

# Genetic Diversity of Local Endemic *Teucrium leucophyllum* Montbret & Aucher ex Bentham. (Lamiaceae) in Turkey

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## ABSTRACT

**Background and Objective:** *Teucrium leucophyllum* is endemic plant species having considerable narrow distribution in Erzincan (Turkey) Region. The aim of this study was to determine genetic diversity levels of *T. leucophyllum* in order to obtain data for its conservation. **Method:** Genomic DNA was isolated from 81 plants using CTAB method. Genetic diversity in four natural populations of *T. leucophyllum* was investigated by ISSR markers. A binary matrix was produced by scoring each amplified fragment as present (1) or absent (0) from each individual. The matrix was used to produce an input file and analyzed using the software programs; POPGENE 1.32 and GenAlex. **Results:** Twenty primers amplified a total of 434 bright and discernible bands of 220–3000 bp, of which 431 bands (PPB: 99.31%) were polymorphic in 4 populations. The genetic diversity was high at the species level; the observed number of alleles ( $N_a$ ) was 1.993, the effective number of alleles ( $N_e$ ) was 1.404, the Nei's genetic diversity ( $H$ ) was 0.263, and the Shannon's information index ( $I$ ) was 0.418. A high level of gene flow ( $N_m$ : 2.208) and low-moderate genetic differentiation ( $G_{st}$ : 0.184) were also observed. Analysis of molecular variance showed that 11% of the total genetic diversity resided among populations, while 89% within the populations. **Conclusion:** Our results indicated that the conservation efforts should aim to preserve the extant populations of this endangered species in its natural habitat. Seeds could be collected from Kayacik and Bagistas populations for *ex situ* conservation practices.

**Keywords:** Genetic diversity, ISSR, PCR, *Teucrium leucopyllum*, Endemic plant.

## INTRODUCTION

Medicinally, *Teucrium* (Lamiaceae) species have a long history of use as diuretic, diaphoretic, tonic, antipyretic, antidiabetic, antispasmodic, and cholagogic purposes. In Turkey, 48 *Teucrium* species occur naturally, of which 18 are endemic.<sup>1</sup> *Teucrium leucophyllum* Montbret & Aucher ex Bentham is a rare and endemic plant species that shows narrow distribution around Erzincan (Turkey) province. According to the IUCN criteria, this species was evaluated as EX (Extinct), however, it has been rediscovered from its original localities.<sup>2,3</sup> At present, only four populations exist in Erzincan. Besides its narrow distribution its populations are under various risks due to

intensive human activities such as mining, grazing and road construction.

Estimation of genetic variation levels in endemic species is crucial to develop reliable conservation strategies. Inter simple sequence repeats (ISSRs) have been widely used for estimating genetic variation at interspecific and intraspecific levels.<sup>4</sup> To date, various studies concerning morphological features and germination characteristics of *T. leucophyllum* were undertaken but no molecular study was conducted.<sup>3,5</sup> Therefore, the aim of this study was to determine genetic diversity levels within and among the four populations of *T. leucophyllum*. The results could

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provide valuable information to establish effective conservation strategies for this rare and endemic plant.

## MATERIALS AND METHODS

### Plant materials and DNA extraction

Plant samples were collected from each populations of *T. leucophyllum* between June and July 2014 (Table 1). A total of 81 plants were sampled for DNA extraction. The identification of species was performed based on the Flora of Turkey and East Aegean Islands.<sup>6</sup> The cetyl trimethyl ammonium bromide (CTAB) method was employed to isolate total genomic DNA from silica dried leaf samples.<sup>7</sup>

### ISSR-PCR and Data analysis

PCR amplifications were performed in a 25  $\mu$ L reaction mixture using gradient thermocycler (Applied Biosystems, CA, USA). After screening 45 ISSR primers, 20 of them that amplified clear and reproducible fragments were selected for further analysis [GAG(CAA)<sub>5</sub>, VHV (GT)<sub>7</sub>G, (GA)<sub>8</sub>YC, (GA)<sub>8</sub>T, (AG)<sub>8</sub>T, (AC)<sub>8</sub>C, (AGC)<sub>6</sub>G, DBD(AC)<sub>7</sub>, (AG)<sub>8</sub>YT, (TG)<sub>8</sub>A, (GACA)<sub>4</sub>, (GTTC)<sub>4</sub>, (GGAT)<sub>4</sub>, (GGGTG)<sub>3</sub>, (CT)<sub>8</sub>G, (TC)<sub>8</sub>C, (CA)<sub>8</sub>RC, (ACC)<sub>6</sub>, (AGC)<sub>6</sub>, (CTC)<sub>6</sub>]. PCR products were run on

1.4% agarose gel containing ethidium bromide (0.5  $\mu$ g/mL) at 90V and digitally photographed.

The amplification products were scored as present (1) or absent (0) for each individual. POPGENE program v1.32) was used to calculate genetic parameters and Nei's genetic distance between individuals.<sup>8</sup> The Analysis of Molecular Variance (AMOVA) was performed with the program GenAlEx 6.5 by resampling 999 times.<sup>9</sup>

## RESULTS AND DISCUSSION

Using 20 ISSR primers, a total of 434 clear and reproducible bands was amplified, of which 431 were polymorphic. ISSR band patterns of 81 individuals from four populations using primer (CTC)<sub>6</sub> was shown in Fig. 1. The percentage of polymorphic bands (PPB) ranged from 40.55% (Pop 4) to 94.47% (Pop 3) with an average of 79.14% at the population level and 99.31% at the species level (Table 2). The observed number of alleles (*N<sub>a</sub>*) ranged from 1.405 to 1.944, while the effective number of alleles (*N<sub>e</sub>*) varied from 1.263 to 1.398. Nei's genetic diversity (*H*) varied from 0.153 to 0.249, with an average of 0.220, and Shannon's information indices (*I*) ranged from 0.228 to 0.392, with an average of 0.345. At species level, *N<sub>a</sub>*, *N<sub>e</sub>*, *H* and *I* were 1.993,

Population locality	Population code	Sample size	Altitude (m)	Longitude (E)/Latitude (N)
İliç/Kayacık 1	Pop 1	23	920-944	37 S 451970 E, 4367486 N
İliç/Kayacık 2	Pop 2	22	950-1350	37 452122 E, 4365875 N
İliç/Bağıştaş-Salihli	Pop 3	33	952-975	37 454137 E, 4361001 N
Kemaliye/Binkoçlu	Pop 4	3	1314	37 455721 E, 4350433 N

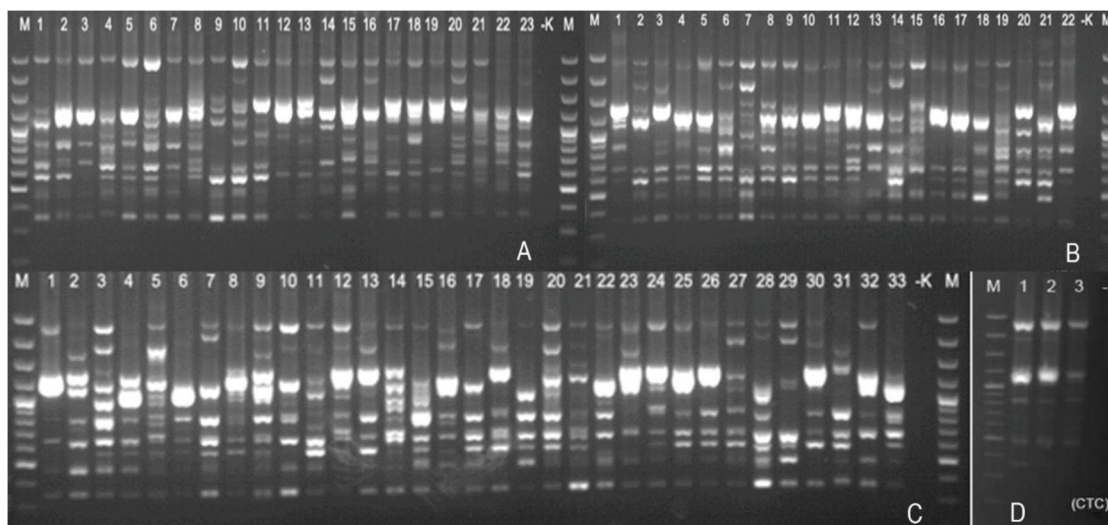


Figure 1: ISSR profiles of 81 individuals (4 populations) of *T. leucophyllum* using (CTC)<sub>6</sub> primer (M: GeneRuler 100 bp Ladder Plus, -K: a negative control).

**Table 2: Genetic diversity of *T. leucophyllum* populations**

Population	Na (sd)	Ne (sd)	H (sd)	I (sd)	PPB (%)
Pop 1	1.900 (0.299)	1.372 (0.301)	0.237 (0.157)	0.375 (0.213)	90.09
Pop 2	1.914 (0.279)	1.398 (0.313)	0.249 (0.158)	0.392 (0.212)	91.47
Pop 3	1.944 (0.228)	1.381 (0.301)	0.243 (0.153)	0.385 (0.204)	94.47
Pop 4	1.405 (0.491)	1.263 (0.359)	0.153 (0.195)	0.228 (0.284)	40.55
Average	1.791 (0.018)	1.353 (0.008)	0.220 (0.004)	0.345 (0.006)	79.14
Species	1.993 (0.082)	1.404 (0.267)	0.263 (0.132)	0.418 (0.171)	99.31

Na: observed number of alleles; Ne: effective number of alleles; H: Nei's gene diversity; I: Shannon's information indexes; PPB: percentage of polymorphic bands; sd: standard deviation.

1.404, 0.263 and 0.418, respectively. The highest variability levels were observed in Pop 2 ( $H_s$ : 0.249,  $I$ : 0.392) whereas lowest levels were observed in Pop 4 ( $H_s$ : 0.153,  $I$ : 0.228).

Generally, a low level of genetic diversity is commonly expected for endemic and geographically restricted species.<sup>10</sup> Interestingly, despite of its narrow distribution *T. leucophyllum* maintains high levels of genetic diversity (PPB: 99.31%,  $H$ : 0.263,  $I$ : 0.418) compared to levels of genetic diversity found in other endemic species. Similarly, numerous findings have been reported for many narrow endemic species maintaining high genetic diversity; for example, *Helenium virginicum* (PPB: % 98.5,  $H$ : 0.324,  $I$ : 0.484), *Lilium regale* (PPB: 97.3%,  $H$ : 0.198,  $I$ : 0.333).<sup>11,12</sup> Hamrick and Godt reported that outcrossing perennial species commonly have higher levels of genetic diversity than selfing and clonal plants.<sup>10</sup> *T. leucophyllum* is perennial plant reproducing both sexually and asexually. We have observed moderate level of seed set but seed germination was very low (13.5%) and can only be increased up to 52.5% with GA<sub>3</sub> application under laboratory conditions.<sup>5</sup> How many seeds germinate in its natural habitats is unknown, however, it was reported that successful establishment of one or a few seedlings should be enough to maintain high levels of genetic variation in plant populations.<sup>13</sup> Several studies proposed that asexual reproduction may promote the maintainance of high genetic diversity in plants.<sup>14</sup> Therefore, it could be speculated that *T. leucophyllum* reproduced sexually in its recent history and substantial amount of its genetic diversity was maintained with its clonal growth and perennial characteristics.

AMOVA analysis (89%) and Shannon's information measure (82.6%) demonstrated that majority of the total genetic variation partitioned within populations.  $G_{st}$  values between 0.05–0.15 indicate a low level of genetic differentiation whereas  $G_{st}$  values above 0.30 indicate high level of genetic differentiation among populations.<sup>15</sup> We have found low-moderate level of genetic differentiation among populations of *T. leucophyllum* ( $G_{st}$ : 0.184). This value is comparatively low from that of non-endemic *Teucrium polium* ( $G_{st}$ : 0.38).<sup>16</sup> However, there are several studies representing high genetic diversity and low population genetic differentiation in rare and endemic plant species such as *Salvia lachnostachys* ( $G_{st}$ : 0.167) and *Magnolia wufengensis* ( $G_{st}$ : 0.171).<sup>17,18</sup> High genetic diversity and low population differentiation in endemic plants have been attributed to a number of factors e.g. inadequate time for lowering genetic diversity following isolation, reduction in population size, recent fragmentation of continuous genetic system, and substantial gene flow.<sup>19,20</sup> In *T. leucophyllum*, we observed high gene flow between populations ( $N_m$ : 2.208). Usually, a gene flow equal or higher than 1 ( $N_m \geq 1$ ) is sufficient to prevent significant population differentiation among populations<sup>21</sup> and this is attributed to the long-distance pollen or seed dispersal ability of plant. Most probably, seed dispersal mode and insect pollination may be contributed to the gene flow among closely located *T. leucophyllum* populations. In our field study, we observed that *T. leucophyllum* grows on crevices of limestone rocks. Habitat specificity of this species makes it vulnerable and to be affected seriously from habitat loss and environmental change. Our previous investigation revealed that *T. leucophyllum* is under threat of human interference, destruction and habitat fragmentation. Therefore, despite of its high levels of genetic diversity, narrow distribution and habitat specificity of *T. leucophyllum* may negatively effect maintenance of its populations in the future.

## CONCLUSION

This study have shown that the endangered status of *T. leucophyllum* is not due to the degree of its genetic variation but its narrow distribution on specific habitat. Conservation efforts for this species should be concentrated on protecting its natural habitat first. In addition, seeds can be collected from populations located in Kayacık and Bağıştaş for *ex-situ* conservation practices.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

None

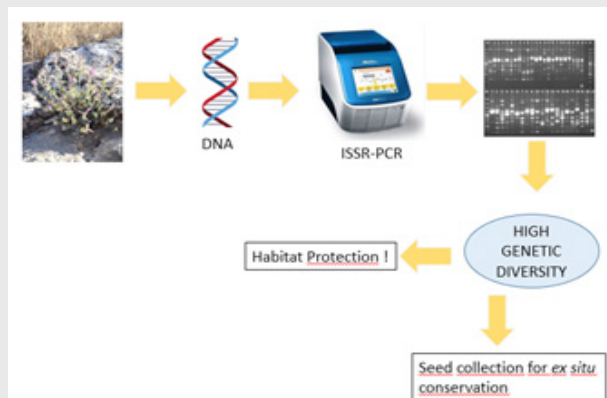
## ABBREVIATION USED

CTAB: Cetyl Trimethyl Ammonium Bromide; ISSR: Inter Simple Sequence Repeat; *PPB*: Percentage of Polymorphic Band; IUCN: The International Union for Conservation of Nature; *N<sub>a</sub>*: the observed number of alleles; *N<sub>e</sub>*: the effective number of alleles; *H*: the Nei's genetic diversity; *I*: the Shannon's information index; *N<sub>m</sub>*: Number of Migrants; *G<sub>st</sub>*: A measure of Genetic differentiation; *EX*: Extinct; DNA: Deoxyribo Nucleic Acid; AMOVA: The Analysis of Molecular Variance; GA3: Gibberellic acid; TUBITAK: The Scientific And Technological Research Council of Turkey.

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## PICTORIAL ABSTRACT



## SUMMARY

- Teucrium leucophyllum* is endemic plant species having considerable narrow distribution in Turkey. Beside, its populations are under various risks due to human activities.
- ISSR analyses showed that *Teucrium leucophyllum* possess high level of genetic diversity.
- Therefore, the endangered status of *T. leucophyllum* is not due to the degree of its genetic variation but its narrow distribution on specific habitat.
- Conservation strategies should be developed for protecting its habitats first and seeds also can be collected for *ex situ* conservation practices.

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