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CHROMOSOME AND POLLEN MORPHOLOGY OF THE RARE ENDEMIC *CENTAUREA LYCOPIFOLIA* BOISS. & KOTSCHY

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Abstract

Chromosome and pollen morphology of *Centaurea lycopifolia* Boiss. & Kotschy were studied. The chromosome number is 2n = 34 with haploid karyotype formula 9m + 9sm. Metaphase chromosome length ranging from 6.16 to 2.23 µm and the total haploid chromosome length was 65, 85 µm. The light and scanning electron microscope investigations revealed spheroidal-subprolate, the amb triangular and tricolporatae pollens in the taxon. Exine ornamentation was tectatae and microechinate-scabrate.

Introduction

The family Asteraceae is one of the largest families in flowering plants consisting of three subfamilies and 14 tribes which differ in respect of morphology, chemistry, cytology, and DNA sequences. Turkey is one of the significant centers of the genus *Centaurea* having 197 species of which139 are endemic (Wagenitz 1975).

A close correlation among karyology, pollen morphology and systematic in the subtribe Centaureinae has been demonstrated where basic chromosome numbers are considered a key character for sectional classification (Garcia-Jacas *et al*. 2006, Romaschenko *et al*. 2004). Members of the Jacea group show descending disploidy with chromosome numbers ranging from x = 12 to x = 7. The basic chromosome number of *Centaurea* is known to be x = 7, 8, 9, 10, 11, 12, 13, 14 and 15 (Gömürgen 2006). Moreover, chromosome numbers and karyotype information are important for the study of evolutionary patterns and thus useful in taxonomy in addition to morphological features (Stebbins 1971).

The pollen morphology of *Centaurea* was first investigated by Wagenitz (1955). More so, the palynological data have been used effectively for taxonomy of the genus in the recent years. Palynological characters of some Turkish *Centaurea* taxa have been described by Pehlivan (1995, 1996) and Gömürgen *et al*. (2009). The rare endemic *C. lycopifolia* Boiss. & Kotschy, is threatened [LR (nt)] according to the Red Data Book of Turkish Plants (IUCN 2001) and thus becomes an important material for detail investigation.

The analysis of karyotype together with pollen morphology have much important impacts for the revision and systematics of this species. Therefore, the purpose of the present study was to provide karyological and palyinological knowledge of *C. lycopifolia* - a rare endemic species in Turkey for the first time.

Material and Methods

Seeds of *Centaurea lycopifolia* were collected from C6 Adana: Kadirli, stony and rocky slopes, 650 m on 23 July 2009, H. Altınözlü 5600. The seeds were germinated at 25º C on moist filter paper in Petri dishes. Actively growing root tips of about

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1 cm in length were excised from the germinating seeds and pretreated in 0.002 M 8-hydroxyquinoline solution for 5 h at 18º C followed by fixation in ethanol-acetic acid (3 : 1) for 24 h. The root tips were then hydrolyzed in 1 N HCl at 60º C for 12 min and washed in distilled water. Staining was carried out in Feulgen for 1 h and squashed with 45% acetic acid. Using a Leica DM 4000B microscope, microphotographs of five good quality metaphase plates were taken and recorded with a Leica digital camera. The long arm (l), short arm (s) and total chromosome lengths (c) of each chromosome were measured on enlarged microphotographs. The relative lengths, arm ratios (r = l/s) and centromeric index (I = 100 s/c) were calculated and used to classify and determine homologous chromosomes. The chromosome nomenclature proposed by Levan *et al.* (1964) is used. A karyogram was constructed by arranging the homologous pairs from largest to smallest. The variation in chromosome length and chromosome arm ratio within the karyotype was estimated by calculating the standard deviation (SD) of these parameters.

Pollen material was obtained from dried flower specimens. The pollen morphology of this taxon was investigated through light and scanning electron microscope. Faegri and Iversen's (1975) terminology was used for naming the exine layers. For light microscopic investigations the method of preparation described by Wodehouse (1935) and Erdtman (1969) was followed. Pollen identifications and counts have done by Prior binocular microscope with a 10x oculor, 10, 40 and 100x plain oil immersion objectives. The exine and intine thickness of pollen were measured using 20 and 50 replicates. From the measurements, a natural mathematical mean is calculated. Microphotographs were taken at the Osmangazi University Science and Art Faculty, Department of Biology by Spot In-SIGHT Color Digital camera and an Olympus type microscope.

For scanning electron microscopy (SEM) investigations, unacetolyzed pollen grains were directly placed onto stubs, sputter-coated with gold, and examined with a Jeol 5600 LV scanning electron microscope (Walker 1974). Terminologies for pollen morphology proposed by Wodehouse (1935), Erdtman (1969), Walker 1974, Faegri and Iversen (1975) and Pehlivan (1995) were used.

Results and Discussion

The somatic chromosome number of *C. lycopifolia* was found to be 2n = 36 without any satellite (Fig. 1).

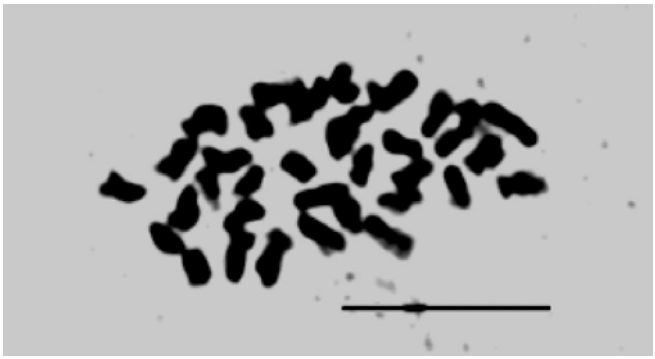

Fig. 1. Somatic metaphase chromosomes of *Centaurea lycopifolia* (2n = 36).
Bar represents 10 μm.

The haploid karyotype formula is n = 9m + 9sm. Long and short arm with SD, total length of the chromosomes, arm ratio (r = l/s) and relative length are given in Table 1. The range of

metaphase chromosome lengths was 6.16 to 2.23 µm. The total length of the haploid set was 65.85 µm. The karyogram and idiogram of haploid chromosome set are presented in Figs 2 - 3. *C. lycopifolia* has a longer choromosome set than some other *Centeaurea* species (Martin *et al*. 2009).

Table 1. Morphometric data on chromosomes of *Centaurea lycopifolia.*

Chromo-some pair	Long arm (µm)	Short arm (µm)	Total length (µm)	Arm ratio (r = l/s)	Centromeric index	Relative length	Centromeric position
I	3.70 ± 0.83	2.46 ± 0.77	6.16 ± 1.28	1.50	39.9	9.35	m
II	3.15 ± 0.69	2.35 ± 0.65	5.50 ± 1.18	1.34	42.7	8.35	m
III	3.12 ± 0.90	1.84 ± 0.42	4.96 ± 1.03	1.70	37.1	7.53	sm
IV	3.34 ± 0.53	1.52 ± 0.59	4.86 ± 1.07	2.20	31.3	7.38	sm
V	2.70 ± 0.71	1.66 ± 0.28	4.36 ± 0.77	1.63	38.1	6.62	m
VI	2.27 ± 0.35	1.84 ± 0.52	4.11 ± 0.81	1.23	44.8	6.24	m
VII	2.06 ± 0.33	1.68 ± 0.32	3.74 ± 0.59	1.23	44.9	5.68	m
VIII	2.37 ± 0.48	1.36 ± 0.31	3.73 ± 0.71	1.74	36.5	5.66	sm
IX	1.93 ± 0.27	1.43 ± 0.34	3.36 ± 0.47	1.35	42.6	5.10	m
X	2.41 ± 0.58	0.94 ± 0.23	3.35 ± 0.48	2.56	28.1	5.08	sm
XI	2.27 ± 0.51	0.92 ± 0.26	3.19 ± 0.43	2.47	28.8	4.84	sm
XII	2.01 ± 0.21	1.00 ± 0.22	3.01 ± 0.33	2.01	33.2	4.57	sm
XIII	1.64 ± 0.14	1.27 ± 0.30	2.91 ± 0.34	1.29	43.6	4.41	m
XIV	1.88 ± 0.25	0.93 ± 0.25	2.81 ± 0.32	2.02	33.1	4.27	sm
XV	1.56 ± 0.21	1.14 ± 0.25	2.70 ± 0.35	1.37	42.2	4.10	m
XVI	1.71 ± 0.32	0.93 ± 0.15	2.64 ± 0.35	1.83	35.2	4.00	sm
XVII	1.45 ± 0.29	0.78 ± 0.14	2.23 ± 0.35	1.86	34.9	3.39	sm
XVIII	1.19 ± 0.12	1.04 ± 0.15	2.23 ± 0.25	1.14	46.6	3.39	m

m = Median, sm = Submedian, ± = Standard deviation.

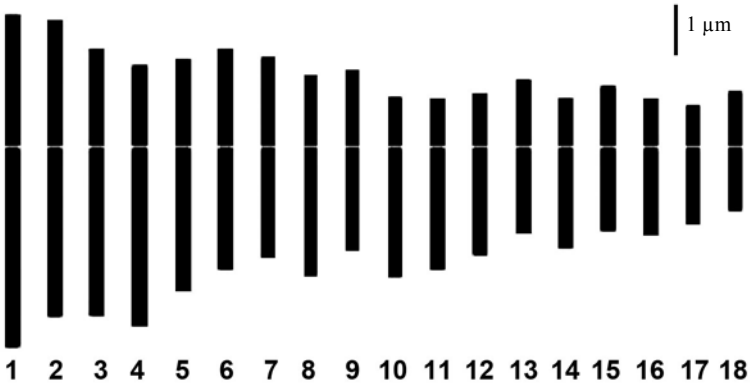

Fig. 2. Haploid idiogram of *Centaurea lycopifolia.*

Chromosome number of *C. lycopifolia* was first reported by Gömürgen (2006), Uysal *et al*. (2009). Garcia Jacas *et al*. (2006) stated that the status of section *Pseudoseridia* was highly dubious and thus evaluated *C. lycopifolia* with the species of section *Cheriolepis.* Uysal *et al*.

(2009) also placed this species in the section *Cheirolepis.* Polyploidy is frequent in the section and its basic chromosome number is x = 9 (Romaschenko *et al*. 2004, Uysal *et al.* 2009). Authors results indicate that somatic chromosome number of *C. lycopifolia* was 2n = 36. They can evaluate this taxon as tetraploid because of the basic chromosome number section *Cheriolepis* These results were in agreement with the previous counts of C. *lycopifolia*. (Gömürgen 2006, Uysal *et al*. 2009) and also in agreement with basic chromosome number of section *Cheriolepis.*

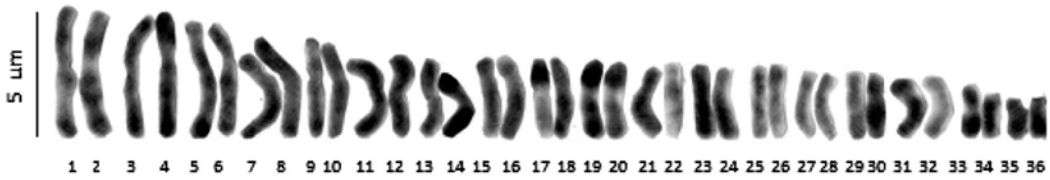

Fig. 3. Diploid karyogram of *Centaurea lycopifolia.*

Mean, SD and variation of palynological measurements are given in Table 2. The pollen grains of *Centaurea lycopifolia* are spheroidal-subprolate, the amb triangular and tricolporatae, P/E = 1.18 (N), 1.10 (A). Ornamentation is tectatae, microechinate-scabrate. The polar axis measured 35.48 µm (N), 32.2 µm (A), and the equatorial axis 30 µm (N), 29.28 µm (A), Amb was

Table 2. Numeric results from palynological measurements in *Centaurea lycopifolia.*

Treatments	P (range) (µm)	E (range) (µm)	P/E	L (range) (µm)	Clg (range) (µm)	Clt (range) (µm)	Plg (range) (µm)	Plt (range) (µm)	t (range) (µm)	Exine (range) (µm)	Intine (range) (µm)
Non-acetolysed	35.48 ± 2.95 (41 - 29)	30 ± 2.20 (35 - 25)	1.18	32.28 ± 2.97 (38 - 25)	26.28 ± 2,39 (32 - 22)	3.6 ± 2.02 (11 - 2)	9.48 ± 2.00 (12 - 6)	7.28 ± 1.88 (10 - 4)	11.44 ± 1.47 (14 - 9)	1.62 ± 0.46 (2 - 1)	1 ± 0 (1 - 1)
Acetolysed	32.2 ± 2.27 (36 - 27)	29.28 ± 2.48 (34 - 26)	1.10	28.46 ± 2.68 (33 - 22)	23 ± 4.70 (28 - 14)	5.96 ± 2.07 (9 - 3)	10.36 ± 2.93 (18 - 5)	7.2 ± 1.61 (10 - 4)	10.64 ± 1.22 (13 - 9)	1.86 ± 0.42 (3 - 1)	-

P = Polar axis, E = Equatorial axis, Clg = Length of colpus, Clt = Width of colpus, Plg = Length of porus, Plt = Width of porus, t = Apocolpium, ± = Standard deviation.

triangular and 32.28 µm (N), 28.46 µm (A) in diameter. The apocolpium was 11.44 µm (N), 10.64 µm (A) in diameter. Exine 1.62 µm (N), 1.86 µm (A). The exine has one layer of columellae beneath spines, microspine length 0.5 µm, and spinule width 1.1 µm. Exine tectate, microechinate-scabrate, 86 spinule in 100 μ^2 and average distance between spinules 0.6 µm (Table 2). Colpi ends are rounded Clg 26.28 µm (N), 23 µm (A), Clt 3.6 µm (N), 5.96 µm (A). The pores are transversely elongated; Plg 9.48 µm (N), 10.36 µm (A), Plt 7.28 µm (N), 7.2 µm (A). The pore latitude is wider than the colpi latitude. The surface ornamentation is microechinate under LM, scabrate under SEM (Fig. 4 a-f).

The results of the light and scanning electron microscope revealed the spheroidal-subprolate and tricolporatae in *C. lycopifolia* taxon. It was also determined that the exine was tectatae, microechinate-scabrate. The pollen morphology of exhibits a close relationship to the other *Centaurea* species inhabiting Turkey. However, pollen surface, exine structure, pollen and amb shape, spinule dimensions and spinule density of this taxon was different from other *Centaurea* species (Pehlivan 1996).

The essential criteria for the determination of the philogenetic relationship of the characteristics of the aperture and exine function of this species has been reported in the literature (Walker 1974).

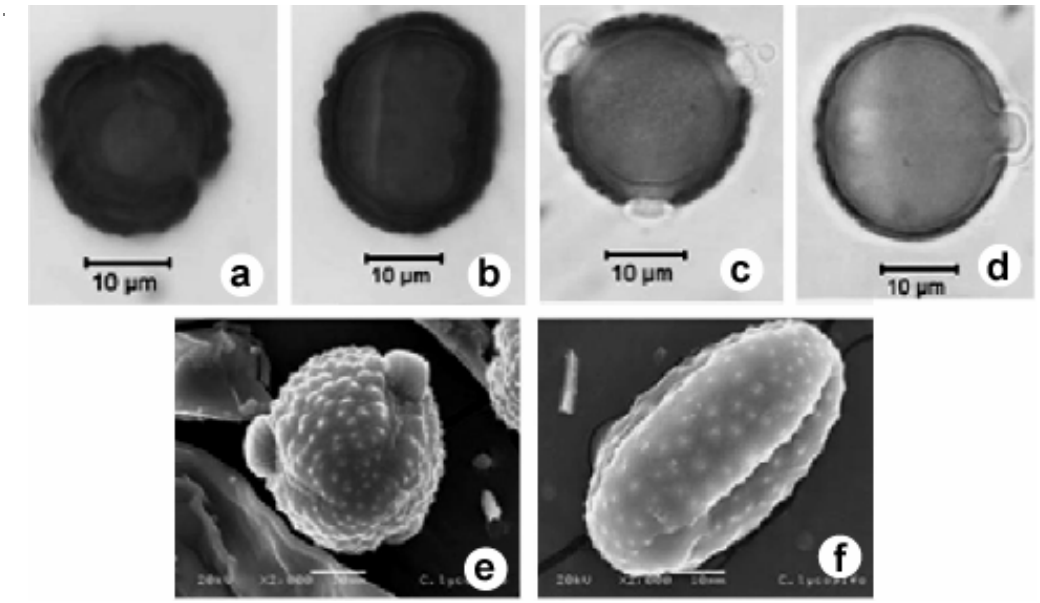

Fig. 4 a-f. Pollen microphotography of *Centaurea lycopifolia.* (a) Polar view of a non acetolysed pollen in light microscope, (b) Equatorial view of a non acetolysed pollen in light microscope. (c) Polar view of an acetolysed pollen in light microscope. (d) Equatorial view of an acetolysed pollen in light microscope. (e) Polar view of a non acetolysed pollen in SEM. (f) Equatorial view of a non acetolysed pollen in SEM.

Analysis of this taxon, it was observed that determined genetic distinctions encompassed differences in the measurements determined, raising objections to the possession of a morphological characteristic passing to the pollen structure of this species (Cronquist 1968). Authors believe that they may have distinguished a criterion in the pollen morphology of *Centaurea lycopifolia* taxon's systematic system which characteristizes ancillary sequence. This study at the same time has also shed light on the exposed systematic-philogenetic relationship of investigated taxon. The determination of the taxon's pollen morphological structure has led them to better consider the usefulness of pollen studies in distinguishing the characteristics possessed by taxon.

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