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A MORPHOLOGICAL, ANATOMICAL AND CARYOLOGICAL STUDY ON ENDEMIC *ORNITHOGALUM ALPIGENUM* STAPF (HYACINTHACEAE)

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Abstract

Ornithogalum alpigenum Stapf is endemic to Turkey. Its morphological, anatomical and caryological properties were investigated. Cross-sections of root, scape and leaf parts of the plant were examined. Raphide were observed in scape cross section. Most of the anatomical properties were similar to the other member of Hyacinthaceae family. Chromosome number was determined as 2n = 2x = 22 in *O. alpigenum.* The mitotic chromosome number and karyotype of the species are reported for the first time in present study.

Key words: Anatomy, Hyacinthaceae, *Ornithogalum alpigenum,* Morphology.

Introduction

The genus *Ornithogalum* L. belonging to Hyacinthaceae family contains over 140 species widespread around the world. The genus is mostly distributed in South Africa and around the Mediterranean (Uysal *et al*., 2005). *Ornithogalum* is represented by 45 species in the flora of Turkey and 17 of these species are endemics (Cullen 1984; Davis *et al*., 1988; Uysal *et al*., 2005; Varol 2008; Düşen & Deniz, 2005; Dalgıc *et al*., 2009). Turkey has a rich plant diversity with a high ratio of Endemism (Akyol *et al*, 2014). *Ornithogalum* is a taxonomically difficult genus, while its morphology is poorly correlated with the variation in chromosome number and karyotype. Since several decades, this taxonomically extremely difficult genus was studied several times for bulb structure and germination-type (Zahariadi, 1962 ; Speta, 1990, 1998), classical cytotaxonomy (Peruzzi & Passalacqua, 2002; Garbari *et al*., 2003; Tornadore *et al*., 2003; Aquaro & Peruzzi, 2006), chemotaxonomy (Øvstedal, 1991), morphometry (Moret *et al*., 1991; Øvstedal, 1991; Raamsdonk & Heringa, 1987; Moret, 1992; Moret & Galland, 1992; Coskuncelebi *et al*., 2002), seed micromorphology (Coskuncelebi *et al*., 2000).

There are no detailed morphological, anatomical and caryological studies on *Ornithogalum alpigenum* Stapf. species. In this research, detailed morphological, anatomical and caryological features of *O. alpigenum* were studied.

Material and Methods

Materials were collected from B1 Manisa Spil Mountain. Specimens were kept in the herbarium at Celal Bayar University. Morphological illustration of the plant taxon was made from fresh and dry specimens following "Flora of Turkey" (Davis, 1988). Morphological measurements were made from root, scape and leaf of fresh plant material. For anatomical studies plant specimens were fixed in 70% ethanol. Hand cut sections were stained with sartur reactive and safranin and photographed with motorized Leica DM 3000 microscope. Measurements of root, stem and leaf cell sizes were taken using ocular-micrometer. Minimum, maximum, mean and standard deviation was determined.

Results

Morphological findings: Scape 7-12 cm long and glabrous. Leaves 3-7 very narrowly linear to filiform with a white line on the upper surface exceeding the scape. Raceme at first ovate, later corymbose. Pedicels with erect 4-7 flowers at anthesis. Perianth segments white inside, white with a green fascia outside, 7-11 mm. Fruiting pedicels arcuate spreading, lowermost 20-40 mm. Capsule unwinged (Fig. 1).

Anatomical structure

Root: Epidermis single-layered covered by thick cuticle on the outer surface of root. Cortex is 5-9 layered and parenchymatous cells are circular or elliptical shaped. Intercellular spaces absent in the cortex. Endodermis single-layered. The wall thickenings of the endodermal cells are three sided towards to cortex. The pericycle has thin-walled cells. The number of protoxylem ridges between the phloem strands are 4-5 (Figs. 2A, B).

Scape: Shape of scape is circle. Outer part of scape is covered by thick cuticle. Epidermis is single-layered with cortical parenchyma under epidermis. Cortical cells are thin walled, parenchymatous and have intercellular spaces. Raphide are present in the cortex. The sclerenchymatic tissue is 5-6 layerd. Vascular bundles located in three row. The number of vascular bundles in the vascular cylinder is 27–30. Vascular bundles are of the collateral type and they begin with sclerenchymatic tissue. In outer circles, the bundles are smaller than the inner bundles. The center of the scapes (the pith region) is composed of parenchymatous cells. Raphides are present in the cortical cells. (Fig. 2C, D, E, F, Fig. 3D, E).

Leaf: The epidermis of leaves is uniseriate in the species and this is surrounded by a thickened cuticle. The adaxial and abaxial epidermal cells are rectangular-oval shaped and the adaxial epidermis cells are larger than the abaxial ones. A mesophytic stoma is present on both sides of the leaves, raphides are found in the mesophyll leaves. 8-12 layered mesophyll is equifacial and differentiated as palisadeand spongy parenchyma. Uniseriate layer ofpalisade parenchyma is present on both of adaxial andabaxial sides. Spongy cells areoval shaped and often broken down in the middle of theleaf.There are very large lacunae in mesophyll. Big and small sized vascular bundles are located in mesophyll (Fig. 3 A, B, C; Table 1).

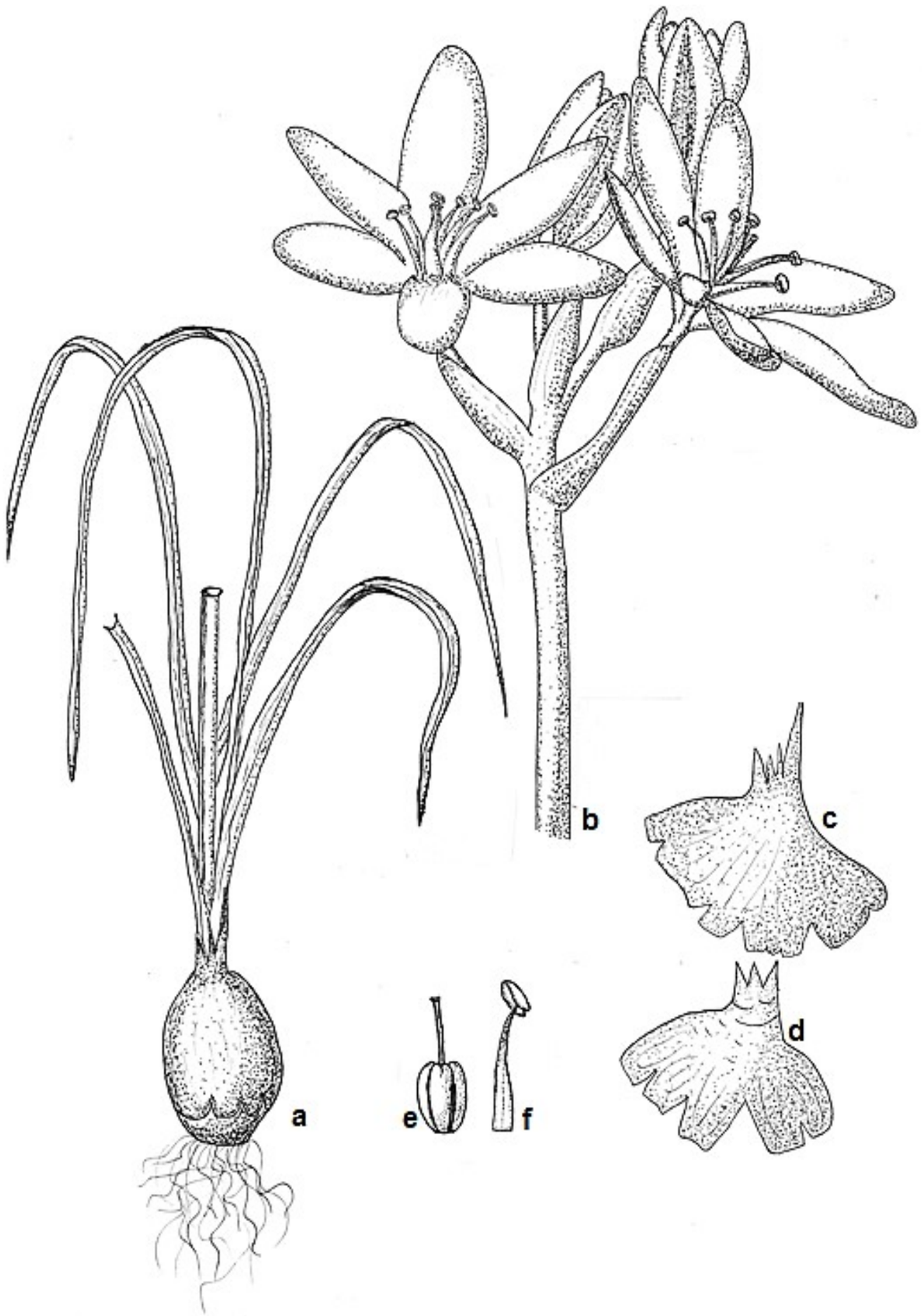

Fig. 1. Morphological illustration of *O. alpigenum*; a: bulb and leaves, b: flowers, c: inner corm cover, d: outer corm cover, e: pistil, f: stamen.

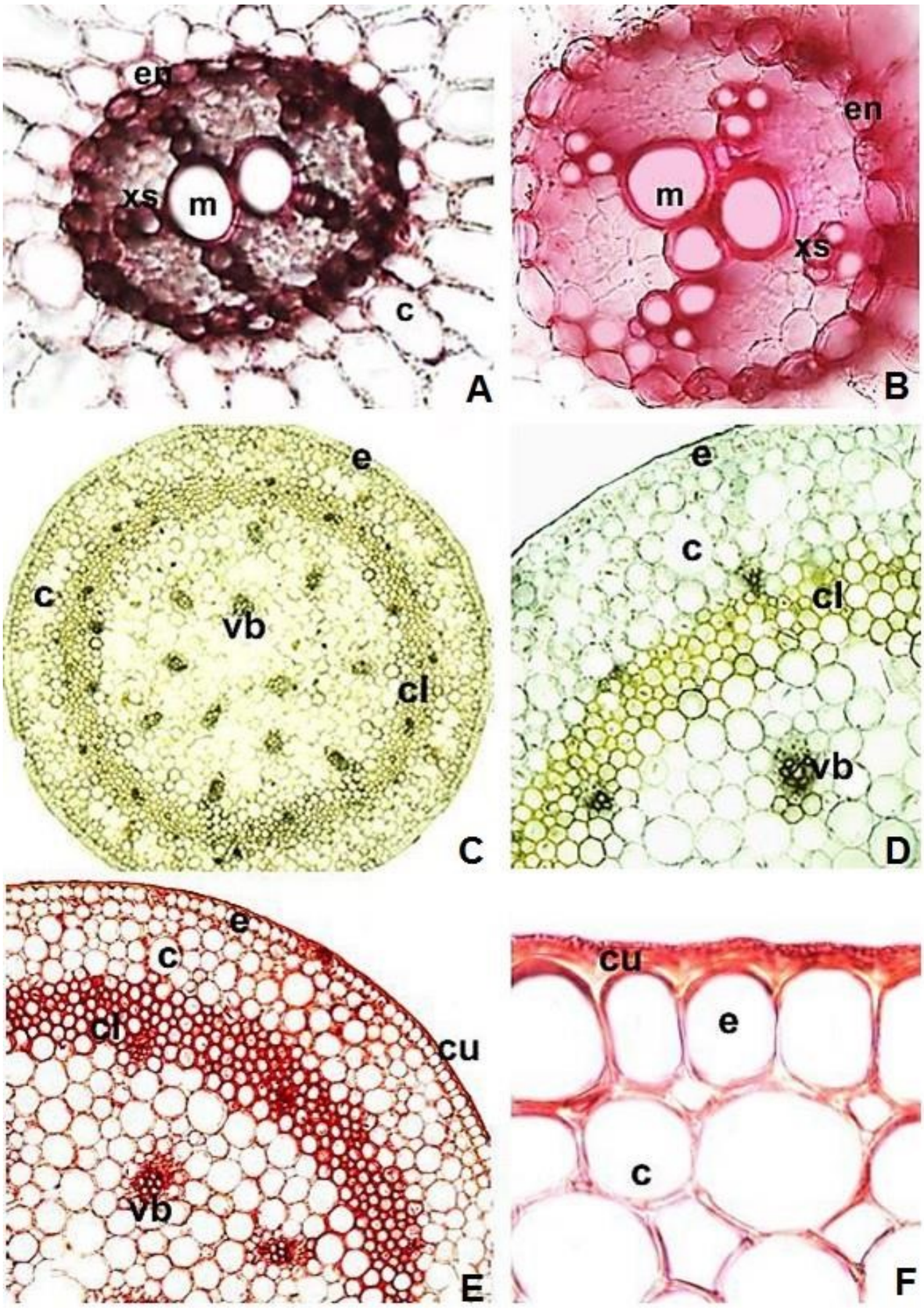

Fig. 2. A, B: Root, C, D, E, F; Scape cross section of *O. alpigenum,* c: cortex, cl: collenchyma, cu: cuticle, en: endodermis, m: metaxylem, p: perisikl, vb: vascular bundle, xs: xylem strand.

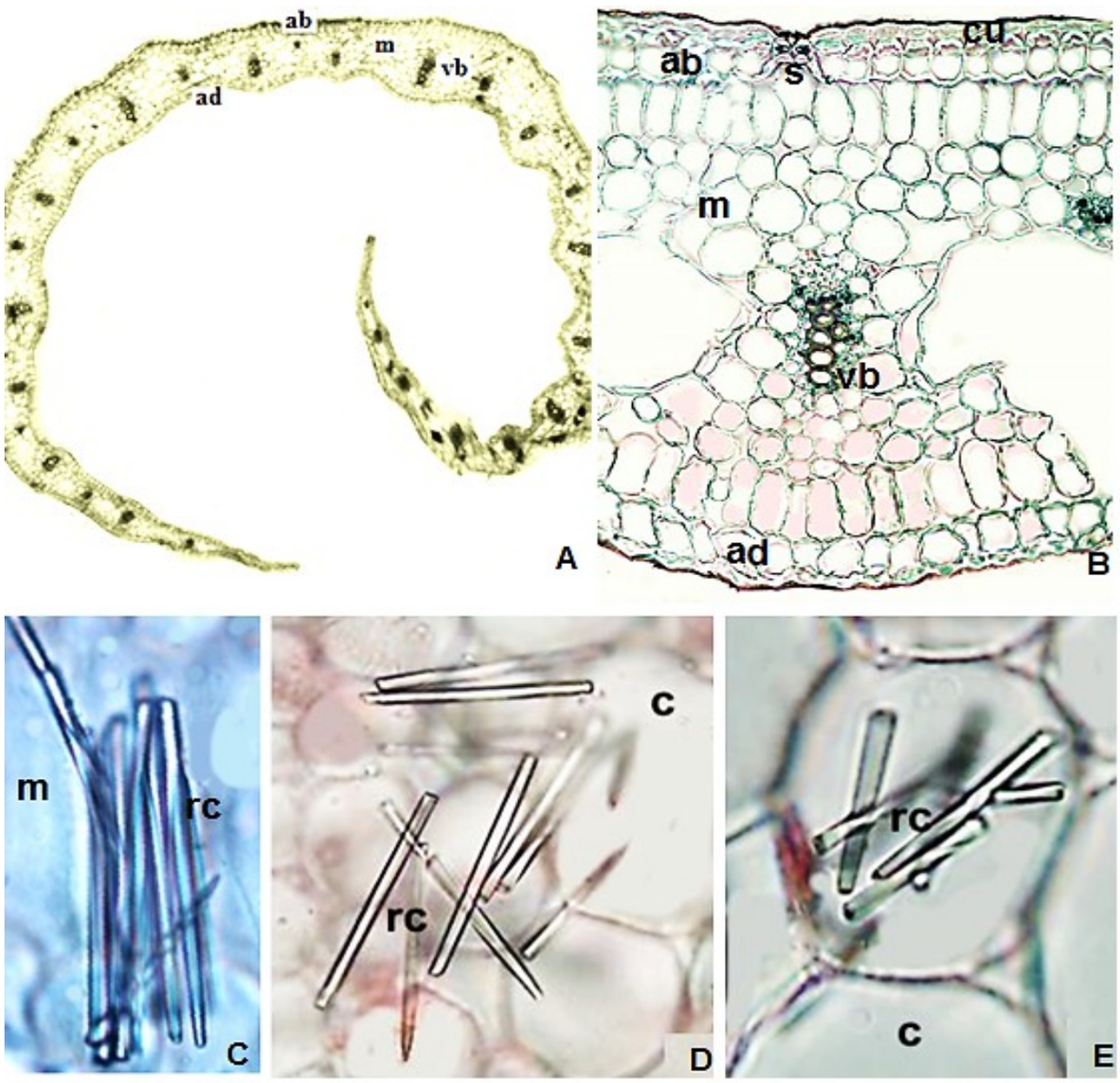

Fig. 3. A, B: Leaf, C: leaf D, E: Scape cross section of *O. alpigenum;* ab: abaxial epidermis, ad: adaxial epidermis, c: cortex, cu: cuticle, m:mesophile, rc: raphide crystals vb:vascular bundle.

Table 1. Anatomical measurements of *O. alpigenum*.

	Width (µm)		Length (µm)	
	Min.-Max.	**Ort ± S.D.**	**Min.-Max.**	**Ort ± S.D.**
Root				
Epidermis	22-43	31±9.0	12-35	27±7.0
Cortex	32-80	59±11		
Endodermis	15-24	19±1.7	12-16	14±1.1
Metaxylem	30-70	42±7.8		
Stem				
Cuticle	7.0-15	10±3.0		
Epidermis	17-35	26±5.2	25-32	29±2.8
Cortex	17-45	27±10		
Pith	32-80	54±21		
Leaf				
Adaxial epidermis	17-32	25±5,9	12-20	15±5.5
Palisade	20-35	31±8.0	50-72	60±12
Spongy	23-39	29±2.0		
Abaxial epidermis	25-48	35±4.1	15-26	21±2.1

Karyological findings: Details of karyotype analyses in the *O. alpigenum* are presented in Table 2 and Figs. 4, 5. Chromosome number is 2n = 2x = 22. 4 pairs metacentricand 7 pairs sub-metacentric chromosomes are present in the species. Centromere of the 4th, 5th, 7th and 11th chromosomes were at the median region; the 1th, 2th, 3th, 6th, 8th, 9th, and 10th chromosomes was at the submedian. No satellites were observed in the karyotype of the species. The size of chromosomes varied from 3.00 µm to 7.00 µm and the longest arm was 4.50 µm and the shortest arm was 1.25µm (Figs. 4 and 5).

Discussion

In this study, we investigated the morphological, anatomical, and caryological characters of *O. alpigenum* an endemic species to Turkey. The cross-sections of roots show a common general view of primer roots. The cortex

parenchyma layer has 5-9 rows in the roots of *O. alpigenum*. Çıtak *et al*. (2014) have reported 5-6 rows in the endemic *O. chetikianum*; however, *O.demirizianum* has 7–8 rows in root cortex. There are 4-5 protoxylem ridgesbetween the phloem strands in *O. alpigenum*root. *O. chetikianum* comprises 5 protoxylem arms, *O. demirizianum* root has 4 number (Çıtak *et al*., 2014).

In our study, the cortex parenchyma has 5-9 rows in *O. alpigenum*scape. The cortex parenchyma has 4–5, 2-3, 3-4, 2-3 rows respectively in *O. chetikianum*, *O. demirizianum, O. nutans a*nd *O. boucheanum* (Çıtak *et al*., 2014, Meriç *et al*., 2011). In most species of subfamily Ornithogaloideae, vascular bundles are in two rows and large bundles alternate with small bundles (Lynch *et al*., 2006). Vascular bundles are in 3 rows in *O. alpigenum.*The number of vascularbundles in the vascular cylinder is 27–30 in *O. alpigenum.* Çıtak *et al*. (2014) have reported that 25-35 vascular bundles in *O. chetikianum*, 20–25 in *O. demirizianum*. Meriç *et al*. (2011) have reported *O. nutans* has 35–39, *O. boucheanum* has 37–43 number vascular bundles in scape cross section. In scape anatomy, the number of layers of cortex parenchyma and the number of vascular bundles in the vascular cylinder can be used as diagnostic characters for distinguishing species.

Lynch *et al*. (2006) reports that Hyacinthaceae has a wide range of variation in the crystal types. There are raphide, styloid and druse crystals in this family (Lynch *et al*., 2006). Raphide crystals present in stem cortex and leaf mesophyll of *O. alpigenum.* The morphology and distribution of crystals within a species are very important, because it is controlled genetically (Franceschi & Nakata, 2005). Thus the constancy of crystal type and distribution can be viewed as a taxonomic character for classification of species.

The mesophyll of *O. alpigenum* consist of 2 different types of parenchymatous cells (cylindrical palisade and oval spongy). *O. chetikianum* and *O. nutans* mesophyll are composed of 2 different types of parenchymatous cells while *O. demirizianum* and *O. boucheanum* has monotypic parenchymatous cell (only cylindrical palisade) (Çıtak *et al*., 2014, Meriç *et al*., 2011).

The chromosome number of *Ornithogalum alpigenum* is 2n = 22, while different chromosome counts are reported in *O. narbonense* (2n = 14, 14 + 1B, 14 + 2B, 14 + 5B, 16, 18, 22, 24, 28, 36, 46, 52, 52 + 2B, 52 + 11B, 54, 88), *O. sorgerae* (2n = 18), and *O. pyrenaicum* subsp. *sphaerocarpum* (2n = 16, 16 + 2B, 17, 18, 24, 32) (Lungeanu, 1972; Murin & Majovsky, 1979; Wittmann, 1985; Tornadore, 1986; Moret, 1987; Galland, 1988; Davis *et al.*, 1988b; Druskovic & Lovka, 1995; Markova & Goranova, 1996).

Table 2. Parameters of mitotic metaphase chromosomes of *O. alpigenum*.

Chromosome No.	Total chromosome length (C) µm	Long arm length (L) µm	Short arm length (S) µm	Arm ratio R: L/S	Centro meric index I: (S/C). 100 µm	Relative size	Centromeric position S.D
1	7.00	4.50	2.50	1.80	35.71	12.00	Sm
2	6.50	4.50	2.00	2.25	30.76	11.25	Sm
3	6.00	4.00	2.00	2.00	33.33	10.38	Sm
4	6.00	3.50	2.50	1.40	41.66	10.38	M
5	5.50	3.25	2.25	1.44	40.90	9.52	M
6	5.00	3.25	1.75	1.85	35.00	8.65	Sm
7	5.00	3.00	2.00	1.50	40.00	8.65	M
8	4.75	3.00	1.75	1.71	36.84	8.22	Sm
9	4.50	3.25	1.25	2.60	27.77	7.79	Sm
10	4.50	3.00	1.50	2.00	33.33	7.79	Sm
11	3.00	1.75	1.25	1.40	41.66	5.19	M

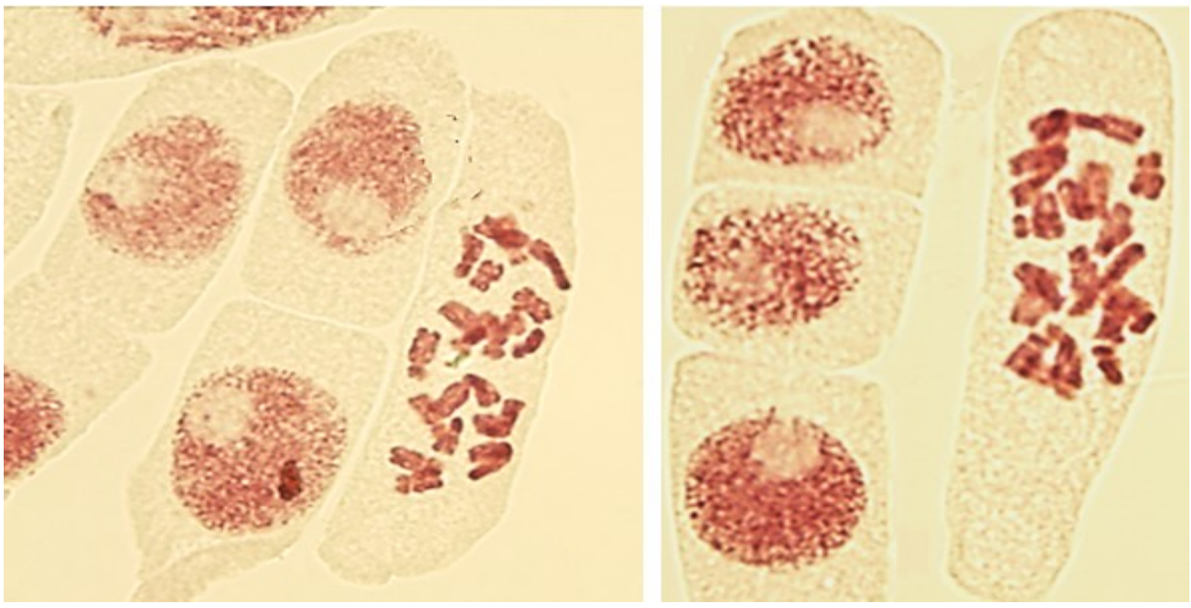

Fig. 4. Microphotograph of somatic metaphases of *O. alpigenum*.

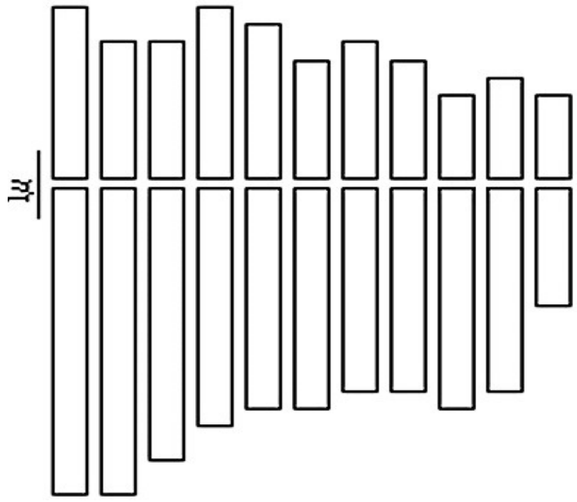

Fig. 5. Chromosome Ideogramof *O. alpigenum*.

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