Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2023, 16(2), 436-452
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2023, 16(2), 436-452
DOI: 10.18185/erzifbed.1260729
Research Article

Genetic Diversity Analyses of *Scrophularia erzincanica* and *Scrophularia fatmae* (Scrophulariaceae) Populations Distributed in Eastern Anatolia of Türkiye

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Received: 06/03/2023 **Revised:** 03/07/2023 **Accepted:** 05/07/2023 **Published:** 31/08/2023

Abstract

Scrophularia fatmae Kandemir & İlhan and *Scrophularia erzincanica* R.R. Mill (Scrophulariaceae) are endemic species to Erzincan/ Turkiye. IUCN categories in *S. erzincanica* and *S. fatmae* are EN and CR. In the present study, we investigated levels of genetic variation and genetic structure of three populations of *S. erzincanica*, and two populations of *S. fatmae* in Erzincan using ISSR markers. For this aim, 10 primers amplified 116 total bands, with 104 (89.6 %) being polymorphic, from five populations composed of 75 individuals. The UPGMA cluster analysis demonstrated a significant correlation between genetic variations and geographic distances. The distribution area and population size of *S. fatmae*, which has adapted to the alpine region, is smaller than S. erzincanica. In addition, *S. fatmae* has a higher tendency to self-pollination. The number of effective pollinators in *S. erzincanica* and *S. fatmae* is three and one, respectively. When we compare *S. fatmae* and *S. erzincanica* species according to the results of the research, it is seen that genetic diversity is higher in *S. erzincanica*. The genetic data obtained as a result of the present study may be used in the development of conservation strategies for other rare and endangered plant species, in addition to *S. erzincanica* and *S. fatmae* species.

Keywords: Endemic plant, ISSR, Population genetic, *Scrophularia erzincanica, Scrophularia fatmae*

Doğu Anadolu Bölgesinde (Türkiye) Yayılış Gösteren *Scrophularia erzincanica* ve *Scrophularia fatmae* (Scrophulariaceae) Popülasyonlarının Genetik Çeşitlilik Analizleri

Öz

Scrophularia fatmae Kandemir & İlhan ve *Scrophularia erzincanica* R.R. Mill (Scrophulariaceae) Erzincan/Türkiye'ye özgü endemik türlerdir. *S. erzincanica* ve *S. fatmae*'de ki IUCN kategorileri sırasıyla EN ve CR'dir. Bu çalışmada, ISSR belirteçlerini kullanarak Erzincan'daki üç *S. erzincanica* popülasyonunun ve iki *S. fatmae* popülasyonunun genetik varyasyon seviyelerini ve genetik yapısı araştırılmıştır. Bu amaçla 10 primer, 75 bireyden oluşan beş popülasyondan 104'ü (%89.6) polimorfik olmak üzere toplam 116 bandı amplifiye edildi. UPGMA küme analizi, genetik varyasyonlar ve coğrafi mesafeler arasında anlamlı bir ilişki olduğunu göstermiştir. Alpin bölgesine uyum sağlamış olan *S. fatmae*'nin yayılış alanı ve populasyon büyüklüğü daha küçüktür. Ek olarak, *S. fatmae*'nin kendi kendine tozlaşma eğilimi daha yüksektir. *S. erzincanica* daha büyük bir popülasyona sahiptir. *S. erzincanica* ve *S. fatmae*'de etkili tozlayıcı sayısı sırasıyla üç ve birdir. Bu sebeplerin de katkısı ve araştırma sonuçlarına göre *S. fatmae* ve *S. erzincanica* türleri karşılaştırıldığında *S. erzincanica*'da genetik çeşitliliğin daha fazla olduğu görülmektedir. Bu çalışma sonucunda elde edilen genetik veriler, *S. erzincanica ve S. fatmae* türlerinin yanı sıra diğer nadir ve tehlike altındaki bitki türleri için koruma stratejilerinin geliştirilmesinde kullanılabilir.

Anahtar Kelimeler: Endemik bitki, ISSR, Populasyon genetiği, *Scrophularia erzincanica, Scrophularia fatmae*

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1.Introduction

Scrophularia L. is one of the largest genera of the Scorophulariaceae family including 220 genera and about 350 species. It contains many species with medicinal and aromatic properties and is used in traditional treatment [1]. The genus *Scrophularia* is represented by 77 taxa (61 species, 1 subspecies, and 15 varieties) in Turkiye. In Turkiye, 37 of these taxa are endemic and the endemism rate is 48% [2].

Scrophularia fatmae Kandemir & İlhan and *Sscrophularia erzincanica* R.R. Mill. (Scrophulariaceae) are rather narrowly distributed species (Fig 1). *S. erzincanica* grows sparsely in the serpentine areas between 1200-2800 m. It has been estimated that the population size of *S. erzincanica* is approximately 2,500 adult individuals. *S. fatmae* is an obligate alpine species that blooms by following the melting snow in the snow beds and is an unusual species with its flamboyant appearance unique to Erzincan, known from Mount Ergan, located in the south of the Erzincan Plain. It was mentioned that the species, which was introduced to the scientific world in 2014, is represented by a single population containing less than 500 individuals at an altitude of 3000 m [3]. In recent years, some studies were carried out related to these species including pollinators, taxonomy, and morphological characters [4,5,6,7]. IUCN category of *S. fatmae* is CR [B2ab(i,ii,iii)] and EN [B2ac(i,iv); C2a(i)] for *S. erzincanica* [8].

Molecular markers have been constantly applied to population genetic and conservation genetic studies [9,10,11,12]. In recent years, the technique of inter-simple sequence repeat amplification (ISSR) has offered a promising new marker system for use in the detection of genetic diversity in population and conservation genetics [13,14,15]. This technique is rapid as well as quite sensitive, and capable of differentiating between closely related individuals [16]. The shortcoming of ISSR markers is that most bands are scored as dominant markers, giving no possibility to distinguish between homozygosis and heterozygosis directly. ISSR markers are likely noncoding loci and dispersed throughout the genome.

Surprisingly, few studies have been done on population genetics, although there has been a great deal of research on the systematics, pollination, and chemical composition of the genus *Scrophularia.* In one of the few studies, *Scrophularia ningpoensis* Hemsl., different cultivars were compared in terms of genetic diversity using genetic markers [17]. The genetic diversity of populations and effective population sizes should be known in conservation studies. Loss of genetic diversity is greater in populations living in isolated, small, and fragmented habitats. In populations with insufficient effective population size, rare alleles are rapidly lost. As a result, determining the genetic diversity of populations is important in planning conservation studies [18].

The importance of genetics in conservation biology is increasing day by day. It is known that biodiversity depends on genetic diversity and when genetic diversity is lost, it is not possible to sustain biodiversity in the long run. Knowing the genetic diversity of populations that need to be protected can provide valuable data in designing conservation programs [19]. No studies have been conducted on the genetic diversity of these two species to date. The present study

aimed to utilize ISSR markers for examining the genetic diversity among the populations of *S. erzincanica* and *S. fatmae*.

2. Material and Methods

2.1. Plant material

Healthy leaf samples used in DNA analyses were collected from 3 different localities for *S. erzincanica* (Fig 1A) from Kemah, between Erzincan-Sakaltutan road and near Sakaltutan Radiolink station, and *S. fatmae* (Fig 1B) was collected from 2 different localities on Ergan Mountain in the years 2019-2020. Fifteen genotypeswere used for each population. Samples were collected in line with the permissions obtained from the General Directorate of Nature Conservation and National Parks. Information about the genotypes and the locations where the samples were collected are given in Tab. 1.

Table 1. Genotypes and locations.

Genotype	Population	GPS coordinates	Altitudes (m)
S. erzincanica 1	Erzincan Sakaltutan Road	4412445 K 37 S 524752 D	1597
S. erzincanica 2	Sakaltutan- Radiolink station	4414900 K 37 S 513634 D	2096
S. erzincanica 3	Sürek Village/Kemah	4376752 K 37 S 488884 D	1126
S. fatmae 1	Ergan Mountain 1	4381830 K 37 S 543902 D	3078
S. fatmae 2	Ergan Mountain 2	4382027 K 37 S 542249 D	2801

Figure 1. *S. erzincanica* (A) and *S. fatmae* (B).

Leaf samples were obtained from healthy individuals with at least a 10 m distance between them. Samples were wrapped in aluminum foils and taken into ice boxes in the field. Then they were stored at -80 ^{0}C.

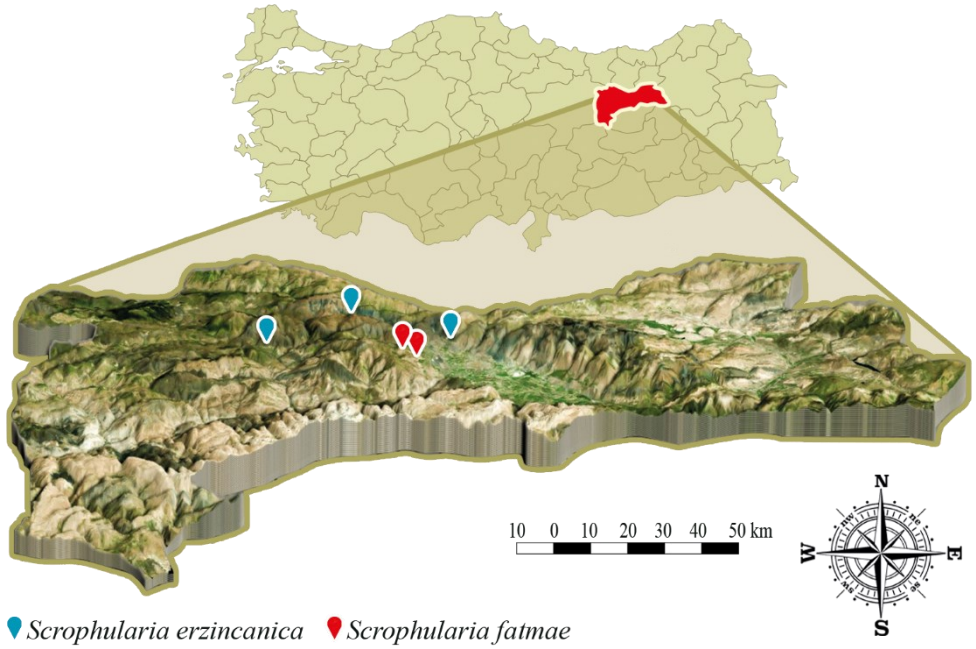

Figure 2. Geographic distribution of species in Erzincan city, Türkiye.

2.2. DNA extraction and ISSR analysis

Genomic DNA was extracted from ground plant material using liquid nitrogen using a method described by Sunar *et al.* [20]. The purity and quantity of genomic DNA were determined spectrophotometrically and confirmed using 0.8 % agarose gel electrophoresis against known concentrations of unrestricted lambda DNA.

45 primers had been used to generate ISSR profiles and 10 primers were chosen for ISSR analyses of genetic diversity (Tab 2). The PCR reactions were carried out in a 25 µl reaction mixture containing 40 ng of template DNA, 1X reaction buffer, 200 Mm of each of the four dNTPs, 1 U of Taq DNA polymerase, 1.5 mM MgCl2 and 0.5 mM of primer. Amplification was performed using a thermal cycler programmed for an initial denaturation step of 5 min at 94◦C followed by 35 cycles of 45 s at 94◦C, 1 min at the specific annealing temperature, and 1 min at 72◦C, ending with a final extension step of 7 min at 72◦C. The PCR products (25 µl) were mixed with 6× gel loading buffer (3 µl) and loaded onto agarose (1.5% w/v) gel electrophoresis in 0.5 XTBE (Tris-Borate- EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µl EtBr/100 ml 1×TBE buffer) for 40 min and visualized under UV in Bio Doc Image Analysis System with Ubisoft analysis package (Cambridge, UK).

Table 2. ISSR primers used in this study and analysis of ISSR-generated banding.

Primer code	Core sequence 5'→3'	Band size (bp)	Annealing temp.	No of bands	No of polymorphic bands	Polymorphic bands (%)
UBC 808	AGA GAG AGA GAG AGA GC	250-1300	52°C	16	14	87.5
UBC 809	AGAGAGAGAGA GAGAGG	700-1280	52°C	13	12	92.3
UBC 811	GAG AGA GAG AGA GAG AC	530-1100	52°C	10	9	90
UBC 823	TCT CTC TCT CTC TCT CC	400-1500	52°C	14	13	92.9
UBC 827	ACA CAC ACA CAC ACA CG	600-1170	52°C	8	7	87.5
UBC 834	AGA GAG AGA GAG AGA GYT	350-980	52°C	10	8	80
UBC 855	ACA CAC ACA CAC ACA CYT	570-1450	52°C	13	11	84.6
UBC 874	CCC TCC CTC CCT CCC T	290-1040	51°C	10	10	100
UBC 881	GGG TGG GGT GGG GTG	350-1170	60°C	13	12	84.6
UBC 895	AGA GTT GGT AGC TCT TGA TC	300-850	42 °C	9	8	88.8
Total		250-1500		116	104	
Average				11.6	10.4	89.6

2.3. Data analysis

ISSR bands were scored as present (1) or absent (0) for each DNA sample. The binary data matrix of the populations was analyzed using POPGENE version 1.31 [21]. The following parameters of genetic diversity were calculated: the observed number of alleles per locus (Na), the effective number of alleles per locus (Ne), Nei's gene diversity (H), Shannon's information index (I), the number of polymorphic loci (NP), percentage of polymorphic loci (PPL), Nei's genetic differentiation index among populations (Gst), total genetic diversity (Ht), genetic diversity within the population (Hs), gene flow estimates between populations (Nm).

To visualize the genetic relationship among populations, a dendrogram was constructed based on Nei's genetic distance (D) by an unweighted pair group method of cluster analysis using arithmetic averages (UPGMA) using the software NTSYS pc2.02 [22].

3. Results and Discussion

3.1. ISSR polymorphisms

45 ISSR primers were initially screened against *S. erzincanica* and *S. fatmae*. Bulk DNA was tested with 45 primers. As a result 35 of those showed low-quality amplication. Ten primers (Tab 2) produced a total of 116 distinct reproducible bands with an average of 11.6 bands per primer. Of the 116 bands obtained, 104 (89.6 %) were polymorphic. The sizes of the amplified products ranged from 250 bp to 1500 bp. The number of bands detected with each primer ranged from 8 (primer UBC 827) to 16 (primer UBC 808) (Tab 2). Banding patterns of the *S. fatmae* (Fig 3) and *S. erzincanica* (Fig 4) genotypes using the primers UBC 834 and UBC 827 are illustrated in figures.

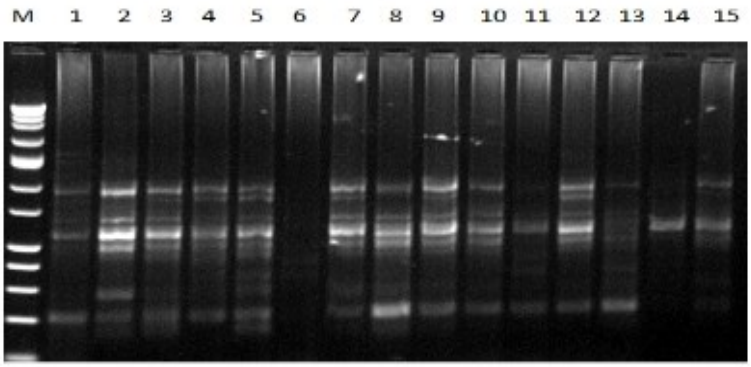

Figure 3. Amplification products generated from *S. fatmae* population using primer UBC 834.

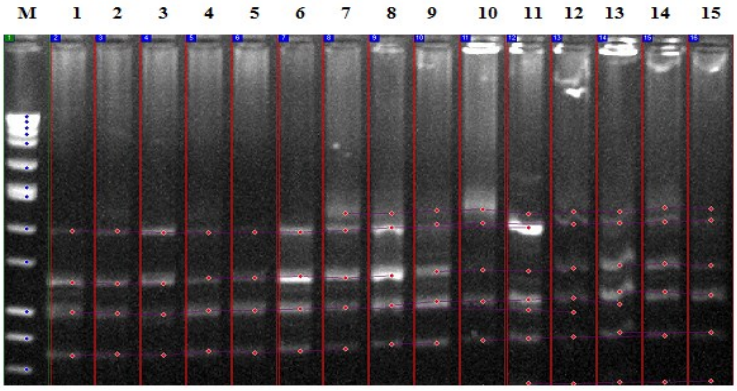

Figure 4. Amplification products generated from *S. erzincanica* population using primer UBC 827.

3.2. Genetic distance, diversity and genetic structure for *S. erzincanica* populations

The observed number of alleles per locus (Na) ranged from 1.5727 to 1.8455 with an average of 1.727. The effective number of alleles per locus (Ne) ranged from 1.3532 to 1.5022 with an average of 1.409. Nei's [23] genetic diversity index (H) ranged from 0.2045 to 0.2947 with an average of 0.2404. Shannon's information index (I) ranged from 0.3043 to 0.4420 with an average of 0.3623. The mean number of polymorphic bands was 80 (72.72%).

The total genetic diversity (Ht), between population genetic diversity (Hs), the coefficient of genetic differentiation (Gst), and the level of gene flow (Nm) were 0.3038, 0.2404, 0.2086, and 1.897 respectively. At the species level, Na, Ne, H, I, NPP, and PPB were 1.9909, 1.5029, 0.3038, 0.4669, 109, and 99.09 % respectively (Tab 3).

Table 3. Genetic diversity of the three populations of *S. erzincanica*.

Population	N	Na ± S	Ne ± S	H ± S	I ± S	NPB	PPB (%)	*H*t	*H*s	Gst	Nm
S. erzincanica (Between Erzincan-Sakaltutan)	15	1.57 ± 0.49	1.35 ± 0.38	0.20 ± 0.20	0.30 ± 0.29	63	7.27				
S. erzincanica (Sakaltutan radiolink station)	15	1.84 ± 0.36	1.50 ± 0.35	0.29 ± 0.17	0.44 ± 0.23	93	84.55				
S. erzincanica (Kemah)	15	1.76 ± 0.42	1.37 ± 0.36	0.22 ± 0.19	0.34 ± 0.26	84	76.36				
Average		1.72	1.40	0.24	0.36	80	72.72	0.30	0.24	0.20	1.8
Species level	45	1.99 ± 0.09	1.50 ± 0.32	0.30 ± 0.14	0.46 ± 0.17	109	99.09				

N, sample number; Na, observed number of alleles per locus; Ne, the effective number of alleles per locus; H, The Nie genetic diversity index; I, Shannon's information index; NPB, Number polymorphic bands, PPB, the percentage of polymorphic bands; Ht, total genetic diversity; *H*s, genetic diversity within the population, Gst; genetic differentiation index among populations; Nm, gene flow estimates between populations.

Estimates of Nei's genetic distance (D) ranged from 0.0372 between populations Sakaltutan Radiolink area and between Erzincan-Sakaltutan populations to 0.1889 between Kemah and Erzincan-Sakaltutan (Tab 4). The UPGMA dendrogram (Fig. 5) shows the 3 populations

divided into clusters and the genetic similarity between the *S. erzincanica* genotypes. The Sakaltutan Radyolink area and between Erzincan-Sakaltutan populations were in one group, and Kemah formed the other group.

Table 4. Nei's unbiased measures of genetic distance among populations of *S. erzincanica*

Population	**Kemah**	**Between Erzincan-Sakaltutan**	**Sakaltutan Radiolink area**
Kemah	****		
Between Erzincan-Sakaltutan	0.1889	****	
Sakaltutan Radiolink area	0.1852	0.0372	****

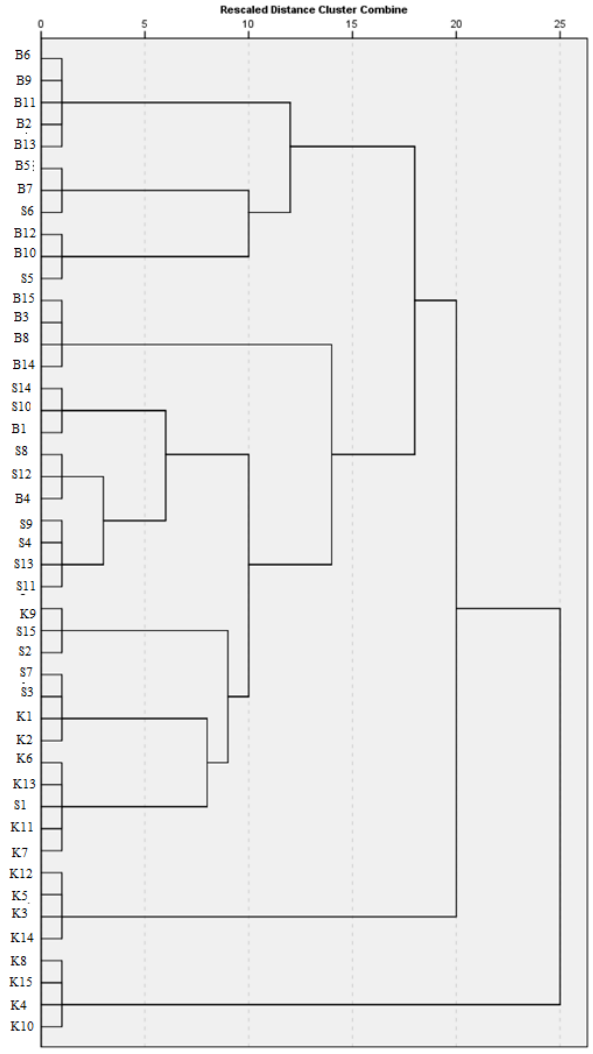

Figure 5. Average linkage Dendrogram of three population genotypes of *S. erzincanica* UPGMA cluster analysis of ISSR data (B= Between Erzincan-Skaltutan genotypes, S= Sakalturan Raidolink Area genotypes, K= Kemah genotypes).

3.3. Genetic distance, diversity and genetic structure for *S. fatmae* populations

Na ranged from 1.6044 to 1.9011 with an average of 1.7528. Ne ranged from 1.3440 to 1.4097 with an average of 1.376. H ranged from 0.2004 to 0.2472 with an average of 0.2238. **I** value ranged from 0.3013 to 0.3843 with an average of 0.3428. NPP average of 68.5. PPB average of 75.2%. HT, HS, Gst, and Nm, were 0.2309, 0.2238, 0.1839, and 1.569, respectively. At the species level, Na, Ne, H, I, NPP, and PPB were 1.9451, 1.3793, 0.2309, 0.3625, 86, and 94.51% respectively (Tab 5). Estimates of Nei's genetic distance (D) ranged from 0.0181 between populations Ergan Mountain I. station and Ergan Mountain II. station (Tab 6). The UPGMA dendrogram (Fig. 6) shows the 2 populations divided into clusters and the genetic similarity between the *S. fatmae* genotypes.

Table 5. Genetic diversity within populations of the two of *S. fatmae* populations

Population	N	Na ± S	Ne ± S	H ± S	I ± S	NPB	PPB %	*H*t	*H*s	Gst	Nm
S. fatmae 1.(E)	15	1.6 ± 0.49	1.34 ± 0.37	0.20± 0.20	0.30± 0.28	55	60.44				
S. fatmae 2.	15	1.90± 0.30	1.40± 0.35	0.24± 0.17	0.38± 0.23	82	90.11				
Average		1.75	1.376	0.22	0.34	68.5	75.2	0.23	0.22	0.18	1.56
Species level	30	1.94± 0.22	1.37± 0.35	0.23± 0.17	0.36± 0.23	86	94.51				

N, sample number; Na, observed number of alleles per locus; Ne, the effective number of alleles per locus; H, The Nie genetic diversity index; I, Shannon's information index; NPB, Number polimorphic bands; PPB, the percentage of polymorphic bands; Ht, total genetic diversity; *H*s, genetic diversity within population; Gst, genetic differentiation index among populations; Nm, gene flow estimates between populations.

With this study, the knowledge of genetic diversity in the populations of the *S. erzincanica* and *S. fatmae* species, which are endemic, was analyzed using the ISSR marker techniques.

Table 6. Nei's unbiased measures of genetic distance among populations of *S. fatmae*

Population	Ergan Mountain station 1	Ergan Mountain station 2
Ergan Mountain Station 1	****	
Ergan Mountain Station 2	0.0181	****

It is very important to understand the genetic diversity within and between populations to provide effective protection for rare and endangered plants. Molecular methods are often used in population genetics studies. Many molecular markers, including ISSR markers [16] are effective tools for detecting genetic diversity and genetic makeup when it comes to endangered species *Changium smyrnioides* H.Wolff [24], *Sinojackia dolichocarpa* C.J.Qi [25], *Primula merrilliana* Schltr. [26] and *Aethionema* W.T.Aiton Species [27].

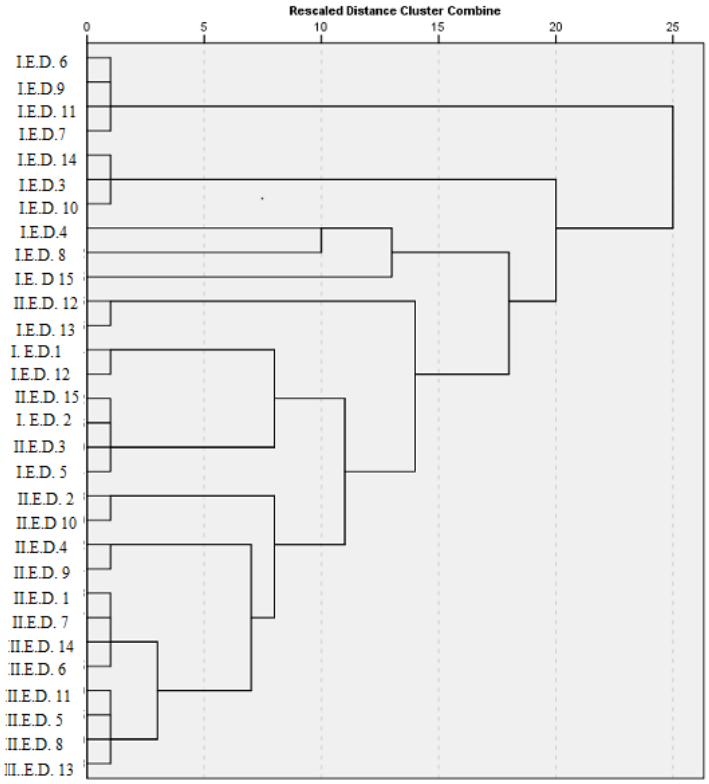

Figure 6. Average linkage dendrogram of two population genotypes of *S. fatmae* UPGMA cluster analysis of ISSR data (I.E.D= Ergan Mountain I. Station genotypes; II.E.D.= Ergan Mountain II. Station genotypes).

Population genetics of some species with a narrow and rare spread from the Scrophulariaceae family have been studied by molecular methods such as AFLP, isoenzyme, and RAPD [28,29,30,31]. In the literature, the results of population genetic studies with species *Verbascum songaricum* Schrenk [32], *Neopicrorhiza scrophulariiflora* (Pennell) D.Y.Hong [33] and *S. ningpoensis* [34], which are endemic in the Scrophulariaceae family, were compared with the species we studied. Accordingly, the observed and expected allele values, Nei's genetic diversity, Shannon index, Gst, and polymorphic locus percentage values are in line with our results (Tab 7).

Table 7. Results of some population genetic studies with species belonging to the family Scrophulariaceae

Value	*Verbascum songaricum*	*Scrophularia ningpoensis*	*Neopicrorhiza scrophulariiflora*	*Scrophularia erzincanica*	*Scrophularia fatmae*
Na	1.76	1.88	1.30	1.72	1.75
Ne	1.54	1.33	1.22	1.40	1.37
H	0.30	0.21	0.12	0.24	0.22
I	0.44	0.346	0.179	0.36	0.34
PLY	76.57 %	88.93 %	30.56 %	72.72 %	75.2 %
Gst		0.43	0.69	0.2686	0.21
Nm		0.64	0.21	1.89	1.56

Ne'i genetic diversity was 0.3038 at the *S. erzincanica* species level and 0.2404 for populations, while *S. fatmae* was 0.2309 at the species level and 0.2238 for populations. The polymorphism rate shown by the ISSR primers used was 99.09% at the *S. erzincanica* species level, 72.72% on average for their populations, while *S. fatmae* was 94.54% at the species level and 75.2% for their population. The Gst value was calculated as 0.2686 in *S. erzincanica* populations and 0.2183 in *S. fatmae* populations (Tab 8). Polymorphism rates, Nei's (1972) genetic diversity, and genetic diversity in terms of Gst values have been higher in *S. erzincanica*. This is mostly due to habitat fragmentation and loss, limited gene flow, and small population size due to geographic isolation. Habitat degradation poses one of the major threats to plants and animals. Habitat fragmentation can cause some negative situations such as population shrinkage, and reducing genetic diversity by increasing in-population reproduction. While generally endemic and endangered plants have a low level of genetic diversity [35], narrowly dispersed species also tend to have a low level of gene diversity [36,37]. In accordance with these

the species we studied have low genetic diversity. In this context, our results were compared with some endemic species in the Scrophulariaceae family (Tab. 7).

The average Shannon index for *S. erzincanica* populations was 0.3623, compared to 0.3428 for *S. fatmae* populations. Gene flow usually occurs by seed transfer, pollen transfer, etc., or by migrating individuals. Hamrick and Murawski [38] average Nm is 0.265 for species that can self-fertilize and spread their seeds and pollen as short distances as 2-3 meters, while 4,750 is reported to be used for species that fertilize from long distances with various seed and pollen carriers and can also spread seeds and pollen over long distances. The gene flow value was calculated as Nm= 1.897 for *S. erzincanica* and Nm= 1.569 for *S. fatmae* (Tab 8). Nm values indicate that the studied species may be species that are fertilized from long distances with various seed and pollen carriers and can also spread seeds and pollen over long distances.

Table 8. Data on genetic diversity of *Scrophularia erzincanica* and *Scrophularia fatmae* species.

Plant Characteristics	***S. erzincanica***	***S. fatmae***
Genetic variation between populations	0.24	0.22
Total genetic diversity	0.30	0.23
Gene flow rate	1.89	1.56
Percentage of polymorphic locus	72.72	75.20
Shannon index	0.36	0.34
Gst value	0.26	0.21

When the dendrogram of S. erzincanica populations was examined, it was observed that geographically distant populations (Kemah and Sakaltutan 1 populations) were also genotyped distant, and geographically close populations (Sakaltutan 1 and Sakaltutan 2 populations) were close in genotype. When the dendrogram formed by the *S. fatmae* populations was examined, it was clustered in 1 group. According to dendrogram data, as the geographical distance between populations increases, so does the genetic distances of the population increase.

According to Nei [23], the genetic distance value between *S. erzincanica* populations ranges from 0.0372 to 0.1889. *S. fatmae* populations were found to be 0.0181. These results suggest high genetic similarity for both species.

Total genetic diversity (HT) for *S. erzincanica* populations was calculated as 0.3038, intra-population genetic diversity (HS) was calculated as 0.2404, total genetic diversity (HT) for *S. fatmae* populations was calculated as 0.2309 and intra-population genetic diversity (HS) was calculated as 0.2238. According to the findings, total genetic diversity and intra-population genetic diversity are higher in *S. erzincanica* than *S. fatmae*.

In *S. erzincanica*, we can attribute the high genetic diversity at the species and population level to the following reasons. The span size of *S. erzincanica* is 1196.7 km^2 and the habitat size is 44 km^2. The spread and habitat size of the *S. fatmae* species was 1.2 km^2. As the population size increases in a plant species, the effectiveness of pollination increases, and the genetic diversity increases accordingly [39]. It is expected for genetic diversity to be higher in *S. erzincanica*, which is represented by larger areas than *S. fatmae*

Considering the upbringing, it can be said that *S. fatmae* is a mandatory limestone, *S. erzincanica* is a mandatory serpentine, *S. fatmae* is a mandatory alpine, *S. erzincanica* is a medium altitude (1000-1500 m), and, at times, a facultative alpine species. At the altitude where *S. fatmae* was found, the activity of the pollinator B. terrestris a pollinator is low. This result is also indicated by studies stating that the density and activity of the pollinator decrease due to the height of alpine plants compatible [40,41].

Biological characteristics, the reproductive system, and reproductive mode are often the main factors affecting genetic diversity in plant populations [42]. Likewise, the pollination system is one of the most important factors affecting genetic diversity in plant species [43]. In a study of the pollinators of *S. fatmae* and *S. erzincanica* [6], it was determined that *S. fatmae* was pollinated by *Bombus niveatus* L., which adapts to alpine conditions. It has been observed that this pollinator visits the flowers only for nectar, the duration of the flower stay is limited to a few seconds. Compared to *S. erzincanica*, it was also determined by the pollinators that the frequency of flower visits was low. In *S. erzincanica*, the taxas *Halictus quadricinctus* Fabricius (Hymenoptera-Halictidae), *Halictus* sp. and *Lasioglossum pauxillum = Halictus pauxillus* Schnck. (Hymenoptera-Halictidae) were found to be pollinators. The purpose of these pollinators is to collect nectar and pollen. The average flower survival time of these pollinators is 14-23 seconds. Sometimes this time can take up to 2 minutes. In *S. erzincanica*, pollinators, unlike *S. fatmae*, have long contact with male and female organs on each visit [6,44]. Cross-pollinated plants have a higher genetic diversity than self-pollinating plants. Cross-pollinated plant species have a genetic diversity between populations, while annual and self-pollinating plants reveal greater in-population diversity [36]. Similarly, it can be said that the genetic diversity of the species is greater because the number of pollinators in *S. erzincanica* and the more intense pollinator activity increases the rate of cross-pollination. According to the research results, the number of pollinators of *S. fatmae* grown at high altitudes is low compared to *S. erzincanica*. The fact that the rate of self-growth in *S. fatmae* and the percentage of flowers turning into fruits are higher than in *S. erzincanica* supports that this species adapts to alpine

conditions. Both types are self-compatible. *S. fatmae* species is more prone to self-pollination. *S. erzincanica* prefers cross-pollination (xenogamy) rather than self-pollination [44]. For these reasons, it has been concluded that the genetic diversity at the species and population level is high in *S. erzincanica*.

4. Conclusion

The indiscriminate collection of endemic and endangered plants from their natural habitats creates a great risk for plant diversity and ecosystems. Revealing the lack of detailed information on the genetic diversity of species with a limited population may help plan sustainable studies for the long-term survival of threatened plants. This is the first report on genetic diversity analysis with important findings for the conservation of endemic and narrowly distributed *S. erzincanica* and *S. fatmae* plants. The results of the studies carried out for this purpose also reveal that the genetic diversity is higher in *S. erzincanica* than in *S. fatmae*. It is thought that the formation of this result may be due to the fact that the ecosystems in which the species live are different in many ways, as well as the different types and species of pollinators that visit the species, as revealed in previous studies. All these factors can be examined in detail as a different study subject.

Ethics in Publishing

Plant samples were collected from nature in accordance with the permission dated 12.01.2018 and numbered 12652 obtained from the General Directorate of Nature Conservation and National Parks.

Author Contributions

Ali Kandemir, Faruk Yıldız, Halil İbrahim Türkoğlu, Engin Kılıç did the field work and helped to draft the manuscript. Nalan Yildirim Doğan carried out the molecular genetic studies and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The research was carried out with the support of TUBITAK's project 218Z002. We would like to thank TUBITAK for its support. In addition, this study is a part of Faruk Yıldız's PhD thesis.

References

[1] Pasdaran A., Hamedi A. (2017). The genus *Scrophularia*: a source of iridoids and terpenoids with a diverse biological activity, Pharmaceutical Biology, 55 (1), 2211-2233.

[2] Uzunhisarcikli M.E., Doğan Güner E., Özbek F., Ekici M. (2019). *Scrophularia vernalis*: a new species record from Turkey and its comparison with *Scrophularia chrysantha* (Scrophulariaceae). Phytotaxa, 397 (1), 91-98.

[3] Kandemir A., İlhan V., Korkmaz M., Karacan S. (2014). *Scrophularia fatmae* (Scrophulariaceae): Doğu Anadolu Bölgesi'nden sıra dışı yeni bir Sıracaotu (*Scrophularia* L.) türü, Bağbahçe Bilim Dergisi, 1 (1), 11-17.

[4] Uzunhisarcikli M.E., Güner E.D., Ekici M. (2018). Synopsis of the genus *Scrophularia* (Scrophulariaceae) in Turkey, Phytotaxa, 333 (2), 151-187.

[5] Babür H., Kandemir A. (2019). Scrophulariaceae (Sıracaotugiller) familyasına ait Erzincan'a özgü bazı türlerin polen morfolojileri, Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 9 (4), 1931-1936.

[6] Kiliç E., Yildiz F., Türkoğlu H.İ., Kandemir A., Doğan N.Y., Bekdemir M. (2020). Sürek Sıracaotu *Scrophularia erzincanica* R.R. Mill (Scrophulariaceae) 'nin polinatörleri ve zararlı böcekleri üzerine bir araştırma, Bağbahçe Bilim Dergisi, 7 (3), 1-11.

[7] Özbek F., Uzunhisarcikli M. E., Ekici M., Güner E.D., Pinar N.M. (2020) Seed morphology of the genus *Scrophularia* L. (Scrophulariaceae) from Turkey and its taxonomic implications, Phytotaxa, 434 (1), 35-64.

[8] Kandemir A., Sevindi C., Korkmaz M., Çelikoğlu Ş. (2015). Erzincan Türkiye'ye özgü endemik bitki taksonlarının IUCN tehdit kategorileri, Bağbahçe Bilim Dergisi, 2 (1), 43-65.

[9] Aagaard J. E., Krutovski K.V., Strauss S.H. (1998) RAPDs and allozymes exhibit similar levels of diversity and differentiation among populations and races of Douglas-fir, Heredity., 81, 69–78.

[10] Chase M.W., Kesseli R.V., Bawa K. (1996). Microsatellite markers for population and conservation genetics of tropical trees, *American Journal of Botany.* 83, 51–57.

[11] Zheng Z.L. (2010). Studies on genetic diversity and construction of fingerprinting of *Magnolia officinalis*, Doctoral dissertation, Fujian Agriculture and forestry university.

[12] Xiong M., Tian S., Zhang Z., Fan D., Zhang Z. (2014). Population genetic structure and conservation units of *Sinomanglietia glauca* (Magnoliaceae), Biodiversity Science, 22 (4): 476-484.

[13] Culley T.M., Wolfe A.D. 2001: Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity.* 86: 545-556.

[14] Mattioni C., Casasoli M., Gonzalea M., Ipinza R., Villani F. (2002). Comparison of ISSR and RAPD markers to characterize three Chilean Nothofagus species, Theor. Appl. Genet., 104, 1064–1070.

[15] Yu H.H., Yang Z.L., Sun B., Liu R.N., Yang X. (2011). Genetic diversity and relationship of the endangered plant *Magnolia officinalis* (Magnoliaceae) assessed with ISSR polymorphisms, Biochem. Syst. Ecol., 39: 71−78.

[16] Zietkiewicz E., Rafalski A., Labuda D. (1994). Genome Finger printing by Simple Sequence Repeat (SSR)-anchored Polymerase Chain Reaction Amplification, Genomics, 20 (2): 176-183

[17] Chen D., Zhang X., Wang Y., Li L. (2012). Genetic diversity of *Scrophularia ningpoensis* based on SCoT analysis, Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica, 37(16), 2368-2372.

[18] Primack, R. B. (2012). Koruma Biyolojisi, Çeviri editörleri Dönmez, A. A. ve Dönmez, E. O., Hacettepe Üniversitesi Yayınları, Ankara.

[19] Cornwell W. K., Pearse W. D., Dalrymple R. L., Zanne A. E. (2019). What we (don't) know about global plant diversity, Ecography, 42(11), 1819-1831.

[20] Sunar S., Aksakal O., Yildirim N., Agar G., Gulluce M., Sahin F. (2009). Genetic diversity and relationships detected by FAME and RAPD analysis among Thymus species growing in eastern Anatolia region of Turkey, Rom. Biotechnol. Lett., 14, 4313–4318.

[21] Yeh F.C., Yang R.C., Boyle T.B., Ye Z.H., Mao J.X. (1997). Popgene, the user-friendly shareware for population genetic analysis, *Molecular biology and biotechnology centre*, University of Alberta, Canada, 10, 295-301.

[22] Rohlf J. (1997). Numerical taxonomy and multivariate analysis system NTSYS-pc v2. 01b, Dept Ecology and Evolution, State University of New York, NY, USA.

[23] Nei M. (1972). Genetic distance between populations, The American Naturalist, 106 (949), 283-291.

[24] Qiu Y.X., Hong D.Y., Fu C.X., Cameron K.M. (2004). Genetic variation in the endangered and endemic species *Changium smyrnioides* (Apiaceae), Biochem. Syst. Ecol., 32 (6), 583–596.

[25] Cao P.J., Yao Q.F., Ding B.Y., Zeng H.Y. (2006). Genetic diversity of *Sinojackia dolichocarpa* (Styracaceae), a species endangered and endemic to China, detected by inter-simple sequence repeat (ISSR), Biochemical Systematics and Ecology, 34 (3), 231–239.

[26] Shao J.W., Chen W.L., Peng Y.Q., Zhu G.P. (2009). Genetic diversity within and among populations of the endangered and endemic species *Primula merrilliana* in China, *Biochem. Syst. Ecol.,* 37 (6), 699-706.

[27] Sunar S., Yildirim N., Sengül M., Ağar G. (2016). Genetic Diversity and Relationships Detected by ISSR and RAPD Analysis Among *Aethionema* Species Growing in Eastern Anatolia (Turkey), Comptes Rentus Biologies, 339 (3-4), 147-151.

[28] Schmidt K., Jensen K. (2000). Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components, American Jour. Botany, 87 (5), 678-689.

[29] Park J., Kim M., Park K.R. (2010). Genetic variation in endangered *Scrophularia takesimensis* (Scrophulariaceae) from Ulleung Island, Botanical Studies*,* 51 (3), 371-376.

[30] Torres E., Iriondo J. M., Pérez C. (2003). Genetic structure of an endangered plant, *Antirrhinum microphyllum* (Scrophulariaceae): allozyme and RAPD analysis, American J. Botany*,* 90 (1), 85-92.

[31] Jiménez J.F., Sánchez-Gómez P., Güemes J., Werner O., Rosselló J.A. (2002). Genetic variability in a narrow endemic snapdragon (*Antirrhinum subbaeticum*, Scrophulariaceae) using RAPD markers, Heredity*,* 89 (5), 387-393.

[32] Selseleha M., Hadiana J., Ebrahimib S.N., Sonbolic A., Georgievd M.I., Mirjalilia M.H. (2019). Metabolic diversity and genetic association between wild populations of *Verbascum songaricum (*Scrophulariaceae), Industrial Crops & Products*,* 137, 112–125.

[33] Liu X.L., Qian Z.G., Liu F.H., Yang Y.W., Pu C.X. (2011). Genetic diversity within and among populations of *Neopicrorhiza scrophulariiflora* (Scrophulariaceae) in China, an endangered medicinal plant, Biochem. Syst. Ecol*.,* 39 (4-6), 297-301.

[34] Yang S., Chen C., Zhao Y., Xi W., Zhou X., Chen B., Fu C. (2010). Association between Chemical and Genetic Variation of Wild and Cultivated Populations of *Scrophularia ningpoensis* Hemsl., Planta Medica*,* 77 (08), 865-871.

[35] Torres-Diaz C., Ruiz E., Gonzalez F., Fuentes G., Cavieres L.A. (2007). Genetic diversity in *Nothofagus alessandrii* (Fagaceae), an endangered endemic tree species of the coastal Maulino forest of central Chile, Annals of Botany, 100 (1), 75–82.

[36] Hamrick J. L., Godt M. J. W., Sherman-Broyles S. L. (1992). Factors influencing levels of genetic diversity in woody plant Species, New Forests., 6*,* 95-124.

[37] He T.H., Rao G.Y., You R.L.S., Zhang D.M. (2000). Genetic diversity of widespread *Ophiopogon intermedius* (Liliaceae s.l.) a comparison with endangered *O. xylorrhizus*, Bio. Conserv*.,* 96 (2), 253–257.

[38] Hamrick J.L., Murawski D.A. (1990). The breeding structure of tropical tree populations, Plant Species Biology*.,* 5 (1), 157-165.

[39] Peterson A., Bartish I.V., Peterson J. (2007). Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L., Plant Ecology, 198 (1), 101-110.

[40] Erhardt A. 1993: Pollination of the edelweiss*, Leontopodium alpinum*, Botanical Journal of the Linnean Society*.,* 111 (2), 229-240.

[41] Körner C. (2003). Alpine Plant Life Functional Plant Ecology of High Mountain Ecosystems (2nd Edition). Berlin: Springer-Verlag Berlin Heidelberg, New York.

[42] Petrova G., Petrov S., Delcheva M., Bancheva S. (2017). Genetic diversity and conservation of Bulgarian endemic *Verbascum tzar-borisii* (Scrophulariaceae), Annales Botanici Fennici, 54 (4-6), 307–316.

[43] Ballesteros-Mejia L., Lima N.E., Lima-Ribeiro M.S., Collevatti R.G. (2016). Pollination mode and mating system explain patterns in genetic differentiation in neotropical plants, PLoS One, 11 (7), e0158660.

[44] Yildiz F. (2021). An investigation of the effects of pollinator varieties, genetic diversity and reproductive success in different pollination applications of *Scrophularia fatmae* Kandemir & İlhan (Scrophulariaceae) and *Scrophularia erzincanica* R. R. Mill. (Scrophulariaceae), PhD Thesis, Erzincan Binali Yıldırım University, https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp