

Morphological and Molecular Diversity Among *Heliotropium greuteri* Samples

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Heliotropium greuteri,
Characterization,
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Abstract: The aims of this study were to characterize *Heliotropium greuteri* H. RIEDL plants for some selected morphological characters, and to investigate their diversity and relationships by using inter-simple sequence repeat (ISSR) markers. Forty-seven *H. greuteri* and one *H. lasiocarpum* genotypes as control were used in this study. The plant materials were collected from the sites of Erciyes University and city of Develi, Kayseri, Turkey, and characterized for plant structure, flower structure, seed characteristics and molecular marker diversity. Plant heights ranged from 13 to 110 cm, averaging 28 cm. Flower diameters ranged from 1.35 to 18.5 mm, averaging 9.78 mm. All traits studied indicated variations, which were indicated potential for improvement as ornamental plant. For ISSR analysis, 13 ISSR primers selected based on initial screening with 20 primers were applied to all samples, which produced 90 polymorphic markers. Dice's genetic similarity values varied between 0.81 and 0.99, which was relatively narrow. Probably this species faced a recent bottleneck or speciation has arisen recently resulting in narrowed diversity. Overall, *H. greuteri* plants indicated potential for ornamental use due to their relatively drought tolerance, fragrance, extended flowering time and honeybee attraction.

Heliotropium greuteri Örnekleri Arasındaki Morfolojik ve Moleküler Çeşitlilik

Anahtar Kelimeler

Heliotropium greuteri,
Karakterizasyon,
ISSR,
Süs Bitkisi Potansiyeli

Öz: Bu çalışmada *Heliotropium greuteri* H. RIEDL bitkisinde seçilen bazı morfolojik karakterler bakımından karakterizasyonunu yapmak, ISSR markırları vasıtasıyla akrabalık ve çeşitliliklerini belirlemek amaçlanmıştır. Bu çalışmada kırk yedi *H. greuteri* genotipi ile kontrol olarak bir *H. lasiocarpum* genotipi kullanılmıştır. Bitki materyalleri Erciyes Üniversitesi ve Kayseri-Develi çevresinden toplanmıştır. Bitki yapısı, çiçek yapısı, tohum özellikleri ile moleküler markır çeşitlilikleri karakterize edilmiştir. Bitki yüksekliği 13-100 cm (ort:28 cm), çiçek çapı 1,35-18,5 mm (ort: 9,78 mm) olarak belirlenmiştir. Elde edilen veriler doğrultusunda; tüm özellikler bakımından varyasyon tespit edilmesi bu bitkinin süs bitkisi olarak geliştirilebilir bir potansiyele sahip olduğunu göstermektedir. ISSR analizlerinde, seçilen 13 primer ile 90 polimorfik bant elde edilmiştir. Dice genetik benzerlik değerleri 0,81-0,99 arasında bulunmuştur. Bu bağlamda bu tür bir darlaşma ile karşı karşıya kalmıştır ya da daralan çeşitlilikle sonuçlanan bir türleşme ortaya çıkmıştır. Genel olarak *H. greuteri* bitkileri kuraklık toleranslarının yüksek olması, kokuları, çiçeklenme sürelerinin uzunluğu ve bal arılarını cezbetme gibi özellikleriyle kurakçıl peyzajda süs bitkisi olarak kullanılmaları için önemli bir potansiyele sahiptir.

1. Introduction

More than 9000 seeded plants are naturally present in Turkey. More than 3.000 of these species are endemic to Turkey [1]. The *Boraginaceae* family is one of the most important among them. This family has 100 genera and 2.000 species in tropical, subtropical and temperate regions. They include herbs, shrubs and trees in the world. Within the *Boraginaceae* family, the *Heliotropium* genus show great differences in many of the biological features including habitat preferences, physiognomy and morphological characteristics. This genus contains almost 300

species [2]. The important diversity center of the genus is South-West and Central Asia. The plants of *Heliotropium* occur in areas with an arid and semi-arid climate, mostly on dry soils, sandy and gravelly deserts, disturbed soils as weeds in cultivated lands and wastelands along riversides [3, 4].

Among the people of Turkey, *Heliotropium greuteri* H. RIEDL is called 'yoghurt otu', bostan otu or 'paslı bambul otu'. This species occurs in few locations of Turkey including Adana, Malatya and Bursa [5]. Based on our observations in the fields of Kayseri province, they benefit from loosened cultivated soils of seed pumpkin fields very common in this region with elevation ranging from 1.000 to 2.200 meter from the sea level in the slopes of Erciyes Mountain. Their plants form very showy larger sized plants in cultivated lands compared to those in non-cultivated areas.

Ornamental, genetic and biochemical properties of the *H. greuteri* plants has not been reported yet. There are several reports of the related species [6, 7]. In example, indicated that *H. europaeum*, a close relative of *H. greuteri*, was host for an important pathogen of tomato causing tomato leaf curl disease and tomato leaf curl Palampur virus [8]. The members of *Heliotropium* L. involved both C-3 and C-4 photosynthetic mechanism, which makes the genus important to elucidate evolution of these mechanisms [9]. In addition, the identification and characterization of ornamental plants with high resistance to drought is very important due to changing climate conditions. It is very important to characterization of alternative ornamental plants that have high resistance to drought and arid conditions. The aims of this study were to characterize *Heliotropium greuteri* H. RIEDL plants for some selected morphological characters and to investigate their diversity and relationships by using ISSR markers.

2. Material and Method

2.1. Plant materials

Forty-seven *Heliotropium greuteri* H. RIEDL and one *H. lasiocarpum* samples were obtained from the two sites of Kayseri, Develi and Kayseri in 2015. These two sites are divided by Erciyes Mountains. The evaluations were performed in-site or in the laboratory of Erciyes University (Table 1).

Table 1. The plants used in this study, the latitudes, longitudes and the regions where they are collected

Plant number	Latitude	Longitude	Region
1	38'41.859	35'32.249	KAYSERİ
2	38'41.897	35'32.238	KAYSERİ
3	38'41.938	35'32.207	KAYSERİ
4	38'41.938	35'32.203	KAYSERİ
5	38'41.935	35'32.191	KAYSERİ
6	38'41.955	35'32.194	KAYSERİ
7	38'41.933	35'32.102	KAYSERİ
8	38'41.924	35'32.104	KAYSERİ
9	38'41.892	35'32.094	KAYSERİ
10	38'41.981	35'32.043	KAYSERİ
11	38'41.959	35'32.191	KAYSERİ
12	38'41.933	35'32.197	KAYSERİ
13	38'41.932	35'32.196	KAYSERİ
14	38'42.327	35'32.017	KAYSERİ
15	38'42.327	35'32.020	KAYSERİ
16	38'42.328	35'32.019	KAYSERİ
17	38'42.301	35'32.051	KAYSERİ
18	38'42.279	35'32.048	KAYSERİ
19	38'42.276	35'32.051	KAYSERİ
21	38'42.274	35'32.022	KAYSERİ
22	38'42.215	35'32.152	KAYSERİ
23	38'42.216	35'32.155	KAYSERİ
24	38'42.228	35'32.169	KAYSERİ
25	38'42.228	35'32.169	KAYSERİ
26	38'42.226	35'32.171	KAYSERİ
27	38'42.225	35'32.172	KAYSERİ
28	38'42.227	35'32.172	KAYSERİ

29	38'42.227	35'32.173	KAYSERİ
30	38'42.227	35'32.174	KAYSERİ
31	38'42.236	35'32.179	KAYSERİ
32	38'42.233	35'32.181	KAYSERİ
33	38'41.956	35'32.187	KAYSERİ
34	38'41.957	35'32.191	KAYSERİ
35	38'41.957	35'32.188	KAYSERİ
36	38'41.956	35'32.190	KAYSERİ
37	38'22.871	35'27.239	DEVELİ
38	38'22.087	35'27.024	DEVELİ
39	38'22.871	35'27.234	DEVELİ
40	38'22.886	35'27.346	DEVELİ
41	38'22.889	35'27.348	DEVELİ
42	38'22.876	35'27.315	DEVELİ
43	38'22.879	35'27.312	DEVELİ
44	38'22.880	35'27.310	DEVELİ
45	38'22.885	35'27.263	DEVELİ
46	38'22.883	35'27.258	DEVELİ
47	38'22.853	35'27.254	DEVELİ
48	38'22.873	35'27.245	DEVELİ
50	38'42.279	35'32.193	KAYSERİ

2.2. Morphological characterization

The plants were evaluated for different phenotypic characteristics such as general appearance by using a likert scale of 1 (not good) to 5 (very good), branching by using a likert scale of 1 (not good) to 5 (very good), plant height (cm) leaf width (mm), leaf length (mm), leaf color measured by using a Konica Minolta Chroma Meter CR-400, flower top diameter (mm), flower bottom diameter (mm), spike length (cm), seed width (mm), seed length (mm), 1000-seed weight (gr).

2.3. Molecular marker analysis

Genomic DNA was isolated from young leaves of field grown plants using modified CTAB method [10]. The quality and quantity of DNA isolated from these leaf samples were determined by agarose gel electrophoresis. The genomic DNAs were subjected to PCR amplification using 13 ISSR primers selected from 20 primers. PCR reaction was performed in a 15 ul volume containing 1.5 ul Taq buffer A (10 mM Tris-HCl, pH 8.3 with 15 mM MgCl₂), 1.2 ul of 2.5 mM dNTPs, 0.2 ul of 3 unit of Taq DNA polymerase, 2 ul (20 ng) of template genomic DNA and 1 ul (5 pM) each of ISSR primers. Cycling conditions used for ISSR PCR amplifications were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min and a final extension step at 72°C for 5 min. The amplified products were resolved on 2% agarose gel at 70–80 V for 3.5–4 h, using TBE (Tris-Boric acid-EDTA) buffer, visualized under UV light after staining with ethidium bromide and photographed using gel documentation system (Kodak EL Logic 200). The size (bp) of the amplicons was determined by comparing their mobility with 100 bp DNA ladder as size standard.

2.4. Data analysis

Morphological data were created based on averages of 10 repetitions. Shapiro-Wilk test was used to test whether the samples had normal distribution, and Levene's test was used to test variance homogeneity. One-way analysis of variance was performed to compare the samples by using SPSS 15.0 package program. For molecular data, only clear and reproducible DNA fragments were scored as 1–0 binary data matrix for the presence and absence of a band, respectively. The cluster analysis among the 48 plants of *Heliotropium* species for morphological and molecular data was performed based on correlation matrix for morphological data and the Dice' similarity matrix for molecular data [11], and unweighted pair-group with arithmetic average method (UPGMA) algorithms nested in NTSYS-pc software (Numerical Taxonomy Multivariate Analysis System) version 2.1 [12].

3. Results

3.1. Morphological characterization

The 48 plant samples including 47 *H. greuteri* and one *H. lasiocarpum* were characterized for their several morphological properties described above. The germination time was between late May and early June. The time of flowering was recorded as mid-June, and lasted until the first frost, usually mid-October. This has great value because this time of year usually lacks actively growing plants in Central Anatolia due to insufficient level of precipitation. It appeared that the flowering period of *H. greuteri* was significantly long, which was important for ornamental purpose and beekeeping. The plant height was measured as 13 cm minimum in plant number 19 in Kayseri and 110 cm maximum in plant number 39 in Develi (Table 2). General plant appearance and branching were scored using a scale 1 (not good) to 5 (very good), with averages of 3.34 and 3.58, respectively. Regarding branching, the least branching value (1) was observed in 4 of the plants while the highest scale (5) was present in 15 of the samples. Branching characteristics differed probably because of plants of different ages and environmental conditions. In some areas, despite removal of upper plant parts by gardeners, the plants were able to reproduce branches. This may indicate presence of cutting tolerance in these potential ornamental plants.

For general appearance of the plants, considerable diversity was observed among the samples, which also indicated potential for selection. 4 of 48 plants had very poor appearance (1), 7 had a rate of 2, 14 of medium, 13 indicated 4, and 10 had the best appearance (5).

Data of leaf size, leaf height, leaf width were presented in Table 2. The minimum value in the measurements made from the leaf blade was measured at the number 40 (5.2 mm), while the maximum value was found at the number 9 (28.9 mm) and the average was found to be 18.6 mm (Table 2). The highest number of leaves was observed in the plant number 9, while the numbers 2, 6, 7, 9, 16, 23, 30 and 32 were not statistically different. No statistically significant difference was observed among the samples 18, 21, 22, 33, 36, 37, 42 and 44 while the lowest value belong to plant number 40.

Leaf length was found to be 8.2 mm minimum in plant number 44 sampled from Develi, maximum 65.3 mm from plant number 9 from Kayseri and the average was 27.5 mm (Table 2). Plant number 9, having the highest value for leaf length, was not statistically different from 2, 14, 15, 17 and 32.

The minimum leaf width was found to be 3.35 mm in plant number 37 from Develi, the maximum value was 27.6 mm in plant number 30 from Kayseri, averaging 12.3 mm (Table 2). Flower upper diameter, flower lower diameter, spike length measurements were also studied. However, no statistically significant differences for these traits were observed among the plants. *Heliotropium greuteri* were also evaluated for seed properties. The largest seed size (2.24 mm) recorded in plant 43, and the smallest seed (1.68 mm) were measured in plant 3.

Correlation analysis among the phenotypic characteristics indicated few meaningful results. A positive correlation was observed between leaf length and leaf blade ($r = 0.89$). Similarly, there was a moderate correlation between the flower lower diameter and the flower upper diameter ($r = 0.55$). There was a positive moderate correlation between general appearance and branching ($r = 0.65$), but no correlation was found between the other morphological characters.

Table 2. The minimum, maximum, mean and standard deviations of the population for the morphological characters studied

	Minimum	Maximum	Mean	Standard Deviation
Leaf blade (mm)	5.16	38.65	18.62	5.46
Leaf width (mm)	3.33	27.61	12.25	3.74
Leaf height (mm)	8.17	65.28	27.45	8.41
internode (cm)	0.50	9.10	2.74	1.21
Plant width (cm)	13.00	110.00	28.59	15.8
Flower upper diameter (mm)	1.35	18.54	9.78	1.73
flower lower diameter (mm)	1.73	8.98	5.14	1.00
spike length (cm)	0.80	10.60	4.39	1.74
Seed width (mm)	0.75	1.68	1.11	0.10
Seed length (mm)	0.99	2.24	1.78	0.17
L*	36.42	68.66	44.86	4.69
a*	-14.70	-2.63	-7.97	2.09
b*	-4.11	-77.5	-14.87	34.31

'L', 'a', 'b' values for leaf color were measured. 'L' has a maximum value of 100 and corresponds to the white color. In the measurements, 'L' value was found to be minimum of 36 and maximum of 69, averaging 45. We can interpret the measurements as medium brightness. Positive and negative 'a' represents red and green colors, respectively. No positive 'a' value was observed among the samples. 'a' values ranged from -14.70 to -2.63, with

the average of -7.97. 'b' values were between -4.11 and -77.5 with the average of -14.87. The negative 'a' value corresponded dark green and bluish gray color due to the negative 'b' values.

3.2. UPGMA analyses of morphological and molecular data

The UPGMA analysis was performed for estimating genetic diversity and relationships among the samples based on morphological and molecular data. Morphological characters (general appearance, branching, plant height, internode, leaf width, leaf length, leaf color, flower upper diameter, flower lower diameter, spike length, seed width, seed length, 1000 seed weight) among the 48 samples were analyzed. The UPGMA dendrogram based on the correlation matrix indicating similarity values between each pair of the samples was generated for the forty-eight samples. This yielded two large groups at the similarity value of 0.43 to 1.00 (figure not shown). There were 20 and 28 individuals in group A and B, respectively.

For molecular analyses of the samples, 13 ISSR primers out of 20 primers produced 101 scorable bands, of which 90 was polymorphic. The most productive primer was (CA)₈R yielding 13 bands while the least productive was (AG)₈YC primer with 4 bands, averaging 7.76 bands per primer. The UPGMA dendrogram based on Dice' similarity matrix resulted in two groups at the similarity level of 0.65 (Fig. 1). The most distinctive one was plant 48, *H. lasiocarpum*. The plants 40 and 48 were sampled from Develi city and very similar with the value of 0.91. Two *H. greuteri* samples, 8 and 31, were clustered separately from the rest of *H. greuteri* samples. These two samples were collected from the same area in Kayseri. The similar results were also observed between 2 and 3 and between 10 and 11, both pairs were sampled from the same location of Kayseri. The Mantel correlation coefficient (*r*) between Dice' similarity and ultrametric distance (obtained from conversion of dendrogram to distance matrix) matrices was 0.82, meaning sufficient representation of the similarity matrix. Morphological and molecular data resulted in different grouping patterns. Probably, the first are affected/ modified by environmental factors such as water availability and nutrients [13].

4. Discussion and Conclusion

In this study, ornamental plant characteristics of forty-eight samples of the *Heliotropium* genus belonging to *Boraginaceae* family, 47 *H. greuteri* and one *H. lasiocarpum* were investigated. All samples were distinguished from each other based on morphological and molecular data. Based on the observations made in this study, *H. greuteri* had good ornamental characteristics because of 1) extended flowering period up to four months, 2) showy plants harmonizing white and green plant parts, 3) fragrant flowers, and 4) considerable drought tolerance after young seedling stage (Fig. 2). They also attract honeybees, particularly during poor vegetation at the end of summer and in the first half of fall season. They can be used as cover plant. We think that it can be successfully used in open areas as well as under trees (in semi-shaded). Particularly, they can produce healthy plants in the borders where they are often exposed to drought stress. On the other hand, due to their non-hairy root structure, they may not be successful in avoiding soil erosion in sloppy areas. Enlargement of flower and leaf sizes may be potential research topics for *H. greuteri*. These characters can be improved by selecting promising genotypes among the collections or applying colchicine for polyploidization. Another research area would be improving germination time and rate, which was poor in our preliminary studies. The effects of seed treatment practices such as priming, hydration and vernalization on germination should be investigated.

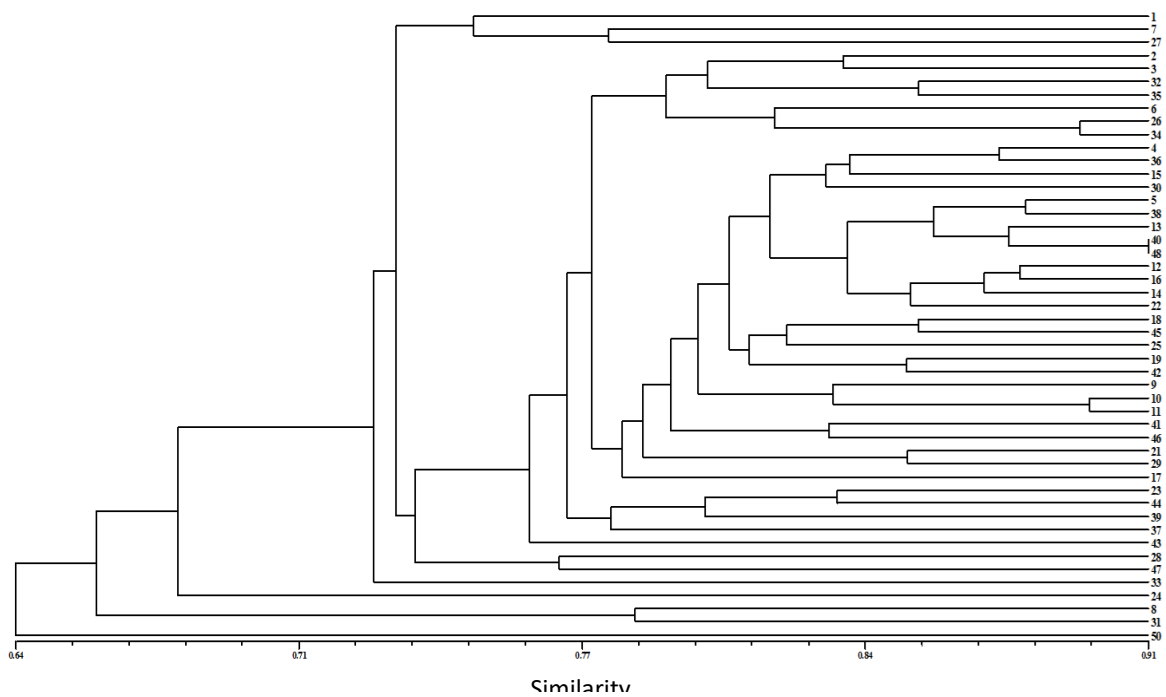


Figure 1. The UPGMA dendrogram of 47 *Heliotropium greuteri* and one *H. lasiocarpum* plants based on the similarity matrix by using 13 ISSR markers



Figure 2. Naturally growing *Heliotropium greuteri* plant in the Kayseri province of Turkey.**Acknowledgement**

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