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Study of population variability of the endemic species *Moehringia grisebachii* Janka (Caryophyllaceae) in Bulgaria

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Abstract

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Moehringia grisebachii Janka is a Balkan endemic species spread on the territory of Bulgaria, Romania and Turkey. In the present study were included 17 populations of *M. grisebachii* from Bulgaria. Morphometric measurements of 24 quantitative traits were performed in all populations. On the base of the data obtained, within population and between population variability was examined. A comparative analysis has been made and similarities and differences have been identified. Dominant in total variability was within populations variability (63.87%). The impact of environmental conditions on measured quantitative traits was reported and as the more important factors the longitude and elevation were pointed out. The results obtained are of importance in the development and updating of the conservation programs for keeping and trust of the genetic diversity and the protected species included in the Red Data Book of Bulgaria.

Keywords: Moehringia grisebachii; population diversity; phenotype

Introduction

Adaptive plasticity in plant species, expressed by their ability to grow and multiply under changed environmental conditions, is due on the one hand to the selection of phenotypic variants and to the interaction of genotype with environmental conditions on the other hand (Alpert & Simms, 2002). Also, phenotypic plasticity can manifest itself as a high degree of specialization in species-friendly environments (Lortie & Aarssen, 1996). It has been found that productive habitats are characterized by a high level of morphological plasticity (Grime et al., 1986; Gafta et al., 2006).

The study of the phenotypic diversity in endemic and threatened species is a main element and prerequisite for disclosure of genetic variation and the evolutionary potential for adapting and surviving in changed environmental conditions. Therefore, phenotypic diversity is a key factor for understanding of the model of genetic variation, the management and protection of genetic resources (Barzev et al., 2010; Lopes et al., 2014; Hristova, 2015; Hongyan et al., 2017).

In Bulgaria, genus *Moehringia* (Caryophyllaceae) is presented by 5 species *Moehringia grisebachii* Janka, *M. jankae* Griseb. ex Janka, *M. muscosa* L., *M. trinervia* L., *M. pendula* (Kuzmanov & Kožuharov, 1966). All of the species are insufficiently investigated.

The object of this study was *Moehringia grisebachii* Janka, an endemic species included in the Red book of Bulgaria, vol.1. Plants and fungi under category „endangered" (Stoyanov**,** 2015). According to literary sources (Grozeva, 2004; Stanev & Delipavlov, 2007, Stoyanov 2015, Grozeva 2016), it is distributed in the Eastern Balkan Range (Sinite Kamani Natural Park above the town of Sliven), Sredna Gora Mts (above

the village of Rozovets) and North Eastern Bulgaria (above the village of Madara, Shumensko). It forms tufts, propagates vegetatively and with seeds. Recent field trials of the species have so far identified 32 populations at an area of 1 to 1720 m^2 and elevation from 285 to 1049 m (Grozeva et al., 2016; Zhelyazkova et al., 2018). The number of specimens ranged from 5 to about 256. *M. grisebachii* forms populations on the cliffs of carbonate sandy limestones, carbonate limestones, granite acid rocks, quartz-porphyry rock formations, conglomerates, sandstone and limestones. As endangered species, it has to be saved as a part of the world biodiversity.

The aim of this investigation was to explore the morphological variability of the *M. grisebachii* and to compare between and within population variability, as well as the impact of geographic coordinates and altitude on the studied parameters.

Material and Methods

Seventeen populations of *M. grisebachii* were investigated (Table 1). Location and altitude for all of them were described. Geographical coordinates were detected by Garmin GPS model, reflecting the central point of each population. Fifteen plants from each population were included in morphological analysis. Height of stem was measured on the spot and all other traits in herbarized plant parts (branches with leaves and flowers, seeds and capsules). The plants were collected during the vegetation period 2017-2019.

Measured were 24 quantitative traits: 1. Height of stem (HS, mm) 2. Basal leaves length (BLL, mm); 3. Basal leaves width (BLW, mm); 4. Basal leaves length/width ratio (BLL/W); 5. Basal leaves petiole length (BLPL, mm); 6. Upper leaves length (ULL, mm); 7. Upper leaves width (ULW, mm); 8. Upper leaves length/width ratio (ULL/W); 9. Upper leaves petiole length (ULPL, mm); 10. Flower diameter (FD, mm); 11. Sepals length (SL, mm); 12. Sepals width (SW, mm); 13. Sepals length/width ratio (SL/W); 14. Petals length (PL, mm); 15. Petals width (PW, mm); 16. Petals length/width ratio (PL/W); 17. Flower petiole length (FPL, mm); 18. Capsule (fruit) length (CFL, mm); 19. Capsule width (CW, mm); 20. Capsule length/width ratio (CL/W); 21. Seed length (SeedL, mm); 22. Seed width (SeedW, mm); 23. Seed length/width ratio (SeedL/W); 24. Seed thickness (STH, mm).

Table 1. Studied popoulations of *Moehringia grisebachii* Janka

Voucher speciments	№ population	Location and geographic coordinates			
		Region	Longitude	Latitude	Altitude (m)
SOA 062305	Mg1	Eastern Balkan Range, Sinite Kamani Natural Park, south of Karandilska polyana	42° 42.828′, N	26° 22.530′, E	956
SOA 062394	Mg2	Eastern Balkan Range, Sinite Kamani Natural Park, near to Karandilska polyana	42° 42.848′, N	26° 22.546′, E	919
SOA 062395	Mg3	Eastern Balkan Range, Sinite Kamani Natural Park, south-east of Karandila	42° 42.851′, N	26° 22.447′, E	971
SOA 062400	Mg4	Eastern Balkan Range, Sinite Kamani Natural Park, bellow of Karandila	42° 42.786′, N	26° 22.360′, E	919
SOA 062396	Mg5	Eastern Balkan Range, Sinite Kamani Natural Park, west of Karandilska polyana	42° 42.818′, N	26° 22.482′, E	965
SOA 062396	Mg6	Eastern Balkan Range, Sinite Kamani Natural Park, east of Micro dam area	42° 42.813′, N	26° 22.605′, E	922 – 975
SOA 062397	Mg7	Eastern Balkan Range, Sinite Kamani Natural Park, north of Micro dam area	42° 42.815′, N	26° 22.647′, E	951
SOA 062390	Mg8	Sredna Gora Mts.,Rozovets, northwest of Pravite Kamani	42° 28.845′, N	25° 05.206′, E	602
SOA 062391	Mg9	Sredna Gora Mts., Rozovets, west of Pravite Kamani	42° 28.929′, N	25° 05.271′, E	725
SOA 062388	Mg10	Sredna Gora Mts, Rozovets,on the way for Pravite Kamani	42° 28.831′, N	25° 05.204′, E	638
SOA 062322	Mg11	Sredna Gora Mts., Rozovets, north of Chepilskata Cheshma	42° 29.067′, N	25° 07.421′, E	845
SOA 062324	Mg12	Sredna Gora Mts., Rozovets, between Orlite Peak and the megalith Popova Turla	42° 28.794′, N	25° 06.975′, E	786
SOA 062389	Mg13	Sredna Gora Mts., Rozovets, rock formation Pravite Kamani	42° 28.935′, N	25° 05.290′, E	731-738
SOA 062323	Mg14	Sredna Gora Mts., Rozovets, the path towards Bratan peak	42° 28.708′, N	25° 07.427′, E	741
SOA 062319	Mg15	Sredna Gora Mts., above village Pesnopoy	42° 29.489′, N	24° 48.011′, E	378
SOA 062316	Mg16	North-Eastern Bulgaria, The Madara national historical-archeological reserve	42° 16.742′, N	27° 07.108′, E	293
SOA 062318	Mg17	North-Eastern Bulgaria, the fortress above village Madara	43° 16.599′, N	27° 07.214′, E	392

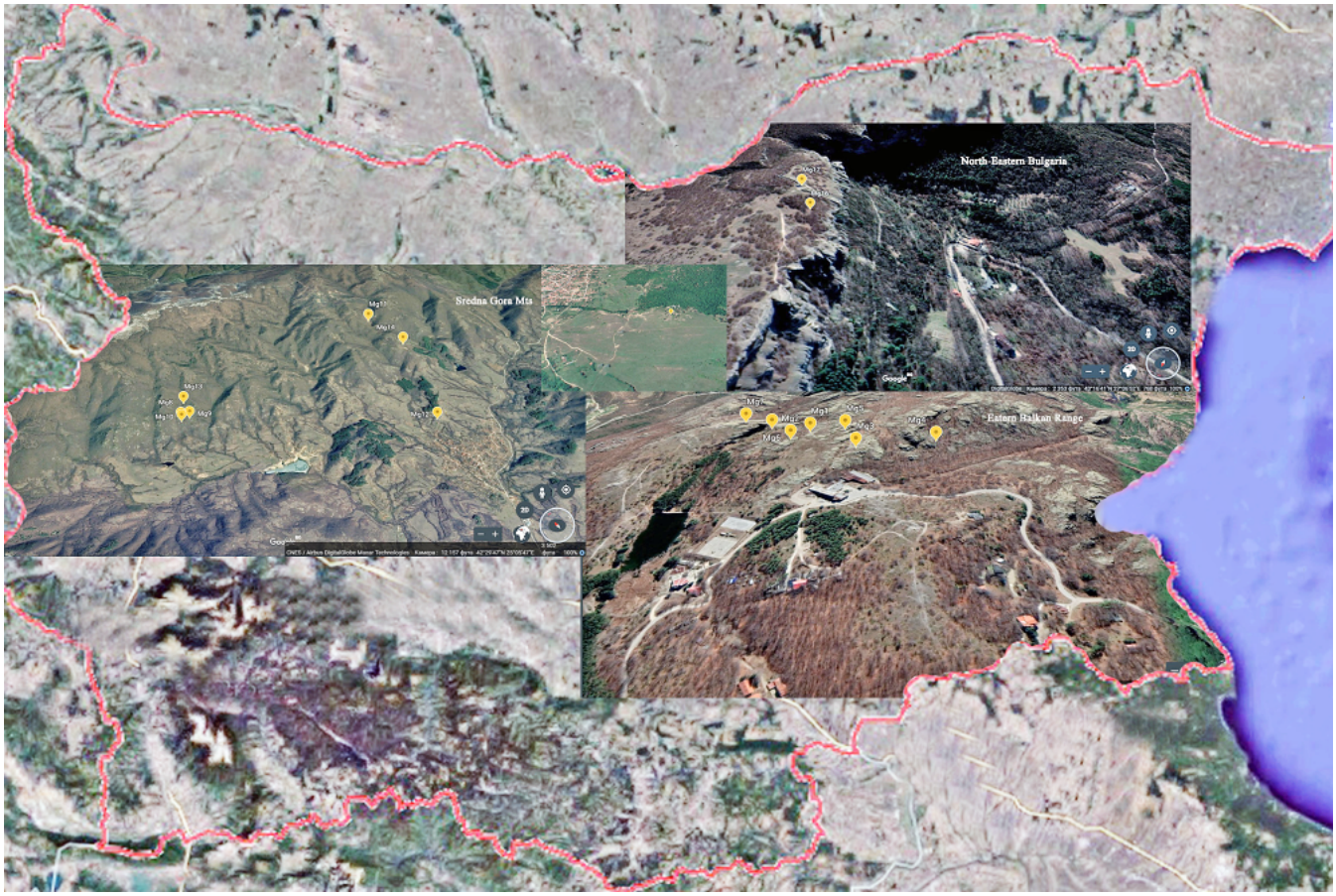

Fig. 1. Distribution of the 17 populations of *Moehringia grisebachii* Janka according to Zhelyazkova (2018)

The voucher specimens were deposited in the herbarium of the Agricultural University in Plovdiv (SOA).

Distribution map of the species in Bulgaria was made using Google Earth (Fig. 1).

Statistics: The analysis of variance was done by ANOVA/MANOVA model. Pearson correlation analysis was performed for calculation the correlation coefficients between the plants trait and geographic factors. Variation coefficient (CV) was determined as follows: CV = S/X. Diversity of 24 phenotypic traits was used for PCA and Cluster analysis on the principle of Squared Euclidean distance. The program Statistica 12, StatSoft was used.

Results and Discussion

Morphometric variability

For all measured 24 traits the mean population variation coefficient (CV) was calculated (Table 2). The data showed that in the studied 24 traits CV varied from 13.14 for seed lenght (SL – Fig. 2) to 118.85 for upper leaves petiole lenght (ULPL – Fig. 3) and the overall mean was 36.35. The comparison between generative and vegetative traits have shown that mean CV calculated for generative traits (CV = 23.01) was more than twice as lower than the one for vegetative traits (VC = 57.81).

Among vegetative traits most variable were petioles of upper (ULPL) and basal leaves (BLPL) while the least variable were height of stem (HS). Acording to the generative traits it was found that the biggest was CV for flower petiole length (FPL) and flower diameter (FD) and the lowest was for sepal (SL) and cupsule (CFL) length. The mean within population CV varied from 27.52 for population from Eastern Balkan Range, Sinite kamani Natural park, east of micro dam area (Mg6) to 48.78 for population from Sredna gora Mts., Rozovets, rock formation Pravite kamani (Mg13).

Analysis of variance

The Analysis of variance on the base of 24 quantitative traits from all populations of *M. grisebachii* revealed that within popuilation variability was bigger than between population variability (Table 3).

Table 2. Variation coefficients of phenotypic traits of the 17 populations of *Moehringia grisebachii* Janka

TRAITS	Mg 1	Mg 2	Mg 3	Mg 4	Mg 5	Mg 6	Mg 7	Mg 8	Mg 9	Mg 10	Mg 11	Mg 12	Mg 13	Mg 14	Mg 15	Mg 16	Mg 17	MEAN
Vegetative																		
HS	37.4	21.5	29.9	32.1	17.0	39.9	27.8	41.2	32.4	21.4	11.3	24.0	26.8	21.8	29.5	32.1	36.6	28.41
BLL	48.3	30.4	30.4	47.7	73.2	34.3	49.3	62.9	43.4	55.0	59.5	50.2	45.3	32.1	36.8	38.9	38.4	45.66
BLW	35.2	49.3	41.4	64.7	56.9	34.4	61.8	45.3	44.8	62.8	68.2	44.3	36.8	25.1	35.0	19.7	42.3	45.18
BLL/W	32.4	55.9	40.3	59.4	45.6	31.7	59.7	46.9	52.1	48.5	36.3	71.2	52.9	37.3	32.8	41.7	48.1	46.62
BLPL	46.0	99.6	68.6	79.3	61.3	61.3	109	82.6	81.9	90.6	42.9	84.3	102	88.0	218	169	89.0	92.61
ULL	44.5	26.5	38.8	37.8	33.5	54.4	53.5	70.1	66.2	78	713	56.5	46.7	69.7	30.6	15.7	33.2	48.61
ULW	35.8	35.6	39.4	34.8	40.9	41.7	63.2	61.8	69.4	67.8	66.8	67.9	48.8	39.4	39.8	27.3	27.3	47.51
ULL/W	55.1	33.5	54.9	55.0	42.9	42.1	43.5	47.8	37.2	56.7	41.7	56.2	52.9	68.9	42.6	30.9	35.0	46.87
ULPL	73.1	105	72.2	84.7	52.4	52.4	107	157.	166	151	39.9	129	162	231.	153	146	138	*118.85*
Generative																		
FD	39.5	49.2	35.9	28.2	42.0	33.2	40.4	29.8	31.1	45.7	34.4	28.6	33.6	34.8	29.2	35.2	50.5	36.53
SL	17.4	10.5	11.9	12.1	6.87	10.9	7.73	24.2	12.7	12.8	11.9	25.9	13.8	12.9	15.3	6.57	9.74	*13.14*
SW	25.1	17.8	18.7	22.4	18.9	46.3	27.6	35.2	22.7	25.6	14.8	32.5	145	24.6	27.5	25.4	16.2	32.17
SL/W	19.8	19.1	22.1	25.6	19.1	44.4	38.7	25.6	26.1	30.4	18.1	42.3	44.2	17.9	28.5	27.8	7.51	26.89
PL	11.8	10.4	11.0	9.49	14.2	6.22	19.8	20.5	11.2	16.8	25.1	18.9	12.7	28.3	12.3	12.4	17.8	15.20
PW	16.1	15.7	14.3	13.3	16.8	12.5	20.2	21.3	156	15.6	29.3	21.8	170.	16.8	15.9	24.8	30.2	35.99
PL/W	27.7	13.0	13.7	9.19	18.3	12.4	21.1	30.1	28.4	23.9	26.1	16.3	32.7	28.9	22.7	24.3	30.3	22.34
FPL	29.4	41.8	37.7	37.4	29.8	33.4	31.6	70.3	49.3	45.6	20.0	38.0	38.2	33.3	66.6	26.1	54.1	40.20
CFL	20.92	12.51	12.67	10.13	12.55	6.85	8.82	18.85	9.29	11.31	18.24	14.41	13.51	21.70	12.58	14.42	14.97	13.74
CW	12.4	7.57	7.97	11.0	8.94	8.28	7.71	26.4	7.46	9.86	17.4	13.5	14.1	27.1	140	16.3	17.2	20.81
CL/W	17.5	12.6	13.5	15.6	13.2	10.1	7.31	33.3	8.59	13.2	16.7	19.5	8.92	18.6	27.7	17.6	19.9	20.82
SEEDL	15.9	12.4	11.4	11.0	11.4	7.09	7.93	16.1	8.77	10.8	18.7	18.1	15.2	30.3	14.7	26.2	21.8	16.15
SEEDW	9.00	11.2	10.0	11.4	9.70	11.8	5.94	32.2	8.95	11.0	19.7	18.0	16.4	36.7	19.1	27.2	20.2	15.20
SEEDL/W	19.9	18.1	16.1	17.1	14.9	14.1	10.7	36.5	9.92	17.0	17.3	27.5	5.74	24.1	31.3	27.1	26.8	16.41
STH	26.2	19.5	28.8	30.6	19.7	10.3	17.8	52.0	14.1	32.5	34.0	36.3	31.1	47.7	20.2	18.1	31.1	19.69
MEAN	29.86	30.3	28.41	31.6	28.3	27.5	35.3	45.3	41.59	39.7	31.6	39.	48.78	42.40	45.9	35.47	35.7	*36.35*

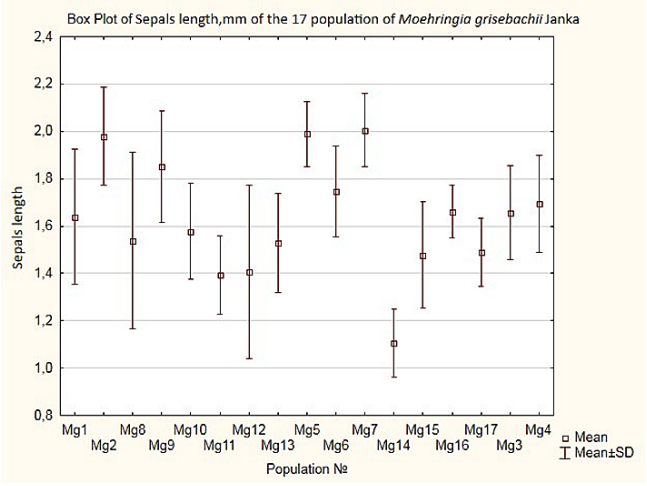

Fig. 2. Variability of sepal length – SL (mean ±SD) of the 17 populations of *Moehringia grisebachii* Janka

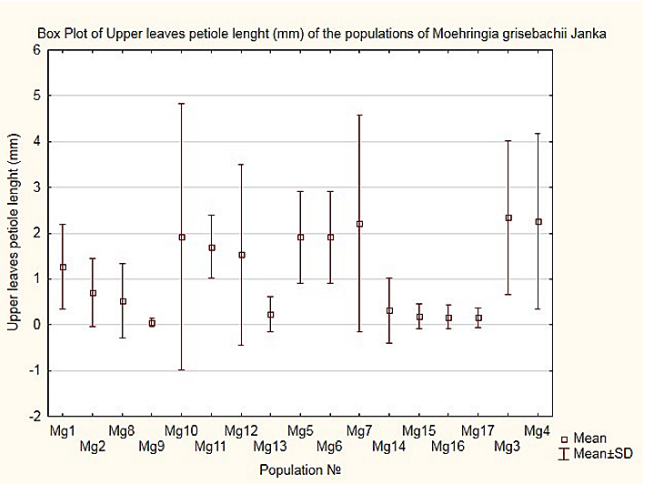

Fig. 3. Variability of upper leaves petiole length – ULPL (mean ±SD) of the 17 populations of *Moehringia grisebachii* Janka

Table 3. Analysis of variance for 24 quantitave traits of the 17 populations of *Moehringia grisebachii* Janka

Traits, mm	SSb	SSw	Mean square		F	% AMONG	% WITHIN
			MSb	MSw			
Vegetative							
HS	157530.8	52758.52	9845.673	182.5554	53.93***	**74.91**	25.09
BLL	2702.2	4302.43	168.890	14.8873	11.34***	38.58	61.42
BLW	10.2	37.73	0.636	0.1306	4.87***	21.28	78.72
BLL/W	3956.4	10939.66	247.275	37.8535	6.53***	26.56	73.44
BLP	2533.6	2448.08	158.349	8.4709	18.69***	**50.86**	49.14
ULL	1746.6	5167.38	109.164	17.8802	6.11***	25.26	74.74
ULW	12.6	34.18	0.785	0.1183	6.64***	26.93	73.07
ULL/W	6181.2	12155.37	386.322	42.0601	9.19***	33.71	66.29
ULPL	220.6	508.21	13.785	1.7585	7.84***	30.27	69.73
Generative							
FD	38.7	185.00	2.416	0.6401	3.77***	17.30	82.70
SL	16.3	14.11	1.017	0.0488	20.81***	**53.60**	46.40
SW	15.8	70.87	0.985	0.2452	4.02***	18.23	81.77
SL/W	246.9	262.93	15.431	0.9098	16.96***	48.43	51.57
PL	10.3	23.10	0.643	0.0799	8.05***	30.84	69.16
PW	8.6	169.02	0.540	0.5849	0.92	4.84	95.16
PL/W	11.8	69.99	0.735	0.2422	3.04***	14.43	85.57
FPL	919.7	1557.93	57.482	5.3908	10.66***	37.12	62.88
CFL	15.8	6.49	0.989	0.0225	44.0***	**70.88**	29.11
CW	17.3	97.82	1.080	0.3385	3.19***	15.03	84.97
CL/W	1.5	9.16	0.094	0.0326	2.89***	14.07	85.93
SEEDL	11.7	3.64	0.729	0.0126	57.88***	**76.27**	23.73
SEEDW	10.4	5.03	0.650	0.0174	37.38***	**67.40**	32.60
SEEDL/W	0.8	12.48	0.051	0.0432	1.18	6.02	**93.98**
STH	2.2	1.56	0.137	0.0054	25.4***	**58.51**	41.49
						36.13	63.87

The results are statistically significant at $P < 0.001$***

The variance analysis showed that the phenotypic variation between populations reached 36.13% against 63.86% calculated for within populations. Seventeen traits had higher variance for within populations variability (Table 3). The other seven traits (HS, BLP , SL, CFL, SEEDL, SEEDW and STH) had higher variance for between populations.

Correlation between phenotypic traits and geographic coordinates

Six traits (BLW, ULL, ULPL, FD, SL, PL) showed a significant positive correlation with longitude, while one trait – flower petiole length (FPL) had a negative correlation with it. The correlation analysis showed significant positive relation between latitude and basal leaves (BLW) and upper leaves (ULW) width, but seed thickness (STH) had a negative correlation with latitude. Elevation negatively correlated to two of the traits seed width (SW) and petals length/width ratio (PL/W), but calculations showed positive correlation with nine traits (BLPL, ULW, ULPL, FD, SL, FPL, SeedL, SeedW and STH).

In sum, the most phenotypic traits varied according to geographic coordinates, as more important factors were longitude and elevation (Table 4).

Principal component and Cluster analysis

PCA was done on the base of 24 quantitarive traits (Table 5). From the five PC derived components, the first component showed 22.71% of the overall variance, the second 17.16%, the third 14.34%, the fourth 11.15% and the fifth 7.52%. The comulative % was 72.88. Three of all traits (BLL, BLL/W, ULL) were found to influence positively on the first component, but six traits (BLPL, CFL CW, SeedL, SeedL/W and STH) influenced negatively. The second component was influenced by petals length (PL) and petals

Table 4. Correlations between phenotypic traits of the 17 populations of *Moehringia grisebachii* Janka and geographic coordinates

Traits	Longitude	Latitude	Elevation
Vegetative			
HS	-0.0073	-0.0470	0.0119
BLL	0.0240	0.0951	-0.0797
BLW	**0.1584****	**0.2098*****	-0.0180
BLL/W	-0.0800	-0.0887	-0.0003
BLPL	-0.0824	-0.0325	**0.1914****
ULL	0.1179*	0.0342	0.0996
ULW	0.1079	**0.1243***	**0.1404****
ULL/W	0.0793	-0.0980	0.0310
ULPL	**0.1921*****	0.0468	**0.2694*****
Generative			
FD	**0.1512****	0.0392	**0.1255***
SL	**0.1188***	-0.0145	**0.1864*****
SW	-0.0038	-0.0375	**-0.1392****
SL/W	**0.2147*****	0.0562	0.0855
PL	**0.1819****	0.1044	0.0048
PW	0.0585	0.0429	-0.0142
PL/W	-0.0114	0.0550	**-0.1836*****
FPL	**-0.1245***	-0.0955	**0.1252***
CFL	**0.1383***	-0.0727	**0.4672*****
CW	**0.1336***	0.0103	0.0947
CL/W	-0.0868	-0.1046	**0.1852****
SeedL	**0.2058*****	0.0152	**0.4043*****
SeedW	**0.1599****	-0.0427	**0.4810*****
SeedL/W	0.0736	0.0337	-0.0660
STH	0.0083	**-0.2181*****	**0.4321*****

The results are statistically significant at P < 0.05*; at P < 0.01**; at P < 0.001***

Table 5. PCA of phenotypic characteristics of 17 populations of *Moehringia grisebachii* Janka

Vegetative Variance	PCA components				
	PC1	PC2	PC3	PC4	PC5
HS	0.258	-0.250	0.176	0.446	0.414
BLL	**0.644**	0.175	0.421	-0.388	0.366
BLW	0.207	0.263	0.296	**-0.605**	0.366
BLL/W	**0.762**	-0.075	0.327	0.031	0.202
BLPL	**-0.527**	0.249	0.271	-0.341	0.143
ULL	**0.568**	-0.465	-0.281	0.371	0.034
ULW	0.014	0.126	**0.640**	**0.538**	0.215
ULL/W	0.450	-0.450	**-0.581**	-0.234	-0.185
ULPL	0.410	-0.212	-0.250	0.536	0.282
Generative Variance					
FD	0.019	-0.474	0.442	0.095	-0.072
SL	0.243	0.603	**0.600**	-0.023	0.234
SW	0.367	0.413	**0.673**	0.127	-0.242
SL/W	-0.191	0.004	-0.400	-0.210	**0.558**
PL	-0.068	**0.753**	-0.444	0.381	0.056
PW	-0.370	**-0.614**	0.336	-0.412	-0.175
PL/W	0.187	**0.732**	-0.381	0.454	0.099
FPL	0.290	-0.112	0.214	0.366	-0.459
CFL	**-0.670**	**0.574**	-0.122	0.009	-0.064
CW	**-0.800**	-0.045	0.289	0.396	0.002
CL/W	-0.237	0.658	-0.340	-0.241	-0.134
SeedL	**-0.806**	-0.170	0.299	0.292	0.043
SeedW	0.211	0.452	0.303	0.025	**-0.588**
SeedL/W	**-0.738**	-0.392	0.101	0.222	0.302
STH	**-0.707**	-0.071	0.031	0.0189	0.039
Eigenvalues	5.45	4.12	3.44	2.68	1.80
% Total variance	22.71	17.16	14.34	11.15	7.52
Cumulative %	22.71	39.87	54.20	65.36	72.88

length/width ratio (PL/W) positively and petals width (PW) negatively. The influence on the third component had ULW, SL, SW (+) and ULL/W (–). Two variances were important in PC 4, as follows: BLW (–) and ULW (+). PC 5 emphasised SL/W (+) and SW (–).

The Morphometric data of the measured 24 traits arranged the 17 populations of *M. grisebachii* into 3 main clusters (Fig. 4). Cluster A is devited into 2 subsclusters. The subcluster A1 involved 8 populations from different floristic regions and an altitude of 378 to 965. The biggest similarity was found between populations Mg2 and Mg3. Subcluster A2 was also heterogeneous and included four populations (Mg12, Mg4, Mg6 and Mg16) from different floristic regions and an altitude of 293 to 975.

Cluster B included 3 populations from Sredna gora Mts., Rozovetts – Mg8, Mg 9 and Mg14 which are located geographically closely at a similar altitude (602-741 m).

In third cluster C two populations again from Sredna gora Mts., Rozovets (Mg 10 and Mg 11) were included.

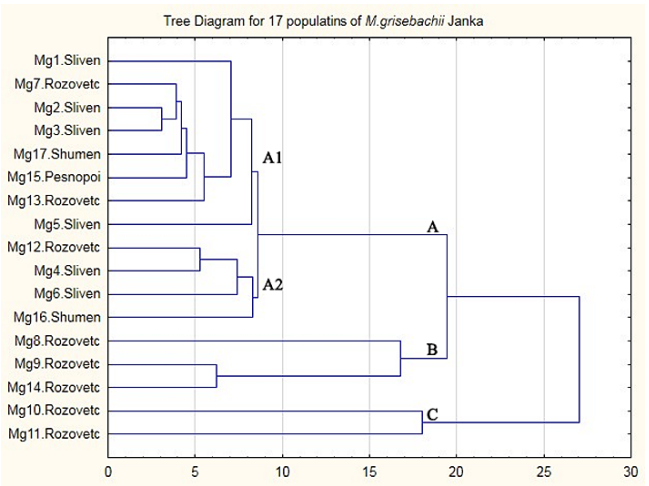

Fig. 4. Dendrogram of CA of the 17 populations of *Moehringia grisebachii* Janka based on 24 traits of vegetative and generative morphological variability

The data obtained showed that in the 17 studied populations of *M. grisebachii* the phenotypic features varied at a hight extent. As we mentioned before the variation of generative traits significantly exceeded that of the vegetative traits.

Among the all 17 popoulations of *M. grisebachii*, the CV of the phenotypic traits in Mg13 (Gora Mts., Rozovets, rock formation Pravite Kamani, N42° 28.935′, E 25° 05.290′, 731-738 m) is the highest (CV = 48.78), which showed the relatively large phenotypic diversity. The lowest was CV (27.52) in Mg6 (Eastern Balkan Range, Sinite Kamani Natural Park, east of micro dam area, N 42° 42.818′ E 26° 22.482′, 922 – 975 m).

From our research it became clear that the phenotypic variance of *M. grisebachii* has reached a significant level in the individual populations and between them. From the vegetative signs height of stem (HS) and basal leaves petiole length (BLPL) had a higher interpopulation variability, and all other signs had higher intrapopulation variability. For reproductive traits – seeds and capsules (SL, CFL, SL, SW and STH), the intrapopulation variety was higher than between the populations.

As a whole, interpopulation variability overreach the intrapopulation variability.

Similar results were reported by Chen et al. (2005) for the endemic *Coelonema draboides.* The date of the molecular RAPD analysis and variance analysis (AMOVA) showed a high level of genetic diversity (84.2%) at the population level, with the between populations variation being only 15.8%. Also Wang et al. (2006) have studied the genetic diversity in 13 populations of the species *Psathyrostachys huashanica* Keng using primers developed for barley. As a result of the analysis, 77.63% genetic variation in subpopulations and 32.37% among populations was established, indicating that genetic differentiation in each subpopulation is higher than that between subpopulations. They suggest that altitude is probably the main factor limiting the gene flow of populations and leading to differentiation of subpopulations.

As a result of evolution processes, plants change their phenotype by showing more plasticity (Coleman, 1994). Phenotypic plasticity is associated with greater adaptability and can penetrate into large areas, as well as to propagate heterogeneous habitats (Sun et al., 2005). Dominant in the general population variability in the studied populations of *Moehringia grisebachii* Janka is the inter-population variety, which we consider to be due to the specificity of their habitats, the uneven distribution of moisture within the individual population, and partly to the differences in environmental conditions. The data from this comprehensive population survey give us reason to believe that there is a certain relationship between the registered variability between some populations and ecological conditions, their size and area. In addition we think that the development of individuals is strongly influenced by humidity and daytime temperatures. The plants develop in the slits of the rocks and their development depends mainly on whether there will be enough water for germination and further development.

According to Hunter (2003) the main factors influencing the phenotypic diversity of plants are the size of the distri-

bution range and its ecological specificity. In addition, the change in plant signs is determined by latitude and temperature differences (Garciâa et al., 2000, Naia et al., 2013; Hongyan et al., 2017). In our study, 12 of 24 phenotypic traits of *M. grisebachii* showed a significant positive correlation with longitude and 11 traits had a positive correlation with elevation, with only 3 indicators showing correlation with latitude.

The results show that differences in the *M. grisebachii* phenotype are more dependent on Longitude and Altitude, when the Latitude affected the phenotypic variation at a lower level.

A number of authors recognize that at high altitudes there is usually a low atmospheric pressure, low CO_2 concentration and more rainy days, which contributes to more precipitation and lower temperatures, which can improve the efficiency of leaf photosynthesis and seed multiplication (Garciâa et al., 2000; Connolly et al., 2003; Li et al., 2014).

Likewise it is well known that natural selection processes, genetic flow and genetic drift, as well as regional and random variations, determinate phenotypic differences in plants (Young et al., 2010; Newsham, 2011; Ellegren & Galtier, 2016; Hongyan et al., 2017).

Cluster analysis has shown that some populations are grouped according to the regions in which they are registered. However, some populations located in remote areas are grouped at random. The results resemble the established dependencies for endemic plants of Tadzhikistan (Nowaka et al., 2011) and *Paulownia fortunei (*Mo et al., 2013) which may be due to the overall effect of geographic, climatic and genetic factors (Aguilar et al., 2008).

Conclusion

The alteration of the genetic or phenotypic structure is a result of a limited flow of genes between populations, which leads to the formation of different genetic characteristics within each population. Despite the small number of individuals and the limited range of *M. grisebachii* in Bulgaria, we have found a significant degree of phenotypic diversity, with the prevailing being the inter-population one. Our results showed that studying phenotypic diversity through variance analysis provided important information that can serve as a basis for keeping strategy and exploitation of the genetic resources in species. The established significant level of phenotypic diversity is the basis for an appropriate strategy for the *in*-situ conservation of the species. The most conservative traits were those that characterize flowers and seeds and they could be used as the most informative taxonomic marks. Also, information about genetic structure of the populations of *M. grisebachii* have to be supplied by DNA markers and comparison with the phenotypic date have to be done.

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