Species relationships in the genus *Helichrysum* Mill. (*Asteraceae*) based on morphological characters in Iran

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ABSTRACT

The present study considers phenetic and cladistic analyses of 20 populations belong to 19 Helichrysum species, trying to reveal their morphological variations and inter-specific relationships in Iran. To evaluate the species relationships, cluster analyses and principal component analysis were done. Statistical analyses indicated that characters as maximum basal leaf width, maximum stem leaf width, involucre form, achene length and achenial papillae, bud prop, longitudinal plicate in phyllary and stem branched had the most important role as diagnostic characters in intera- genus variation. In general two clusters provided, H. makranicum (Rech. f. & Esfand.) Rech. f. due absence rest buds placed in first cluster and remaining species formed the second cluster. In the second cluster, H. araxinum Takht. ex Kirp., H. athanaton Georgiadou & Rech. f., H. artemisioides Boiss. & Hausskn. and H. davisianum Rech. f. with slim stems formed the first sub cluster and H. plicatum DC., H. polyphyllum Ledeb., H. pseudoplicatum Nab.- by having phyllaries with longitudinal plicate-, H.psychrophilum Boiss., H. graveolens (M. B.) Sweet., H. armenium DC., H. aucheri Boiss., H. glandoliferum Schultz. Bip., H. globiferum DC., H. leucocephalum Boiss., H. oligocephalum DC., H. oocephalum Boiss., H. pallasii (Spreng.) Ledeb., and H. rubicundum (C. Kooch) Bornm., populations -with wide rosette leaf- have been placed together in second sub cluster.

Key words: *Helichrysum* Mill., morphological variations, Iran.
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INTRODUCTION

The genus *Helichrysum* Mill. belongs to the tribe Gnaphalieae of family Asteraceae. It is comprised of 500-600 species [1, 2], which are distributed mainly in the African continent, Madagascar and Eurasia regions [3]. The *Helichrysum* species have homogamous or heterogamous capitulum with hermaphrodite florets that outnumber the female florets. The phyllaries of the florets have a fenestrated stereome with a flat, generally smooth or toothed receptacle. Similarly, the cypselae are glabrous or covered with duplex or twin hairs. The pappus is free at the base and is monomorphic. It usually has uniseriate with scabried to plumose bristles [4].

Helichrysum is a large and taxonomically difficult genus, and its circumscription has undergone notable changes, because the traditional concept of the genus included an assemblage of numerous heterogeneous taxa [5]. According to Flora Iranica [6] 19 *Helichrysum* species have been reported in Iran. There are few reports on systematic study of

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Helichrysum species from Iran [7], therefore, the present study considers phenetic and cladistic analyses of 20 plant populations belong to 19 *Helichrysum* species, trying to reveal their morphological variations and inter-specific relationships.

MATERIALS AND METHODS

Morphometric studies were performed on 20 plant populations of 19 *Helichrysum* species.

Three specimens from each population were randomly selected and used for morphometric studies. From the specimens collected, 5 readings were taken for each character. Details of the localities and the voucher numbers are provided in Table 1. Voucher specimens are deposited in TARI and SBUH. In total 5 quantitative and 15 qualitative morphological characters were studied (Table 2).

Table 1- *Helichrysum* species studied their localities and voucher numbers

Species	Voucher number	Locality
H. araxinum Takht. ex Kirp.	HSBU 201200	West Azerbaijan: Makou
H. armenium DC.	HSBU 201201	Hamadan: Heydareh
H. artemisioides Boiss. & Hausskn.	TARI 74	Arak: Mahalat, Baqer- abad
H. athanaton Georgiadou & Rech. f.	TARI 62142	Isfahan: Semirom, Dehe vanak
H. aucheri Boiss.	HSBU 201202	West Azerbaijan: Nagadeh
H. davisianum Rech. f.	HSBU 201203	Yazd: Shirk ooh, Bala-Deh
H. glanduliferum Schultz- Bip.	HSBU 201204	West Azerbaijan: Oshnavieh
H. globiferum Boiss.	HSBU 201205	West Azerbaijan: Khoy, Ghare-tappeh
H. graveolens (M. B.) Sweet.	TARI 21658	Golestan: Gorgan, Golestan Natural Park
H. leucocephalum Boiss.	HSBU 201206	Fars: Estahbanat: Dare- Morghak
H. makranicum (Rech. f. & Esfand.) Rech. f.	TARI 39250	Hormozgan: Bashagard: Senderk- Darre Pahn
H. oligocephalum DC.	HSBU 201207	Tehran: Darakeh
H. oocephalum Boiss.	HSBU 201208	Khorasan: Neyshaboor, Boujan
H. pallasii (Spreng.) Ledeb.	HSBU 201209	Tehran: Dizin
H. plicatum DC.	HSBU 201210	West Azerbaijan:Uroumia, Solouk
H. polyphyllum Ledeb.	HSBU 201211	West Azerbaijan:Uroumia, Solouk
H. pseudoplicatum Nab.	HSBU 201212	Kordestan: Sanandaj, Chehel- cheshmeh
H. psychrophilum Boiss.	TARI 74	West Azerbaijan:Uroumia, Mavaneh
H.rubicundum (C. K.och) Bornm.	HSBU 201213	East Azerbaijan: Tabriz
H.rubicundum (C. K.och) Bornm.	HSBU 201214	Zanjan: Qeydar, Soltanieh

Table 2- Morphlogical characters and their codings

Character No.	Character (Abbreviation for graphics)	Codes/units
1	Stem form	1: erect, 2: procumbent
2	Stem thickness	1: thin, 2: medium, 3: thick
3	Resting bud	1: absent, 2= present
4	Indumentum basal leaf	1: Tomentose, tomentose- glandular 2- glandular, 3= lanate, 4: floccos- lanate
5	Indumentum stem leaf	1: Tomentose, tomentose- glandular, 2: tomentos- lanate, 3: glandular
6	Involucre form	1: Conical, 2: non conical
7	Number of female flower per capitulum	1: 0 , 2:> 0
8	Ratio of innermost phyllaries length to outermost phyllaries	1: $\geq$ 4, 2: $\leq$ 3
9	Achenial papillae	1: duplex, 2: multi cellular
10	reflexed Corolla lobes	1: revolute, 2: non revolute
11	Stem branched	1:absent, 2= present
12	Pappus bristle	1: plumose, 2: smooth
13	Phyllaries apex colour	1: Brown wish, 2: non brown wish
14	Bud prop	1: absent, 2: horizontal, 3: erect
15	Longitudinal plicate in phyllary	1: whit plicate, 2: without plicate
16	Achene length	mm
17	Corolla length	mm
18	involucrum width	mm
19	Maximum basal leaf width	mm
20	Maximum medial leaf width	mm

In order to group the populations with morphological similarities cluster analysis using UPGMA (Unweighted Paired Group with Arithmetic Average) and WARD (Minimum Variance Spherical Cluster) was performed.

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Clustering and ordination plot analyses were performed by NTSYS ver. 2. (1998). In order to determine the most variable morphological characters among the species studied, factor analysis based on principal components analysis (PCA) was performed [8].

RESULTS AND DISCUSSION

Cophenetic correlation obtained for different clustering method, showed the highest value for UPGMA dendrogram (Fig. 1).

In general, two clusters were obtained. The *H. makranicum* based on absence of rest buds, placed in first cluster, while all remaining species formed the second cluster.

In second cluster, *H. araxinum* Takht. ex Kirp*., H. athanaton, H. artemisioides* and *H. davisianum* with slim stems fromed the first sub cluster and the second major sub cluster has two sub clusters. *H. plicatum, H. polyphyllum* and *H. pseudoplicatum*, formed first sub cluster, while *H.psychrophilum*, *H.graveolens, H.armenium, H. aucheri, H. glandoliferum, H.globiferum. H. leucocephalum, H. oligocephalum, H. oocephalum, H. pallasii*, and *H. rubicundum* populations have been placed together in second sub cluster.

In order to identify the most variable morphological characters among the species studied, PCA analysis revealed that the first four factors comprise about 71% of total variance. In the first factor with about 30% of total variance, characters like maximum basal leaf width, maximum medial leaf width, involucre form, achene length and achenial papillae possessed the highest positive correlation. In the second factor with about 23% of total variance, characters as bud prop, longitudinal plicate in phyllary and stem branched possessed the highest positive correlation. In the third factor with about 10% of total variance, characters like involucrum width stem form, Pappus bristle and phyllaries apex color possessed the highest positive correlation. In the fourth factor with 9% of total variance, characters like stem thickness and indumentum basal leaf possessed the highest positive correlation (Table 3).

Table 3- Contribution of the variables to components 1 (PC1), 2 (PC2), 3 (PC3), 4 (PC4)

Variable	PC1	PC2	PC3	PC4
1	-0.1818	0.3572	0.7271	-0.1906
2	-0.473	0.3961	-0.1874	0.4685
3	0.3176	0.1611	-0.2443	-0.2255
4	-0.7693	0.2608	0.1261	0.3203
5	0.3895	0.3569	0.01782	-0.2137
6	0.6264	-0.5057	0.1739	0.1003
7	0.07352	-0.4912	0.03067	-0.3945
8	0.09458	0.8902	0.07034	0.03595
9	0.3978	-0.1929	0.05427	0.3094
10	0.3176	0.1611	-0.2443	-0.2255
11	-0.6569	0.3745	-0.2959	-0.3312
12	-0.2114	0.4407	0.4771	-0.07111
13	-0.3176	-0.1611	0.2443	0.2255
14	0.5164	0.8087	0.00254	-0.1246
15	0.2923	0.7093	-0.3155	0.1033
16	0.5306	-0.2971	0.1249	-0.5633
17	0.3176	0.1611	-0.2443	-0.2255
18	-0.384	0.1867	0.8485	-0.08147
19	0.7911	-0.4544	0.2981	0.03267
20	0.7449	0.04827	0.1453	0.5682

According to our study based on morphological characters, our results are partly in agreement with taxonomic treatment of the genus in Flora Iranica. First cluster was composed of *H. makranicum*, which is one of the clearly circumscribed species found in Iranian. It differs from other species in absence rest buddies.

In the first sub cluster, *H. araxinum.* and *H. athanaton* base of stem form, indumentum basal leaf, achenial papillae, Pappus bristle, involucrum width, longitude plicate in phyllary characters, placed close to each other and *H. artemisioides* and *H. davisianum* differ from other species in having characters as achene length, branches stem, maximum basal and medial leaf width. In second subcluster, *H. plicatum, H. polyphyllum* and *H. pseudoplicatum*,

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based on presence of phyllary whit longitudinal plicate, indicated more similarity. In this study, due to presence of buddy vertical prop, *H. armenium* is separated of *H. auchery, H. ribicundum, H. leucocephalum* and *H. oocephalum.* All of these results supported by Flora Iranica and show that multivariate methods based on vegetative and reproductive characters allow the differentiation of Iranian *Helichrysum* species.
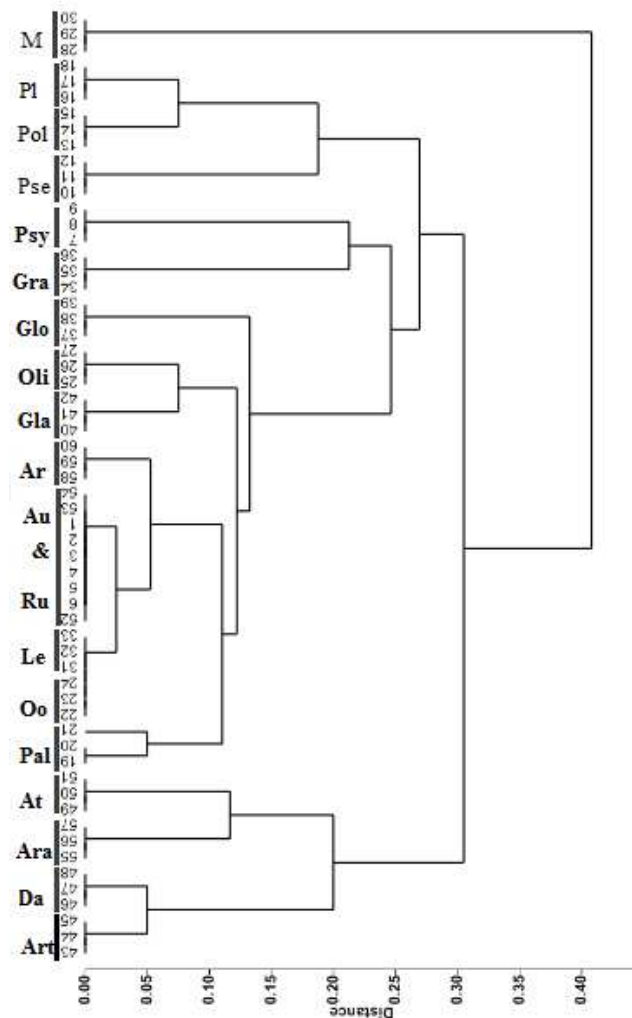

Fig.1- UPGMA clustering of *Helichrysum* species based on morphological characters

Although as it is observed in dendrogram (Fig. 2), the results of morphological data not shown clearly separation two species *H. leucocephalum* and *H.oocephalum.* Based on the our studies, *H. leucocephalum* grows mainly in center and south of Iran and *H.oocephalum* found in Khorasan and Hormozgan province of Iran and according to Flora Iranica and genetic studies (unpublished data), *H. leucocephalum* and *H.oocephalum* are distinguishable species. It seems these morphological characters are not sufficient for distinguishing these species.

H. rubicundum species founds in different regions of west, west- north and center of Iran with a wide range of ecological conditions, while *H.aucheri* grows in small area of *H. rubicudum* niche, in west Azerbaijan, As it is observed in dendrogram (Fig. 2), the results of morphological data not showed any separation within populations of these species.

Except flowering stage, which *H. rubicundum* often is distinguishable due its involucres color, in other stages of life cycle, -maybe due phenotypic plasticity in this species- identification of these species is difficult [9]. It seems common and general methods of morphological studies are not capable to revealing this kind of variation, so it is

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better to use molecular approaches. Or micro morphology, especially seed characters -which are less affected by habitat conditions-, may provide useful characters for evaluating of variation in these taxons. Otherwise according to Flora of Turkey [10] these species are two subspecies of *H. arenarium* (L.) Moench, so the present study maybe confirms the result of Flora of Turkey.

Summing up, the results of analysis based on vegetative and reproductive traits support the identity of Iranian *Helichrysum* species and their relationships, although more focus are needed for this knowledge.

CONCLUSION

Statistical analysis based on morphological characters indicated the most diagnostic characters in intera-genus variation. By clustering method, in general two clusters provided that some extend support the identity of Iranian *Helichrysum* species and their relationships.

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