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CHROMOSOME NUMBER, KARYOTYPE ANALYSIS AND POLLEN MORPHOLOGY OF TURKISH ENDEMIC *TORDYLIUM ELEGANS* (BOISS. & BAL.) ALAVA & HUB.-MOR. (APIACEAE)

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Abstract

The chromosome number, karyotype and pollen analysis of Turkish endemic species *Tordylium elegans* (Boiss. & Bal.) Alava & Hub.-Mor. are reported in this paper for the first time. The somatic chromosome number is determined as 2n = 4x = 16. It is a tetraploid species and the basic chromosome number is x = 4. Haploid karyotype formula is 4sm + 3m + 1T. The pollen morphology of *T. elegans* was examined under light and scanning electron microscope. Pollen of *T. elegans* are radially symmetrical, isopolar, tricolporate, perprolate and equatorially-constricted.

Introduction

The Apiaceae (Umbelliferae) are mostly temperate herbs almost always with umbellate inflorescences comprising about 300 genera and 3,000 species. The relationship at higher level within Apiaceae is not clear particularly in the largest subfamily Apioideae. Molecular phylogenetical analysis systems were also used for clarifying the evolutionary history of subfamily Apioideae (Downie *et al*., 2000; Downie *et al*., 2001; Ajani *et al*,. 2008). Apiaceae has 4 endemic genera and 140 endemic species in Turkey (Pimenov & Leonov, 2004). This means that in Asiatic Turkey Apiaceae has not only the highest concentration of endemism at species-level but also the species diversity in Asia and probably in the world. The genus *Tordylium* L., includes 17 species in Turkey and 9 of them are endemic to Turkey (Alava, 1972; Duman, 2000). *T. elegans* (Boiss. & Bal.) Alava & Hub.-Mor. is one of these endemic species of Turkey. It is an East Mediterranean element. It grows on rocky places, fields, roadsides, from sea level to 2140 m.

Chromosome number has been recorded only in 10 *Tordylium* species (Tamaschjan, 1933; Garde & Garde, 1954; Runemark, 1968; Al-Eisawi & Jury, 1988; Al-Eisawi, 1989; Capineri *et al*., 1978; Dobes *et al*., 1997; Silvestre, 1978; Silvestre, 1993; Strid & Franzén, 1981; Geldykhanov, 1986; Baltisberger & Baltisberger, 1995; Vogt & Aparicio, 1999; Constance *et al*., 1971, 1976). However, there are no reports on the chromosome number and karyotype of *T. elegans*. According to Anderson (1937) cytological evidence, can do more than discriminate between species. Cytological evidence has assisted clarifying taxonomic relationships in a large number of genera. As a rule, systematic and cytological approaches have led to conclusions, which were in general agreement (Frankel, 1941). Cytological characteristics and pollen morphology have a significance exceeding that of ordinary morphological characters, so chromosome number identification and pollen analysis of the species are therefore significant. The purpose of the present study is to determine chromosome number to analyze the chromosome and pollen morphology of endemic *T. elegans*.

Material and Methods

Seeds and samples of *T. elegans* were collected from Osmaniye, Kadirli, around Ciccik Village, 150-200 m, near roadsides in 2006 by the last author. Voucher specimens were deposited at Hacettepe University Herbarium (H.Ü.).

Karyotype: The study was performed on actively growing root tips. The seeds were germinated at room temperature (20°C) on moist filter paper in Petri dishes. Preparations were made according to the method given by Gömürgen *et al*., (2005). Digital photos of five well spread metaphase plates were taken by the Nikon Eclipse E600 light microscope. The chromosomes in the karyotype were ordered by decreasing length. The detection of the homologous chromosomes and the determination of their position in the karyograms were carried out following the method proposed by Levan *et al*., (1964). The measurements obtained from ten-long and short arm length-metaphase plates allowed the construction of the idiograms of the taxon. Permanent slides are stored in the Department of Biology, Hacettepe University, Ankara.

Pollen: Pollen slides were prepared according to Erdtman's (1960) method. The LM studies were examined under Olympus CX41 microscope with the aid of an apochromatic oil immersion objective and periplan eyepieces. Measurements were based on 50 pollen grains. Mean, standard deviation and variation of measurements were calculated according to Sokal & Rohlf (1995).

For scanning electron microscopy (SEM), the pollen grains were put on stubs, sputter-coated with gold plate and examined under a Jeol JSM-6060 scanning electron microscope.

The terminology used here is that of Erdtman (1952).

Results and Discussion

Chromosomes: The somatic chromosome number of *T. elegans* is 2n = 16 (Fig. 1). Detailed chromosome parameters [long and short arm and their SD, total length of the chromosomes, arm ratio (r = ı/s), centromeric index (i = 100 × s/c)] are given in Table 1. The total chromosome length varies between 1.26 – 3.15 µm. It has a short chromosome set. Total haploid chromosome length is 17.67 µm. The difference between the longest and the shortest chromosome is 1,89 µm. Chromosome pairs 1, 2 and 7 have median, 3, 4, 5 and 6 have submedian and the 8th one has terminal centromere position. Terminal chromosomes (T) are the shortest pairs. The karyotype formula is 2n = 4x = 1T + 3m + 4sm. The basic number is x = 4. *T. elegans* is a tetraploid (2n = 4x = 16). This is the first chromosome count and karyotype analysis for this species. Karyogram and haploid chromosome set idiogram are given in Figs. 2-3.

Table 1. Chromosome parameters in *T. elegans* (2n=16). m=median, sm=submedian, T=terminal.

Chromosome pairs	Chromosome length (μm)			Arm ratio r=L/S	Centromeric index i=100xs/c	Relative length (%)	Centromere position
	Long arm (±SD)	Short arm (±SD)	Total				
I	1.93 (±0.615)	1.22(±0.127)	3.15	1.59	38.73	17.83	m
II	1.59 (±0.456)	0.99 (±0.220)	2.58	1.61	38.37	14.60	m
III	1.59 (±0.326)	0.84 (±0.225)	2.43	1.89	37.17	13.75	sm
IV	1.45 (±0.368)	0.81 (±0.181)	2.26	1.79	35.84	12.79	sm
V	1.35 (±0.367)	0.80 (±0.175)	2.15	1.69	37.21	12.17	sm
VI	1.30 (±0.345)	0.71 (±0.109)	2.01	1.83	35.32	11.38	sm
VII	1.06 (±0.298)	0.77 (±0.127)	1.83	1.38	42.08	10.35	m
VIII	1.26 (±0.333)	0.00 (±0.00)	1.26	∞	0.00	7.13	T

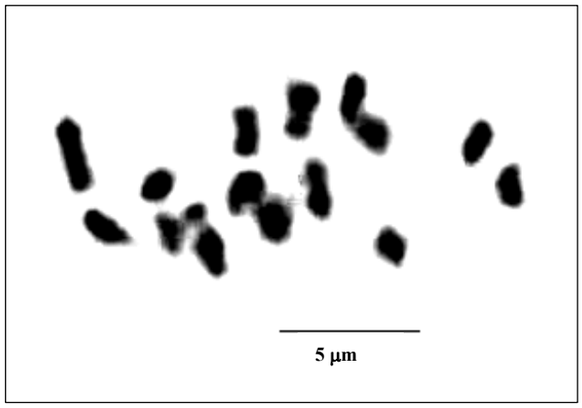

Fig. 1. Somatic metaphase chromosomes of *T. elegans* (2n=16).

Fig. 2. Karyogram of *T. elegans.*

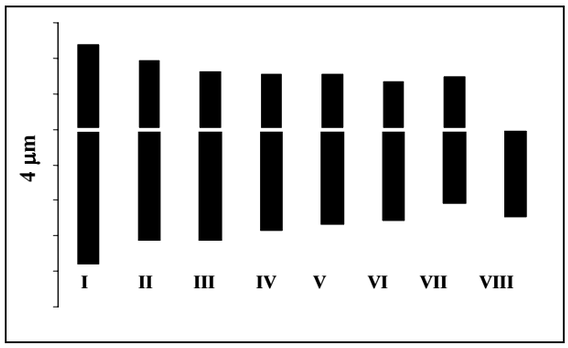

Fig. 3. Haploid idiogram of *T. elegans*.

Chromosome count of *Tordylium* species based on previous studies is given in Table 2. Although the first chromosome count of *T. maximum* was given as 2n = 22 by Tamaschan (1933), later researchers have reported that this species has 2n = 20 chromosomes (Runemark, 1968; Silvestre, 1978; Strid & Franzén, 1981; Geldykhanov, 1986; Baltisberger & Baltisberger, 1995; Dobes *et al*., 1997). Chromosome number of *T. aegaenum, T. apulum, T. pestalozzae, T. syriacum, T. cordatum, T. aegyptiacum* were given as n = 10, 2n = 20 (Garde & Garde, 1954; Runemark, 1968; Constance *et al*., 1976; Capineri *et al*., 1978; Al-Eisawi & Jury, 1988; Al-Eisawi, 1989; Vogt & Aparicio, 1999; Silvestre, 1993). *T. officinale*, has 2n=18 chromosomes (Silvestre, 1993). *T. hirtocarpum* has 2n = 8 chromosomes (Runemark, 1968). This is the lowest chromosome number reported for *Tordylium* species.

The most important studies about chromosome number of *Tordylium* genus were that of Runemark (1968) and Al-Eisawi & Jury (1988). According to Runemark (1968) the basic chromosome number of this group is x = 10. It is difficult to explain the presence of 2n = 8 (*T. hirtocarpum*). If basic chromosome number is x = 10 *T. hirtocarpum* must be derived from haploid number by loosing two chromosomes and it must be sterile but Al-Eisawi & Jury (1988) state that *T. hirtocarpum* is fertile. Constance *et al*. (1971) state chromosome number of *T. trachycarpum* (Boiss.) [Syn: *Ainsworthia trachycarpa* Boiss.] as n = 8 but later on another studies they gave chromosome number as n = 9 and n = 10 for this species (Constance *et al*., 1976). Al-Eisawi & Jury (1988) state the basic chromosome number as x = 4, because of the presence chromosome number 2n = 16 (*T. trachycarpum* (Boiss.) Al-Eisawi & Jury [Syn: *Ainsworthia trachycarpa,* n = 8] in this group. According to Al-Eisawi & Jury (1988) *Ainsworthia* are congeneric with the genus *Tordylium*. 2n = 16 is tetraploid and 2n = 20 is pentaploid species, derived from crossing between tetraploid with 2n = 16 and hexaploid 2n = 24 or it is derived from one or the other loosing or gaining four chromosomes. The authors accept that the basic number in the family range from x = 4 to x = 11. Our results (*T. elegans* 2n = 16) also support Al-Eisawi & Jury's (1988) idea of basic number x = 4.

Pollen: Pollen grains were found to be radially symmetrical, isopolar, tricolporate, perprolate and equatorially-constricted (dumb-bell shaped). The polar axis measured 29.83 μm and the equatorial axis 12.68 μm. Amb was triangular and 12.52 μm in diameter. The apocolpium was rather wide; colpi ends could not be seen in polar area.

In polar view, the exine was 4.39 μm thick at mesocolpia. The sexine was thicker than the nexine; sexine 3.41 μm, nexine 0.98 μm at mesocolpia. In equatorial view, the exine was 1.90 μm, the sexine 1.00 μm and the nexine 0.90 μm at apocolpia. At the end of the carina, the exine was 2.55 μm, the sexine 1.57 μm and the nexine 0.98 μm. In equatorial area, the exine was 3.23 μm, the sexine 2.09 μm and the nexine 1.14 μm. The carina was 5.25 μm and costa 1.67 μm thick.

Colpi ends were rounded, the margin was even and colpi short were not reaching to polar area; Clg 18.25 μm, Clt narrower than 0.98 μm. Pores transversely elongated; Plg 2.00 μm, Plt 2.78 μm. The pore latitude was wider than the colpus latitude.

Table 2. Previous chromosome counts for *Tordylium* L., species.

Species	Chromosome number	References
T. maximum L.	2n=22	Tamamschjan, 1933
	2n=20	Runemark, 1968, Geldykhanov, 1986, Baltisberger & Baltisberger, 1995, Strid & Franzén, 1981, Silvestre, 1978, Dobes *et al*., 1997
T. apulum L.	2n=20	Runemark, 1968, Capineri *et al*., 1978
T. pestalozzae Boiss.	2n=20	Runemark 1968
T. syriacum L.	n=10	Vogt & Aparicio, 1999
	2n=20	Garde & Garde, 1954, Silvestre, 1993
T. aegyptiacum Lam	n=10	Al-Eisawi & Jury, 1988
T. aegaeum Runem.	2n=20	Runemark, 1968
T. officinale L.	2n=18	Runemark, 1968
	n=9	Silvestre,1993
T. cordatum (Jacq.) Poir.	2n=20	Garde & Garde, 1954
	n=10	Constance *et al*., 1976
T. hirtocarpum Cand.	2n=8	Runemark, 1968
T.trachycarpum (Boiss.)	n=8	Constance *et al*., 1971
[*Synonym: Ainsworthia trachycarpa* Boiss.]	n=8	Al-Eisawi & Jury, 1988
	n=9	Constance *et al*., 1976
T.elegans (Boiss. & Bal)	n=10	Constance *et al*., 1976
Alava & Hub.-Mor	2n=16	Present study

The surface ornamentation was rugulate-striate under SEM (Fig. 4), perforate under LM (Fig. 5). Mean, SD and variation of palynological measurements are given in Table 3.

Al-Eisawi & Jury (1988) divided *Tordylium* species into 4 main groups according to pollen morphology. One of these groups was characterised having is dumb-bell shaped, equatorially constricted pollen and with discontinuous carinae. The pollen of *T. elegans* are small sized (Al-Eisawi & Jury, 1988). Which correspond to our findings.

Cerceau-Larrival (1963) classified the pollen grains of the sub-tribe Tordylinae under 'The equatorially-constricted type'. According to her study, this pollen type has a size range from 50-70 µm; the exine assumes a proportion such that in the equatorial zone, the pollen grain becomes winged on account of carina of the exine, the ectoapertures get smaller and are often scarcely visible (Al-Eisawi & Jury, 1988). The pollen of *T. elegans* are also equatorially-constricted and winged on account of carina of the exine. However, the pollen of *T. elegans* are smaller than Cerceau-Larrival's (1963) 'The equatorially-constricted type'.

According to Al-Eisawi & Jury (1988), *Ainsworthia* is congeneric with the genus *Tordylium*. Palynological characters of *T. elegans* and *A. trachycarpa* show very distinc-similarities such as pollen size, shape and exine ornamentation. Besides, chromosome number of both species is 2n = 16. Palynological characters and chromosome numbers of these species endorse each other.

Table 3. Numeric results from palynological measurements in *T. elegans*.

P/E	2.35
P ± std (range) µm	29.83 ±2.51 (25.48-36.24)
E ± std (range) µm	12.68 ±1.21 (9.80-15.68)
Amb ± std (range)µm	12.52 ±1.16 (9.80-14.70)
Exine at msc. µm	4.39 (3.92-5.88)
Exine at apc. µm	1.90 (1.47-1.96)
Exine at end of car. µm	2.55 (2.45-2.94)
Exine at equa. area µm	3.23 (2.94-3.92)
Carina µm	5.25 (4.0-6.84)
Costa µm	1.67 (1.47-1.96)
Clg µm	18.25
Clt µm	<0.98
Plg/ Plt	0.72
Plg µm	2.00
Plt µm	2.78
Aperture number/type	3-colporate

P=polar axis, E=equatorial axis, Amb=diameter in polar view, msc.=mesocolpia, apc.=apocolpia, car.=carina, equa.=equatorial, Clg=length of colpus, Clt=width of colpus, Plg=length of porus, Plt=width of porus.

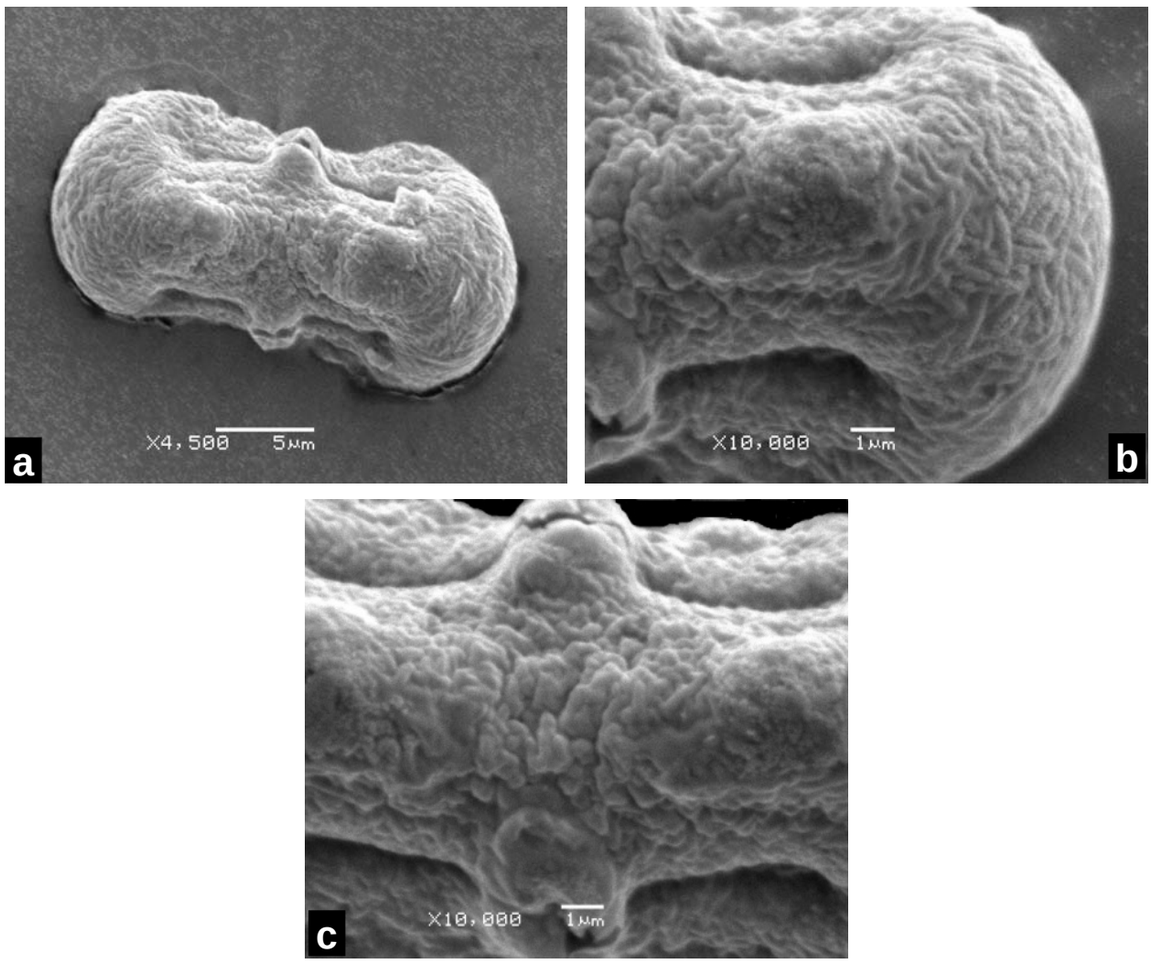

Fig. 4. SEM micrographs of pollen grains of *T. elegans*. (a) equatorial view, (b) exine ornamentation, (c) detailed view of apertures.

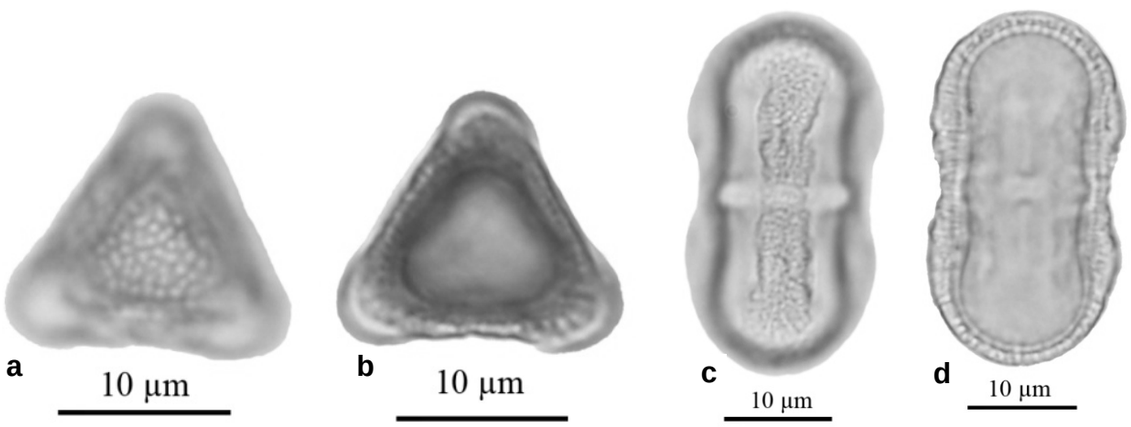

Fig. 5. LM micrographs of pollen grains of *T. elegans*. (a)-(b) polar view (a=low focus, b=high focus), (c)-(d) equatorial view (c=low focus, d=high focus).

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