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Karyotype studies of endemic species *Moehringia grisebachii* (Caryophyllaceae) from Sredna Gora Mts, Bulgaria

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Abstract

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The chromosome number and karyotype of the Balkan endemic species *Moehringia grisebachii* are described for the first time. Plant material was collected from 10 populations spread in Sredna Gora Mts., Bulgaria. Basic chromosome number x = 12 and diploid chromosome number 2*n* = 24 have been established. Metacentric and submetacentric chromosomes have been registered with the metacentric ones being dominant. Differences have been found among the studied populations in the chromosome size and morphology. Microphotographs of somatic metaphase plates and idiograms of all studied populations are presented.

Key words: Moehringia grisebachii; chromosome number; karyotype; Sredna Gora; Bulgaria

Introduction

The family Caryophyllaceae is composed of 85–90 genera and approximately 2200 species, living in a wide range of habitats (Bittrich, 1993; Heywood, 1998; Simpson, 2010). Based on the morphological characteristics the family is subdivided into three subfamilies; Paronychioideae, Alsinoideae and Caryophylloideae (Bittrich, 1993). The genus *Moehringia* belongs to the *Alsinoideae* subfamily and numbers 31 species distributed throughout temperate areas (Fior et al., 2006). Most of the species are locally or regionally endemic, due to their habitat specificity, inhabiting rock crevices. Only a few species, usually of woodland habitats, have a wide distribution area (Fior & Karis, 2007). In the Bulgarian flora *Moehringia* is presented by 5 species, among which is the Balkan endemic species *Moehringia grisebachii.* The species is included in the Red book of Bulgaria, vol. 1. Plants and fungi (Stoyanov, 2015) in the category "endangered". Its area includes the Eastern part of the Balkan Peninsula (E Bulgaria, Romanian Dobrudzha and Turkey-in-Europe (Kuzmanov & Kozhuharov, 1966; Koeva, 1984; Stoyanov, 2015). Based on data by Zhelyazkova et al. (2018) *M. grisebachii* forms populations in North-Eastern Bulgaria (above the village of Madara, Shumensko), Eastern Balkan Range (Sinite Kamani Natural Park), Sredna Gora Mts (between the village of Rozovets and Bratan peak and above the village Pesnopoy), on carbonate limestones, granite acid rocks, quartz-porphyry rock formations, conglomerates, sandstones and limestones at an elevation from 285 to 1049 m. Until now the Bulgarian populations of the species have not been the subject of a karyological study. The main focus of the investigation is the distribution of the species in Bulgaria (Zhelyazkova et al., 2018) and the status of its populations from the territory of Sinite Kamani Natural Park (Grozeva et al., 2004; 2016).

The objective of the present study is to establish the chromosome number and to describe the karyotype of *M. grisebachii* from the populations of the species spread in Sredna gora Mts.

Materials and Methods

Seeds were collected during the vegetation periods of 2017 and 2018 year. A total of 10 native populations of *M. grisebachii* located in Sredna Gora Mts. have been studied (Table 1).

The chromosome numbers and karyotypes have been reported on lasting preparations of metaphase root apex plates of seeds germinated in laboratory conditions collected in the natural habitats of the species. Root tips were treated with 0.5% Colchicin for 3,5 hours and then fixed in Clarke's fixation agent for 10-16 h at 4ºC according to the methodology of Yıldız et al. (2008). Then the root tips were stained with 1% aceto-orcein for 24 hours, squashed in 45% acetic acid and mounted in Malinol. The photographs were made with Olympus U-TVO.5XC-3 camera and Olympus BX51 microscope, Japan.

The chromosome type was determined using the cenmtrifugal index of Grif & Agapova (1986). The average length of the chromosomes; the sum of the length of the haploid chromosome number (hcl) were measured and the karyotype formula was indicated with at least five metaphase plates measured from each population. The karyograms and idiograms were obtained using the Adobe Photoshop CS6 software. The herbarium specimens of the karyologically researched plants have been deposited at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences.

To assess the karyotype symmetry the following have been used: mean centromeric asymmetry $M_{CA} = A \times 100$ (Peruzzi & Eroğlu, 2013), where A is degree of asymmetry of karyotype proposed by Watanabe et al. (1999); coefficient of variation of chromosome length $CV_{CL} = A_2 \times 100$ (Paszko, 2006), where A_2 is interchromosomal asymmetry index A_2 by Romero-Zarko (1986).

To establish the karyotype similarity among the various studied populations Cluster analysis (CA) has been employed using the Single Linkage method. In CA the following karyotypic and mitotic characters have been included: short arm; long arm; arm ratio; mean length of chromosomes; a total length of shortest chromosome; a total length of longest chromosome; haploid chromosome length (Hcl); coefficient of variation of chromosome length (CV_{CL}); mean centromeric asymmetry (Mca).

The data obtained were processed variationally with software STATISTICA 12, Stat Soft Inc. (2012). To group the studied populations by karyotype similarity Cluster analysis (CA) was used.

Results and Discussions

In all *M. grisebachii* populations diploid chromosome number $2n = 24$ and main chromosome number x = 12 have been found (Table 2). Our data correlated to those presented by a number of researchers (Tischler, 1950; Dmitrieva et al., 1976; Májovský et al., 1976; Lövkvist & Hultgård., 1999; Simon & Blanché, 2016), who reported main number x = 12 and most common chromosome number $2n = 24$ for the *Moehringia* genus. In scientific literature up to this moment there is no data about the chromosome number of *M. grisebachii.* For the rest of the Bulgarian representatives of the genus *Moehringia* karyological data have been reported for three species: *M. muscosa* L. – $2n = 24$ (Májovský et al., 1976; Simon & Blanché, 2016), *M. trinervia* L. – $2n = 24$ (Dmitrieva et al., 1976; Lövkvist & Hultgård, 1999) and *M. pendula* – $2n = 48$ (Arohonka, 1982; Nikolov, 1991; Tiniakou, 1996).

Table 1. Localition of the studied population of *Moehringia grisebachii* Janka

No	Locality	2*n*	Longitude	Latitude	Altitude (m)
Mg1	Sredna Gora Mts., above village Rozovets at the first corner after the path towards Bratan peak	24	42° 28.708′N	25° 07.427′E	741
Mg2	Sredna Gora Mts., rock formation along the old Roman road north of Chepilskata Cheshma	24	42° 29.067′N	25° 07.421′E	845
Mg3	Sredna Gora Mts., rock formation at the first peak on the way to the megalith Popova Turla, Orlite Peak	24	42° 28.714′N	25° 06.885′E	741
Mg4	Sredna Gora Mts., rock massifs in the west part of Orlite Peak	24	42° 28.783′N	25° 06.896′E	773
Mg5	Sredna Gora Mts., rock formations between Orlite Peak and the megalith Popova Turla	24	42° 28.794′N	25° 06.975′E	786
Mg6	Sredna Gora Mts., rock formations on the way from village Rozovets to the rock formation Pravite Kamani	24	42° 28.831′ N	25° 05.204′E	638
Mg7	Sredna Gora Mts., rock formation Pravite Kamani	24	42° 28.935′N	25° 05.290′E	738
Mg8	Sredna Gora Mts., rock formations northwest of rock formation Pravite Kamani	24	42° 28.845′N	25° 05.206′E	602
Mg9	Sredna Gora Mts., rock formations west of rock formation Pravite Kamani	24	42° 28.929′N	25° 05.271′E	725
Mg10	Sredna Gora Mts., above village Pesnopoy, area Usoykata	24	42° 29.489′N	24° 48.011′E	378

Table 2. Karyomorphometric data for the studied populations of *Moehringia grisebachii* Janka, chromosome size variation (µm) – short (S) and long (L), total haploid chromosome length (hcl, µm), interchromosomal asymmetry indices (A_2); coefficient variation of chromosome length (CV_{cl}), degree of asymmetry of karyotype (A), mean centromeric asymmetry (M_{ca})

Population	Karyotype formula	S	L	Hcl	A_2	CVcl	A	Mca
Mg1	2n = 24m	1.10	3.38	21.46	0.04	4.43	0.005	0.50
Mg2	2n = 22m + 2sm	1.70	4.10	33.65	0.05	4,75	0.007	0.69
Mg3	2n = 18m + 6sm	1.46	3.89	30.01	0.03	3.30	0.01	1.21
Mg4	2n = 16m + 8sm	1.32	4.58	26.14	0.03	3.48	0.01	1.22
Mg5	2n = 22m + 2sm	1.34	4.95	26.43	0.04	3.59	0.009	0.86
Mg6	2n = 20m + 4sm	1.06	4.08	24.22	0.04	3.92	0.01	1.06
Mg7	2n = 24m	1.40	3.80	29.54	0.03	2.95	0.008	0.83
Mg8	2n = 20m + 4sm	0.77	2.80	20.61	0.05	4,75	0.009	0.85
Mg9	2n = 22m + 2sm	1.09	2.72	20.59	0.05	4.61	0.01	1.15
Mg10	2n = 22m + 2sm	1.47	4.72	28.97	0.03	3.14	0.008	0.79

*numbering of populations follows the indications in Table 1

The karyotype registered for the 10 studied populations of *M. grisebachii* – above village Rozovets at the first corner after the path towards Bratan peak (Mg1), rock formation along the old Roman road north of Chepilskata Cheshma (Mg2), rock formation at the first peak on the way to the megalith Popova Turla, Orlite Peak (Mg3), rock massifs in the west part of Orlite Peak (Mg4), rock formations between Orlite Peak and the megalith Popova Turla (Mg5), rock formations on the way from village Rozovets to the rock formation Pravite Kamani (Mg6), rock formation Pravite Kamani (Mg7), rock formations northwest of Pravite Kamani (Mg8), rock formations west of Pravite Kamani (Mg9), above village Pesnopoy, area Usoykata (Mg10) consisted of metacentric and submetacentric chromosomes with the metacentric ones being dominant (Figs 1-2). Satellites were not recorded. The average length of chromosomes in the different populations varied from 1.72 µm in Mg8 to 2.80 µm in Mg2. The size of the shortest chromosome was from 0.77 to 1.70 µm and of the longest one – from 2.72 to 4.95 µm and the total sum of the haploid chromosome length varied from 20.59 to 33.65 µm (Table 2). The correlation between the longest and the shortest chromosome was from 2.4:1 in Mg2 to 3.85:1 in Mg6, respectively. Differences in the size of the shortest and the longest chromosome varied from 1.63 µm in Mg9 to 3.61 µm in Mg5. Differences have been found among the studied populations in the chromosome morphology, too. For two of the populations (Mg1 and Mg7) a karyotype of 12 pairs of metacentric chromosomes was registered (Fig. 1, A-B; Fig. 2, A-B). For four of the populations (Mg2, Mg5, Mg9 and Mg10) 1 pair of submetacentric chromosomes and, respectively, 13 pairs of metacentric ones were found (Fig. 1, C-D, I-J; Fig. 2, E-H). In both populations – Mg6 and Mg8 two pairs of submetacentric chromosomes were registered in the karyotype (Fig. 1, K-L; Fig. 2, C-D). In popula-

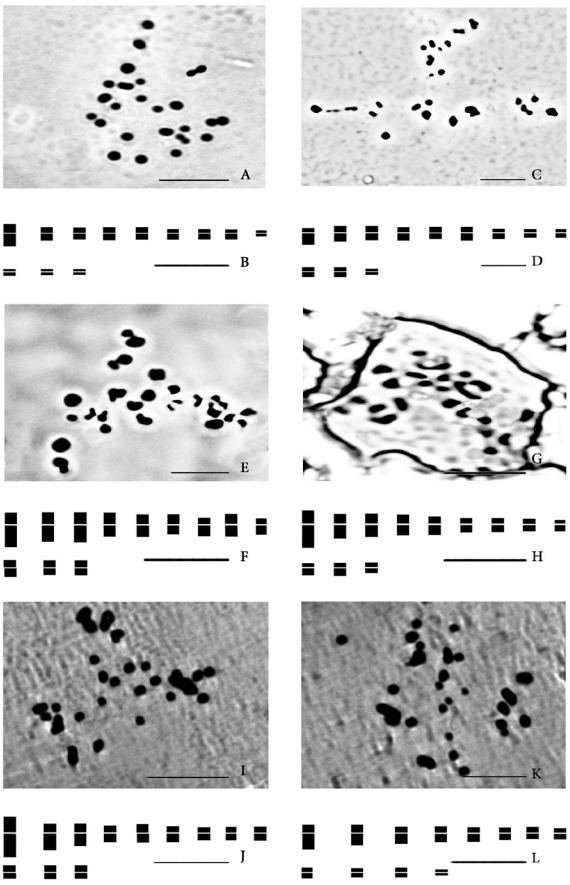

Fig. 1. Microphotographs of root tip mitosis and idiograms of *Moehringia grisebachii* Janka: *A, B* – population Mg1; *C, D* – population Mg2; *E, F* – population Mg3; *G, H* – population Mg4; *I, J* – population Mg5; *K, L* – population Mg6; scale bar 10 µm

*numbering of populations follows the indications in Table 1.

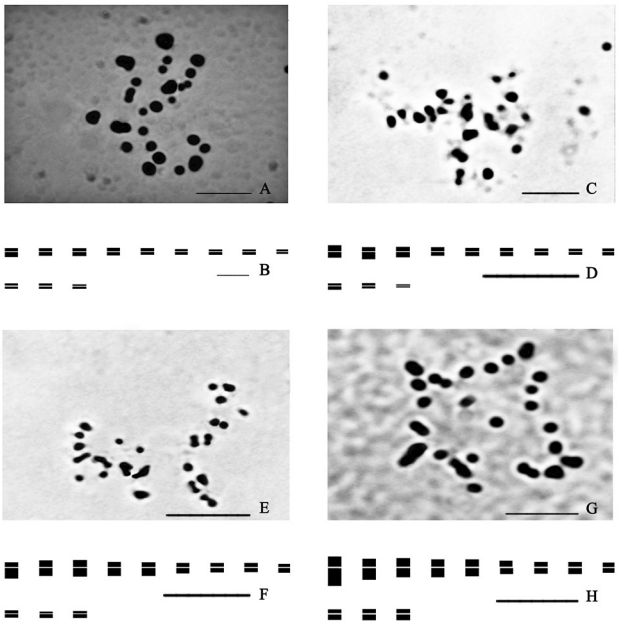

Fig. 2. Microphotographs of root tip mitosis and idiograms of *Moehringia grisebachii* Janka: *A, B* – population Mg7; *C, D* – population Mg8; *E, F* – population Mg9; *G, H* – population Mg10; scale bar 10 µm
*numbering of populations follows the indications in Table 1.

tion Mg3 karyotype of 9 pairs of metacentric and 3 pairs of submetacentric chromosomes were recorded (Fig. 1, E-F), while in population Mg4 the submetacentric chromosome pairs were four (Fig. 1, G-H). According to asymmetry indices, the highest values CV_{CL} = 4.75 were registered for two of the populations Mg2 and Mg8, while the lowest CV_{CL} = 3.14 was for population Mg10. Concerning the mean centromeric asymmetry the highest value M_{CA} = 1.22 was recorded for population Mg4 and the lowest M_{CA} = 0.5 for population Mg1. The t-tests showed significant difference ($p < 0.05$) for CV_{CL} and M_{CA} among the studied populations.

Based on the degree of karyotype similarity, the 10 studied populations of *M. grisebachii* are grouped at metric distance of 4.5 (Fig. 3). Nine of the populations form 2 clusters. The first cluster includes 3 populations (Mg8, Mg9, Mg1), for which the lowest values of the total sum of the haploid chromosome length and the mean length of chromosomes were found. Greater similarity was registered between the two populations – Mg8, Mg9, respectively, which form a subcluster and therefore have closer values of the two indicators – total sum of the haploid chromosome lenght and mean length of chromosomes. The second cluster includes a total of 6 populations (Mg3, Mg7, Mg10, Mg4, Mg5, Mg6).

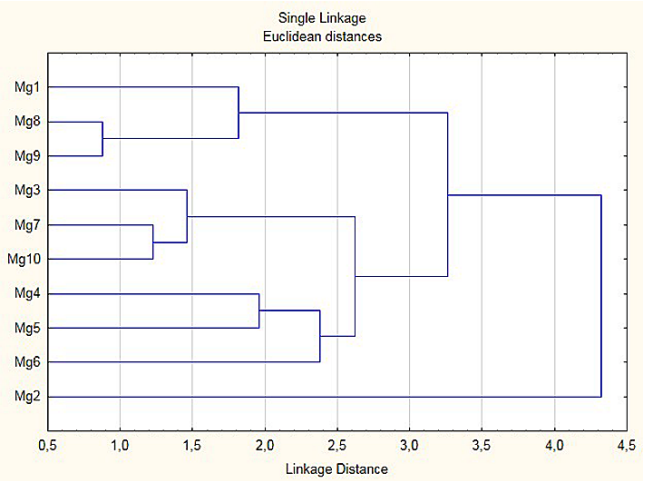

Fig. 3. Dendrogram of the cluster analysis of the studied populations of *Moehringia grisebachii.*
*numbering of populations follows the indications in Table 1.

The greatest similarity concerning the traits describing their karyotype, have the first 2 populations (Mg3, Mg7). They form a subcluster and for them the highest values of the total sum of the haploid chromosome length and the mean length of the chromosomes were registered (Table 2). Greater similarity is observed between other two populations – Mg4, Mg5, which also have similar values of these two indicators and form a second subcluster within the cluster boundaries (Fig. 3). The greatest differences compared to all populations has the population of Mg2, for which the highest values for the length of the shortest chromosome and for the total sum of the haploid chromosome length were found. This population is located at the highest altitude as well (Table 1).

Conclusion

M. grisebachii populations from Sredna Gora Mts have diploid chromosome number $2n = 2x = 24$ and a karyotype of metacentric and submetacentric chromosomes with the metacentric ones being the dominant. The total sum of haploid chromosome length varies from 20.59 to 33.65 ***µm µ***m and population Mg4 has the highest value for mean centromeric asymmetry (Mca). The average chromosome dimension is from 1.72 to 2.80 µm with the shortest chromosomes being from 0.77 to 1.70 µm, and the longest ones from 2.72 to 4.95 µm. The highest values of coefficient of variation of chromosome length (CVcl) were registered for the populations Mg2 and Mg8. For the first time the chromosome number is specified and the karyotype of *M. grisebachii* from populations in Sredna Gora Mts, Bulgaria is described.

Currently karyological studies of *M. grisebachii* in populations in North-Eastern Bulgaria and Eastern Balkan Range continue. The data analysis from all studies will allow tracing the karyological variability of the species in its Bulgarian populations.

References

Arohonka, T. (1982). Chromosome counts of vascular plants of the island Seili in Nauvo, southwestern Finlan. *Annales Universitatis Turkuensis*, Serie A 2, Biol.-Geogr., *3*, 1-12.

Bittrich, V. (1993). Caryophyllaceae. FloweringPlants. Dicotyledons. Springer Berlin Heidelberg, 206-236.

Dmitrieva, S.A., Parfenov, V.I. & Schvec, I. V. (1977). The karyological characterization of some species of the Belorussian flora. *Vesci Akad. Navuk Belarusk SSR Ser. Bi jal. Sel'skagasp. Navuk*, *19*, 82-91.

Fior, S. & Karis, P. (2007). Phylogeny, evolution and systematics of *Moehringia*(Caryophyllaceae) as inferred from molecular and morphological data: a case of homology reassessment. *Cladistics*, *23*, 362-372.

Fior S., Karis, P., Casazza, G., Minuto, L. & Sala, F. (2006). Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast matK and nuclear ITS rDNA sequences. *American Journal of Botany*, *93*, 399-411.

Grif, V.G. & Agapova, N.D. (1986). Methods of description of plant karyotypes. *Botanicheskii Zhurnal, 71*, 550-553.

Grozeva, N., Georgieva, M. & Vulkova, M. (2004). Flowering plants and ferns. In: Stoeva, M. (ed.), *Biological diversity of Sinite Kamani Nature Park*. Kontrast, Stara Zagora, 9-112 (Bg).

Grozeva, N., Gerdzhikova, M., Todorova, M., Panayotova, G. Dohchev, D. & Tsutsov, K. (2016). The Balkan Endemics *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka in Sinite Kamani Natural Park, Bulgaria. *Trakia Journal of Sciences*, *14*(2), 163-170.

Heywood, V.H. (1998). Flowering plants of the world. Oxford, Oxford University Press.

Koeva, Y. (1984). *Moehringia grisebachii* Janka – In: Velchev, V. (ed.), *Red Data Book of the Republic of Bulgaria*, Volume. 1, Plants. BAS, Sofia. pp 96.

Kuzmanov, B. & Kozhuharov, S. (1966). Genus *Moehringia* L. In: Yordanov, D. (ed.). *Flora of the People's Republic Bulgaria*, Vol. 3, Aedibus Academiae Scientiarum Bulgaricae, Serdicae, pp. 541-549 (Bg).

Lövkvist, B. & Hultgård, U. M. (1999). Chromosome numbers in south Swedish vascular plants. *Opera Botanica*, *137*, 1-42.

Majovsky, J., et al. (1976). Index of chromosome numbers of Slovakian Flora. (Part 5). *Acta Facultatis Rerum Naturalium Universitatis Comenianae Bot*, *25*, 1-18.

Nikolov, N. A. (1991). Chromosome numbers of Bulgarian angiosperms from North Pirin Mountain: Reserve "Bajuvi Dupki-Dhzindhzirica". *Fitologija, 41*, 70-75.

Paszko, A. (2006). A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution, 258*, 39-48.

Peruzzi, L. & Eroğlu, H.E. (2013). Karyotype asymmetry: again, how to measure and what to measure? *Comparative Cytogenetics*, *7*(1), 1-9.

Romero-Zarco, C. (1986). A new method for estimating karyotype asymmetry. *Taxon*, *35*, 526-530.

Simon, J., & Blanché, C. (2016). Karyological knowledge of the Catalan vascular flora inferred from *CromoCat*, database.

Simpson, M.G. (2010). Plant systematics. 2nd ed. CA, Academic Press, San Diego.

Stoyanov, S. (2015) *Moehringia grisebachii* Janka. In: Peev, D. et al. (eds.) *Red Data Book of the Republic of Bulgaria*. Volume 1. Plants and Fungi. BAS & MoEW, Sofia, pp. 533.

Tiniakou, A. (1996). Mediterranean chromosome number reports 6 (773-778). *Flora mediterranea*, *6*, 333-337.

Tischler, G. (1950). Die chromosomenzhalen der GefäBpflanzen Mitteleuropas. s'- Gravenhage: *W. Junk*, 263.

Watanabe, K., Yahara, T., Denda, T. & Kosuge, K. (1999). Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): Statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. *Journal* of *Plant Research.*, *112*(2), 145-161.

Yıldız, K., Minareci, E., Çirpici, A. & Dadandı, M.Y. (2008). A karyotypic study on *Silene*, section Siphonomorpha species of Turkey. *Nordic Journal of Botany, 26*, 368-374.

Zhelyazkova, M., Grozeva, N., Gerdzhikova, M. & Terzieva, S. (2018). The Balkan endemics *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka in Bulgaria. *Trakia Journal of Sciences*, *4*, 261-269.

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