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Karyological studies on the four *Ornithogalum* L. (Asparagaceae) taxa from Eskişehir (Central Anatolia, Turkey)

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In the current study, karyotypes of four taxa in the genus of *Ornithogalum* L. were examined. These taxa grow naturally in Eskişehir (Central Anatolia). Studies were carried out on somatic chromosomes, using the root tips squash technique and based on staining with acetic orcein stain, for the most actively dividing meristematic cells of the collected root tips. Their counts were based on chromosomes observed during mitotic division. For species included in the *Ornithogalum* L. (Asparagaceae) genus, *O. montanum* Cirillo has $2n = 14$ with basic chromosome number $x = 7$; *O. comosum* L. has $2n = 20$, basic chromosome number $x = 10$, *O. pyrenaicum* L. has $2n = 24$, basic chromosome number $x = 12$; and *O. sigmoideum* Freyn & Sint. has $2n = 14$, basic chromosome number $x = 7$; it was found that they have diploid chromosomes. *O. montanum* and O. *sigmoideum* chromosome numbers are $2n = 14$ (diploid races), but their basic karyotypes are different, including chromosome size, and position of centromere. Mean chromosomal length of the all taxa subject to the study is 1.20–7.63 µm. Chromosomal morphologies of all taxa include median, submedian and subtelocentric chromosome pairs. In addition to, karyograms and idiograms of all studied taxa were done.

Keywords: chromosome numbers; *Ornithogalum* taxa; Asparagaceae; karyotype analysis; Eskişehir; Turkey

Introduction

According to recent phylogenetic studies, the genus *Ornithogalum* L., belonging to the Asparagaceae family (APG III 2009), is distributed almost all around the world. It is especially widely distributed in Europe, around the Mediterranean and Balkan Peninsula, Asia (reaching Afghanistan in the East), Africa (except the Tropic of Cancer) and Madagascar (Zahariadi 1980; Uysal et al. 2005; Martínez-Azorin et al. 2010a, 2010b, 2011; Meriç et al. 2011). *Ornithogalum* is a taxonomically difficult genus, while its morphology is poorly correlated with the variation in chromosome number and karyotype (Garbari et al. 2003; Dalgıç and Özhatay 1997; Meriç et al. 2011; Mousavi et al. 2013).

The number of species included in *Ornithogalum*, and hence its circumscription and taxonomy, is still a matter of controversy (Martínez-Azorin et al. 2009, 2010a, 2010b, 2011). *Ornithogalum* comprises about 120–130 species (Landstrom 1989), or even 200 according to Obermeyer (1978). However, Müller-Doblies and Müller-Doblies (1996) recognized 123 species from southern Africa, forty of which were described as new taxa. Therefore, the total number of species in the world could be 200–300 or higher (Martínez-Azorin et al. 2007, 2010a). On the basis of floral and reproductive characters, 17 subgenera have been recognized in the *Ornithogalum* genus (cf. Baker 1873; Zahariadi 1980; Obermeyer 1978; Martínez-Azorin et al., 2007).

It was reported in the revision conducted by Cullen (1984) for the Flora of Turkey that *Ornithogalum* was represented by 23 taxa in Turkey. However, a few new taxa were added afterwards (Cullen 1984; Özhatay 2000; Düşen and Deniz 2005; Uysal et al. 2005; Özhatay and Kültür 2006; Varol 2008; Bağcı et al. 2009, 2011; Doğru-Koca and Yıldırımlı 2010). Today, *Ornithogalum* is represented by about 50 taxa in the flora of Turkey, 20 of these species are endemics and endemism ratio is *c*.40% (IUCN 2001). Anatolia is an important diversification center of *Ornithogalum* genus with about 50 taxa (Cullen 1984; Davis et al. 1988; Meriç et al. 2011). This genus is represented in Eskişehir by 14 taxa in two subgenera (subgen. Ornithogalum and subgen. Beryllis) (Ocak et al. 2010).

As already pointed out by Peruzzi et al. (2011) and Bedini et al. (2010, 2012a, 2012b, 2012c), plant chromosome number databases are a useful tool for systematic comparisons of geographical or taxonomical groups of plants (Peruzzi et al. 2012). Karyotypes are an important distinguishing feature in biosystematics. Karyotype features including chromosome number, structure and behavior can be used as taxonomic evidence for biosystematic classification at the level of species, genus and even family (Stace 1980; Odah and Oran 2007). In addition, interspecific variation in the karyotype, for example in chromosome number, differences in size and proportion among chromosome arms, and the position of satellite regions, has proved to be an important source of cytological data, and is considered as a complementary tool to other taxonomic studies, such as morphology, anatomy and palynology (Stace 1980; Odah and Oran 2007).

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Anatomical, morphological, palynological, karyological, taxonomic, systematic, pharmacological, chorological and ethnobotanical studies, as well as studies of the antioxidant activity and conservational status have been conducted in *Ornithogalum* genus (Jensen 1962; Czapik 1968; Ashley 1979; Cullen 1984; Davis et al. 1988; Stedje 1988; Griesbach et al. 1993; Özhatay et al. 1996; Dalgıç and Özhatay 1997; Barbujian and Pigliucci 1989; Çoşkunçelebi et al. 2000, 2002; Özhatay 2000; Düşen and Sümbül 2002, 2003; Herrman 2002; Garbari et al. 2003; Düşen and Deniz 2005; Uysal et al. 2005; Martínez-Azorin et al., 2007, 2009, 2010a, 2010b; Varol 2008; Bednorz and Czarna 2008; Bağcı et al. 2009, 2011; Dalgıç et al. 2009; Manning et al. 2009; Peruzzi et al. 2009; Ebrahimzadeh et al. 2010; Çiler et al 2011; Hua 2011; Maria 2011; Meriç et al. 2011; Heydarian et al 2012; Karagüzel et al. 2012).

We aimed to find karyological features in addition to morphological characters to distinguish *O. comosum*, *O. montanum*, *O. sigmoideum* and *O. pyrenaicum* species. We hope that this study will make contributions to the taxonomy of the *Ornithogalum* genus.

Materials and methods

Plant materials

The samples of *Ornithogalum* were collected from Eskişehir (Central Anatolia, Turkey) province and its close environs, for this study in 2006–2007 (Figure 1). Taxonomic descriptions of the plants were made according to Cullen (1984), Zahariadi (1980) and Davis et al. (1988). In order to ensure a systematic study of the material obtained, herbarium samples were prepared and these samples were protected as herbarium samples at the OUFE (Eskişehir Osmangazi University Herbarium Center).

Localities of collected plant samples (origin of the examined taxa)

(1) *O. comosum* L.: Turkey, B3 Eskişehir; Bozan reforestation site, 39°47′55.4″ N, 31°07′13.7″ E, 878 m, 17 June 2006, OUFE: 16587.
(2) *O. montanum* Cirillo: Turkey, A3 Eskişehir; Emresultan-Sazak road, 40°04′07.0″ N, 31°23′43.5″ E, 399 m, 5 May 2006, OUFE: 16583.
(3) *O. sigmoideum* Freyn & Sint.: Turkey, B3 Eskişehir; Tandır village, 39°55′27.6″ N, 30°41′ 33.3″ E, 1273 m, 23 April 2006, OUFE: 16584.
(4) *O. pyrenaicum* L.: Turkey, A3 Eskişehir; Laçin-Mayıslar road, 40°01′3.4″ N- 30°43′35.1″ E, 234 m, 5 May 2006, OUFE: 16585.

Karyological studies

Karyotype studies were carried out on somatic (on mitotic metaphase) chromosomes using the root tip squash technique, as described by Azzioui et al. (1990), Griesbach (1998) and Vosa (1997). The root tips were pretreated with 8-hydroxyquinoline solution at 4°C for 6 h. After washing with distilled water, the material was fixed in fresh Carnoy's solution (3:1 ethanol and glacial acetic acid) overnight at 4°C and stored in 70% ethanol at 4°C. This material was hydrolyzed with 1 N HCl for 10 min at 60°C, washed with distilled water, stained with 2% acetic orcein, and squashed in 45% acetic acid. Chromosome measurements including long arm, short arm, total length of chromosome set, arm ratio index and relative chromosome length were made from at least five enlarged well-spread metaphase cells of same population for each taxon. The homologous chromosomes were paired by their similarity in size and shape. The best five metaphase plates were used to construct the respective ideogram according to the relative chromosome length. Chromosomes were classified using the nomenclature established in Levan et al. (1964). The preparations were photographed by using a Kameram™ digital camera (Micro System Computer Aided Microscope Systems Co.Ltd. Maslak/Istanbul) and a Nikon 80i type microscope (Nikon Instruments, Melville, NY) in our department.

Results

In this study, chromosome numbers and karyotype morphology are given for four *Ornithogalum* taxa.

O. comosum L.

From cytological evaluations made with meristematic cells of *O. comosum*, it was found that the plant is comprised of diploid plants with $2n = 20$ chromosomes and the principal chromosome number is $x = 10$ (Figure 2a). No mitosis anomaly was determined in meristematic cells. On the mitotic metaphase of the meristematic cells, karyotype and idiograms were prepared since the chromosomes were on the sample plane and they segregated (Figures 3a, 4a). It was determined by karyotype analysis that this taxon has a chromosome number of $2n = 20$ (Figure 2a). The shortest chromosome length is 2.84 μm, and the longest is 8.3 μm. Of the metaphase chromosomes, five pairs are of the subtelocentric (subterminal) type, four pairs are submetacentric (sub-median) and one pair is metacentric (median). Chromosome proportional lengths were measured as 3.16–8.78 μm. The centromeric index values vary between 16.65 and 52.27 μm, and the arm index values vary from 0.02 to 0.91 μm (Table 1). The chromosomes have no satellites. The results of the karyological analysis are presented in Table 1 and Figures 2a, 3a and 4a. Our results of chromosome counts and karyotype morphology agree with the previous counts and karyotype reported by Özhatay (2002).

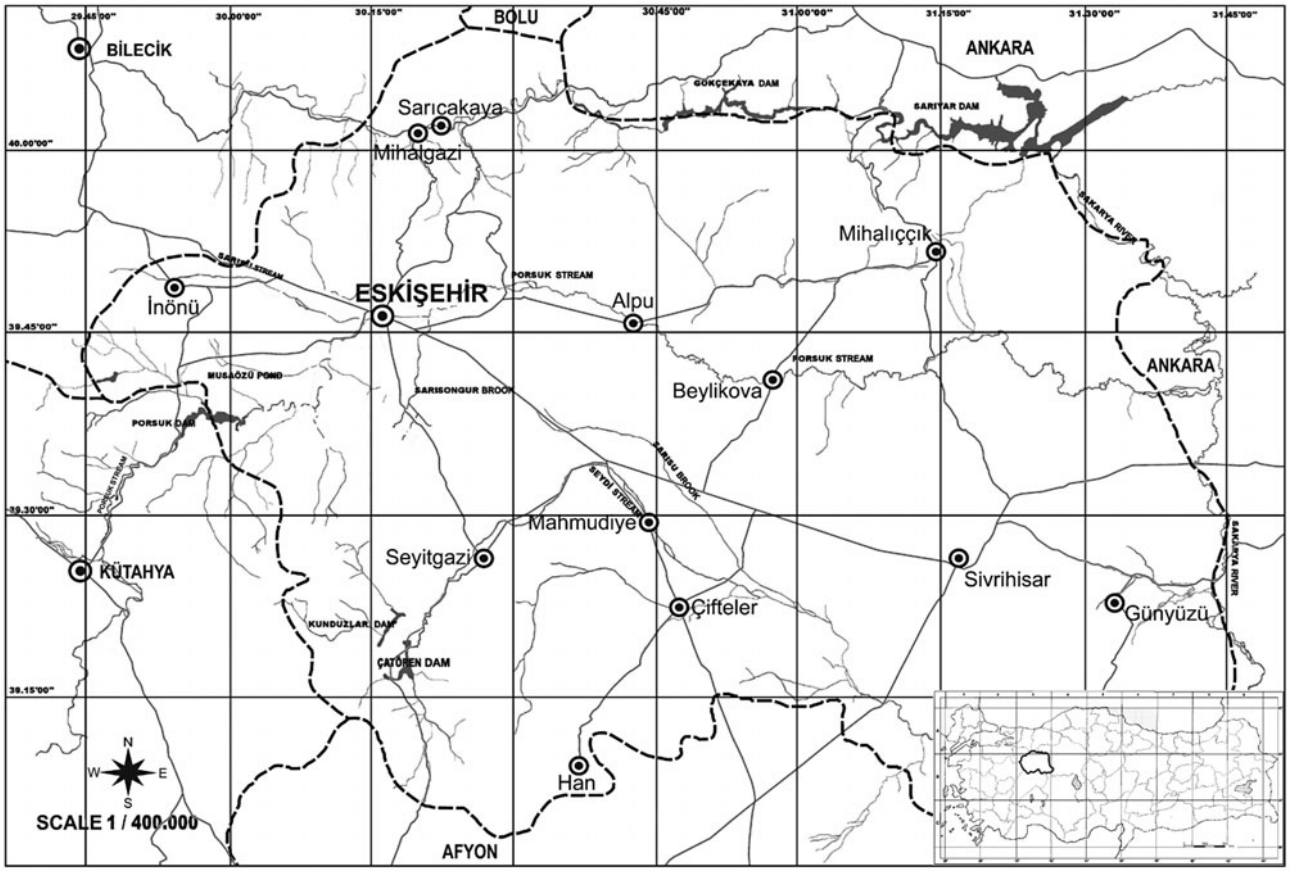


Figure 1. Map of the area where plant samples were collected.

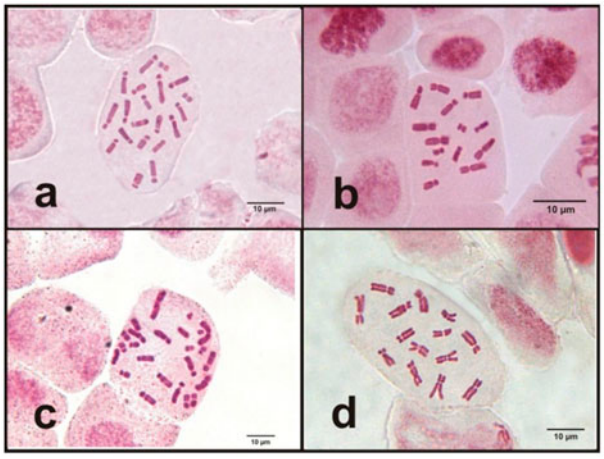


Figure 2. (Color online) Somatic metaphase in investigated *Ornithogalum* taxa: (a) *O. comosum* $2n = 20$; (b) *O. montanum* $2n = 14$; (c) *O. pyrenaicum* $2n = 24$; (d) *O. sigmoideum* $2n = 14$. Bar = 10 µm.

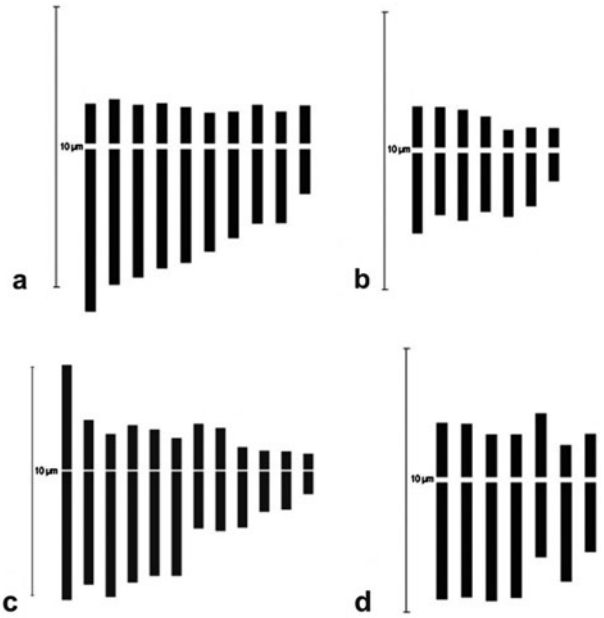


Figure 3. Idiograms of somatic chromosomes in investigated *Ornithogalum* taxa: (a) *O. comosum* $2n = 20$; (b) *O. montanum* $2n = 14$; (c) *O. pyrenaicum* $2n = 24$; (d) *O. sigmoideum* $2n = 14$. Bar = 10 µm.

O. montanum Cirillo

From cytological evaluations made with meristematic cells of *O. montanum*, it was found that the plant is comprised of diploid plants with $2n = 14$ chromosomes. The principal chromosome number is $x = 7$ (Figure 2b).

No mitosis anomaly was determined in meristematic cells. On the mitotic metaphase of the meristematic cells,

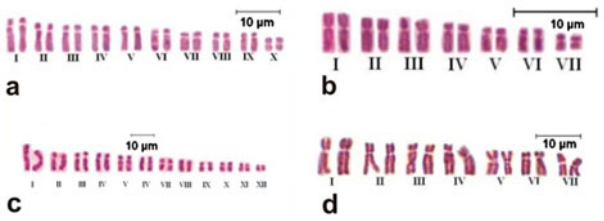


Figure 4. Karyograms of somatic chromosomes in investigated *Ornithogalum* taxa: (a) *O. comosum* $2n = 20$; (b) *O. montanum* $2n = 14$; (c) *O. pyrenaicum* $2n = 24$; (d) *O. sigmoideum* $2n = 14$. Bar = 10 µm.

karyotype and idiograms were prepared since the chromosomes were on the sample plane and they segregated (Figures 3b, 4b). It was determined by karyotype analysis that this taxon has a chromosome number of $2n = 14$ (Figure 2b). The shortest chromosome length is 1.56 µm, and the longest is 4.75 µm. Of the metaphase chromosomes, one pair is of the subtelocentric (subterminal) type, four pairs are submetacentric (sub-median) and two pairs are metacentric (median). Chromosome proportional lengths were measured at 1.73–5.42 µm. The centromeric index values vary between 20.61 and 52.28 µm, and the arm index values vary from 0.22 to 0.7 µm (Table 1). The chromosomes have no satellites. The results of the karyological analysis are presented in Table 1 and Figures 2b, 3b and 4b.

O. pyrenaicum L.

From cytological evaluations made with meristematic cells of *O. pyrenaicum*, it was found that the plant is comprised of diploid plants with $2n = 24$ chromosomes.

Table 1. Measurements (µm) of somatic chromosomes of investigated *Ornithogalum* taxa.

Taxa	Chromosome pair number	ChL (µm)			PL (µm)			AI			CI			Type
		A	Min	Max	A	Min	Max	A	Min	Max	A	Min	Max	
O. comosum	I	7.84	7.39	8.30	8.36	7.93	8.78	0.24	0.15	0.35	19.87	16.65	23.09	st
	II	6.58	6.24	6.92	7.01	6.69	7.34	0.37	0.26	0.49	27.35	24.19	30.51	st
	III	6.05	5.63	6.47	6.45	6.19	6.70	0.29	0.17	0.42	22.72	19.43	26.01	st
	IV	5.86	5.52	6.20	6.24	5.88	6.61	0.32	0.17	0.48	24.81	21.44	28.18	sm
	V	5.55	5.29	5.81	5.91	5.65	6.18	0.35	0.02	0.68	35.36	33.03	37.69	st
	VI	5.04	4.53	5.55	5.37	5.00	5.74	0.30	0.20	0.42	23.65	19.89	27.41	st
	VII	4.46	4.74	4.68	4.75	4.27	5.23	0.29	0.21	0.37	22.60	19.34	25.86	sm
	IIX	4.35	3.96	4.74	4.63	4.37	4.90	0.31	0.22	0.41	24.02	20.37	27.67	sm
	IX	4.12	3.64	4.61	4.39	3.74	5.05	0.37	0.30	0.45	27.12	22.86	31.38	sm
	X	3.08	2.84	3.32	3.28	3.16	3.40	0.84	0.78	0.91	45.39	38.51	52.27	m
O. montanum	I	4.64	4.53	4.75	5.16	4.90	5.43	0.57	0.53	0.62	36.45	36.33	38.97	sm
	II	4.20	3.89	4.50	4.67	4.33	5.00	0.47	0.44	0.50	30.39	20.61	24.73	m
	III	3.68	3.44	3.91	4.09	3.94	4.24	0.66	0.62	0.71	39.92	28.26	33.54	sm
	IV	3.31	3.16	3.47	3.69	3.37	4.01	0.50	0.47	0.53	33.34	22.06	28.10	sm
	V	3.00	2.64	3.36	3.34	3.09	3.59	0.26	0.22	0.29	20.48	43.82	52.28	st
	VI	2.88	2.50	3.27	3.21	3.04	3.37	0.29	0.23	0.35	22.52	22.52	24.96	sm
	VII	1.84	1.56	2.11	2.06	1.74	2.39	0.54	0.50	0.58	34.98	38.49	43.57	m
O. pyrenaicum	I	10.50	9.39	11.60	8.35	8.22	8.47	1.20	1.09	1.30	43.75	41.69	45.81	m
	II	7.28	6.14	8.43	5.58	5.44	5.71	2.19	2.08	2.29	30.12	27.12	33.12	sm
	II	7.05	5.38	8.72	5.49	5.38	5.69	3.41	3.30	3.51	21.71	20.70	22.72	st
	IV	6.78	5.41	8.15	5.37	5.24	5.49	2.49	2.39	2.59	27.06	25.03	29.09	sm
	V	6.28	4.86	7.70	5.18	4.97	5.38	2.53	2.42	2.63	27.46	26.40	28.52	sm
	VI	5.96	4.28	7.64	4.62	4.52	4.73	3.30	3.19	3.40	22.75	21.55	23.95	st
	VII	4.46	3.17	5.75	3.40	3.29	3.50	1.17	1.07	1.27	44.26	43.24	45.28	m
	IIX	4.39	2.60	6.18	3.43	3.33	3.54	1.31	1.16	1.46	40.85	39.66	42.04	m
	IX	3.48	1.92	5.04	3.12	3.02	3.23	2.50	2.39	2.60	28.16	27.11	29.21	sm
	X	2.91	1.50	4.32	2.56	2.56	2.66	1.66	1.55	1.76	36.74	33.04	40.44	sm
	XI	2.68	0.69	4.67	2.10	1.99	2.20	1.59	1.49	1.69	37.15	35.07	39.23	m
	XII	1.60	1.47	2.72	1.22	1.11	1.32	1.35	1.29	1.40	40.86	36.84	44.74	m
O. sigmoideum	I	7.80	7.60	8.00	4.71	4.61	4.82	0.60	0.58	0.63	32.63	31.42	33.85	sm
	II	6.64	6.40	6.87	4.01	3.71	4.32	0.29	0.27	0.32	27.2	25.92	28.48	sm
	III	6.51	6.41	6.62	3.93	3.29	4.58	4.45	0.63	0.70	24.31	23.7	24.93	sm
	IV	6.22	6.09	6.34	3.76	3.27	4.24	0.33	0.29	0.38	41.08	41.03	41.13	sm
	V	5.70	5.58	5.81	3.44	2.77	4.11	0.94	0.89	1.00	46.28	44.7	47.86	m
	VI	5.20	5.09	5.30	3.14	2.29	3.99	0.31	0.29	0.34	26.16	25.81	26.51	st
	VII	4.20	3.04	5.26	2.54	2.12	2.96	0.70	0.66	0.73	31.71	31.27	32.15	m

Abbreviations: ChL, chromosome length; PL, proportional length; AI, arm index; CI, centromere index; A, average; st, subterminal; m, median; sm, submedian.

The principal chromosome number is $x = 12$ (Figure 2c). No mitosis anomaly was determined in meristematic cells. On the mitotic metaphase of the meristematic cells, karyotype and idiograms were prepared since the chromosomes were on the sample plane and they segregated (Figures 3c, 4c). It was determined by karyotype analysis that this taxon has a chromosome number of $2n = 24$ (Figure 2c). The shortest chromosome length is 0.68 µm, and the longest is 11.59 µm. Of the metaphase chromosomes, two pairs are of the subtelocentric (subterminal) type, five pairs are submetacentric (sub-median) and five pairs are metacentric (median). Chromosome proportional lengths were measured at 1.11–8.47 µm. The centromeric index values vary between 20.7 and 45.81 µm, and arm index values vary from 1.06 to 3.5 µm (Table 1). The chromosomes have no satellites. The results of the karyological analysis are presented in Table 1 and Figures 2c, 3c and 4c.

O. sigmoideum Freyn & Sint

From cytological evaluations made with meristematic cells of the *O. sigmoideum*, it was found that the plant is comprised of diploid plants with $2n = 14$ chromosomes. The principal chromosome number is $x = 7$ (Figure 2d). No mitosis anomaly was determined in meristematic cells. On the mitotic metaphase of the meristematic cells, karyotype and idiograms were prepared since the chromosomes were on the sample plan and they segregated (Figures 3d, 4d). It was determined by karyotype analysis that this taxon has a chromosome number of $2n = 14$ (Figure 2d). The shortest chromosome length is 3.04 µm, and the longest is 7.99 µm. Of the metaphase chromosomes, one pair is of the subtelocentric (subterminal) type, four pairs are submetacentric (sub-median) and two pairs are metacentric (median). Chromosome proportional lengths were measured at 2.12–4.81 µm. The centromeric index values vary between 23.7 and 47.86 µm, and arm index values vary from 0.26 to 0.99 µm (Table 1). The chromosomes have no satellites. The results of the karyological analysis are presented in Table 1 and Figures 2d, 3d and 4d.

Discussion

In the last few decades, karyological studies have played an important role in solving taxonomic problems. Chromosome number variation and karyological analysis, play very important role for an understanding of plant evolution and diversification, especially the relationship between each of the ploidy level, biogeography and origin of the basic chromosome number (Badr et al. 1997; Abou-El-Enain 2002; Arslan et al. 2012). The differences in the number of the chromosome numbers are useful in evaluating the taxonomic position of a plant (İsmailoğlu and Meral 2011).

The current study examined cytotaxonomic features of four species of the *Ornithogalum* genus, which is a member of Asparagaceae family growing naturally in Eskişehir. Chromosome number and karyotype were analyzed for *O. comosum* L., *O. pyrenaicum* L., *O. montanum* Cyr. and *O. sigmoideum* Freyn & Sint. For karyotype analysis, root tips were used which were obtained by germination of bulbs. The results show that *O. montanum* is $2n = 14$, *O. comosum* is $2n = 20$, *O. pyrenaicum* is $2n = 24$ and *O. sigmoideum* is $2n = 24$, indicating that they are diploid plants.

The chromosome numbers of species are consistent with chromosome numbers of some species which grow outside Turkey, as investigated previously by some researchers. No mitosis anomaly was found.

A lot of chromosome count studies were performed in *O. comosum*, which is also subject to the current study, are 14, 16, 18, 18+B and 20 (Federov 1974; Özhatay 2002; Bedini et al. 2010). The results of this study are consistent with the results of the study conducted by Özhatay (2002). In the current study the chromosome number was $2n = 20$ (Figure 2d). Karyograms and idiograms were studied and morphological details of chromosomes were obtained (Figures 3d, 4d). Here a detailed karyotype analysis is given for the first time. Based on chromosome analysis, it was observed that chromosome pairs II and X were metacentric, while chromosome pairs I, III, IV and VI were submetacentric and chromosome pair V was subtelocentric (Table 1).

A lot of chromosome count studies were performed in *O. montanum*, which is also subject to the current study, are 14, 18, 18+B, 30 (Federov 1974; Özhatay 2002; Bedini et al. 2010). In the current study the chromosome number was $2n = 14$ (Figure 2b). A difference in the number of chromosomes is considered as normal even in different populations of the same species. Here, a detailed karyotype analysis is given for the first time. Karyograms and idiograms were studied and morphological details of chromosomes were obtained (Figures 3b, 4b). Based on chromosome analysis, it was observed that chromosome pairs II and VII were metacentric, while chromosome pairs I, III, IV and VI were submetacentric and chromosome pair V was subtelocentric.

In previous studies, the reported chromosome numbers of *O. pyrenaicum* are 16, 16+B, 18 and 24 (Federov 1974; Özhatay 2002; Bedini et al. 2010). The results of this study are consistent with the results of Özhatay (2002). In the current study, chromosome number was $2n = 24$ (Figure 2c). Karyograms and idiograms were studied and morphological details of chromosomes were obtained (Figures 3c, 4c). Here, a detailed karyotype analysis is given for the first time. Based on chromosome analysis, it was observed that chromosome pairs II and VII were metacentric, while chromosome pairs I, III, IV and VI were submetacentric and chromosome pair V was subtelocentric (Table 1).

In previous studies, the reported chromosome numbers of *O. sigmoideum* are 12, 14, 16, 16+B 18, 20, 24 and 34 (Federov 1974; Özhatay 2002; Bedini et al.

2010). In the current study, chromosome number was $2n = 14$ (Figure 2a). Difference in the number of chromosomes is considered as normal even in different populations of the same species. Karyograms and idiograms were studied and morphological details of chromosomes were obtained (Figures 3a, 4a). Here, a detailed karyotype analysis is given for the first time. Based on chromosome analysis, it was observed that chromosome pairs V and VII were metacentric, while chromosome pairs I, II, III and IV were submetacentric and chromosome pair VI was subtelocentric (Table 1).

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