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Genetic diversity and structure analysis of 'Ak Sakı' and 'Kara Sakı' apple cultivars growing in Erzincan/Türkiye

Original Article

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Abstract:

In this study, 'Ak Sakı' and 'Kara Sakı' apple cultivars were collected from different locations in Erzincan province, Türkiye, and genetic diversity was determined using the Start Codon Targeted (SCoT) marker technique. The SCoT marker technique was chosen because its gene targeting, long primer, and high annealing temperature make it more effective than other marker techniques. Using ten SCoT primers, 60 bands were obtained, and 42 of them were polymorphic. The polymorphism rate was determined to be 70%. The UPGMA (Unweighted Pair Group Method with Arithmetic mean) dendrogram created using the PAUP 4.0b10 program consists of two clades. The genetic distance between apple cultivars varies between 0.13462 and 0.45614. Principal Component Analysis (PCA) results were compatible with the UPGMA dendrogram. With the SCoT marker technique, genetic diversity among apple cultivars can be determined in a shorter time and with more reliable results.

Key words:
apple, genetic diversity, SCoT, Türkiye

Apstrakt:

Analiza genetske raznovrsnosti i strukture sorti jabuka "Ak Sakı" i "Kara Sakı" koje rastu u Erzindžanu/Turska

U ovoj studiji, sorte jabuka 'Ak Sakı' i 'Kara Sakı' prikupljene su sa različitih lokaliteta u provinciji Erzindžan, Turska, koristeći tehniku markera startnog kodona (SCoT) za određivanje genetske raznovrsnosti. Tehnika SCoT odabrana je zbog ciljanog delovanja na gene, dužine prajmera i visoke temperature vezivanja, što je čini efikasnijom od drugih tehnika markera. Korišćenjem deset SCoT prajmera dobijeno je 60 traka, od kojih su 42 bile polimorfne. Procenat polimorfizma utvrđen je na 70%. UPGMA dendrogram (Unweighted Pair Group Method with Arithmetic mean), kreiran korišćenjem programa PAUP 4.0b10, sastoji se od dve klade. Genetska udaljenost između sorti jabuka varira između 0.13462 i 0.45614. Rezultati analize glavnih komponenti (PCA) bili su kompatibilni sa UPGMA dendrogramom. Tehnikom SCoT može se u kraćem vremenskom periodu i sa pouzdanijim rezultatima odrediti genetska raznovrsnost među sortama jabuka.

Ključne reči:
jabuka, genetska raznovrsnost, SCoT,Turska

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Introduction

The apple (*Malus* × *domestica* Borkh.), a member of the Rosaceae family, originates from the region between the Caspian Sea and the Black Sea. It is one of the most economically and culturally significant fruit species globally (Han et al., 2020; Geană et al., 2021; Chen et al., 2021; Fotirić Akšić et al., 2022). Due to its ecological compatibility and high nutritional value, it is popular among both producers and consumers (Eberhardt et al., 2000; Boyer and Liu, 2004; Hyson, 2011; Sarkate et al., 2017). 'Ak Sakı' is a variety grown in the Erzincan province of Türkiye and is the most commonly grown and readily consumed apple. In the region 'Ak Sakı' and 'Kara Sakı' ecotypes are known. It was also registered under the name 'Ak Sakı' on 03.05.1990. (Öztürk et al., 2013). The 'Kara Sakı' apple is a native apple variety of Erzincan, resembling the Amasya apple, but with a slightly lighter and brighter color and a mildly tart taste (Doğan, 2001). Its aroma is the most important feature that makes it the consumer's preference. To date, more than 300 volatile compounds have been detected in apples

(Dixon and Hewett, 2000; Aprea et al., 2011; Yang et al., 2021). Also, apples provide high amounts of carbohydrates, vitamins and bioactive compounds, such as phytosterols, β-carotene and phenolic compounds (Wu et al., 2020; Feng et al., 2021). Various apple phytochemical compounds with antioxidant activity and anticancer properties reduce the risk of chronic and degenerative diseases (Boyer and Liu, 2004; Inroga et al., 2021). Molecular markers are widely utilized in many plant species and are one of the most essential methods for analyzing genetic diversity (Yang et al., 2023). SCoT markers are simple and reliable markers designed to produce of gene-targeted markers. Compared to many other marker systems, the SCoT system provides more information about the universality and biological properties of plants (Yilmaz and Ciftci, 2021). The SCoT marker approach is more effective than other random markers because of its long primer and high annealing temperatures (Alzahrani et al., 2023). SCoT markers have been used in genetic diversity and phylogenetic relationships analysis in many plant species (Guo et al., 2016; Jalilian et al., 2018; Etminan et al., 2018; Vivodík et al., 2019; Zarei and Erfani-Moghadam, 2021; Vivodík et al., 2023). In this study, the genetic diversity and structure of the 'Ak Sakı' and 'Kara Sakı' apple cultivars, distributed in Erzincan – Türkiye, were analyzed using ten SCoT markers.

Materials and Methods

Plant Materials, Genomic DNA Isolation and PCR

'Ak Sakı' and 'Kara Sakı' cultivars were collected from different localities of Erzincan-Türkiye. For both cultivars, the collection was made from approximately seven centers. Genomic DNA isolation was performed using green leaves with a commercial kit (GeneMark Catalog No: DP022). Selected ten SCoT primers (Collard and Mackill, 2009) for PCR amplifications, PCR components and the protocol used are given in **Tab. 1**. PCR products were run on 1.0% agarose gel. In gel imaging, GeneRuler (Cat#: GMM100) between 100 bp and 3000 bp was used. Gel images of PCR results using the SCoT 11 primer are shown in **Fig. 1**.

Start Codon Targeted (SCoT) Analysis

Following the SCoT analyses, the DNA bands were scored by giving the value "1" in the presence of DNA, "0" in the absence of DNA and "?" or "9" for the missing cases. The UPGMA phylogenetic tree was drawn using the program PAUP 4.0b10 (Swofford, 2001). Pairwise distance was created with the same program. The genetic distance matrix between apple cultivars was calculated and shown in **Tab. 2**. The JMP Statistical Software which presents the distances between individuals in a two-dimensional diagram, was used to perform the PCA analysis. In addition, major allele frequency, Nei's H (gene diversity) and PIC (polymorphism

Table 1. SCoT primers name, sequences and PCR components used for PCR amplification conditions

SCoT Primers	DNA Sequences(5'-3')	Tm °C	PCR components	PCR Amplification (35 cycle)
SCoT 1	CAACAATGGCTACCACCA	54 °C		
SCoT 2	CAACAATGGCTACCACCC	56 °C		
SCoT 3	CAACAATGGCTACCACCG	56 °C		
SCoT 4	CAACAATGGCTACCACCT	54 °C		
SCoT 5	CAACAATGGCTACCACGA	54 °C	1 µL genomic DNA 1 µL primer, 5 µL master mix (Cat. No: RP02-II-400, RP02-II-2000, 0.75 U of Taq DNA polymerase, reaction buffer, 2 mM $MgCl_2$, 250 µM dNTPs and enzyme stabilizer) and 18 µL dH_2O.	
SCoT 6	CAACAATGGCTACCACGC	56 °C		95 °C/1min.
SCoT 8		54 °C		95 °C/30 sec
	CAACAATGGCTACCACGT			54-56 °C/30sec.
SCoT 9	CAACAATGGCTACCAGCA	54 °C		72 °C/1 min.
SCoT 10	CAACAATGGCTACCAGCC	56 °C		72 °C/5 min.
SCoT 11	AAGCAATGGCTACCACCA	54 °C		

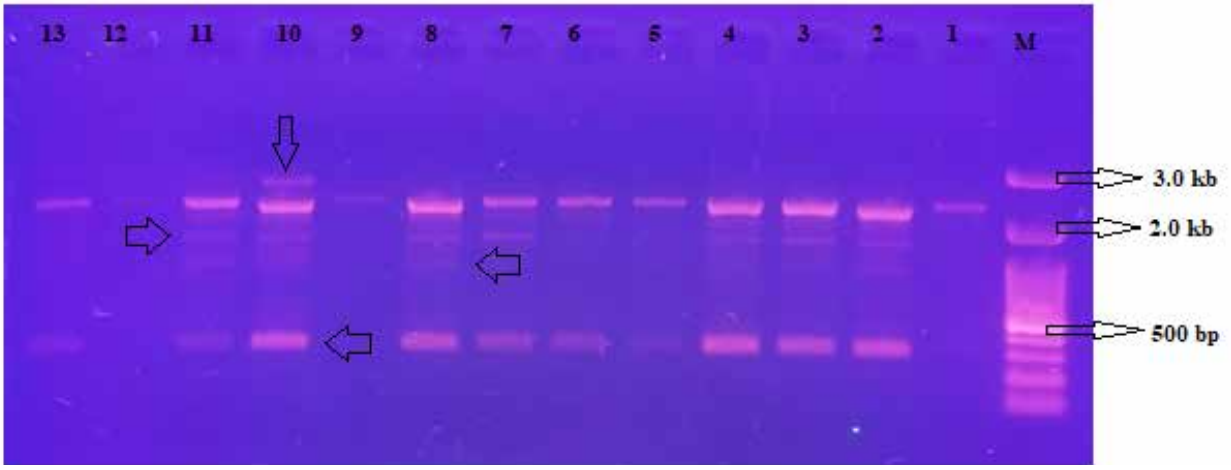

Fig. 1. Gel image of bands amplifed with SCoT 11 primer: 1: 'Ak Sakı' (Elma village), 2: 'Ak Sakı' (Yaylabaşı), 3: 'Kara Sakı' (Çağlayan), 4: 'Ak Sakı' (Çağlayan), 5: 'Ak Sakı' (Karatuş), 6: 'Kara Sakı' (Elma village), 7: 'Kara Sakı' (Karakaya), 8: 'Ak Sakı' (Karakaya), 9: 'Kara Sakı' (Karatuş village), 10: 'Ak Sakı' (Pişkidağ), 11: 'Ak Sakı' (Üzümlü), 12: 'Kara Sakı' (Yaylabaşı), 13: 'Kara Sakı' (Üzümlü)

Table 2. Monomorphic and polymorphic band numbers of SCoT primers

Primers	Total bands	Monomorphic bands	Polymorphic bands
SCoT 1	8	6	2
SCoT 2	11	2	9
SCoT 3	7	1	6
SCoT 4	5	1	4
SCoT 5	5	1	4
SCoT 6	7	1	6
SCoT 8	1	1	0
SCoT 9	3	1	2
SCoT 10	4	1	3
SCoT 11	9	3	6
Total	60	18	42

information content) values were calculated using the PowerMarker software package version 3.25 (Liu and Muse, 2005). Using the statistical program Structure 2.3.4, a structure test was utilized to classify members of various populations (Pritchard et al., 2000).

Results and discussion

For SCoT analysis, ten primers were used. A total of 60 bands were obtained. 42 of these bands were polymorphic and the polymorphism rate was 70%. The highest number of bands was obtained from the SCoT 2 primer and the lowest number of bands was obtained from the SCoT 8 primer (**Tab. 2**). "PIC", "H" and "major allele frequency" values of each primer are given in **Tab. 3**. The mean PIC value was determined at 0.2878, while the average H value and the mean major allele frequency were determined at 0.3581 and 0.7413, respectively.

Table 3. Major allele frequency, gene diversity (H) and polymorphism information content (PIC) values

Primers	Major Allele Frquency	Gene Diversity (H)	PIC
SCoT 1	0.6643	0.446	0.3465
SCoT 2	0.6014	0.4794	0.3645
SCoT 3	0.6783	0.4364	0.3412
SCoT 4	0.7692	0.355	0.292
SCoT 5	0.8392	0.2699	0.2335
SCoT 6	0.6573	0.4505	0.349
SCoT 8	0.9091	0.1653	0.1516
SCoT 9	0.8601	0.2406	0.2117
SCoT 10	0.8531	0.2506	0.2192
SCoT 11	0.5804	0.4871	0.3684
Mean	0.7413	0.3581	0.2878

The UPGMA dendrogram was created and it consists of two main clades (**Fig. 2**). Clade A consists of 'Ak Sakı' (Elma village), 'Ak Sakı' (Karatuş village), 'Kara Sakı' (Elma village), 'Kara Sakı' (Üzümlü), 'Kara Sakı' (Karatuş village), 'Kara Sakı' (Yaylabaşı), 'Kara Sakı' (Karakaya) apple cultivars. Clade B consists of 'Ak Sakı' (Yaylabaşı), 'Kara Sakı' (Çağlayan), 'Ak Sakı' (Çağlayan), 'Ak Sakı' (Karakaya), 'Ak Sakı' (Üzümlü) and 'Ak Sakı' (Pişkidağ) apple cultivars. According to **Tab. 4**, the

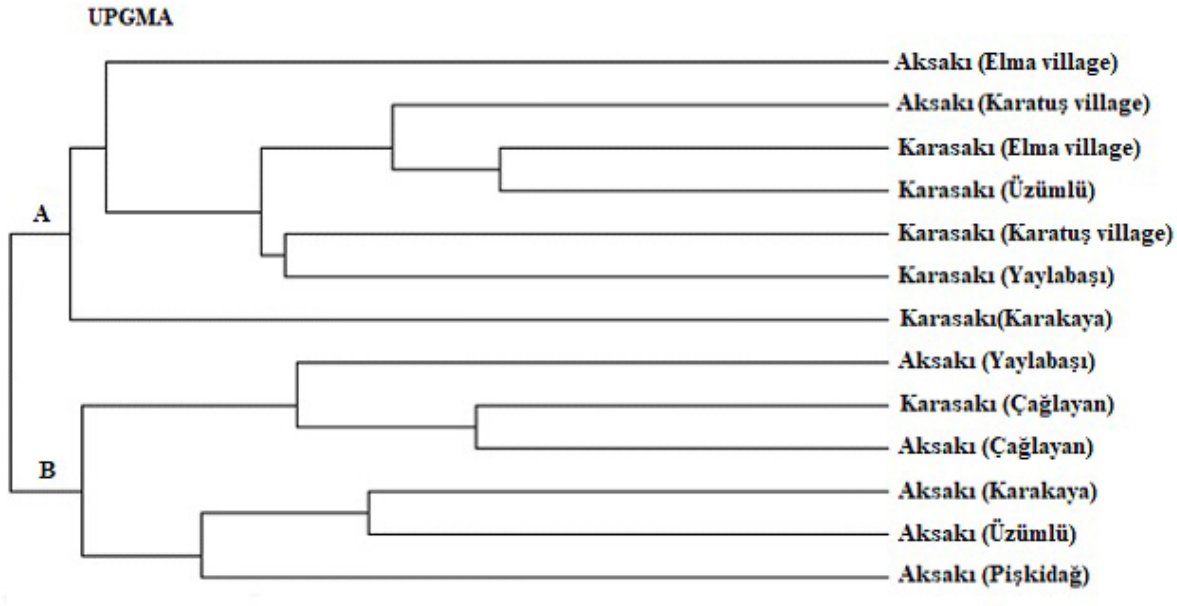

Fig. 2. The UPGMA tree generated using ten SCoT markers

lowest distance (0.13462) was found between the 'Kara Sakı' (Yaylabaşı) and 'Kara Sakı' (Üzümlü), 'Kara Sakı' (Elma village) and 'Kara Sakı' (Üzümlü) cultivars. The highest distance (0.45614) was found between the 'Ak Sakı' (Üzümlü) and 'Kara Sakı' (Yaylabaşı) cultivars. The graphical results and eigenvalues obtained on the two-dimensional plane according to PCA analysis are given in **Fig. 3**. The first three eigenvalues explained 64.755% of the total variance in the cultivars. As a result of the analysis, 13 samples were reduced to two dimensions. The first dimension consists of seven cultivars and the second one consists of six (eigen > 1). The clustering in the two-dimensional graph obtained from the PCA analysis partially showed parallelism with the dendrogram results. Çokran et al. (2019) determined the genetic diversity of 30 local 'Misket' apple genotypes using AFLP, SSR, and RAPD markers. In their study, AFLP, SSR and RAPD markers amplified a total of 423 bands and obtained 205 polymorphic bands, while 30 RAPD primers obtained 207 bands, 91 of which were polymorphic (40.1%), and 10 SSR primers obtained 33 bands and 26 polymorphic (78.78%) bands. Five AFLP combinations obtained 183 bands, 88 of which were polymorphic (48.08%). Sevindik et al. (2018) determined the genetic diversity of apple genotypes in Ardahan province with the ISSR-PCR technique. The authors found the polymorphism rate to be 60% in their study results. Kaya et al. (2015) used RAPD markers to perform molecular genotyping on apples collected from Van province, Türkiye. As a result of their study, they found the polymorphism rate to be 89.29%. Khachtib et al. (2024) determined the genetic diversity of 29 apple cultivars in different regions of Morocco using ISSR-PCR technique. They obtained 177 bands from 15 ISSR primers, 156 of which were polymorphic. Also, the mean values of PIC, Rp, I and H were determined as 0.46, 4.58, 0.43 and 0.28, respectively. In conclusion, they revealed that ISSR markers could be useful in detecting genetic diversity in this fruit crop. Najar et al. (2023) have collected samples from the North Kashmir region and screened 62 apple genotypes using ten SSR markers. In their study, they amplified a total of 77 alleles with an average polymorphism percentage of 87.5%, PIC of 0.71 and resolving power (RP) of 3.58. Dar et al. (2020) have determined the genetic diversity of 19 apple varieties from the Kashmir region using ten RAPD markers. In their study, they detected a total of 70 polymorphic bands with a polymorphism percentage of 83.33. The same study suggested that these results can be implemented in apple-related conservation and breeding programs. In our study, the cultivar structure of individuals was estimated using the Structure test. Structure analysis of 13 cultivars was performed using ten SCoT primers. Our results showed a clear peak point for ΔK at K = 3. At the first level of clustering (ΔK at K = 3), although 'Ak Sakı' and 'Kara Sakı' apple cultivars were divided into three subpopulations; all 13 cultivars were considered as mixtures. (**Fig. 4**). This suggests significant gene flow with high exchange rates and different allele combinations between populations, in agreement with the results reported by Najar et al. (2023) for *Malus*×*domestica* germplasm from North Kashmir, India.

Table 4. Pairwise genetic distance matrix obtained from ten SCoT primers

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13
'Ak Sakı' (Elma village)	-	0.25	0.32727	0.36667	0.28333	0.31579	0.28333	0.4	0.26667	0.33333	0.41667	0.24561	0.26963
'Ak Sakı' (Yaylabaşı)	15	-	0.16364	0.25	0.26667	0.31579	0.26667	0.31667	0.31667	0.31667	0.3	0.42105	0.38462
'Kara Sakı' (Çağlayan)	18	9	-	0.14545	0.21818	0.26923	0.30909	0.2	0.27273	0.30909	0.21818	0.38462	0.32692
'Ak Sakı' (Çağlayan)	22	15	8	-	0.25	0.21053	0.28333	0.23333	0.3	0.36667	0.28333	0.35088	0.26923
'Ak Sakı' (Karatuş)	17	16	12	15	-	0.17544	0.3	0.25	0.25	0.28333	0.3	0.2807	0.17308
'Kara Sakı' (Elma village)	18	18	14	12	10	-	0.26316	0.33333	0.21053	0.19298	0.17544	0.2807	**0.13462**
'Kara Sakı' (Karakaya)	17	16	17	17	18	15	-	0.25	0.25	0.31667	0.3	0.36842	0.25
'Ak Sakı' (Karakaya)	24	19	11	14	15	19	15	-	0.4	0.3	0.18333	0.4386	0.30769
'Kara Sakı' (Karatuş village)	16	19	15	18	15	12	15	24	-	0.23333	0.31667	0.21053	0.17308
'Ak Sakı' (Pişkidağ)	20	19	17	22	17	11	19	18	14	-	0.18333	0.33333	0.26923
'Ak Sakı' (Üzümlü)	15	18	12	17	18	10	18	11	19	11	-	**0.45614**	0.28846
'Kara Sakı' (Yaylabaşı)	14	24	20	20	16	16	21	25	12	19	26	-	**0.13462**
'Kara Sakı' (Üzümlü)	14	20	17	14	9	7	13	16	2	14	15	7	-

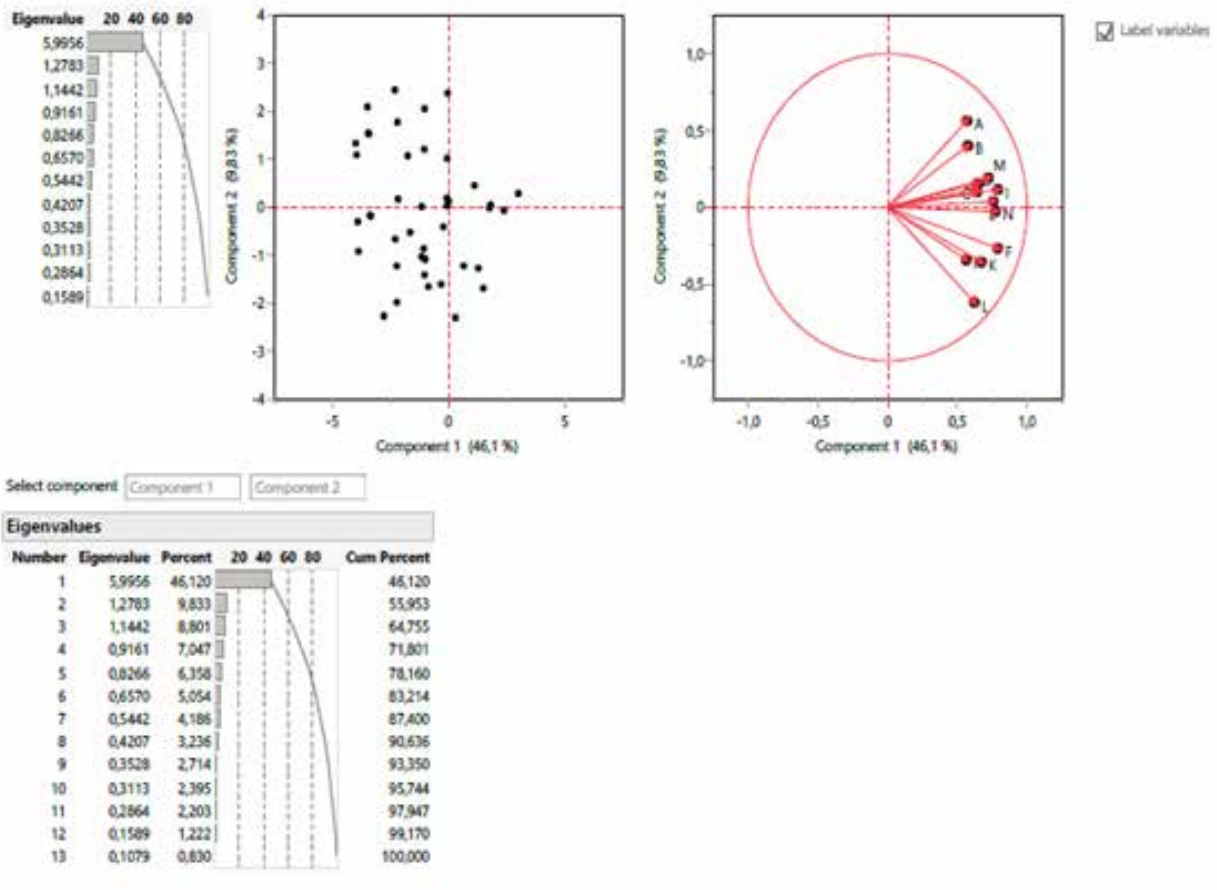

Fig. 3. Two dimensional graph and eigen values created as a result of Principal Component analysis with SCoT marker

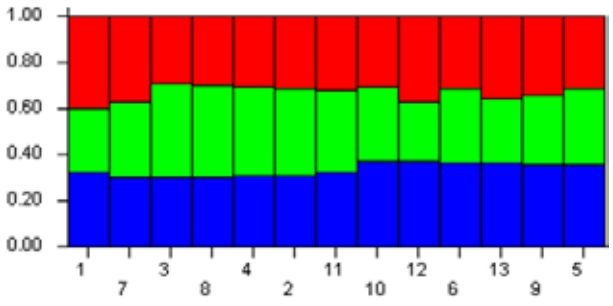

Fig. 4. Population structure analysis of apples

Conclusion

The study revealed a 70% polymorphism using ten SCoT primers, demonstrating the genetic diversity of the 'Ak Sakı' and 'Kara Sakı' apple cultivars. The PCA analysis results were consistent with the UPGMA dendrogram, and the cluster analysis of genetic structure indicated that the apple cultivars are best represented by three genetic groups (ΔK=3). In addition, the results obtained will enable the cultivars to be used more effectively in future breeding programs.

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