



Research Article

Karyological Examination of *Ornithogalum orthophyllum* Ten. and *Ornithogalum oligophyllum* E. D. Clarke

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Abstract: In this study, two species of *Ornithogalum* L. genus (*Ornithogalum orthophyllum* Ten. and *Ornithogalum oligophyllum* E. D. Clarke), one of the important genus of the Asparagaceae family, were examined in terms of karyological aspects. The plant materials used in the study were collected from the natural distribution areas of the plants around Elazığ 2019. Some of the collected samples were turned into herbarium samples and stocked in the FU herbarium. Bulbs obtained from the collected samples were used in cytotoxic studies. As a result of the studies carried out in dividing somatic cells, chromosome counts and karyotype analyzes of the species were made and their ideograms were drawn. Chromosome number were determined as $2n=20$ and karyotype formula $M+3m+3sm+3st$ in *Ornithogalum oligophyllum*, *Ornithogalum orthophyllum* species as $2n=14$ and karyotype formula as $3m+2sm+2st$.



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Keywords: Ornithogalum; Chromosome numbers; Cytotaxonomy; Karyotype.

1. Introduction

The genus *Ornithogalum* L., belonging to the family Asparagaceae, includes about 200 species worldwide (Manning et al., 2004, 2009). The members of the genus are mostly distributed in South Africa and the Mediterranean region (Uysal et al., 2005), and the region where the genus shows maximum richness is primarily South Africa and then Turkey (Wendelbo, 1984). In the revision study conducted by Uysal in our country in 2014, it was reported that the genus was represented by 68 taxa distributed in 4 subgenera (*Beryllis*, *Caruelia*, *Ornithogalum* and *Myogalum*) and 4 sections (*Ornithogalum*, *Alatiformis*, *Pulviniformis* and *Oreogalum*) (Uysal et al., 2014). Of these 68 taxa, 33 are endemic, with an endemism rate of approximately 50%.

Ornithogalum species, generally known as “Akyıldız”, have also been given names such as “Kurt soğanı, Tükrük otu, Köpek soğanı, Kurtkirişi, Karga soğanı, Akbaldır” among the public (Baytop, 2007). Some *Ornithogalum* species have ethnobotanical importance among the public and are used both as food and medicinal plants (Aslan, 2022). The bulbs of the *Ornithogalum* genus, which have had economic value since Discorides, have been used as an emetic and also in the treatment of boils (Baytop, 1984). Also, some species of the genus have been used in the traditional treatment of diabetes, heart disease, hepatitis, and even some types of cancer (Ferth and Kopp, 1992; Ghannamy et al., 1987; Al-Qura'n 2009; Şabudak and Oyman, 2002).



Ornithogalum is a taxonomically difficult genus, with morphology poorly correlated with differences in chromosome number and karyotype (Garbari et al., 2003; Dalgıç and Özhatay, 1997; Meriç et al., 2011; Mousavi et al., 2013). Karyotypes are important distinguishing features in biosystematics. Karyotype features, including chromosome number, structure, and behavior, can be used as taxonomic evidence for biosystematic classification at the species, genus, and even family level (Stace, 1980; Odah and Oran, 2007).

Cullen and Ratter (1967) conducted taxonomic and cytological studies on the species of the Turkish *Ornithogalum* genus and reported the chromosome numbers of different populations of *Ornithogalum* species in their studies. According to these reports, the *Ornithogalum* genus contains a wide variety in terms of basic chromosome number. Different researchers have reported different chromosome numbers in *O. baeticum* species as $2n = 18, 27, 52$ (Löve and Kjellquist, 1973; Van Raamsdonk, 1986; Ferth, 2001). According to Neves and De (1952), this complex karyology of *Ornithogalum* is associated with the high frequency of ploidy, aneuploidy, translocation and deletion. This study aims to contribute to subsequent taxonomic studies by determining the chromosome numbers of two species of the *Ornithogalum* (*O. oligophyllum* and *O. orthophyllum*) genus, one of the important genera of our country, distributed in Elazığ, and performing karyotype analyses.

2. Materials and Methods

2.1. Plant materials

In this study, *O. oligophyllum* and *O. orthophyllum* species belonging to the *Ornithogalum* genus were collected in flowering and bulbous form from their natural habitats in Elazığ between April and June 2019. In addition, some of the samples were preserved as herbarium material suitable for karyological examinations. *O. oligophyllum* was collected from Baskil (1500-1900 m), and *O. orthophyllum* from Harput (1450 m).

2.2. Chromosome observation and analysis

The karyological studies are conducted on the meristematic cells of seven bulbs of each species. The bulbs were germinated at 23 °C. The actively growing root tips were pretreated with 0.05% colchicine for 4 h at 23 °C. 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 8 min, then rinsed in tap water for 3–5 min. Finally, they were stained in a Feulgen reagent for 1 h and mounted in 45% acetic acid (Kiran et al., 2022). 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, were measured and arm ratio, relative length, and centromere index were calculated. The karyotype formula was determined based on the centromere position using a system of Levan et al. (1964). The idiograms of these taxa are arranged in decreasing lengths according to the chromosome size in the metaphase (Martin et al., 2009).

3. Results

In this study, karyological characteristics of two species belonging to the genus *Ornithogalum* (*O. oligophyllum* and *O. orthophyllum*) were determined. From 7 metaphase cells in each species; somatic chromosome number, chromosome length range, haploid chromosome length, arm ratio, relative length, centromere index, centromere position and karyotype formula were determined and presented in Tables. Metaphase chromosome appearance and idiograms are shown separately for each species.

Ornithogalum oligophyllum E.D. Clarke

The somatic chromosome number in *O. oligophyllum* species was determined as $2n=2x=20$, and the karyotype formula as $M+3m+3sm+3st$. It was observed that chromosome number II was median point (M), chromosomes numbered II, IX and X were median (m), chromosomes numbered III, VI and VIII were sub-median (sm), and chromosomes numbered IV, V and VII had sub-terminal (st) centromeres. It was determined that total chromosome length of the species was 7.57-17.94 μm , haploid chromosome length was 112.87 μm , arm ratio (L/S) was 1.00-4.02 μm , centromeric index was 0.19-0.50, and relative length was between 6.70-15.89 (Table 1). Karyotype symmetry as 1B according to Stebbins, intrachromosomal asymmetry index (A1) was 0.46, and interchromosomal asymmetry index (A2) was 0.29. The chromosome appearance of the species in the metaphase plane is given in Figure 1A, and the haploid ideogram is given in Figure 1B.

Table 1. Chromosome measurements of *O. oligophyllum* species.

Chr. No	TLC*	L	S	L/S	CI	RL	CP
1	17.9	8.9	8.9	1.0	0.5	15.8	median point
2	15.9	9.5	6.3	1.4	0.4	14.0	median
3	12.2	8.4	3.8	2.1	0.3	10.8	submedian
4	11.7	9.1	2.5	3.5	0.2	10.4	subterminal
5	10.4	8.3	2.0	4.0	0.1	9.2	subterminal
6	10.9	7.5	3.4	2.1	0.3	9.6	submedian
7	9.2	7.0	2.2	3.2	0.2	8.1	subterminal
8	8.5	6.2	2.2	2.7	0.2	7.5	submedian
9	8.3	5.0	3.2	1.5	0.3	7.4	median
10	7.5	3.8	3.6	1.0	0.4	6.7	median

TLC*: Total length of the chromosome (μm), L: Length of the long arm (μm), S: Length of the short arm (μm), L/S: Arm ratio (μm), CI: Centromeric index, RL: Relative length (%), CP: Centromeric position.

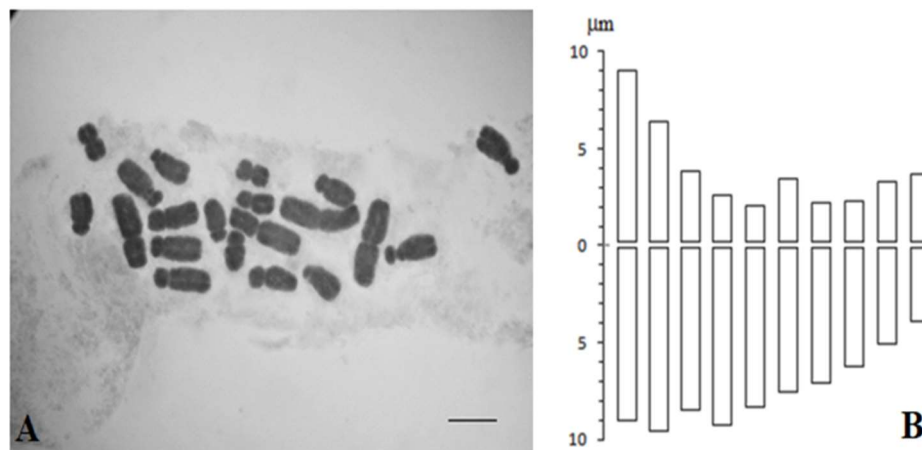


Figure 1. *O. oligophyllum* species **A.** Chromosome appearance of Metaphase (Scale bar:10 μm) **B.** Haploid ideogram.

Ornithogalum orthophyllum Ten.

The somatic chromosome number in *O. orthophyllum* species was determined as $2n=2x=14$, and the karyotype formula as $3m+2sm+2st$. It was observed that chromosome number chromosomes numbered I, IV and VII were median (m), chromosomes numbered II, and V were sub-median (sm), and chromosomes numbered III, and VI had sub-terminal (st) centromeres. It was determined that total chromosome length of the species was 3.49-8.90 μm , haploid chromosome length was 54.89 μm , arm ratio (L/S) was 1.22-3.70 μm , centromeric index was 0.21-0.44, and relative length was between 6.35-17.78 (Table 2). Karyotype symmetry as 3B according to Stebbins, intrachromosomal asymmetry index (A1) was 0.48, and interchromosomal asymmetry index (A2) was 0.26. The chromosome appearance of the species in the metaphase plane is given in Figure 2A, and the haploid ideogram is given in Figure 2B.

Table 2. Chromosome measurements of *O. orthophyllum* species.

Chr. No	TLC*	L	S	L/S	CI	RL	CP
1	9.7	5.9	3.8	1.5	0.3	17.7	median
2	9.1	5.9	3.2	1.8	0.3	16.7	submedian
3	8.9	7.0	1.8	3.7	0.2	16.2	subterminal
4	8.7	5.4	3.2	1.6	0.3	15.9	median
5	7.5	5.5	1.9	2.8	0.2	13.7	submedian
6	7.2	5.2	2.0	2.5	0.2	13.2	subterminal
7	3.4	1.9	1.5	1.2	0.4	6.3	median

TLC*: Total length of the chromosome (μm), L: Length of the long arm (μm), S: Length of the short arm (μm), L/S: Arm ratio (μm), CI: Centromeric index, RL: Relative length (%), CP: Centromeric position.

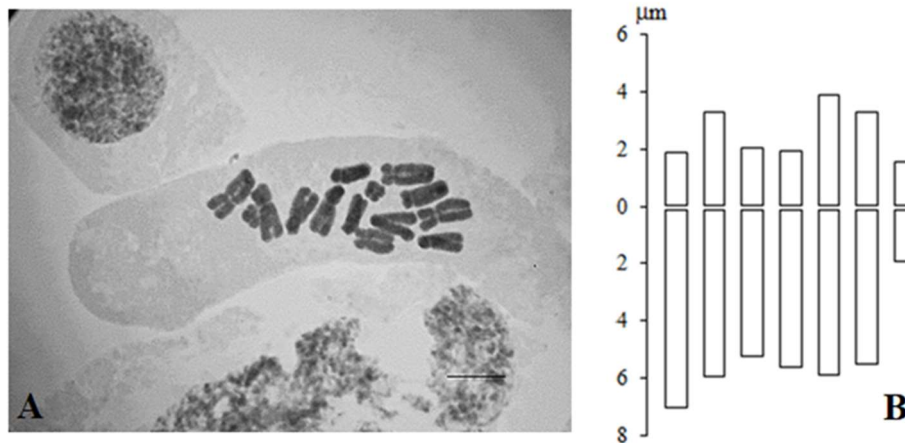


Figure 2. *O. orthophyllum* species **A.** Chromosome appearance of Metaphase (Scale bar:10 μm) **B.** Haploid ideogram.

4. Discussion and Conclusions

Among the studied species; *O. orthophyllum* has the lowest chromosome length and haploid chromosome length, while *O. oligophyllum* has the highest chromosome length and haploid chromosome length. The lowest value of arm ratio was found in *O. oligophyllum*, while *O. orthophyllum* had the highest value. The species with the lowest and highest relative length was determined as *O. orthophyllum*. While the chromosomes of the studied species generally have median (m), submedian (sm) and subterminal (st) centromeres, chromosomes with dotted median (M) centromeres were also found in *O. oligophyllum*.

According to the literature review on *O. oligophyllum* species; Cullen and Ratter (1967) reported the chromosome number of this species as $2n=20, 24$. Johnson et al. (1991) reported the chromosome number of this species as $2n=16, 20, 24, 24+2B, 40$, and 80. Özhatay and Johnson (1996) determined that the chromosome number of the species is $2n=12, 12+0-3B, 18$ as a result of their studies on the species. Dalgıç and Özhatay (1997) added the information that the species has $2n=18$ chromosomes to the literature. In study Özhatay (2002) reported the chromosome number of the species as $2n = 16, 18, 20, 24, 24 + 2, 40, 80$. In 2014, Uysal et al. (2014) reported the chromosome number of the species as $2n = 18$. In this study, it was also reported that all chromosomes consisted of subtelocentric, submetacentric and metacentric chromosomes and the basic chromosome number was $x = 9$. As a result of our study, it was determined that the chromosome number of this species was 20, which is consistent with some literature information.

In their study on *O. orthophyllum* species, Özhatay and Johnson (1996) reported the chromosome number of the species as $2n=14.28$, while Dalgıç and Özhatay (1997) reported the chromosome number of the species as $2n=14$. In study Özhatay (2002) reported the chromosome number of the species as $2n = 14, 16, 28$. Garbari et al. (2003) determined the chromosome number of the species as $2n = 18$. Uysal et al. (2014) determined the chromosome number of the species as $2n = 16$ and $2n = 32$. According to the karyotype analyses performed in the same study, it was also reported that all of the chromosomes of the species were submetacentric and subtolocentric. It was determined that the species had diploid and tetraploid chromosome numbers and the basic chromosome number was $x=8$. As a result of our study, the chromosome number of the species was found to be $2n=14$, as in some literature (Özhatay and Johnson, 1996; Dalgıç and Özhatay, 1997; Özhatay, 2002).

As a result, in this study, two species (*O. oligophyllum* and *O. orthophyllum*) belonging to the genus *Ornithogalum*, which is one of the important genera of our country and distributed in Elazığ province, were karyologically examined and it was aimed to provide an in the future contribution to the taxonomic studies to be carried out on these species in the future.

Conflicts of Interests

The authors have no conflict of interest to declare.

Financial Disclosure

Author declare no financial support.

Statement contribution of the authors

In this study, the collection of plant material were carried out by SÖ, GD; karyotype analyses of by YK, planning of this study, identification of the plants and writing of the Article were done by GD.

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