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Original scientific article

MOLECULAR MARKERS ANALYSIS OF ENDEMIC *BORNMUELLERA* HAUSSKN. SPP. (BRASSICACEAE) IN TÜRKİYE

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In this study, molecular characterisation of Türkiye's endemic species *Bornmuellera cappadocica* (Willd.) Cullen & T.R. Dudley, *Bornmuellera glabrescens* (Boiss. & Balansa) Cullen & T.R.Dudley, *Bornmuellera kiyakii* Aytaç & Aksoy and *Bornmuellera angustifolia* (Hausskn. ex Bornm.) Cullen & T.R.Dudley was carried out using ten RAPD and ten ISSR primers. In RAPD-PCR analysis, 66 bands were obtained and the polymorphism rate was 96.96%. In the ISSR-PCR analysis, 119 bands were obtained and the polymorphism rate was 95.79%. In the UPGMA (Unweighted Pair Group Method with Arithmatic Mean) dendrogram based on RAPD-PCR, *B. angustifolia* and *B. glabrescens* were found to be a sister group, and *B. kiyakii* and *B. cappadocica* were a sister group. Principal Component Analysis (PCA) analysis based on RAPD-PCR were compatible with the UPGMA dendrogram. In the UPGMA dendrogram based on ISSR-PCR, *B. kiyakii* and *B. glabrescens* were found to be sister groups, and *B. cappadocica*

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was closely related to this group. PCA analysis based on ISSR-PCR were compatible with the UPGMA dendrogram. As a result, both RAPD and ISSR results a high rate of polymorphism were obtained. The results were compared with previous sequence-based studies, morphological, anatomical and palynological studies.

Keywords: *Bornmuellera*, RAPD, ISSR, molecular markers, Türkiye

INTRODUCTION

Türkiye has an important place in the world in terms of plant species diversity because it has a different geographical and climatic structure and is located at the intersection of three gene centres. Endemic species are those that are found only in a particular region or area of the world and do not grow anywhere else in the world. Compared to its neighbours, Türkiye ranks first in terms of species richness and endemic species (İPEK and GÜRBÜZ, 2010). This is due to the fact that the climate, soil and elevations are different under the influence of geological and geomorphological structures, resulting in plant diversity and a large number of endemic species in Türkiye (EKİM *et al.*, 2000; GÜNER and AKÇİÇEK, 2014). The Brassicaceae family is one of the largest Angiosperm families, consisting of 348 genera and 4065 species (ATASAGUN, 2022). In Türkiye Brassicaceae family have 97 genera and 571 species (ERTUĞRUL *et al.*, 2023). Some members of this family include economic and important industrial oilseeds, spices, cultivated and ornamental plants, edible vegetables and some forage crops (SIRALI *et al.*, 2013; AVATO and ARGENTIERI, 2015; GIDIK *et al.*, 2016; CHEN *et al.*, 2016; ZHANG and JING, 2022). The genus *Bornmuellera* Hausskn. was discussed by GREUTER (1986) and Türkiye has four species, all of which are endemic (OKAN *et al.*, 2024). In the past, morphological, anatomical, palynological, phytochemical and phylogenetic studies of *Bornmuellera* species have been carried out (MARIN *et al.*, 1997; WARWICK *et al.*, 2008; REŠETNIK *et al.*, 2013; FIRAT and BAŞER, 2015; GÖNEN *et al.*, 2019; OZUDOĞRU and MUMMENHOFF, 2020; KARAİSMAİLOĞLU, 2020; OKAN *et al.*, 2024). Molecular marker techniques are widely used to analyse genetic diversity in many plant species (FİLİZ *et al.*, 2014). In RAPD (Randomly Amplified Polymorphic DNA) , DNA fragments are amplified with primers having random nucleotide sequence and polymorphism is determined (WILLIAMS *et al.*,1990; ÖZŞENSOY and KURAR, 2012; EL-HAGGAR *et al.*, 2023). Another fast, cost-effective, highly discriminative molecular marker is ISSR (Inter Simple Sequence Repeats) technique (MARAŞ-VANLIOĞLU *et al.*, 2020). The major advantage of the ISSR marker technique is that the genome sequence does not need to be known for the construction of primers. Furthermore, the simultaneous evaluation of several loci makes it a rapid marker (KHORSHIDI *et al.*, 2017). Both RAPD and ISSR markers require only a small amount of DNA and their experimental procedures are easy to carry out (KHABIYA *et al.*, 2024). In this study, RAPD and ISSR primers were used to determine the genetic diversity of four endemic *Bornmuellera* species distributed in Türkiye.

MATERIALS AND METHODS

Plant samples, genomic DNA isolations and PCR amplifications

Endemic *Bornmuellera cappadocica, Bornmuellera glabrescens, Bornmuellera kiyakii* and *Bornmuellera angustifolia* species were collected from the localities mentioned in the study of OKAN *et al.* (2024). Fresh leaves were used for DNA isolation (OKAN *et al.*, 2024). The RAPD, ISSR primer sequences, PCR components and PCR protocol are given in Table 1. After

the PCR process, the samples were run on 1.5% agarose gel electrophoresis and visualized in a UV transilluminator. In the study, 100 bp DNA ladder (Cat#: GMM100) was used. Example gel images of RAPD and ISSR results are shown in Figure 1.

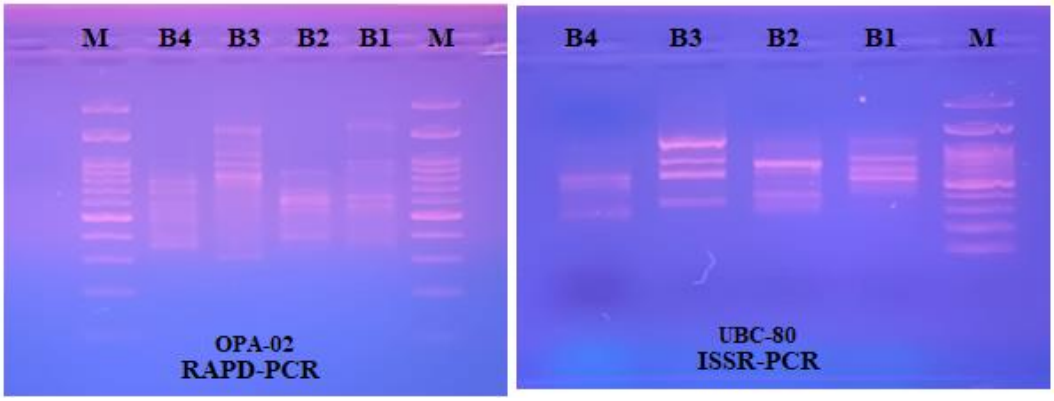

Figure 1. OPA-02 and UBC-880 primers gel photos. M: Marker B1: *Bornmuellera cappadocica* B2: *Bornmuellera glabrescens* B3: *Bornmuellera kiyakii* B4: *Bornmuellera angustifolia*

Table 1. Primers used in the RAPD-PCR reactions and their melting temperature (Tm)

RAPD Primers	DNA Sequences (5'-3')	Tm °C	PCR Components	PCR Amplification (35 Cycles except final extension step)
OPA-02	5' - TGCCGAGCTG - 3'	34 °C	1 µL genomic DNA 1 µL primer, 4 µL master mix 5 x FIREPol® (FIREPol® DNA polymerase, 5 x Reaction Buffer B, 12.5 mM $MgCl_2$, 1 mM dNTPs of each) and 19 µL dH_2O	94°C/2 min 94°C/1 min 32-34°C/1 min 72°C/1 min 72°C/10 min
OPA-05	5' - AGGGGTCTTG - 3'	32 °C		
OPA-13	5' - CAGCACCCAC - 3'	34 °C		
OPA-15	5' - TTCCGAACCC - 3'	32 °C		
OPJ-10	5'-AAGCCCGAGG-3'	32 °C		
OPA-18	5'- AGGTGACCGT - 3'	32 °C		
OPA-20	5' - GTTGCGATCC - 3'	32 °C		
OPJ-08	5' -CATACCGTGG- 3	32 °C		
OPA-07	5'-GAAACGGGTG-3'	32 °C		
OPA-03	5'-AGTCAGCCAC-3'	32 °C		

RAPD and ISSR-PCR Analysis

RAPD and ISSR-PCR gel images were analysed and "1" was written if a band was present, "0" if not, and "9" for missing data. A UPGMA dendrogram based on euclidean's similarity coefficients was constructed using MVSP 3.22 (KOVACH, 2007). Also, PCA analysis of *Bornmuellera* species were generated with this program.

Table 2. Primers used in the ISSR-PCR reactions and their melting temperature (Tm)

ISSR Primers	DNA Sequences (5'-3')	Tm °C	PCR Components	PCR Amplification (35 Cycles except final extension step)
UBC-810	5'-GAGAGAGAGAGAGAGAT-3'	50°C	1 µL genomic DNA 1 µL primer, 4 µL master mix 5 x FIREPol® (FIREPol® DNA polymerase, 5 x Reaction Buffer B, 12.5 mM $MgCl_2$, 1 mM dNTPs of each) and 19 µL dH_2O	94 °C /1 min 94 °C /1 min 50-53 °C /1 min 72 °C /1 min 72 °C /10 min (final extension: 1 cycle)
UBC-819	5' - GTGTGTGTGTGTGTGTA -3	50°C		
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50 °C		
UBC-836	5'-AGAGAGAGAGAGAGAGYA-3'	52 °C		
UBC-826	5'-ACACACACACACACACC-3'	52 °C		
UBC-834	5'-AGAGAGAGAGAGAGAYT-3'	52 °C		
UBC-853	5' - TCTCTCTCTCTCTCTCRT -3'	52 °C		
UBC-856	5'–ACACACACACACACACYA-3'	52 °C		
UBC-855	5'-ACACACACACACACACYT-3'	52 °C		
UBC-880	5'-GGAGAGGAGAGGAGA-3'	53 °C		

RESULTS AND DISCUSSION

In RAPD-PCR analyses, 66 bands were obtained and the polymorphism rate was 96.96%. In the RAPD analysis, primers OPJ-10, OPA-05 and OPA-15 didn't give amplification. The maximum band was obtained from OPA-02 (Table 3).

According to RAPD analysis, the similarity index (Jaccard's coefficient) is between 0.133 and 0.256. A UPGMA dendrogram was constructed to determine the phylogenetic relationship between the species, and in this dendrogram *B. angustifolia* and *B. glabrescens* emerged as a sister group, and *B. kiyakii* and *B. cappadocica* as a sister group (Figure 2). The UPGMA dendrogram was compatible with the groupings in the PCA analysis (Figure 3).

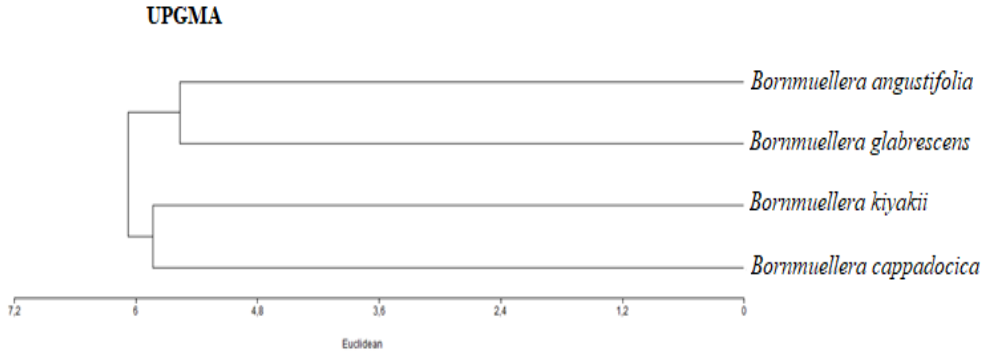

Figure 2. UPGMA tree generated using RAPD-PCR data

Table 3. Monomorphic and polymorphic band numbers of RAPD and ISSR primers

Primers (RAPD)	Total bands	Monomorphic bands	Polymorphic bands
OPA-02	19	0	19
OPA-03	6	1	5
OPA-07	10	0	10
OPA-15	8	0	8
OPA-18	9	0	9
OPA-20	12	0	12
OPJ-08	2	1	1
TOTAL	66	2	64
Primers (ISSR)	Total bands	Monomorphic bands	Polymorphic bands
UBC-807	12	1	11
UBC-810	11	1	10
UBC-819	12	0	12
UBC-826	9	2	7
UBC-834	18	1	17
UBC-836	12	0	12
UBC-853	12	0	12
UBC-855	15	0	15
UBC-856	7	0	6
UBC-880	11	0	11
TOTAL	119	5	114

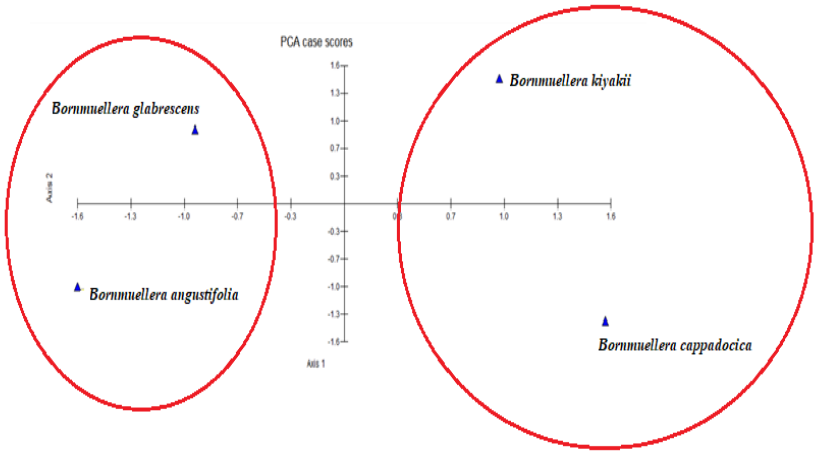

Figure 3. Pricipal component analyses of RAPD-PCR using MVSP 3.22 software

In ISSR-PCR analyses, 119 bands were obtained and the polymorphism rate was 95.79%. The highest number of bands was obtained from the UBC-834 primer, and the lowest number of bands was obtained from the UBC-856 primer (Table 3). According to ISSR analysis, the similarity index (Jaccard's coefficient) is between 0.265 and 0.337. In the UPGMA dendrogram, *B. kiyakii* and *B. glabrescens* were found to be sister groups, and *B. cappadocica* was closely related to this group (Figure 4). The UPGMA dendrogram was compatible with the groupings in the PCA analysis (Figure 5).

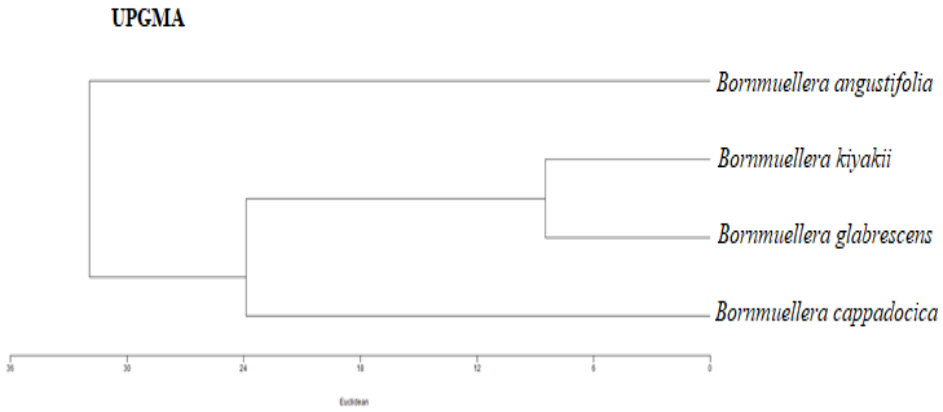

Figure 4. UPGMA tree generated using ISSR-PCR data

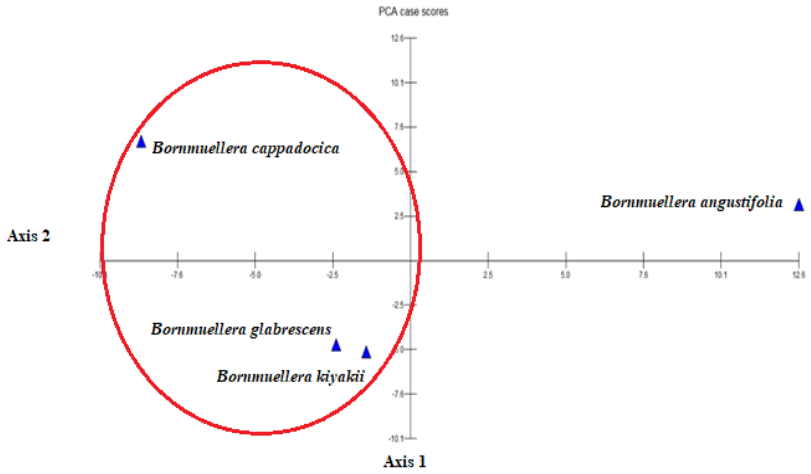

Figure 5. Pricipal component analyses of ISSR-PCR using MVSP 3.22 software

However, RAPD and ISSR results were incompatible. OKAN *et al.* (2024) revealed the phylogenetic analysis of the species in this study with nrDNA ITS and cpDNA (*trn*L intron, *trn*L-F, *rbc*L and *trn*Q-*rps*16) sequences. In their analysis, ITS found *B. angustifolia* and *B. glabrescens* close together, and in their *trn*L intron and *trn*L-F analysis, they found *B. angustifolia* and *B. glabrescens* as a group, *B. kiyakii* and *B. cappadocica* as a group. ITS, *trn*L intron and *trn*L-F results were compatible with RAPD-PCR results. OKAN *et al.* (2024) found *B. cappadocica*, *B. kiyakii* and *B. angustifolia* to be a group in the *rbc*L analysis, and *B. cappadocica* and *B. kiyakii* to be close to each other in the *trn*Q-*rps*16 analysis. In our ISSR results, *B. kiyakii*, *B. glabrescens* and *B. cappadocica* together were determined. OZUDOĞRU and MUMMENHOFF (2020), detected *B. angustifolia* and *B. glabrescens* species together, and *B. kiyakii* and *B. cappadocica* species together in ITS and *trn*L-F analyses. The results of their study were compatible with our RAPD-PCR results. According to AYTAÇ and AKSOY (2000), *B. kiyakii* is closely related to *B. angustifolia* and both species have very characteristic small leaves. GONEN *et al.* (2019) found that *B. kiyakii* and *B. glabrescens* species are similar to each other in terms of pollen morphology, but there are some differences in the anatomical characters of the fruits, morphological characters and seed micromorphology of the samples.

CONCLUSION

As a result, in this study, molecular marker analysis of endemic *Bornmuellera* species in Türkiye was performed using RAPD and ISSR-PCR techniques and the genetic relationship between the species was revealed. Ten primers were used in both RAPD and ISSR analyses and showed a high rate of polymorphism (96.96%. and 95.79%). The results were compared with

previous morphological, anatomical, palynological and DNA sequence-based studies. It was found that the results obtained using the RAPD technique were more compatible with previous studies than those obtained using the ISSR technique. In addition, the results of this study will be a reference for the molecular systematic analysis of endemic *Bornmuellera* species and for future genetic studies on different plants.

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ANALIZA MOLEKULARNIH MARKERA ENDEMIČNE VRSTE *BORNMUELLERA* HAUSSKN. SPP. (BRASSICACEAE) U TURSKOJ

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Izvod

U ovoj studiji, molekularna karakterizacija endemske vrste Turske *Bornmuellera cappadocica* (Villd.) Cullen & T.R. Dudlei, *Bornmuellera glabrescens* (Boiss. & Balansa) Cullen & T.R.Dudlei, *Bornmuellera kiiakii* Aitac & Aksoi i *Bornmuellera angustifolia* (Hausskn. ek Bornm.) Cullen & T.R.Dudlei je izvedeno korišćenjem deset prajmera RAPD i deset ISSR prajmera. U RAPD-PCR analizi, dobijeno je 66 traka i stopa polimorfizma je bila 96,96%. U ISSR-PCR analizi dobijeno je 119 traka i stopa polimorfizma je bila 95,79%. U UPGMA (Metoda grupe bez ponderisanih parova sa aritmetičkom sredinom) dendrogramu zasnovanom na RAPD-PCR, utvrđeno je da su *B. angustifolia* i *B. glabrescens* jedna sestrinska grupa, a *B. kiiakii* i *B. cappadocica* druga sestrinska grupa. Analiza glavnih komponenti (PCA) zasnovana na RAPD-PCR bila je kompatibilna sa UPGMA dendrogramom. U UPGMA dendrogramu zasnovanom na ISSR-PCR-u, *B. kiiakii* i *B. glabrescens* su sestrinska grupa, a *B. cappadocica* je bila usko povezana sa ovom grupom. PCA analize zasnovane na ISSR-PCR bile su kompatibilne sa UPGMA dendrogramom. Kao rezultat, i RAPD i ISSR rezultati su bili sa visokom stopom polimorfizma. Rezultati su upoređeni sa prethodnim studijama zasnovanim na sekvencama, morfološkim, anatomskim i palinološkim studijama.

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