

GENETIC DIVERSITY ANALYSIS OF *TEUCRIUM POLIUM* POPULATIONS IN AYDIN/TÜRKİYE BASED ON RAPD-PCR

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ABSTRACT

In this study, genetic diversity analysis of eight *Teucrium polium* L. populations distributed in Aydın province of Türkiye was conducted using ten RAPD markers. As a result of the study, a total of 45 bands were obtained, of which eight were monomorphic and 37 were polymorphic. The total polymorphism rate was determined as 82.22%. UPGMA dendrogram consists of two clades. The lowest similarity (-0.100) was observed between the Koçarlı and Hamitler village populations, while the highest coefficient (0.646) was observed with the Çakmar (road) and Hamitler village populations. As a result, RAPD marker technique can be a useful marker for revealing the genetic diversity of *Teucrium polium* populations. In addition, this results it will be a references for different marker and sequence-based studies in the future.

Keywords: *Teucrium polium*, genetic diversity, RAPD, Aydın

INTRODUCTION

The *Lamiaceae* family consists of 258 genera and more than 6000 species (Valerio *et al.*, 2021). *Lamiaceae* family, which includes medicinal plants rich in secondary metabolites, species with great economic value due to their potential as ornamental plants, culinary plants, and production of valuable bioactive compounds, contains a large number of species mostly seen in Asia and the Mediterranean (Çelik *et al.*, 2021; Aebisher *et al.*, 2021; Gonçalves *et al.*, 2021). The genus *Teucrium* L belongs to *Lamiaceae* family is mainly distributed in the Mediterranean region (Sadeghi *et al.*, 2021). *Teucrium polium* contains various components such as terpenoids, flavonoids and iridoids (Bahramikia & Yazdanparast, 2012). *T. polium* is a medicinal plant used in folk medicine for various purposes such as anti-inflammatory, antimicrobial, antitumor, anti-nociceptive, hypoglycemic, anti-hypertensive and anti-hyperlipidemic effects (Gharaibeh *et al.*, 1988; Ardestani & Yazdanparast, 2007; Sevindik *et al.*, 2016; Elmasri *et al.*, 2016). Biotechnology is known as a popular branch of science that relies on biological traits to obtain products with desired positive traits in all living organisms with genetic material (Eren, 2021). DNA markers have been developed to provide reliable results for genetic identification of varieties and genotypes (Karakaya *et al.*, 2022). The current molecular markers used in genetic variation identification are RFLP, PCR-RFLP, RAPD, ISSR, SSR, and they are preferred because they are simpler, cheaper, and faster (Aydın & Özden, 2021). Among molecular methods or markers, RAPD is very responsive to detecting variability among individuals of a species. The RAPD method is inexpensive and can work with limited sample

sizes. Moreover, RAPD can amplify and target genomic regions with potential and diverse markers (Vivodik *et al.*, 2022). The RAPD-PCR technique in many plants has been used to examine genotype and population polymorphisms and genetic diversity of species (Vierling *et al.*, 1992; Iqbal *et al.*, 1997; Sensoy *et al.*, 2007; Pinheiro *et al.*, 2012; Doğru Çokran *et al.*, 2019; Balážová *et al.*, 2021; Sekar *et al.*, 2021; Aydın & Özden, 2021; Qian & Mehri *et al.*, 2021; Bi *et al.*, 2021; Vivodik *et al.*, 2022; Yin *et al.*, 2022; Al-Khayri *et al.*, 2022; etc.). In this study, genetic relationships among some *Teucrium polium* populations distributed in Aydın province were determined using RAPD-PCR marker technique.

MATERIALS AND METHODS

Plant Materials and Genomic DNA Isolation

Eight populations of *Teucrium polium* populations were collected from different districts of Aydın province. gDNA was isolated using a commercial kit (GeneMark catalog no: DP022). Extracted genomic DNA samples were stored at -20 °C.

PCR and agarose electrophoresis

The RAPD primers selected for PCR amplifications are given in Table 1 below.

Table 1 RAPD primer sequences, Tm, RAPD- PCR amplification conditions and amplification results

Primers	DNA sequences	Tm °C	PCR Amplification (35 Cycles except final extension step)	Amplification
OPA-15	5'-TTCCGAACCC-3'	32	94 °C/2 min	+
OPA-20	5'-GTTGCGATCC-3'	32		+
OPA-02	5'- TGCCGAGCTG-3'	34	94 °C/1 min	+
OPA-13	5'- CAGCACCCAC-3'	34		+
OPA-16	5'-AGCCAGCGAA-3'	32	32-34 °C/1 min	-
OPA-18	5'-AGGTGACCGT-3'	32		+
OPA-05	5'-AGGGGTCTTG-3'	32	72 °C/1 min	+
OPJ-10	5'-AAGCCCGAGG-3'	32		-
OPA-03	5'-AGTCAGCCAC-3'	32	72°C/10min(final extension: 1 cycle)	-
OPA-07	5'-GAAACGGGTG-3'	32		-

PCR applications were performed using a Thermocycler Gradient device. Ready mix was used as an alternative route for the PCR reaction. Amplification was performed with 0.5 µL genomic DNA, 1 µL primers, 10 µL master mix and 8.5 µL

ddH₂O in a PCR tube. The PCR programs used were generated separately for each primer in accordance with the TM values of the primers used in the studies based on referenced articles. 1.5% agarose gel electrophoresis was applied to separate the

bands formed as a result of PCR. To do this, 1.5 g agarose was weighed and dissolved in 100 mL 1 X TBE (Tris-Boric EDTA) buffer by boiling in a microwave oven. The mixture was cooled, 1.25 µL EtBr was added and casted. Then 5 µL of PCR product and 1 µL of loading dye (6X DNA loading dye) were added, stained and loaded into the wells with a micropipette. To determine the size of the PCR products, 5 µL of DNA size marker (1 kb DNA ladder) was loaded into an empty well and the samples were run at 100 volts for 90 min. The gel was then taken to the gel imaging device, the bands were observed under UV light and the data were recorded by taking photographs with the help of the gel imaging device's computer program and camera.

RAPD-PCR analysis

After RAPD-PCR analyses, DNA bands were assigned a value of "1" if DNA was present and "0" if DNA was absent, and the polymorphism rate was determined by scoring the DNA bands. The genetic diversity of the *Teucrium polium* samples used in the study was analyzed using MVSP 3.22 program (Kovach, 2007).

RESULTS AND DISCUSSION

As a result of RAPD-PCR analysis, 10 RAPD primers were used, six of them yielded positive results and 4 of them displayed negative results. In scoring the bands, a total of 45 bands were obtained, of which 37 were polymorphic and eight were monomorphic. Polymorphism rate was 82.22%. The genetic distance matrix between the UPGMA tree and Principal component analysis (PCA) of *Teucrium polium* populations were generated using the MVSP 3.22 analysis program. The UPGMA tree consisted of two clusters. Cluster 1 consisted of Çakmar (road) and

Hamitler village populations, while cluster 2 consisted of two subgroups. Subgroup A consisted of only Didim population, while subgroup B consisted of Aydın-Centre, Danişment village, Çine (road), Koçarlı and Çakmar (mountain) populations (Figure 1).

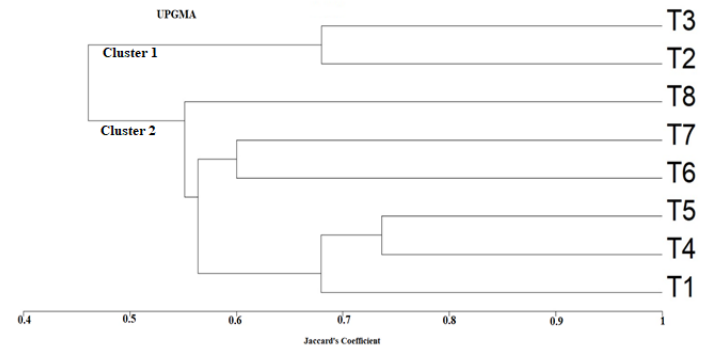


Figure 1 The UPGMA tree generated using RAPD data (T1: Çakmar (mountain), T2: Çakmar (road), T3: Hamitler village, T4: Aydın-Centre, T5: Danişment village, T6: Çine (road), T7: Koçarlı, T8: Didim populations)

Using the MVSP 3.22 program, the PCA image in Figure 2 was obtained. As a result of the analysis, three groups were obtained. The UPGMA dendrogram and PCA analysis were compatible with each other.

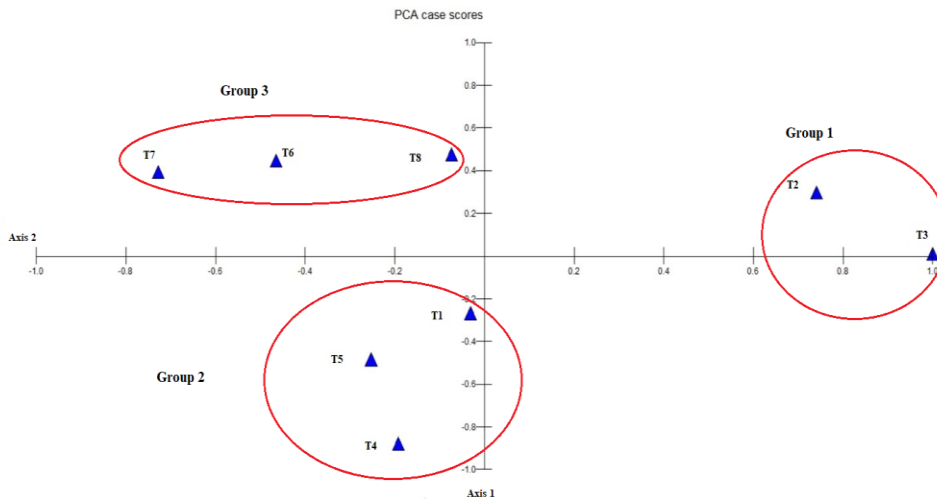


Figure 2 Principal component analyses of *Teucrium polium* populations using MVSP 3.22 software (T1: Çakmar (mountain), T2: Çakmar (road), T3: Hamitler village, T4: Aydın-Centre, T5: Danişment village, T6: Çine (road), T7: Koçarlı, T8: Didim populations)

According to RAPD molecular data, the lowest similarity (-0.100) was observed between the Koçarlı and Hamitler village populations, while the highest coefficient (0.646) was observed with the Çakmar (road) and Hamitler village populations (Table 2).

Table 2 Similarity matrix between the *Teucrium polium* populations

Populations	T1	T2	T3	T4	T5	T6	T7	T8
Çakmar (mountain)	1							
Çakmar (road)	0.409	1						
Hamitler	0.348	0.646	1					
Aydın-Centre	0.416	0.202	0.263	1				
Danişment	0.339	0.273	0.311	0.449	1			
Çine (road)	0.346	0.245	0.070	0.226	0.399	1		
Koçarlı	0.316	0.159	-0.100	0.167	0.365	0.467	1	
Didim	0.440	0.334	0.249	0.226	0.303	0.377	0.378	1

Pesaraklu et al. (2013) determined the genetic diversity of six *Teucrium polium* populations collected from different locations in Iran utilizing RAPD markers. In their study, 61 bands were obtained from 8 RAPD primers and they determined the polymorphism rate as 89.71%. In their study, genetic similarity between genotypes was determined between 0.33 and 0.58. Boulila et al. (2010) evaluated the genetic diversity of seven diploid and polyploid *Teucrium polium* populations in five bioclimatic areas in Tunisia utilizing RAPD markers. They obtained 141 bands in RAPD-PCR results and found 129 of them to be polymorphic. Baghizadeh et al.

(2017) determined the genetic diversity of 15 *Teucrium polium* genotypes collected from Kerman province of Iran by RAPD analysis. In the study, 182 bands were obtained from 15 RAPD primers and 169 (93%) of them were polymorphic. They also determined the genetic similarity of the genotypes to be between 0.37 and 0.72. Norouzi Ghare Tapeh et al. (2018) determined the genetic diversity of 77 *Teucrium polium* populations collected from different regions of Iran using ISSR marker technique. They obtained 198 bands from 18 ISSR markers, 184 of which were polymorphic and the polymorphism rate was 92.9%. Also, Polymorphic Information Content (PIC), Shannon's Information index (I), and Number of effective alleles (Ne) have been determined as 0.39, 0.526 and 1.6, respectively. Esfandyari et al. (2018) revealed the genetic diversity of 17 *Teucrium polium* ecotypes collected from Kermanshah (Iran) with 15 ISSR primers. In their study, 82 bands were obtained from 12 ISSR primers and 80 of them were polymorphic. The average number of bands for each primer was 6.83. Mohajer-Tabrizi et al. (2020) extracted genetic diversity parameters of 16 *T. polium* populations in Alboz mountain range of Iran using ISSR technique. As a result of the study, the means of Polymorphism (%P), Nei's genetic diversity (H) and Shannon's Information Index (I) were found to be 33.24%, 0.118 and 0.179, respectively, while AMOVA analysis revealed a large genetic variation (77%) within the population and a relatively high genetic differentiation (Gst: 0.311) and gene flow (Nm: 1.107). Farshadfar et al. (2020) used the SCoT marker to determine genetic variation among 17 *Teucrium polium* genotypes collected from Kermanshah province, Iran. In their study, they obtained 48 polymorphic bands in all SCoT primers. They calculated the average PIC=0.361, MI=1.525, EMR=4.197, polymorphism percentage 98.485% and RP=2.837 indices for all primers. In the studies conducted in previous years, the polymorphism rate was high as indicated in our study.

CONCLUSION

The present study suggests novel applications for our country in many aspects. RAPD analysis revealed a high rate of polymorphism and genetic diversity in *Teucrium polium* populations. As a result of the study, 45 bands were obtained from 10 RAPD primers, and the polymorphism rate was 82.22%. In this respect, determining the molecular characteristics of *Teucrium polium* populations growing in the region will contribute to both science and future economic studies. In addition, phylogenetic relationships and genetic distances between populations will be revealed and taxonomic problems will be solved. It will also be a reference for future phylogenetic studies.

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