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Karyotype Analysis of Five *Bellevalia* Lapeyr. Species (Asparagaceae) from Turkey

Hüseyin Eroğlu[1], Süleyman Mesut Pınar[2], Gulden Dogan[3]*, M. Yavuz Paksoy[4] and Yasar Kiran[3]

[1] Van Yüzüncü Yıl University, Faculty of Sciences, Department of Biology, Tuşba-Van, Turkey
[2] Van Yüzüncü Yıl University, Van School of Health, Tuşba-Van, Turkey
[3] Firat University, Science Fac., Biology Department, Elazig, Turkey
[4] Department of Environmental Engineering, Faculty of Engineering, Munzur University, Tunceli, Turkey

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Summary The chromosome numbers and karyotypes of five species of the genus *Bellevalia* distributed in Turkey were analyzed. These taxa are *B. anatolica* B. Mathew & Özhatay, *B. fominii* Woronow, *B. kurdistanica* Feinbrun, *B. rixii* Wendelbo, *B. turcica* Pınar & Eroğlu. Three of them (*B. anatolica, B. rixii* and *B. turcica*) are endemic in Turkey. The chromosome numbers of all studied taxa were determeined as $2n=2x=8$. Haploid chromosome lengths varied from 57.78 μm (*B. fominii*) to 68.17 μm (*B. anatolica*) among species. Karyotype analysis showed that *Bellevalia* taxa generally have median (m), submedian (sm), and subterminal (st) chromosomes, but not subterminal chromosomes in *B. fominii* species.

Keywords Asparagaceae, *Bellevalia*, Chromosome number, Karyotype, Turkey.

The genus *Bellevalia* is distributed on three continents: Asia, Europe, and Africa. The continent with the richest number of species in Asia. Especially Anatolia and Iran, as well as Transcaucasia, Palestine, and Syria are the areas where the *Bellevalia* genus is distributed. Considering the numerical distribution of the genus *Bellevalia*, 35 species are distributed in Asia, eight species in Africa, and seven species in Europe. In addition, 38 species are found in the Irano-Turanian, 16 species in the Mediterranean, and one species in the Saharo-Sindian Phytogeographic region (Feinbrun 1939). In the monograph study on the genus *Bellevalia*, it was stated that 14 of the 45 taxa were distributed in Anatolia (Feinbrun 1940). According to Wendelbo (1984) in Turkey, 18 species, seven of which are endemic; According to the List of Plants of Turkey, it is 24 (Tugay 2012); There are 35 species in total, with new species identified in recent years. (Uzunhisarcıklı *et al.* 2013, Gürdal *et al.* 2014, Yıldırım *et al.* 2014, Karabacak *et al.* 2014, 2015, Pınar *et al.* 2016, Şahin *et al.* 2016, Fidan 2019, Yıldırım and Sefalı 2020).

The number, size, and shape of chromosomes can be used to characterize the karyotype of plants (Jahan *et al.* 2014). The genus *Bellevalia* is characterized by a basic chromosome number ($x=4$). Most representatives of the genus have a set of $2n=2x=8$ chromosomes in the complement. Chromosome complement in *Bellevalia* seems to be stable, having a typical karyotype occurring in almost all species studied. However, polyploidy has also been observed in some studies. According to the literature, it has also been determined that some species of this genus are triploid, tetraploid and hexaploid (Özhatay *et al.* 1991a, b, Johnson 2003, Persson 2006, Yaylacı *et al.* 2009, Uzunhisarcıklı *et al.* 2013, Gürdal *et al.* 2014, Karabacak *et al.* 2014, 2015, Pınar *et al.* 2016).

This paper aims to clearly define the chromosome numbers and morphology of the five *Bellevalia* species and to bring the karyotype data to the literature.

Materials and methods

Plant materials of five *Bellevalia* species were collected from natural habitats during the fruiting season between 2019 and 2021. The species name, localities, geographical position, altitude, and voucher number are presented in Table 1. The voucher specimens were de-

Table 1. Localities and voucher numbers of the studied *Bellevalia* taxa.

Taxa	Locality	Voucher number
B. anatolica	B7/Tunceli: Tüllük Village, 1435 m	Eroğlu, 1666
B. fominii	B9/Van: Gevaş, 1887 m	Eroğlu, 1680
B. kurdistanica	B7/Tunceli: Ovacık, 1400 m	Dogan, 2590
B. rixii	B9/Van: Başkale, Güzeldere, 2632 m	Eroğlu, 1610
B. turcica	C5/Adana: Pozantı, 1515 m	Pınar, 6502

* Corresponding author, e-mail: gdogan@firat.edu.tr
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posited at the Herbarium of Firat University.

The karyological studies are conducted on the meristematic cells of seven bulbs of each species. The bulbs were germinated at 25°C. The actively growing root tips were pretreated with 0.05% colchicine for 4 h at room temperature. Afterward, the root tips were fixed with Carnoy fixative (1 : 3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolyzed in 1 M HCl at 60°C for 10–15 min, then rinsed in tap water for 3–5 min. Finally, they were stained in the Feulgen reagent for 1 h and mounted in 45% acetic acid (Kiran *et al.* 2012). Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope and an Olympus Camedia C-4000 digital camera.

The number of somatic chromosomes, chromosome length range, haploid chromosome length, arm ratio, and relative length were measured. The karyotype formula was determined based on the centromere position using a system of Levan *et al.* (1964). The ideograms of these taxa are arranged in decreasing lengths according to the chromosome size in the metaphase (Martin *et al.* 2009).

Results and discussion

The chromosome number, chromosome length range, haploid chromosome length, arm ratio, relative length, and karyotype formula were determined from 14 metaphase cells in each species and given in Table 2. Metaphase chromosomes are shown in Fig. 1 and idiograms in Fig. 2.

The somatic chromosome number of *B. anatolica* was $2n=2x=8$ (Fig. 1A). The karyotype formula

Table 2. Karyological features of the studied *Bellevalia* taxa.

Taxa	2*n*	Chromosome length (μm)		Haploid chromosome length (μm)	Arm ratio		Relative length (%)		Karyotype formula
		Min	Max		Min	Max	Min	Max	
B. anatolica	8	13.79	22.41	68.17	1.24	4.00	20.22	32.88	2m+sm+st
B. fominii	8	10.19	17.43	57.78	1.12	2.76	19.31	33.02	2m+2sm
B. kurdistanica	8	11.67	20.00	59.10	1.33	3.91	19.75	33.83	2m+sm+st
B. rixii	8	12.05	18.82	60.68	1.23	3.40	19.86	31.02	2m+sm+st
B. turcica	8	11.96	20.17	61.88	1.49	3.78	19.32	32.60	m+2sm+st

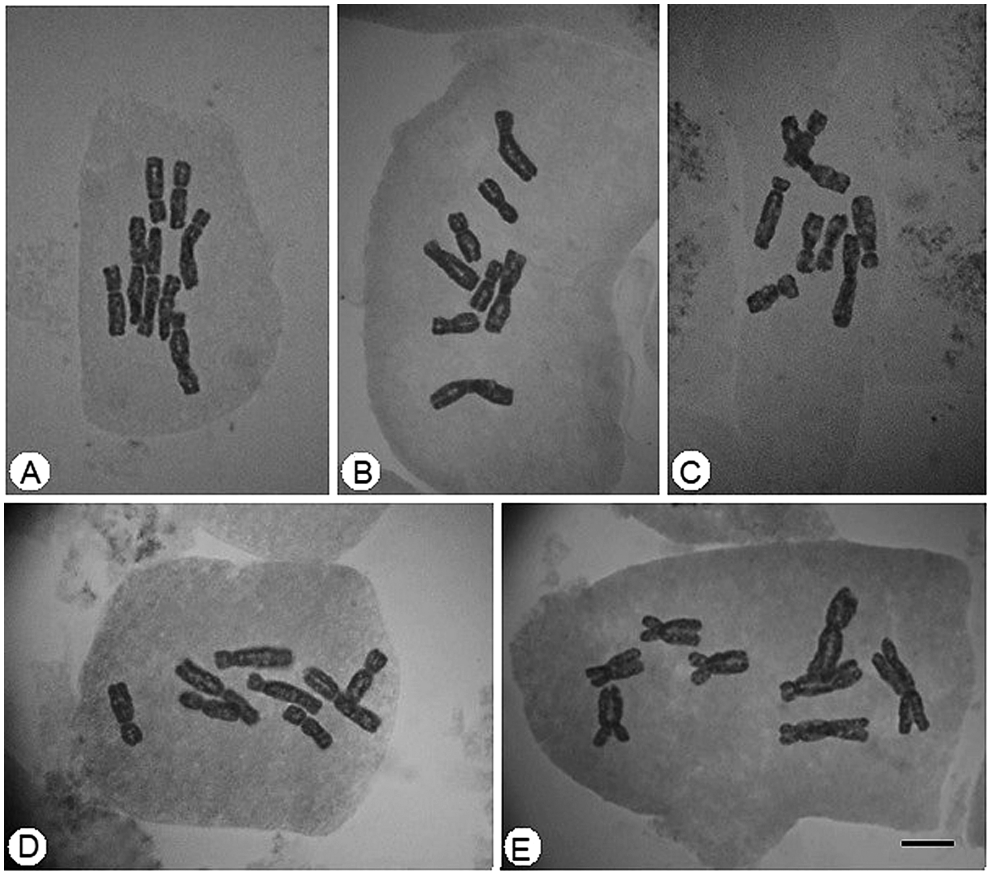

Fig. 1. Metaphase chromosomes of studied *Bellevalia* taxa. A. *B. anatolica*, B. *B. fominii*, C. *B. kurdistanica*, D. *B. rixii*, E. *B. turcica* Scale bars=10 μm.

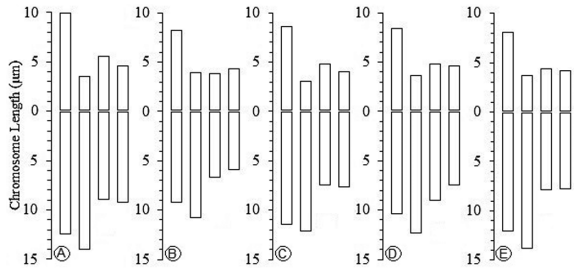

Fig. 2. Haploid idiograms of studied *Bellevalia* taxa. A. *B. anatolica*, B. *B. fominii*, C. *B. kurdistanica*, D. *B. rixii*, E. *B. turcica*.

was 2m+sm+st. The chromosome length ranged from 13.79 to 22.41 μm and the haploid chromosome length was 68.17 μm. Arm ratios were 1.24–4.00 and relative lengths were 20.22–32.88% (Table 2). The idiogram is given in Fig. 2A. The somatic chromosome number of *B. fominii* was $2n=2x=8$ (Fig. 1B). The karyotype formula was 2m+12sm. The chromosome length ranged from 10.19 to 17.43 μm and the haploid chromosome length was 57.78 μm. Arm ratios were 1.12–2.76 and relative lengths were 19.31–33.02% (Table 2). The idiogram is given in Fig. 2B. The somatic chromosome number of *B. kurdistanica* was $2n=2x=8$ (Fig. 1C). The karyotype formula was 2m+sm+st. The chromosome length ranged from 11.67 to 20.00 μm and the haploid chromosome length was 59.10 μm. Arm ratios were 1.33–3.91 and relative lengths were 19.75–33.83% (Table 2). The idiogram is given in Fig. 2C. The somatic chromosome number of *B. rixii* was $2n=2x=8$ (Fig. 1D). The karyotype formula was 2m+sm+st. The chromosome length ranged from 12.05 to 18.82 μm and the haploid chromosome length was 60.68 μm. Arm ratios were 1.23–3.40 and relative lengths were 19.86–31.02% (Table 2). The idiogram is given in Fig. 2D. The somatic chromosome number of *B. turcica* was $2n=2x=8$ (Fig. 1E). The karyotype formula was m+2sm+st. The chromosome length ranged from 11.96 to 20.17 μm and the haploid chromosome length was 61.88 μm. Arm ratios were 1.49–3.78 and relative lengths were 19.32–32.60% (Table 2). The idiogram is given in Fig. 2E.

Chromosome lengths show wide variation among species. The mean chromosome length varies between 10.19 μm (*B. fominii*) and 22.41 μm (*B. anatolica*). Haploid chromosome length ranges from 57.78 μm (*B. fominii*) to 68.17 μm (*B. anatolica*). The arm ratio was determined minimum *of* 1.12 in *B. fominii*, and maximum of 4.00 in *B. anatolica*, whereas the lowest relative length was observed in *B. fominii* (19.31%) and the highest relative length was found in *B. kurdistanica* (33.83%).

To date, only one study has been conducted on the chromosome number of the *B. anatolica*. In this study conducted by Johnson (1994), the chromosome number of the species was reported as $2n=8$. Our investigations also determined the same $2n=8$. Numerous studies have been conducted on *B. fominii* and the somatic chromosome number of this species is generally reported as $2n=8$ (Delaunay 1926, Fedorov 1969, Özhatay *et al.* 1991a, b, Johnson and Brandham 1997, Bothmer and Wendelbo 1981, Nersesian 2001, Johnson 2003).

According to the literature, the chromosome number of *B. kurdistanica* species has been reported as $2n=8$, 16 (Al-Mudarris 1973, Bothmer and Wendelbo 1981, Johnson and Brandham 1997). The somatic chromosome number of *B. rixii* has been reported as $2n=8$ (Özhatay *et al.* 1991a, b, Johnson 2003). The chromosome number of *B. turcica*, which was introduced to the scientific world as a new species for Turkey by Pınar and Eroğlu (2016), was determined as $2n=8$ in the same publication.

Although the genus *Bellevalia* is closely related to *Muscari*, *Hyacinthus*, and *Hyacinthella*, sharing with them considerable morphological similarity, its karyotype is unique and distinguishable. The 'basic karyotype' of *Bellevalia* consists of $x=4$, with long chromosomes (Bothmer and Wendelbo 1981). With this study, we aim to contribute to the search for karyologically understudied species to expand the karyological knowledge of the genus *Bellevalia*.

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