGCGACCCACA	58	
SCoT-23	CACCATGGCTACCACCAG	58	
SCoT-26	ACCATGGCTACCACCGTC	58	
SCoT-28	CCATGGCTACCACCGCCA	61	
SCoT-31	CCATGGCTACCACCGCCT	61	
SCoT-32	CCATGGCTACCACCGCAC	61	
SCoT-34	ACCATGGCTACCACCGCA	58	
SCoT-35	CATGGCTACCACCGGCCC	63	

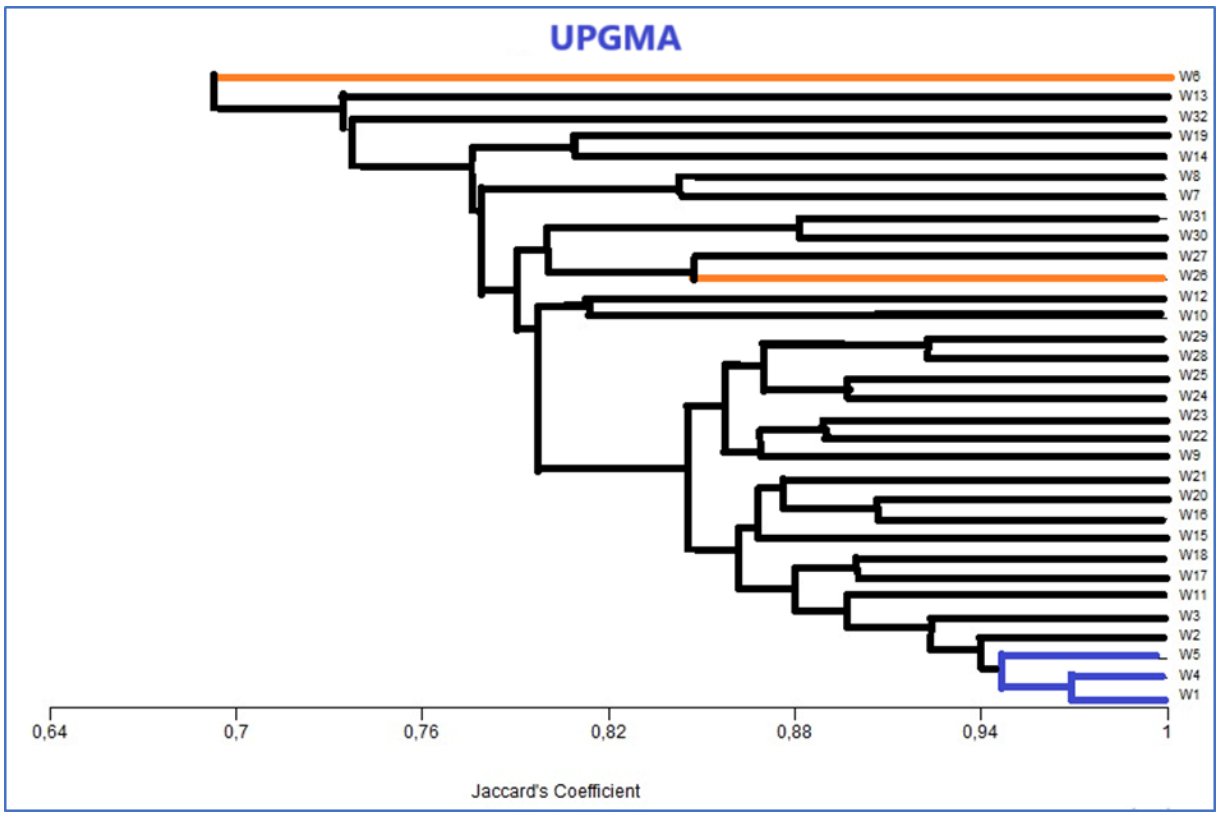

Figure 1. UPGMA Dendrogram of Genetic Similarity Between SCoT Markers and 32 Walnut Genotypes.

Table 3. Pairwise Genetic Distance Matrix Obtained from 15 SCoT Primers.

	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32
W1	1,000																															
W2	0,947	1,000																														
W3	0,939	0,931	1,000																													
W4	**0,970**	0,947	0,924	1,000																												
W5	0,933	0,925	0,902	**0,962**	1,000																											
W6	0,695	0,674	0,688	0,708	0,713	1,000																										
W7	0,776	0,756	0,785	0,789	0,782	0,754	1,000																									
W8	0,828	0,821	0,811	0,856	0,863	0,754	0,843	1,000																								
W9	0,853	0,846	0,850	0,853	0,846	0,672	0,797	0,837	1,000																							
W10	0,787	0,793	0,782	0,800	0,806	0,680	0,768	0,781	0,836	1,000																						
W11	0,909	0,902	0,864	0,924	0,887	0,698	0,756	0,795	0,807	0,808	1,000																					
W12	0,778	0,770	0,786	0,791	0,784	0,669	0,758	0,718	0,813	0,813	0,785	1,000																				
W13	0,735	0,714	0,702	0,748	0,727	0,690	0,711	0,712	0,727	0,710	0,768	0,771	1,000																			
W14	0,789	0,769	0,758	0,817	0,795	0,739	0,770	0,813	0,756	0,768	0,769	0,772	0,739	1,000																		
W15	0,885	0,835	0,853	0,870	0,835	0,754	0,798	0,811	0,823	0,781	0,823	0,772	0,798	0,813	1,000																	
W16	0,902	0,866	0,856	0,902	0,866	0,718	0,775	0,829	0,827	0,814	0,841	0,805	0,732	0,817	0,888	1,000																
W17	0,902	0,895	0,857	0,902	0,881	0,720	0,750	0,803	0,801	0,788	0,870	0,792	0,762	0,791	0,859	0,891	1,000															
W18	0,896	0,888	0,851	0,896	0,888	0,688	0,758	0,797	0,822	0,769	0,836	0,800	0,756	0,785	0,824	0,856	0,900	1,000														
W19	0,799	0,778	0,780	0,812	0,778	0,720	0,752	0,752	0,752	0,778	0,765	0,768	0,736	0,810	0,823	0,841	0,814	0,808	1,000													
W20	0,902	0,881	0,843	0,888	0,867	0,706	0,763	0,789	0,842	0,815	0,842	0,806	0,721	0,791	0,859	0,906	0,878	0,871	0,843	1,000												
W21	0,873	0,880	0,856	0,859	0,838	0,677	0,775	0,815	0,855	0,814	0,813	0,791	0,732	0,775	0,858	0,862	0,848	0,870	0,827	0,891	1,000											
W22	0,857	0,836	0,854	0,830	0,836	0,659	0,731	0,785	0,882	0,769	0,784	0,787	0,742	0,718	0,841	0,803	0,805	0,826	0,740	0,818	0,859	1,000										
W23	0,874	0,881	0,843	0,860	0,867	0,693	0,763	0,803	0,856	0,829	0,828	0,820	0,762	0,791	0,831	0,848	0,864	0,885	0,786	0,878	0,877	0,890	1,000									
W24	0,837	0,830	0,806	0,837	0,816	0,694	0,738	0,752	0,875	0,791	0,818	0,824	0,764	0,738	0,835	0,838	0,826	0,833	0,789	0,840	0,824	0,836	0,883	1,000								
W25	0,873	0,852	0,842	0,859	0,838	0,651	0,735	0,748	0,827	0,786	0,841	0,791	0,719	0,722	0,815	0,847	0,821	0,842	0,771	0,835	0,820	0,831	0,863	0,897	1,000							
W26	0,759	0,752	0,754	0,746	0,713	**0,593**	0,683	0,672	0,752	0,709	0,738	0,783	0,692	0,696	0,766	0,772	0,733	0,781	0,720	0,733	0,772	0,782	0,760	0,790	0,844	1,000						
W27	0,821	0,813	0,789	0,821	0,787	0,635	0,734	0,735	0,802	0,746	0,788	0,792	0,732	0,748	0,775	0,808	0,782	0,831	0,758	0,795	0,808	0,791	0,837	0,827	0,836	0,847	1,000					
W28	0,867	0,846	0,878	0,867	0,859	0,698	0,811	0,795	0,877	0,780	0,835	0,841	0,740	0,742	0,823	0,841	0,815	0,836	0,779	0,815	0,827	0,853	0,870	0,875	0,869	0,794	0,858	1,000				
W29	0,910	0,889	0,880	0,910	0,903	0,677	0,786	0,812	0,851	0,811	0,865	0,815	0,731	0,759	0,840	0,900	0,872	0,852	0,795	0,872	0,857	0,855	0,872	0,863	0,871	0,783	0,846	0,922	1,000			
W30	0,806	0,812	0,802	0,820	0,812	0,700	0,774	0,787	0,786	0,772	0,786	0,790	0,744	0,774	0,831	0,792	0,754	0,788	0,756	0,767	0,792	0,775	0,822	0,811	0,806	0,785	0,837	0,857	0,817	1,000		
W31	0,793	0,799	0,774	0,806	0,799	0,672	0,774	0,773	0,773	0,800	0,786	0,790	0,744	0,760	0,802	0,792	0,780	0,774	0,742	0,767	0,792	0,762	0,836	0,811	0,792	0,756	0,823	0,814	0,817	0,882	1,000	
W32	0,742	0,735	0,764	0,756	0,775	0,714	0,719	0,734	0,721	0,704	0,734	0,736	0,672	0,705	0,748	0,754	0,715	0,710	0,702	0,729	0,687	0,722	0,742	0,758	0,754	0,729	0,754	0,776	0,752	0,767	0,782	1,000

PCA (Principal Component Analysis) is an important tool in genetic diversity studies because this analysis helps us better understand the genetic structure of different genotypes and allows us to extract meaningful patterns from large datasets. PCA groups genetic materials around specific components or key traits. This is particularly useful for visually revealing similarities and differences between different species, varieties, or populations. By using PCA plots, genetically similar genotypes can be placed in the same cluster, while larger distances can be shown between different genotypes. By identifying the main components in genetic data, it facilitates the grouping of genotypes with similar traits. Such analyses are useful in understanding genetic relationships between species or varieties. PCA helps identify genetic resources that need to be conserved by analyzing genetic diversity between different species or populations. Therefore, PCA is a valuable tool for genetic diversity studies because it analyzes large amounts of data meaningfully, uncovers relationships and provides information that can be used in areas such as biodiversity conservation, breeding and genetic resource management (Table 3, Figure 2) (Nachimuthu et al., 2014). Based on the SCoT molecular data, a PCA plot as shown in Figure 2 was obtained using the MVSP 3.22 program. The analysis revealed a broad range of variation. The UPGMA dendrogram and PCA analysis were

consistent with each other. The genetically closest and most distant genotypes were highlighted in the PCA plot. PCA is widely used as an effective dimensionality reduction method for multivariate datasets (Mishra et al., 2017). Numerous studies have been conducted by researchers to determine the genetic diversity of different walnut genotypes. The findings obtained from this study align with similar studies. Tabasi et al. (2020) investigated the relationship between genetic distance and geographical distance in 20 Iranian walnut populations, including 3 wild and 17 cultivated populations, by using the discriminatory power of SCoT markers. The results showed that SCoT markers have a strong discriminatory power and can effectively distinguish between the studied populations. This marker can be used for evaluating genetic diversity, identifying genotypes and DNA fingerprinting of Iranian walnut populations. Güney et al. (2021) used 45 SSR markers to determine the genetic diversity of 91 *Juglans regia* L. genotypes and identified 390 alleles. The average PIC value was calculated to be 0.68 and the genetic distances were divided into five main groups using UPGMA, PCoA and Structure-based clustering methods. Tenche-Constantinescu et al. (2024) created an inventory of 150 trees from five different populations and molecular analyses revealed an average polymorphism rate of 49.44% using SCoT markers. Zarinkolah et al. (2025) obtained a total of 166 bands using 16 SCoT primers, with 2 polymorphic bands from the SCoT-19 primer and 19 polymorphic bands from the SCoT-15 primer. The average PIC value was 0.30 and PCA and AMOVA results revealed a relatively high level of genetic diversity among the studied genotypes. Sağbaş et al. (2023) used 20 SCoT markers in hawthorn genotypes and found PIC values ranging from 0.251 to 0.297. Jalilian et al. (2018) used twelve SCoT primers in pear samples and calculated an average PIC value of 0.58. Seresht et al. (2023) used SCoT markers in 50 pomegranate genotypes, with a PIC value of 0.39. In line with the results of similar studies, it can be concluded that SCoT markers can provide valuable information regarding the genetic relationships among walnut genotypes, which can be utilized in future walnut breeding and conservation programs.

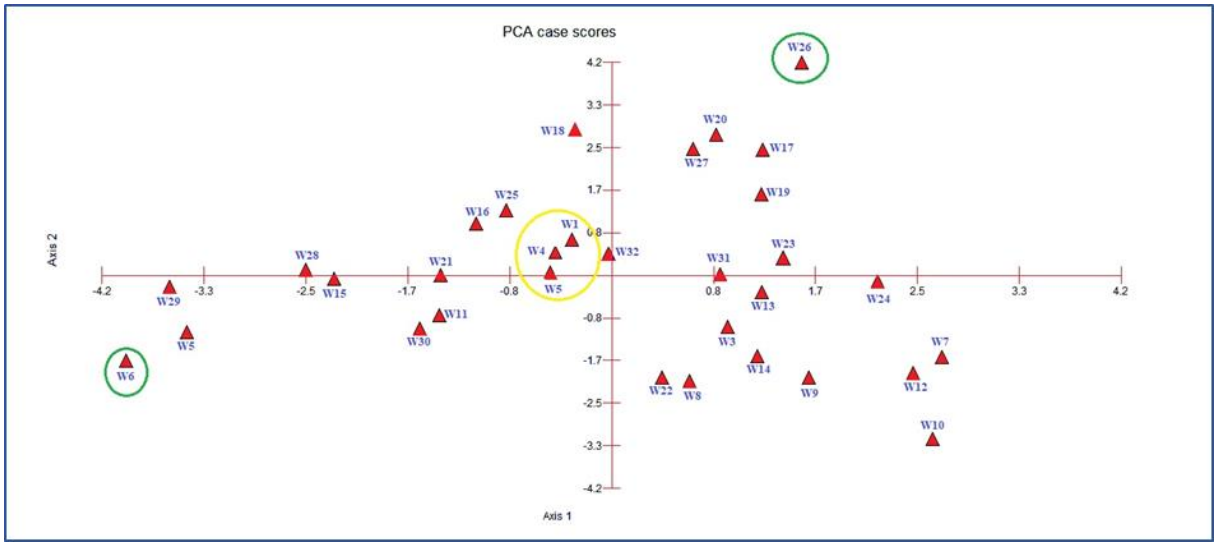

Figure 2. According to SCoT Markers Pricipal Component Analyses of 32 Walnut Populations Using MVSP 3.22 Software (Kovach Computing Services, Pentraeth, Wales, UK)

CONCLUSION

In this study, the genetic diversity of some *Juglans regia* L. germplasm was analyzed using SCoT marker groups. A polymorphism rate of 77.9% was obtained from 136 bands generated by 15 markers. The lowest genetic similarity (0.593) was observed between the W6 and W26 samples, while the highest genetic similarity (0.970) was found between the W1-W4 samples and (0.962) between the W4-W5 samples. Principal Component Analysis (PCA) revealed a homogeneous distribution and wide variation among the genotypes. The UPGMA dendrogram and PCA analysis were consistent with each other. The SCoT analysis conducted on walnut genotypes provides valuable information for determining genetic diversity levels, understanding population structure and selecting individuals with superior traits. Such studies contribute not only to the conservation of genetic resources but also to the development of new cultivars with high yield, disease resistance and enhanced adaptation to climate change in future breeding programs. This study concludes that SCoT markers can provide useful insights into the diversity among walnut genotypes and that these insights could be applied in future walnut breeding and conservation programs.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare no conflict of interest.

Author contribution

Methodology, G.O. and Y.C.; formal analysis, G.O. and Y.C.; investigation, Y.C.; resources, Y.C.; writing-original draft preparation, G.O. and Y.C.; writing-review and editing, Y.C.; All authors have read and agreed to the published version of the manuscript.

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