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KARYOLOGICAL AND MORPHOLOGICAL VARIATIONS WITHIN *Bassia hirsuta* (L.) ASCH. IN BULGARIA

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Abstract: This study was performed to determine karyological and morphological variability within and between populations of *Bassia hirsuta* in Bulgaria and to reveal their ecological preferences and detailed morphological characteristics. The studied Bulgarian *B. hirsuta* populations have diploid chromosome number 2n=18. The interpopulation variability which could be due to differences in ecological conditions is dominant. Vegetative traits are more variable than generative ones. The least variable are those that characterize the seed and could be used as highest value traits. The data given in the present study are supplementary to the data given about the species in Flora of the People's Republic of Bulgaria and can be used both for correct identifications of the specimens and for getting familiar with species characteristics.

Key words: *Bassia hirsuta*, karyology, morphology, chorology

Bassia hirsuta (L.)'nın Bulgaristan Populasyonlarındaki Karyolojik ve Morfolojik Varyasyonlar

Özet: *Bassia hirsuta*'nın Bulgaristan'daki populasyonlarındaki karyolojik ve morfolojik varyasyonu belirlemek ve populasyonların ekolojik tercihlerini ve detaylı morfolojik karakterlerini ortaya koymak amacıyla yapılmıştır. Çalışmada kullanılan *B. hirsuta*'nın Bulgaristan populasyonlarındaki diploid kromozom sayısı 2n=18'dir. Ekolojik şartlara bağlı olabilen populasyonlar arası varyasyon baskın durumdadır. Vejetatif özellikler, generatif özelliklere göre çok daha fazla varyasyon göstermektedir. En az varyasyon gösteren özellikler tohumla ilgili olanlardır ve önem dereceleri yüksek özellikler olarak kullanılabilirler. Bu çalışmada elde edilen bulgular, *B. hirsute* ile ilgili olarak "Flora of the People's Republic of Bulgaria"da verilen bilgileri tamamlayıcı niteliktedir ve hem örneklerin doğru bir şekilde teşhis edilmeleri hem de türün özelliklerinin daha yakından tanınması amacıyla kullanılabilir.

Anahtar kelimeler: *Bassia hirsuta*, karyoloji, morfoloji, koroloji.

Introduction

Bassia hirsuta (L.) Asch. is the only one representative of the genus *Bassia* All. in the Bulgarian flora. The species occurs in saline sands and clays along the Bulgarian Black Sea Coast. It is one of the characteristic taxa in the pioneer halophytic communities inhabiting hyper saline coastal lakes and salt pans (Tzonev and Gussev 2011). The main threat that the populations of the species face is the increased anthropogenic impact which often leads to disturbance of the ecological equilibrium of coastal ecosystems, and in some cases, direct damage to plants. Currently, the species is protected under the Biological Diversity Act (2007) and is included in the Red Book of Bulgaria under the category „Endangered" (Stoeva 2011). Over the past decade the populations of *Bassia hirsuta* have not been subject to a comprehensive study. There are some data about species' distribution (Grozeva et al. 2004; Grozeva 2005; Tzonev et al. 2008), the area and the size of its populations (Grozeva 2004) and its chromosome number (Grozeva and Stoeva 2006; Grozeva 2013).

The aim of this study is to determine the karyological and morphological variability within and between populations of *Bassia hirsuta* in Bulgaria and to reveal their ecological preferences and detailed morphological characteristics.

Materials and Methods

Five *Bassia hirsuta* populations were investigated near saline lakes; Shablensko and Varnensko lakes from Northern Black Sea Coast and Pomoriysko and Atanasovsko lakes and Poda protected area from Southern Black Sea Coast, Bulgaria. The map to the locations of the studied areas and more detailed information about sampling points are shown on Figure 1 and Table 1.

30 plants from each population were used for the study. Morphological and karyological analyses of the sampled populations were performed. The heights of stems of the plants were measured on siteand other morphological measurements were done using herbarium plants. 18 quantitative characters were

included in the morphological analysis: 1. Height of stem; 2. Length of basal leaf; 3. Width of basal leaf; 4. Length/width ratio; 5. Length of basal leaf petiole; 6. Length of upper leaf; 7. Width of upper leaf; 8. Length/width ratio; 9. Length of upper leaf petiole; 10. Length of inflorescence; 11. Diameter of bisexual flower; 12. Length of flower petiole; 13. Diameter of female flower; 14. Length of flower petiole; 15. Length of seed; 16. Width of seed; 17. Length/width ratio; 18. Thickness of seed. A total of 11 qualitative traits were also recorded: 1.Shape of stem; 2. Colour of stem; 3. Shape of lamina; 4. Colour of leaf lamina; 5. Type of inflorescence; 6. Colour of perianth; 7. Degree of perianth connation; 8. Presence of keel on petals; 9. Colour of seed; 10. Colour of pericarp; 11. Indumentum.

Fig. 1. Map showing the locations along North and South Black Sea Coast where *B. hirsuta* populations were studied

The mean values and coefficients of variation (CV, %) were calculated for each character (Table 2). The relative contribution of intra- and interpopulation variation to the overall variation of each characteristic of the studied populations was evaluated by one-way ANOVA (Table 2). The Unweighted Pair-Group Average (UPGA) Hierarchical Cluster Analysis (HCA) was applied to the matrix with the Euclidean distances between the populations of *B. hirsuta* in order to study the morphological pairwise similarities and the hierarchical grouping structure. The statistical program Statistica 12 (StatSoft) was used for the analysis. For a more detailed study of the morphology of indumentum and generative organs, the Scanning Electron Microscope method (SEM) was used. The electron microscopy tests were conducted in the laboratory for X-ray analysis of the Faculty of Chemistry and Pharmacy of Sofia University. Chromosome numbers and karyotypes were reported based on permanent preparations of metaphase root apex plates of seeds collected from natural habitats of the species and germinated in laboratory conditions. The root tips were treated and squashed according to the relevant methods (Grozeva 2007). The chromosomal type was determined after the centromere index I = s/s+l, according to the classification proposed by Grif and Agapova (1986). Three metaphase plates were measured from each population. The voucher specimens are kept in the herbarium of Bulgarian Academy of Sciences (SOM).

Table 1. Studied populations of *B. hirsuta*.

Population №	2 *n*	Locality
№ 801	18	Northern Black Sea Coast, near Schablensko lake, on sand dunes, at 0 m, 43º35,24`N, 28º33,18`E, halophytic community dominated by *B. hirsuta*
№ 802	18*	Northern Black Sea Coast, Varnensko lake, periodically flooded coastal alluvial, at 79 m, 43º11,09`N, 27º 50,16`E, along coastline with *Atriplex tatarica*
№ 807	18**	Southern Black Sea Coast, Pomoriysko lake, sandy terrains near Salt museum, at 1 - 2 m, 42º35,14`N, 27º 36,07`E, halophytic community dominated by *B. hirsuta* and *Suaeda maritime*
№ 804	18**	Southern Black Sea Coast, Atanasovsko lake, at the northern end of the lake near the road Burgas - Pomorie , at - 1.5 m, 42º35,14`N, 27º 36,07`E, halophytic community dominated by *Salicornia europaea*
№ 809	18	Southern Black Sea Coast, Poda protected area near the town of Burgas, saline areas in the western part of the area , at 15 m, 42º27,02`N, 27º 27,00`E, halophytic community dominated by *Salicornia europaea*.

* - data published by Grozeva (2013)
**- data published by Grozeva & Stoeva (2006)

Results

Karyology

The karyological analysis showed that the diploid chromosome number 2*n* = 18 was found in all studied populations (Table 1). The karyotype of the all populations consists of meta- and submetacentric chromosomes (Fig. 2). The karyotypes from Shablensko, Varnensko and Atanasovsko lakes possess a pair of satellites, which are attached to the submetacentric chromosemes (Figs. 2 C-E).

Table 2. Mean (first line), coefficient of variation, CV, % (second line) and percentage of the interpopulation variation in the overall morphological variation (SSb) of *B. hirsuta* populations for each of the 18 observed characters.

Population Character №	Atanasovsko lake	Pomoriysko lake	Poda protected area	Varnensko lake	Schablensko lake	SSb
1.	29.25	38.24	38.81	41.67	42.08	71.83
	33.11	20.31	19.56	15.85	15.25	
2.	8.54	10.26	10.10	10.14	10.15	64.46
	31.99	25.10	22.50	19.87	18.38	
3.	0.92	1.00	1.03	1.02	1.07	53.19
	20.66	23.93	17.71	18.28	14.96	
4.	9.61	10.88	10.09	10.32	9.68	54.71
	35.02	40.11	27.40	32.25	23.89	
5.	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	
6.	4.87	4.49	4.51	5.18	5.26	62.03
	18.42	39.74	33.58	30.11	29.16	
7.	0.95	0.87	0.82	0.94	0.95	52.24
	21.97	22.74	19.43	17.39	15.33	
8.	5.45	5.72	5.44	5.74	5.81	61.72
	33.71	56.77	45.58	38.55	44.02	
9.	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	
10.	142.87	177.05	173.20	201.40	206.41	66.94
	40.09	30.40	29.61	21.93	22.67	
11.	1.72	1.72	1.73	1.73	1.74	75.70
	15.37	16.46	14.01	15.42	14.68	
12.	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	
13.	1.65	1.66	1.71	1.72	1.72	77.85
	11.47	14.64	12.37	13.34	10.63	
14.	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	
15.	1.72	1.69	1.68	1.69	1.67	61.01
	3.55	4.26	4.05	4.96	4.62	
16.	1.24	1.22	1.22	1.23	1.23	63.07
	6.03	4.12	4.63	3.77	4.51	
17.	1.40	1.38	1.38	1.37	1.36	52.42
	7.07	5.68	4.80	6.34	5.11	
18.	0.40	0.39	0.41	0.40	0.42	55.43
	14.40	13.97	10.33	14.17	13.10	

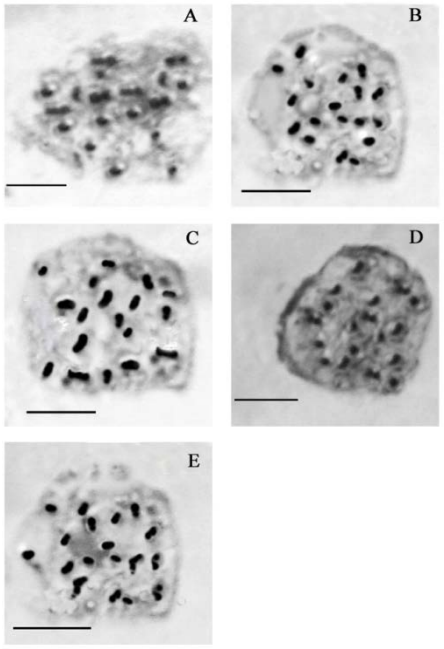

Fig. 2. Microphotographs of metaphase plates of *B. hirsuta* populations in A - Pomoriysko lake; B - Poda protected area; C - Schablensko lake; D - Varnensko lake; E - Atanasovsko lake. (Scale bars = 10 µm)

Morphology

The intrapopulation variation was estimated on the basis of the coefficient of variation (Table 2). The comparison of variation coefficients revealed that they are different both for the various characters within each population and among populations. The data showed that vegetative traits have higher level of variability than generative traits in all populations. The results of ANOVA (Table 2) demonstrated that interpopulation variability in all studied populations is dominant in total variability. The dendrogram generated by UPGA cluster analysis based on the morphological pairwise similarities (Euclidean distances between population centroids) showed that four of the studied populations - Varnensko, Schablensko and Pomoriysko lakes and Poda protected area are grouped in two clusters A and B (Fig. 3, Table 3). The least similarity is determined for the population from Atanasovsko Lake. The results of the SEM analyses (Figs. 4, 5) and of the qualitative study of the traits are presented in general for the species morphological characteristics since no significant differences between the five studied populations were found.

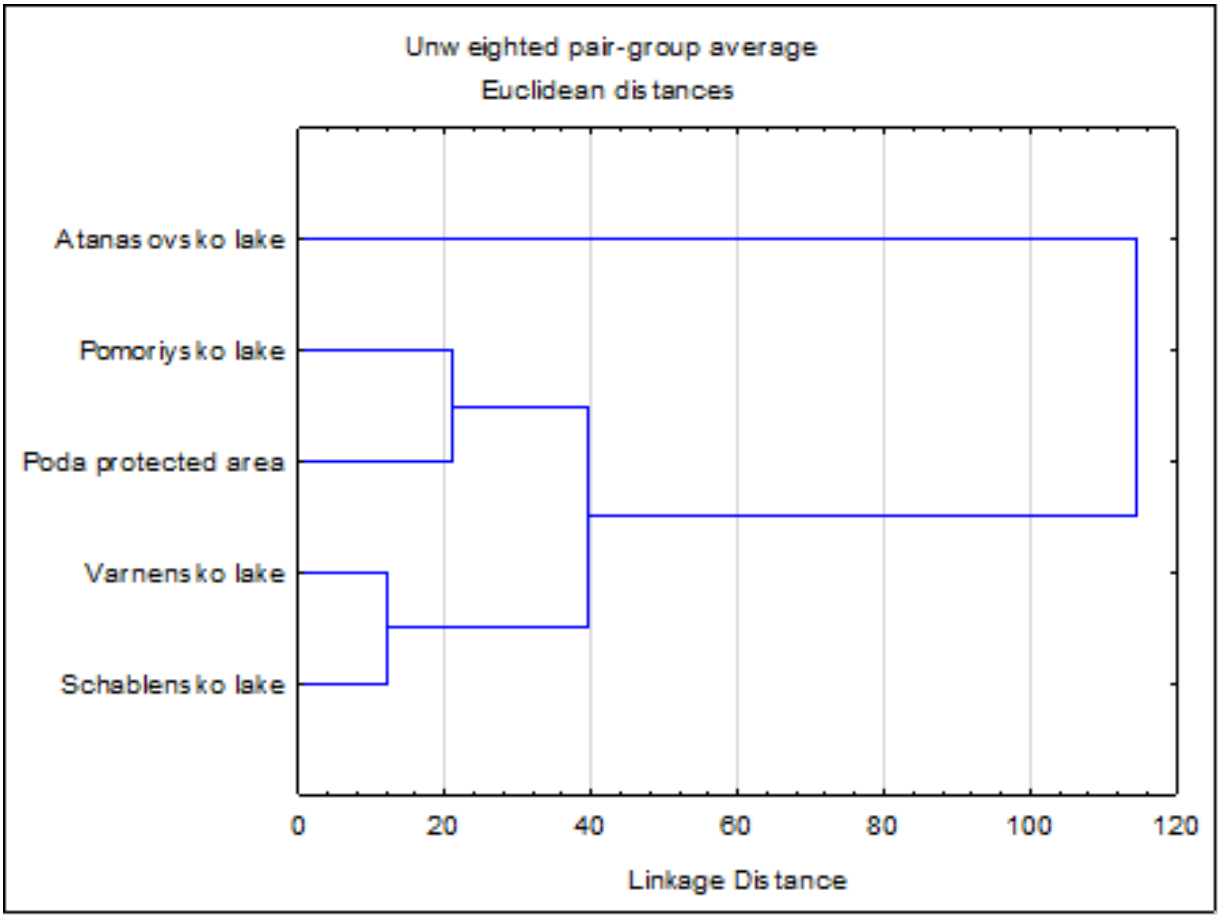

Fig. 3. Dendrogram of the cluster analysis of the *B. hirsuta.*

Table 3. Values of Euclidean distances between the pairs of studied *B. hirsuta* populations based on 18 characters**.**

Population	Atanasovsko Lake	Pomoriysko Lake	Poda protected area	Varnensko Lake
Pomoriysko Lake	97.1			
Poda protected area	100.0	21.0		
Varnensko Lake	128.0	43.0	32.0	
Schablensko Lake	133.0	48.0	35.0	12.0

Morphological characteristics

Annual, moderate or densely covered with multicellular unbranched spiky hairs, with length up to 1,2 mm (Figs. 4 A-C). Stem (15)29.25-42.08(65) cm, erect, angular, branched at bases, sometimes turning yellow with maturity (Table 2, Figs. 6). Leaves alternate, sessile, fleshy. Leaf lamina, green or greyish-green (Figs. 6, 7). Basal leaves (7)8.54-10.26(15) mm × (0.8)0.92-1.07(1.5) mm [length/width ratio (8)9.6-10.88(14)], linear, entire with acute apex and cuneate base (Table 2, Fig. 6). Upper leaves (3)4.49-5.26(8) mm × (0.7)0.82-0.95(1.5) mm [length/width ratio (4.2)5.44-5.81(6.5)], linear or filiform, entire with acute apex and cuneate base (Table 2, Fig. 7). Flowers sessile, 2-3(5) in small glomerules, forming terminal spikes (Fig. 7). Bisexual flowers (1.6)1.72-1.74(1.8) mm in diameter and female flowers (1.6)1.65-1.72(1.8) mm in diameter (Table 2). Tepals (perianth segments) (2)3(5), entire, with acute apex, free nearly to the base, usually keeled, densely covered with hairs (Figs. 4 D-F), enlarging in the fruit. Stamens 3-5 (Figs 4 E-F). Pollen grains 20-25 µm in diameter (Fig. 4 G). Stigmas 2. Pericarp light-yellowish, semi-transparent, not adherent to the seed, with scale-like structure and thickness of 7-10 µm (Figs. 4 H, 5 A-C). Seeds horizontal, ±lenticular (1.6) 1.67-1.72(1.8) × (1.2)1.22-1.24 (1.3) mm, [length/width ratio 1.36-1.4(1.42)] (Table 2, Fig. 5 D). Testa (seed coat) dark brown to black, the sculptural cover on both sides in the shape of shallow longitudinal and shallower transverse notches turning into±elongated loops (Fig. 5 D).

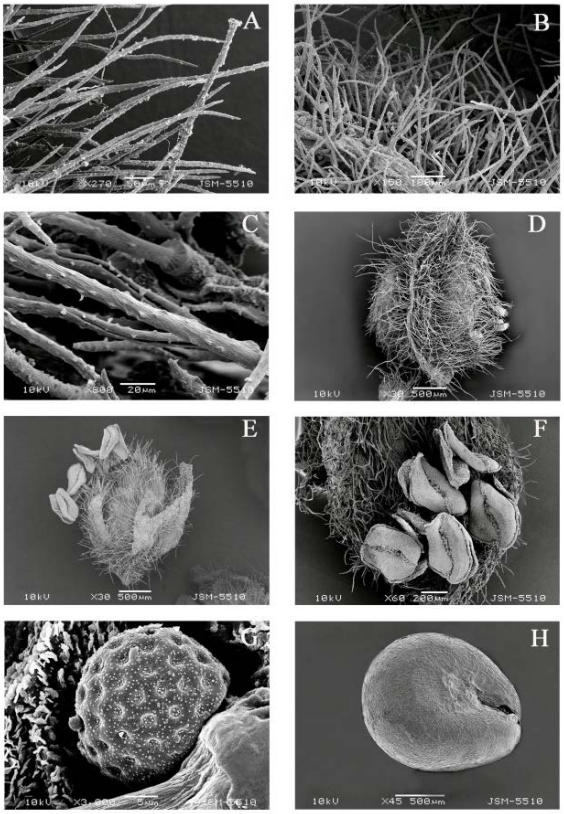

Fig. 4. Scanning electron micrographs of *B. hirsuta*: A - B hairs on stem; C - hairs on flower; D - female flower; E - F bisexual flower; G - pollen grain; H - view of the fruit from lower surface.

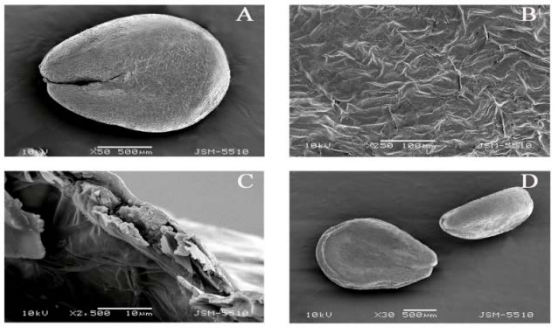

Fig. 5. Scanning electron micrographs of *B. hirsuta*: A - view of the fruit from upper surface; B - C pericarp; D - seeds.

Fig. 6. *Bassia hirsuta* - general view (photo M. Srebreva).

Fig. 7. *Bassia hirsuta-branch with leaves and inflorescences (photo M. Srebreva).*

Discussion

Population variability

The results of the present study on *Bassia hirsuta* which revealed that vegetative traits are more variable than generative ones (Table 2) confirm the data of previous studies on *Chenopodiaceae* species in Bulgaria (Grozeva and Cvetanova 2008, 2011, 2013). The most variable are the traits characterizing the inflorescence length, the leaves (length/width ratio) and the stem height. The least variable are the ones that characterize the seed. The results from the ANOVA showed that the intrapopulation variation is less pronounced than interpopulation variation for all 18 characters (Table 2). It relates to the smaller number and area of the population, and the uniform ecological conditions within its borders. The comparison of the values of the Euclidean distances (Table 3) revealed that interpopulation differences are the greatest between the populations from Atanasovsko and Schablensko lakes, which is in correspondence with their geographical remoteness and different ecological conditions (Table 1). The highest similarities were found between the populations from Schablensko and Varnensko lakes (Table 3, Fig. 3). These are in correspondence with their geographical proximity and similar ecological conditions (Table 1). A high degree of similarity for the total of qualitative traits is also revealed for the other two populations from Pomoriysko Kake and Poda protected area. (Table 3, Fig. 3). This may be associated with the geographical proximity, the small differences between the population habitats and the similarity of karyotypes (Table 1, Figs. 2 A, B). Populations which mostly differ from the other studied populations are those sampled from Atanasovsko Lake. The plants from the population have smaller lower stems and smaller basal leaves. This population is the smallest in terms of size and area. The reason of that could be the high soil salinity in Atanasovsko Lake. The dominant taxa in the community in Atanasovsko Lake is *Salicornia europaea* which grows on higher soil salinity than *B. hirsuta* (Todorova et al. 2014). The differences reported in the present study in morphological traits of the population can be attributed to adverse soil conditions. However, the variability is still in the range of *B. hirsuta's* variability.

Ecology

B. hirsuta is a dominating or asectation species in halophytic communities in coastal salt marshes at the Black Sea Coast. The most common accompanying species in the communities are *Suaeda maritima* Dumort, *Salicornia europaea* L., *Petrosimonia brachiata* Bunge, *Salicornia ramosissima* J. Woods, *Salsola tragus* L., *Atriplex tatarica* L., *Lactuca saligna* L. etc. A limiting factor for its distribution is soil salinity level.

Conclusion

The studied Bulgarian populations of *B. hirsuta* have diploid chromosome number 2*n*=18. The results obtained correspond to the data reported by the other authors (Winge 1917; Wulff 1937; Zakharyeva 1985; Lomonosova 2005). The interpopulation variability is dominant within the total variability, which is due mainly to differences in ecological conditions. Vegetative traits are more variable than generative ones. The least variable are those that characterize the seed and could be used as highest value traits. The data are supplementary to the data given about the species in Flora of the People's Republic of Bulgaria and they can be used both for correct identifications of the specimens and for getting familiar with species characteristics.

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