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Investigation of Genetic Polymorphism in Selected Thyme (*Thymus vulgaris* L.) Accessions Using SCoT Molecular Markers

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HIGHLIGHTS

- In this study, thirty-two thyme genotypes were used as plant material.
- Determining genetic polymorphism is highly important for the species' adaptation to nature, enhancing its commercial value, developing high-quality products, and breeding programs.
- Molecular characterization of some thyme genotypes included in the study was conducted to reveal their genetic diversity and to ensure the sustainable conservation of natural resources.

Abstract

Identifying plant species and evaluating their variation should not be based solely on morphological characteristics. Such studies should be expanded through biochemical and DNA analyses. In this context, the aim of our study is to reveal the genetic diversity of some thyme (*Thymus*) samples naturally growing in the flora of Divriği district of Sivas province, and to contribute to the sustainable conservation of natural resources as well as to determine their agricultural utilization potential. Additionally, this study aims to explore the use of SCoT (Start Codon Targeted) markers in evaluating the diversity among different thyme genotypes. The plants were collected from their natural habitats, and DNA was extracted from fresh leaves using the CTAB method. A total of 36 SCoT markers were screened in the samples and the 14 most polymorphic markers were selected for analysis. In this study, a total of 169 bands were obtained from the 14 SCoT primers used to determine the genetic relationships among the thyme samples. Among these, 141 bands were polymorphic, resulting in a polymorphism rate of 83.43%. The data obtained allowed for the construction of a dendrogram grouping the analyzed ecotypes according to their similarity indices. According to the UPGMA dendrogram and the correlation matrix generated from the SCoT molecular data, the lowest similarity (0.326) was observed between samples G12 and G17, while the highest similarity (0.854) was found between G8 and G10. As a result, it was demonstrated that evaluating variation in *Thymus* species and establishing similarity indices using SCoT markers is possible, providing a solid basis for advanced molecular genetic analyses.

Keywords: *Thymus vulgaris L.*; SCoT marker; polymorphism

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1. Introduction

Thyme, which holds a significant place both in global cuisine and traditional medicine, is a perennial aromatic plant belonging to the *Lamiaceae* family (Gavaric et al. 2015). Thyme species are rich in essential oils, phenolic compounds, flavonoids, vitamins, and minerals. Due to these chemical constituents, thyme exhibits strong antioxidant, antimicrobial, antifungal, anti-inflammatory, and anticancer properties (Shabnum and Wagay 2011). Thyme is widely utilized in the food industry both in fresh and dried forms as a spice, while its essential oils are extensively used in the cosmetic, pharmaceutical, and disinfectant industries. Additionally, thyme tea and extracts are commercially valuable functional products (Miraj and Kiani 2016). Thyme (*Thymus vulgaris* L.) is one of the most commonly used aromatic plants worldwide due to its high economic value, aromatic characteristics, and medicinal effects (Prasanth Reddy et al. 2014). Given their wide ecological distribution and adaptability to diverse environmental conditions, thyme species exhibit a high degree of genetic variation (Basch et al. 2004). Although the Sivas province is not highly suitable for large-scale thyme cultivation, local thyme species are found naturally within the flora of districts such as Zara, Divriği, and İmranlı, where they are collected on a limited scale for traditional use. Natural populations in these areas display morphological and chemical variation due to ecological differences, which presents a valuable opportunity for the conservation of local genetic resources (Koyuncu et al. 2010; Celep and Dirmenci 2017; Fafal et al. 2024). Local thyme species generally possess richer aroma profiles and are more commonly preferred in traditional medicine (Gouyon et al. 1986). This variation, known as genetic polymorphism, is critically important for the sustainability, adaptability, and breeding programs of the species (Vernet et al. 1986). Genetic polymorphism enables thyme species to develop tolerance to different climate, soil, and environmental conditions (Rustaiee et al. 2013). This diversity allows plants to be more resilient against stress factors such as drought, diseases, pests, and climate change. Genetic variation observed particularly in thyme species growing naturally in semi-arid regions supports their adaptive capacity.

Genetic diversity directly influences the quantity and composition of essential oil constituents. The levels of compounds such as carvacrol, thymol, p-cymene, and γ-terpinene can vary significantly among genotypes. These differences have a direct impact on product quality, commercial value, and the potential applications of the plant (Amiot et al. 2005). In this regard, genetic polymorphism is a fundamental factor in determining aromatic quality. The main objectives of thyme breeding programs are to enhance traits such as high yield, disease resistance, essential oil content, and specific aroma profiles (György et al. 2020). Genetic polymorphism enables the selection and development of such traits. Identifying genetically distinct individuals is crucial for the development of superior lines in breeding programs (Thompson et al. 2004). The genetic diversity found in natural thyme populations is of great importance for the conservation of local species. Conserving genetically rich species plays a strategic role in ensuring ecosystem sustainability and meeting future agricultural demands (Rustaiee et al. 2013). Therefore, the molecular identification and conservation of local varieties are essential components of sustainable agricultural practices (El-Demerdash et al. 2019). Techniques such as ISSR, RAPD, AFLP, SCoT and SSR used in the detection of genetic polymorphism are widely employed to elucidate the genetic structure of thyme species and to analyze inter- and intra-genotypic relationships (Sayed Ibrahim et al. 2024). These techniques allow for more precise species identification and classification (Khalil et al. 2012; Elsherbeny and Eldemerdash 2019).

The aim of this study is to determine the genetic polymorphism among different thyme genotypes collected from gardens in the Divriği district of Sivas and to reveal the genetic variation present. The findings are expected to contribute to the conservation of thyme germplasm in the region and support future breeding efforts.

2. Materials and Methods

Fresh leaves were collected from 32 different thyme samples belonging to the *Thymus vulgaris* L. germplasm, cultivated in farmer gardens in the Divriği district of Sivas. Information related to the thyme samples is presented in Table 1.

Table 1. Data of thyme genotypes characterized.

Genotype Code	Taxonomy	Collected Location	Genotype Code	Taxonomy	Collected Location
G1	*Thymus vulgaris* L.	Sivas/Divriği	G17	*Thymus vulgaris* L.	Sivas/Divriği
G2	*Thymus vulgaris* L.	Sivas/Divriği	G18	*Thymus vulgaris* L.	Sivas/Divriği
G3	*Thymus vulgaris* L.	Sivas/Divriği	G19	*Thymus vulgaris* L.	Sivas/Divriği
G4	*Thymus vulgaris* L.	Sivas/Divriği	G20	*Thymus vulgaris* L.	Sivas/Divriği
G5	*Thymus vulgaris* L.	Sivas/Divriği	G21	*Thymus vulgaris* L.	Sivas/Divriği
G6	*Thymus vulgaris* L.	Sivas/Divriği	G22	*Thymus vulgaris* L.	Sivas/Divriği
G7	*Thymus vulgaris* L.	Sivas/Divriği	G23	*Thymus vulgaris* L.	Sivas/Divriği
G8	*Thymus vulgaris* L.	Sivas/Divriği	G24	*Thymus vulgaris* L.	Sivas/Divriği
G9	*Thymus vulgaris* L.	Sivas/Divriği	G25	*Thymus vulgaris* L.	Sivas/Divriği
G10	*Thymus vulgaris* L.	Sivas/Divriği	G26	*Thymus vulgaris* L.	Sivas/Divriği
G11	*Thymus vulgaris* L.	Sivas/Divriği	G27	*Thymus vulgaris* L.	Sivas/Divriği
G12	*Thymus vulgaris* L.	Sivas/Divriği	G28	*Thymus vulgaris* L.	Sivas/Divriği
G13	*Thymus vulgaris* L.	Sivas/Divriği	G29	*Thymus vulgaris* L.	Sivas/Divriği
G14	*Thymus vulgaris* L.	Sivas/Divriği	G30	*Thymus vulgaris* L.	Sivas/Divriği
G15	*Thymus vulgaris* L.	Sivas/Divriği	G31	*Thymus vulgaris* L.	Sivas/Divriği
G16	*Thymus vulgaris* L.	Sivas/Divriği	G32	*Thymus vulgaris* L.	Sivas/Divriği

2.1. Genomic DNA Isolation and PCR

DNA extraction from thyme 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 14 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′)	Tm °C	PCR Reaction Mixture Content	PCR Amplification
SCoT 1	CAACAATGGCTACCACCA	54	4 µL DNA (20 ng) 1 µL primer 10 µL PCR master mix (Eco Tech, Cat No: ET5) 10 µL dH_2O	94 °C - 3 min 94 °C - 1 min 54–63 °C 1 min 72 °C - 1 min 72 °C - 8 min 35 cycles
SCoT 12	ACGACATGGCGACCAACG	58		
SCoT 14	ACGACATGGCGACCACGC	61		
SCoT 16	ACCATGGCTACCACCGAC	58		
SCoT 20	ACCATGGCTACCACCGCG	61		
SCoT 21	ACGACATGGCGACCCACA	58		
SCoT 22	AACCATGGCTACCACCAC	56		
SCoT 24	CACCATGGCTACCACCAT	56		
SCoT 26	ACCATGGCTACCACCGTC	58		
SCoT 28	CCATGGCTACCACCGCCA	61		
SCoT 30	CCATGGCTACCACCGGCG	63		
SCoT 31	CCATGGCTACCACCGCCT	61		
SCoT 33	CCATGGCTACCACCGCAG	61		
SCoT 36	GCAACAATGGCTACCACC	56		

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 thyme 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.

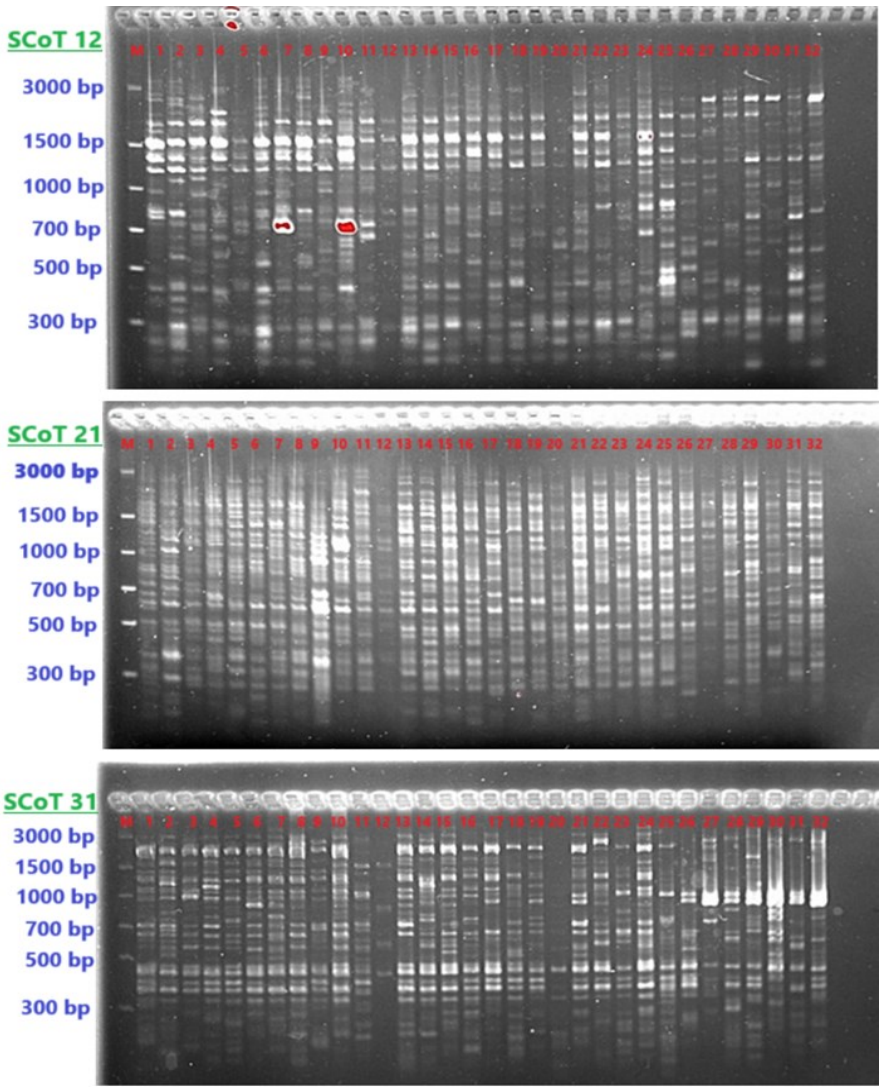

Figure 1. Gel image of bands amplifed with SCoT 12, SCoT 21 and SCoT 31 primers: M: generuler.

In this study, a total of 169 bands were obtained from 14 SCoT primers used to determine the genetic relationships among thyme samples. Among these, 141 bands were polymorphic, resulting in a polymorphism rate of 83.43%. According to the UPGMA dendrogram and the correlation matrix generated from the SCoT molecular data, the lowest similarity (0.326) was observed between samples G12 and G17, while the highest similarity (0.854) was observed between samples G8 and G10 (Figure 2, Table 3).

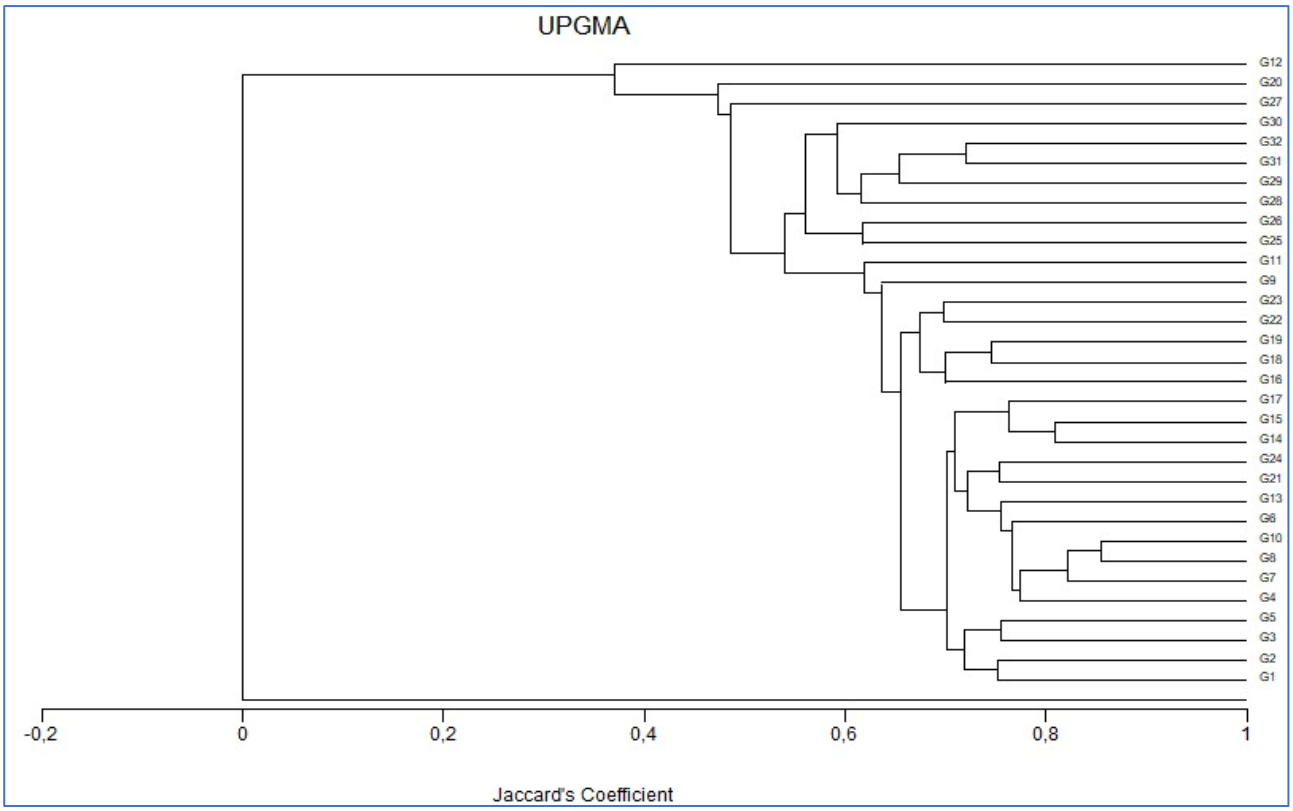

Figure 2. UPGMA dendrogram of genetic similarity between SCoT markers and 32 Thyme genotypes.

Table 3. Pairwise genetic distance matrix obtained from 14 SCoT primers.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28	G29	G30	G31	G32
1	1,000																															
2	0,752	1,000																														
3	0,733	0,738	1,000																													
4	0,686	0,752	0,734	1,000																												
5	0,669	0,736	0,756	0,770	1,000																											
6	0,660	0,703	0,671	0,771	0,694	1,000																										
7	0,717	0,771	0,716	0,793	0,752	0,766	1,000																									
8	0,697	0,760	0,707	0,769	0,707	0,779	0,851	1,000																								
9	0,639	0,662	0,664	0,645	0,676	0,643	0,676	0,704	1,000																							
10	0,723	0,764	0,687	0,760	0,721	0,747	0,792	0,854	0,660	1,000																						
11	0,617	0,585	0,654	0,624	0,654	0,610	0,608	0,648	0,623	0,698	1,000																					
12	0,375	0,336	0,382	0,346	0,378	0,362	0,353	0,360	0,438	0,358	0,423	1,000																				
13	0,688	0,740	0,676	0,736	0,698	0,735	0,732	0,792	0,637	0,784	0,628	0,348	1,000																			
14	0,645	0,724	0,634	0,651	0,635	0,649	0,703	0,718	0,583	0,687	0,563	0,336	0,733	1,000																		
15	0,698	0,752	0,697	0,736	0,720	0,699	0,743	0,769	0,634	0,736	0,636	0,377	0,785	0,809	1,000																	
16	0,593	0,651	0,640	0,705	0,713	0,643	0,676	0,680	0,599	0,638	0,600	0,361	0,706	0,652	0,743	1,000																
17	0,681	0,689	0,669	0,697	0,658	0,671	0,692	0,707	0,594	0,709	0,607	0,326	0,733	0,729	0,796	0,713	1,000															
18	0,614	0,595	0,638	0,678	0,662	0,664	0,662	0,678	0,609	0,636	0,610	0,397	0,669	0,650	0,714	0,733	0,748	1,000														
19	0,588	0,603	0,590	0,631	0,662	0,640	0,686	0,678	0,618	0,657	0,595	0,383	0,657	0,626	0,691	0,669	0,699	0,746	1,000													
20	0,388	0,407	0,436	0,471	0,463	0,435	0,446	0,448	0,456	0,428	0,432	0,358	0,448	0,463	0,471	0,484	0,463	0,610	0,538	1,000												
21	0,616	0,660	0,662	0,712	0,720	0,687	0,743	0,699	0,634	0,691	0,579	0,377	0,702	0,697	0,736	0,669	0,708	0,714	0,691	0,504	1,000											
22	0,617	0,662	0,688	0,681	0,664	0,655	0,688	0,680	0,589	0,649	0,613	0,402	0,660	0,664	0,692	0,672	0,712	0,719	0,669	0,574	0,716	1,000										
23	0,555	0,593	0,591	0,657	0,664	0,630	0,652	0,612	0,620	0,614	0,635	0,381	0,603	0,582	0,610	0,659	0,570	0,709	0,617	0,595	0,645	0,698	1,000									
24	0,679	0,721	0,702	0,729	0,713	0,703	0,773	0,750	0,638	0,778	0,617	0,369	0,695	0,667	0,682	0,616	0,725	0,660	0,684	0,432	0,754	0,709	0,638	1,000								
25	0,482	0,524	0,496	0,507	0,532	0,525	0,524	0,513	0,474	0,483	0,485	0,390	0,524	0,532	0,539	0,581	0,543	0,605	0,504	0,491	0,550	0,583	0,552	0,521	1,000							
26	0,507	0,538	0,522	0,521	0,536	0,507	0,507	0,487	0,511	0,497	0,535	0,370	0,517	0,536	0,531	0,537	0,503	0,560	0,463	0,457	0,531	0,529	0,607	0,524	0,617	1,000						
27	0,512	0,500	0,504	0,450	0,496	0,436	0,437	0,449	0,504	0,438	0,504	0,375	0,448	0,442	0,460	0,473	0,474	0,462	0,441	0,391	0,493	0,489	0,475	0,453	0,518	0,564	1,000					
28	0,496	0,486	0,557	0,479	0,548	0,507	0,466	0,487	0,500	0,467	0,548	0,392	0,497	0,504	0,511	0,538	0,462	0,574	0,532	0,509	0,555	0,565	0,597	0,483	0,538	0,556	0,581	1,000				
29	0,479	0,552	0,536	0,556	0,550	0,542	0,563	0,530	0,538	0,471	0,504	0,351	0,541	0,528	0,556	0,588	0,539	0,611	0,546	0,527	0,579	0,664	0,623	0,571	0,593	0,570	0,513	0,628	1,000			
30	0,543	0,529	0,523	0,479	0,504	0,518	0,529	0,497	0,536	0,507	0,524	0,363	0,507	0,493	0,500	0,504	0,493	0,527	0,508	0,481	0,500	0,485	0,545	0,514	0,539	0,570	0,552	0,559	0,574	1,000		
31	0,545	0,583	0,592	0,577	0,582	0,596	0,553	0,561	0,561	0,571	0,574	0,355	0,582	0,510	0,546	0,542	0,571	0,630	0,569	0,516	0,577	0,633	0,606	0,581	0,578	0,606	0,516	0,664	0,661	0,625	1,000	
32	0,565	0,581	0,545	0,565	0,559	0,617	0,593	0,589	0,594	0,591	0,548	0,359	0,559	0,548	0,554	0,507	0,580	0,583	0,591	0,440	0,586	0,563	0,556	0,636	0,527	0,531	0,500	0,556	0,648	0,610	0,720	1,000

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 thyme genotypes. Shayan et al (2025) investigated the level of polymorphism in a thyme population using ISSR and SCoT markers, and reported that SCoT was more effective in detecting polymorphism in *Thymus daenensis*. In another study evaluating the genetic diversity among different *Thymus* species using SCoT and ISSR analyses, 39 bands (24 polymorphic – 60.52% polymorphism) were obtained from five SCoT primers, and 23 bands (14 polymorphic – 60.86% polymorphism) were obtained from five ISSR primers (Alqahtani et al. 2020). Beicu et al (2020) analyzed 13

natural thyme ecotypes using four SCoT markers (SCoT 11, SCoT 14, SCoT 35, and SCoT 36). As a result, 117 alleles were amplified with an average of 29.25 alleles per primer. All bands were found to be polymorphic. The variance analysis revealed an average PIC value of 0.370 and an average polymorphism index (PI) of 10.57. Ahmed et al. (2022) used five RAPD, six ISSR, and six SCoT primers to assess the genetic diversity levels in four plant species belonging to the Lamiaceae family. Using RAPD primers, a total of 41 amplified bands were identified with a polymorphism rate of 73.71%. ISSR primers generated a total of 31 bands with a polymorphism rate of 54.83%, while SCoT primers produced 43 bands with a polymorphism rate of 39.53%. Karagöz et al (2022) successfully characterized the population structure of 70 oregano genotypes using SCoT markers. A total of 109 polymorphic fragments were generated with 10 SCoT primers, and the average PIC value was calculated as 0.36.

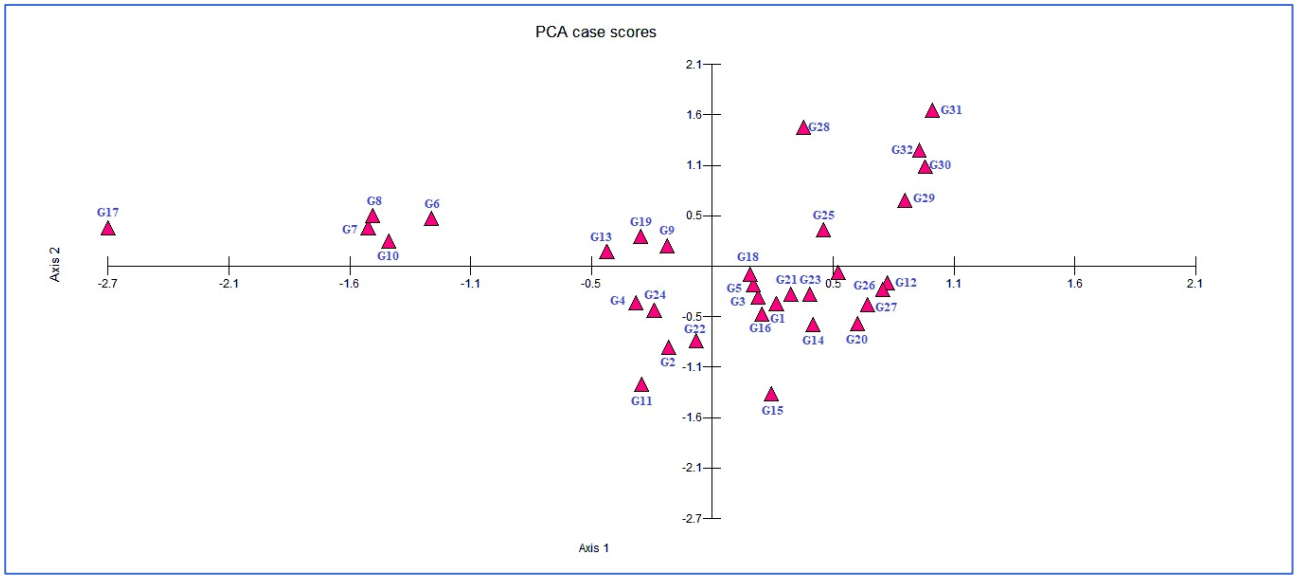

Figure 3. According to SCoT markers pricipal component analyses of 18 Thyme genotypes using MVSP 3.22 software.

4. Conclusions

Biochemical and DNA analyses are conducted to reveal variations within populations. In this study, the genetic diversity of some thyme (*Thymus*) samples naturally growing in the flora of Divriği district of Sivas province was investigated. In this way, the potential use of SCoT markers in evaluating the diversity among different thyme genotypes was also explored. The data obtained enabled the construction of a dendrogram grouping the analyzed ecotypes according to their similarity indices, and revealed a significant level of variation within the population. As a result, it was demonstrated that variation in *Thymus* species can be effectively evaluated using SCoT markers, and that these findings can contribute to the sustainable conservation of natural resources as well as the determination of their agricultural utilization potential. This indicates that the study provides a basis for advanced molecular genetic analyses. Our results suggest that evaluating genetic diversity and population structure in natural populations can provide comprehensive information for the conservation of endemic and endangered species, and can be effectively used in future breeding programs.

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