



Genetic Variation Analysis of Selected Grape (*Vitis vinifera* L.) Accessions Through SCoT Markers

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HIGHLIGHTS

- In this study, 45 grape genotypes were used as plant material.
- SCoT markers can be successfully used, either alone or in combination with other existing DNA markers, to study interspecific or intraspecific genetic variation.
- The molecular identification and conservation of local grape varieties are highly important for sustainable agricultural practices.

Abstract

Molecular techniques such as ISSR, RAPD, SCoT, and SSR are widely used to identify genetic polymorphism, reveal genetic structures, and analyze inter-varietal relationships in grape cultivars. In this context, the aim of our study was to reveal the genetic diversity of some local grape (*Vitis vinifera*) genotypes cultivated or naturally found in the Divriği district of Sivas province, contribute to the sustainable conservation of natural genetic resources, and determine the agricultural production potential of these genotypes. Additionally, the potential use of Start Codon Targeted (SCoT) markers in evaluating the diversity among different grape genotypes was investigated. Plant materials were collected from vineyards, and DNA was isolated from fresh leaves using the CTAB method. A total of 36 SCoT markers were screened in the samples, and 12 markers with the highest polymorphism rate were selected for analysis. In this study, a total of 119 bands were obtained from the 12 SCoT primers used to determine the genetic relationships among the grape genotypes. Among these, 83 bands were polymorphic, resulting in a polymorphism rate of 69.74%. The molecular data obtained allowed for the construction of a dendrogram (phylogenetic tree) grouping the analyzed grape genotypes according to their similarity indices. According to the UPGMA dendrogram and the correlation matrix generated from the SCoT molecular data, the lowest genetic similarity (0.382) was observed between genotypes G6 and G38, while the highest similarity (0.786) was found between genotypes G37 and G39. In conclusion, it was demonstrated that SCoT markers can be effectively used to assess genetic variation and establish similarity indices among grape genotypes. This molecular approach provides a robust basis for advanced genetic research and future breeding programs.

Keywords: *Vitis vinifera* L.; genetic diversity; molecular marker.

Citation: Sönmez E, Çilesiz Y (2025). Genetic variation analysis of selected grape (*Vitis vinifera* L.) accessions through SCoT markers. *Selcuk Journal of Agriculture and Food Sciences*, 39(2), 418-426. <https://doi.org/10.15316/selcukjafsci.1685921>

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Received date: 28/04/2025

Accepted date: 19/06/2025

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1. Introduction

Grapes, which hold an important place in both our kitchens and traditional medicine, are a perennial fruit species belonging to the *Vitaceae* family (Braidot et al. 2008). Grape varieties are rich in phenolic compounds, flavonoids, vitamins, minerals, and organic acids (Lebon et al. 2008). Thanks to these chemical components, grapes exhibit strong antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. In addition to being consumed fresh or dried, grapes are widely used in various forms such as juice, vinegar, molasses, wine, and cosmetic products (Pellegrino et al. 2005). Derivative products like grape seed extract also hold commercial value as functional foods and natural health supplements. *Vitis vinifera* L., due to its high economic value, nutritional content, and medicinal effects, is one of the most widely cultivated fruit species globally. Due to its broad geographical distribution and ability to adapt to different environmental conditions, grape species exhibit a high level of genetic diversity (Franks et al. 1998; Goufo et al. 2020). Although the Sivas province, particularly the districts of Zara, Divriği, and İmranlı, shows limited suitability for large-scale viticulture, local grape varieties naturally or traditionally grown in these areas still exist (Trouillas et al. 2011). These varieties differ morphologically and biochemically depending on the ecological conditions of the region (Martínez et al. 2006). This presents significant potential for the conservation and evaluation of local genetic resources. Local grape varieties are generally preferred for their more intense aroma profiles and region-specific taste characteristics, often tied to traditional production and consumption practices. This diversity, defined as genetic polymorphism, is critically important for the sustainability, environmental adaptation, and breeding of the species (This et al. 2006; Riaz et al. 2018). Genetic polymorphism enables grape varieties to develop tolerance to different climate and soil conditions, and resistance to pests and diseases. The genetic diversity demonstrated by local varieties grown especially in arid and semi-arid regions enhances the adaptability of these plants (Aradhya et al. 2003; Nicolas et al. 2016).

Genetic diversity directly affects the quality traits of grape fruits. Compounds such as tannins, resveratrol, and anthocyanins may vary among genotypes (Aradhya et al. 2013). These differences directly influence many traits such as fruit quality, shelf life, processability, and commercial value. Therefore, genetic polymorphism plays a fundamental role in determining the quality standards of grapes for both fresh consumption and industrial processing (Zdunić et al. 2013). The primary goals of grape breeding programs include improving yield, resistance to diseases and pests, fruit quality, aroma profile, and tolerance to stress conditions. Genetic polymorphism enables the selection and improvement of such traits. Identifying genetically distinct individuals is crucial for determining superior lines in breeding studies (Ekhvaia et al. 2014; Kaya et al. 2023). Molecular techniques such as ISSR, RAPD, SCoT, and SSR are commonly used to determine genetic polymorphism in grape varieties, to reveal genetic structures, and to analyze relationships between different varieties (Korbin et al. 2002; Almadanim et al. 2007). These techniques allow for more precise and reliable variety identification and classification. These molecular marker techniques have a wide range of applications including classification of grape genotypes, understanding population structure, calculating genetic distance, and selecting superior individuals in breeding programs. Thus, scientifically-based strategies can be developed for the conservation of local varieties and sustainable agricultural production (Souframanien and Gopalakrishna 2004; Collard and Mackill 2009; Poyraz 2016). The aim of this study is to determine the genetic polymorphism among different grape genotypes collected from vineyards in the Divriği district of Sivas province and to reveal the existing genetic diversity. The findings are expected to contribute to the conservation of grape genetic resources in the region and to future breeding efforts.

2. Materials and Methods

Fresh leaves were collected from 45 different grape samples belonging to the *Vitis vinifera* L., germplasm, cultivated in farmer gardens in the Divriği district of Sivas. Information related to the grape samples is presented in Table 1.

Table 1. Data of grape genotypes characterised.

Genotype Code	Fruit Skin Color	Taxonomy	Province	Collected Location	Longitude	Latitude	Altitude (m)
G1	Yellow	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G2	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G3	Yellow	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G4	Colourful	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G5	Yellow	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G6	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G7	Colourful	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G8	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G9	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G10	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G11	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G12	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G13	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G14	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G15	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G16	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G17	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G18	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G19	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G20	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G21	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G22	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G23	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G24	Yellow	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G25	Yellow	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G26	Yellow	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G27	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G28	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G29	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G30	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G31	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G32	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G33	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G34	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G35	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G36	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G37	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G38	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G39	Black	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G40	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G41	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G42	Red	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G43	Colourful	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G44	Colourful	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150
G45	Colourful	<i>Vitis vinifera</i> L.	Divriği	Sivas	39°22'39.7"N	38°06'53.1"E	1150

2.1. Genomic DNA Isolation and PCR

DNA extraction from grape samples was performed based on the CTAB protocol described by Doyle and Doyle (1990). The concentrations of stock DNA were measured using a MaestroNano Pro spectrophotometer (MN913A, MaestroGen), and the DNA stocks were diluted to a final concentration of 5 ng/μL. The selected SCoT primers, PCR reaction mixture content and PCR cycling conditions used for PCR amplifications are presented in Table 2. For electrophoresis of the PCR products, a 2% agarose gel prepared in Tris-borate-EDTA (TBE) buffer was used. DNA bands were subsequently visualized under UV light. A total of 36 SCoT primers were screened and 12 primers that exhibited polymorphic patterns were selected for further analysis (Table 2).

Table 2. SCoT primers used for PCR amplification.

SCoT Primers Code	DNA Sequences (5'-3')	T _m °C	PCR Reaction Mixture Content	PCR Amplification
SCoT 2	CAACAATGGCTACCACCC	56	4 μL DNA (20 ng) 1 μL primer 10 μL PCR master mix (Eco Tech, Cat No: ET5) 10 μL dH ₂ O	94 °C - 3 min 94 °C - 1 min 56–61 °C 1 min 72 °C - 1 min 72 °C - 8 min 35 cycles
SCoT 12	ACGACATGGCGACCAACG	58		
SCoT 18	ACCATGGCTACCACCGCC	61		
SCoT 19	ACCATGGCTACCACCGGC	61		
SCoT 20	ACCATGGCTACCACCGCG	61		
SCoT 21	ACGACATGGCGACCCACA	58		
SCoT 22	AACCATGGCTACCACCAC	56		
SCoT 23	CACCATGGCTACCACCAG	58		
SCoT 26	ACCATGGCTACCACCGTC	58		
SCoT 28	CCATGGCTACCACCGCCA	61		
SCoT 32	CCATGGCTACCACCGCAC	61		
SCoT 33	CCATGGCTACCACCGCAG	61		

2.2. 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 coefficient was generated using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm in the MVSP 3.22 software (Kovach 2007). The application of MVSP 3.22 to the SCoT molecular data enabled the generation of a genetic similarity matrix. In addition, principal component analysis (PCA) was performed among grape populations using the MVSP 3.22 software.

3. Results and Discussion

The gel images of PCR results for the primers, which produced the most polymorphic bands, are shown in Figure 1 are shown. A total of 14, 11, 10, and 13 polymorphic bands were obtained from the primers SCoT-12, SCoT-21, SCoT-28, and SCoT-32, respectively, while the lowest number of bands (6) was obtained from the primer SCoT-26. In polymorphism analysis, the number of bands is a highly important indicator in the assessment of genetic diversity. Whether the number of bands is high or low reflects how informative and sensitive the molecular marker used (e.g., SCoT, ISSR, RAPD) is. As the number of bands increases, the resolution, accuracy, and reliability of genetic polymorphism analyses also improve. This directly affects the quality of scientific research and the success of applied agricultural and breeding programs. A primer or marker that produces a high number of polymorphic bands can reveal genetic differences more precisely. Therefore, such markers are more preferred in breeding programs and in the conservation of genetic resources (Pan ve ark. 2024).

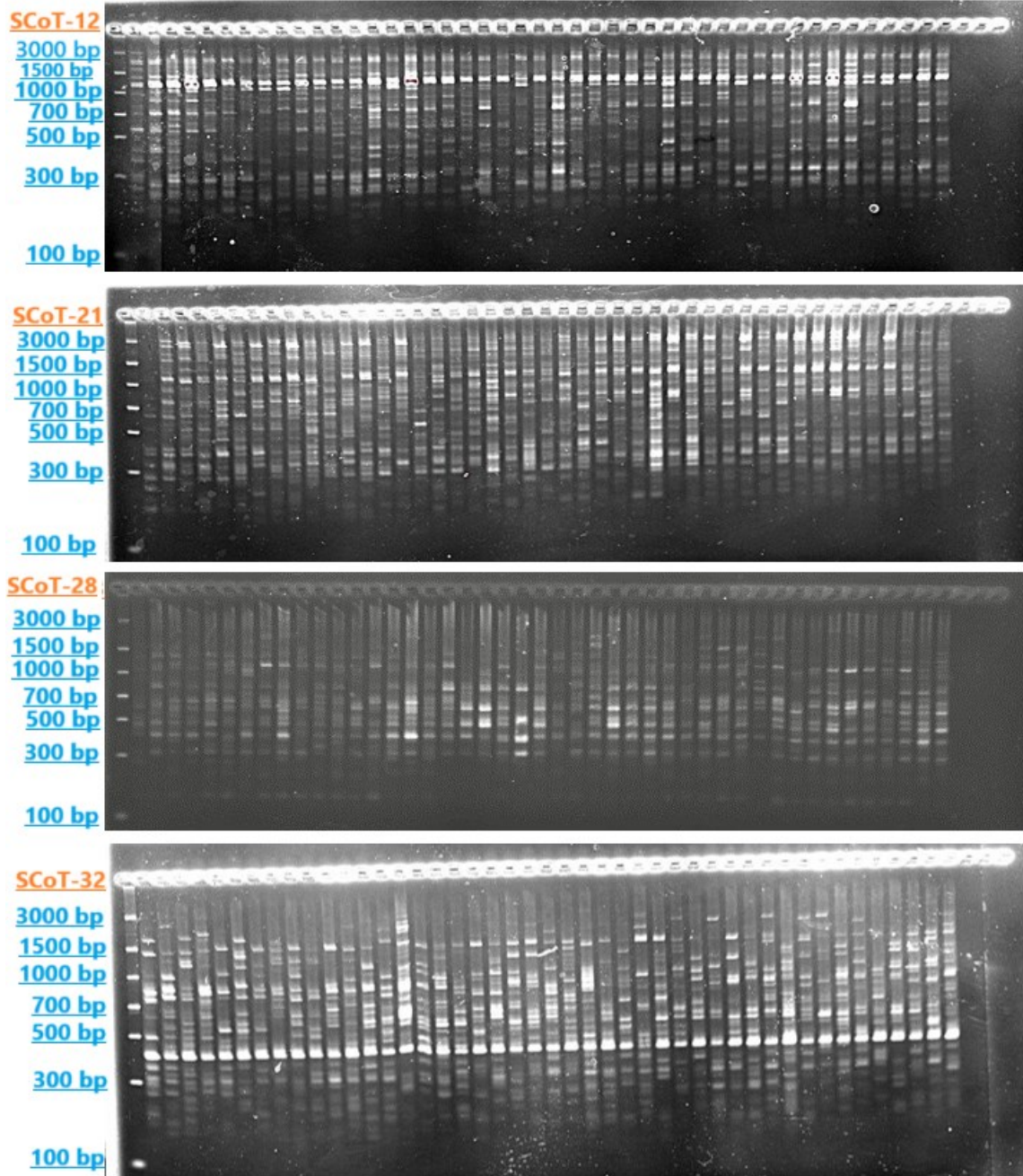


Figure 1. Gel image of bands amplified with SCoT 12, SCoT 21, SCoT 28 and SCoT 32 primers: M: generuler.

In this study, a total of 119 bands were obtained from 12 SCoT primers used to determine the genetic relationships among grape samples. Among these, 83 bands were polymorphic, resulting in a polymorphism rate of 69.74%. According to the UPGMA dendrogram and the correlation matrix generated from the SCoT molecular data, the lowest similarity (0.382) was observed between samples G6 and G38, while the highest similarity (0.786) was observed between samples G37 and G39 (Figure 2, Table 3).

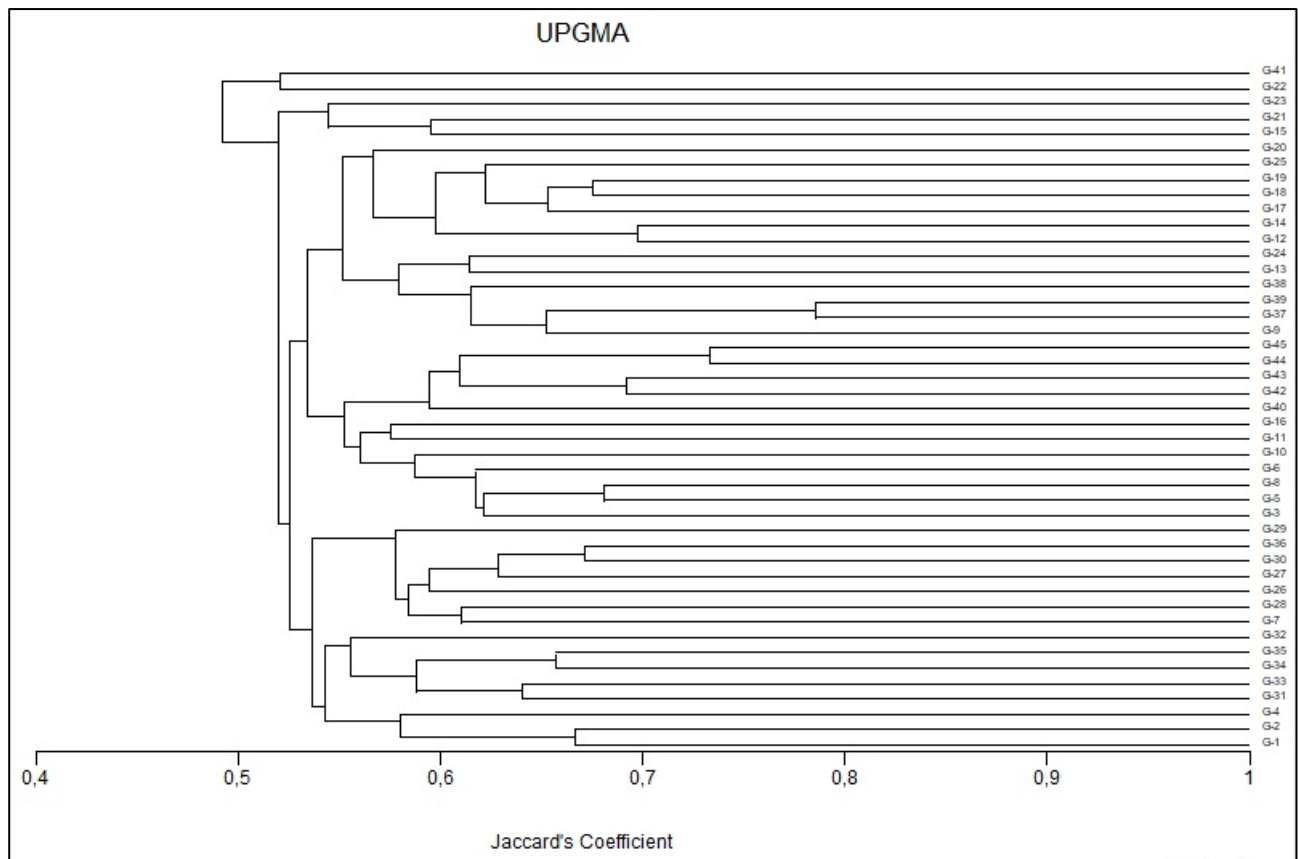


Figure 2. UPGMA dendrogram of genetic similarity between SCoT markers and 45 grape genotypes.

Table 3. Pairwise genetic distance matrix obtained from 12 SCoT primers.

[illegible]

Based on the SCoT molecular data, a PCA image shown in Figure 3 was obtained using the MVSP 3.22 software. The UPGMA dendrogram and the PCA analysis were consistent with each other. Genotypes that are genetically similar were positioned close to one another in the PCA plot. PCA is an effective dimensionality reduction method for multivariate data sets (Mishra et al. 2017). Numerous similar studies have been conducted by researchers to determine the genetic diversity of different grape genotypes. Guo et al. (2012) selected 17 informative primers from 36 SCoT primers to evaluate the genetic relationships among 64 grape varieties. A total of 131 bands were obtained, of which 93.1% were polymorphic; the average polymorphism information content (PIC) value was calculated as 0.82. Ibrahim et al. (2016) genotyped the leaves of seven grape varieties differing in agronomic and morphological traits using Start-Codon Targeted (SCoT) and Simple Sequence Repeats (SSR) markers. A total of 24 SCoT primers produced 362 bands with a 77.10% polymorphism rate and an average PIC value of 0.04. Yue et al. (2019) reported that 36 SCoT primers generated a large number of polymorphic bands, and the amplification results showed clear and distinct bands. The amplification efficiency and polymorphism rate were both determined to be 100%. A total of 221 bands were obtained from the 36 primers, of which 175 were polymorphic. The average polymorphism rate was 80.3%,

and the average genetic similarity coefficient was found to be 0.916. UPGMA cluster analysis of 51 materials showed that the materials were grouped into three clusters when the genetic distance reached 0.856. Tamimi et al. (2023) conducted a study on 123 plants from 25 table grape cultivars using SCoT markers. A total of 48 SCoT bands were obtained from all the studied samples, and only one private band was observed in two cultivars. A low to moderate level of genetic polymorphism was detected in the populations; however, AMOVA (Analysis of Molecular Variance) results indicated that the cultivars differed significantly in their genetic content.

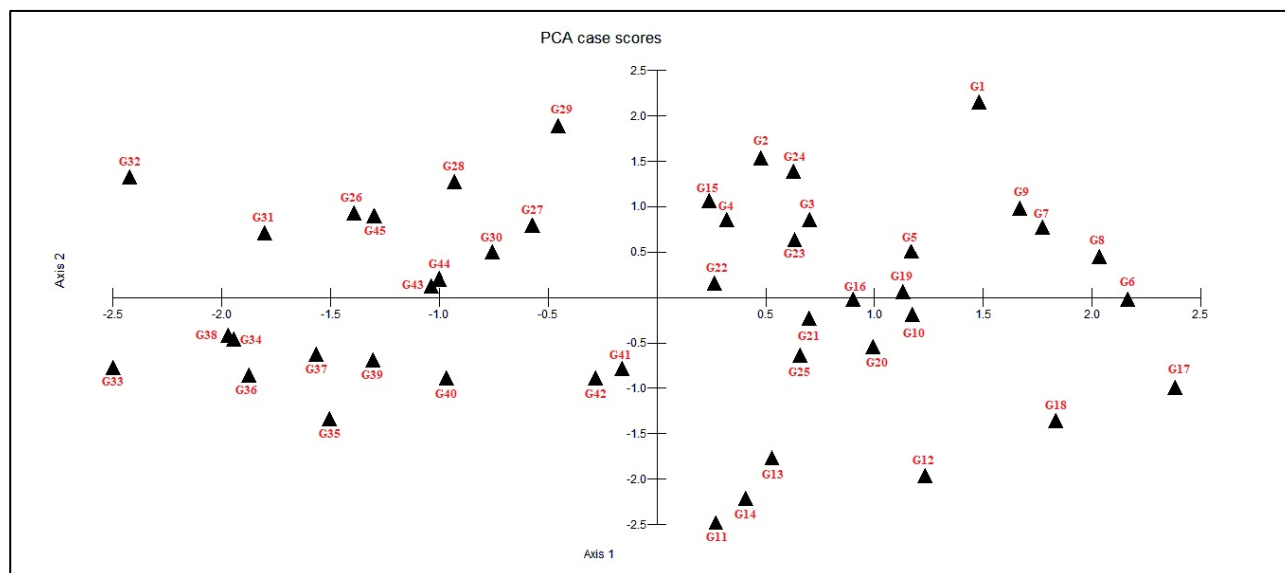


Figure 3. According to SCoT markers principal component analyses of 45 grape genotypes using MVSP 3.22 software

In their study evaluating the genetic diversity of three different grape cultivars, Hameed et al (2020) reported that RAPD primers produced the highest polymorphism rate (67%). This rate was determined as 36% for ISSR primers and 44% for SCoT primers. Each of the three marker systems generated similar dendrograms (phylogenetic trees). However, the genetic distances obtained with RAPD were higher compared to those obtained with SCoT and ISSR. Igwe et al (2022), in their study, reported 326 bands from SCoT primers, with a polymorphism information content (PIC) value of 0.9421. In the study by Bashandy et al (2020), molecular analyses were conducted using Start Codon Targeted (SCoT) and Inter-Simple Sequence Repeats (ISSR) markers. The detected polymorphism rate was 68.38% for SCoT and 41.84% for ISSR. The PIC value was 0.29 for SCoT and 0.19 for ISSR, making SCoT markers more informative. On the other hand, the dendrogram obtained using ISSR markers demonstrated a better clustering pattern compared to that of the SCoT markers. Kajkolah et al (2023) analyzed 178 cultivated *Vitis vinifera* grape accessions to assess genetic diversity and population structure using Start Codon Targeted (SCoT) molecular markers. A total of 35 alleles were detected, indicating that populations exhibited varying levels of genetic variation from low to high. Population genetic analyses revealed that the studied populations differed significantly in genetic content and exhibited a high degree of genetic admixture. When the results of the literature review are compared with our study, it is concluded that similar findings were obtained. Moreover, it was determined that SCoT markers are widely preferred and provide reliable results in the determination of genetic diversity in grape species.

4. Conclusions

The conservation of genetically rich species is critically important for ensuring ecosystem sustainability and meeting future agricultural production needs. Therefore, the molecular identification and preservation of local grape varieties is a necessary and fundamental step for sustainable agricultural practices. In our study, variations among 45 grape genotypes were detected using SCoT markers. Genotypes that are genetically close or distant from each other were clearly identified based on the data from the correlation matrix. Moreover, the genetic variation within the population was distributed homogeneously in both the UPGMA dendrogram and

the PCA plot, with a polymorphism rate of 69.74%. As a result, it was demonstrated that SCoT markers can be successfully used either alone or in combination with other existing DNA markers for studying inter- and intraspecific genetic variation, and they are effective in distinguishing genetically similar materials.

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

Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgments: This study is derived from the master's thesis titled " Investigation of genetic diversity of some grape (*Vitis vinifera*) germplasm grown in Sivas/Divriği using SCoT markers", conducted by the first author at Sivas University of Science and Technology.

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