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Chromosome numbers and karyotypes of some taxa of genus *Artemisia* (Asteraceae, Anthemideae) subgenus *Dracunculus* (Bess.) Rydb

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Abstract — Chromosome numbers and karyotypes of four taxa of genus *Artemisia* subgenus *Dracunculus* were investigated. Chromosome numbers of three varieties (var. *campestris*, var. *marschalliana* and var. *araratica*) of *Artemisia campestris* were found as 2n=4x=36. In the present work, chromosome number of *A. campestris* var. *araratica* and detailed karyotypes of *A. campestris* var. *marschalliana* and *A. scoparia* are presented for the first time. The chromosome complement of *A. campestris* var. *marschalliana* (2n=4x=36) consists of 28 median and eight submedian chromosomes. That of *A. scoparia* (2n=2x=16) consists of 16 median- centromeric chromosomes. We believe that these karyological data will enhance the karyological knowledge of subgenus *Dracunculus* and will prove to be an important source of information for new researches relating to genus.

Key words: *Artemisia*, chromosome numbers, *Dracunculus*, karyotype, Turkey.

INTRODUCTION

The genus *Artemisia* L. is one of the largest of the Asteraceae, with more than 500 species according to different researchers (MCARTHUR and PLUMMER 1978; MABBERLEY 1990; LING 1991a, b; BREMER and HUMPHRIES 1993; LING 1995 a, b; KADEREIT and JEFFREY 2007; FUNK *et al.* 2009). It is an eminent wind pollinated cosmopolitan genus, chiefly found in temperate regions of mid to high latitudes of the northern hemisphere, settled in arid and semiarid climates areas and has only a small number of representatives in southern hemisphere (VALLÈS and MCARTHUR 2001).

After different systematic reshufflings (MCARTHUR *et al*. 1981; LING 1991a, b; LING 1995a, b; KORNKVEN *et al.* 1998; TORRELL *et al.* 1999; FUNK *et al.* 2009), the genus was alienated into 5 large assemblages which have been treated at sectional or subgeneric level; *Absinthium* (Tourn.) DC., *Artemisia* Tourn. (=*Abrotanum* Besser), *Dracunculus* Besser, *Seriphidium* Besser and *Tridentatae* (Rydb.) McArthur (TORRELL *et al*. 1999; and references therein). LING (1991a) segregated *Seriphidium* from *Artemisia* as a separate genus. WATSON *et al*. (2002) once again integrated *Seriphidium* with *Artemisia*. But still an unambiguous depiction of natural classification within *Artemisia* has not been established. This confusion is particularly problematic in the case of subgenus *Dracunculus*, because the demarcation of the group is variable depending on the authors consulted (SHISHKIN and BOBROV 1995).

Numerous karyological surveys have been made on the genus *Artemisia*, enhancing the available cytogenetic and karyological data (KAWATANI and OHNO 1964; VALLÈS 1987; GABRIELIAN and VALLÈS 1996; TORRELL *et al*. 1999; TORRELL and VALLÈS 2001; TORRELL *et al*. 2001; VALLÈS and MCARTHUR 2001; VALLÈS *et al.* 2001a, b; HOSHI *et al*. 2003; KREITSCHITZ and VALLÈS 2003; RABIEI *et al*. 2003; VALLÈS *et al.* 2005; GARCIA *et al.* 2006; İNCEER and HAYIRLIOĞLU-AYAZ 2007; PELLICER *et al*. 2007a, b; PELLICER *et al*. 2008; CHEHREGANI and MEHANFAR 2008; CHEHREGANI and HAJISADEGHIAN 2009; ATRI *et al.* 2009; NASERI *et al*. 2009; NAZIRZADEH *et al*.

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2009; CHEHREGANI *et al.* 2010). Nevertheless, the chromosome number of some species of the genus has yet been unknown or doubtful.

The most common basic chromosome number of the genus is x=9, and less frequent basic number is x=8. Polyploidy has been recognized as a common phenomenon in the genus, which also has aneuploidy (KAWATANI and OHNO 1964; MALAKHOVA 1990). The highest ploidy level found to date in *Artemisia* is 16x (*Artemisia medioxima* Krasch. ex Poljakov, 2n=16x=144) (PELLICER *et al.* 2007b). Polyploidy has played a central role in the evolution and speciation in higher plants, which are estimated from 35 to 80% of all species (STEBBINS 1971; SOLTIS and SOLTIS 2000). Thus, the cytological study provides essential information to examine phylogenetic relationships and speciation in higher plant (HOSHI *et al.* 2006).

This study aims to contribute towards a better understanding of the cytogenetic classification of *Artemisia* in the light of karyological data.

MATERIAL AND METHODS

The present study was carried out on the following taxa: *Artemisia campetris* L. var. *campestris*, *A. campestris* L. var. *marschalliana* (Spreng.) Poljak., *A. campestris* L. var. *araratica* (Novopokr.) Poljak. and *A. scoparia* Waldst. and Kit. Herbarium vouchers of all species studied are deposited in the herbarium of Department Biology, Fırat University, Turkey.

Ripe achenes were collected from natural populations and were germinated on wet filter paper in Petri dishes in an incubator at 20±1°C for several days. Achenes germinated (1-1.5 mm) were pretreated with saturated paradichlorobenzene at room temperature for 4 h before they were fixed in ethanol:glacial acetic acid (3:1) at room temperature for 24 h. Subsequently, the materials were transferred to 70% ethanol and stored at 4°C. Root-tip meristems obtained were hydrolyzed in 1 N HCl for 15- 18 minutes at 60°C, stained in Feulgen for 1-2 h. at room temperature, squashed and mounted in a drop of 45% acetic acid (SHARMA and GUPTA 1982). The best metaphase plates were photographed with a digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope. Thereafter, chromosome morphologies and karyograms were constructed. LEVAN *et al*. (1964) system was used in determining centromere location.

Average chromosome measurements were calculated on 10 metaphase plates. The quantitative values were obtained from chromosome character measurements. They are chromosome number, total chromosome length (TCL), long arm length (L), short arm length (S), arm ratio (r=L/S), centromeric index (CI=100x S/TCL), relative length (RL=TCL / total haploid length x 100), and chromosome type.

To assess the existence of previously published chromosome counts in the species studied, we used the most common indexes of plant chromosome numbers (cited in VALLÈS *et al*. 2001a, b), previous publications (GARCIA *et al*. 2006; VALLÈS *et al*. 2005), as well as on-line chromosome number databases, the chromosome number databases, 'Index to Plant Chromosome Numbers' (Missouri Botanical Garden, http://mobot.mobot.org/W3T/Search/ipcn.html) and 'Index to Chromosome Numbers in the Asteraceae' (WATANABE 2010; http://www-asteraceae.cla.kobeu.ac.jp/index.html).

RESULTS AND DISCUSSION

The classification at the genus level of Asteraceae is very dynamic - every year at least 10 new genera are described and many more are resurrected or moved into synonymy (BREMER 1994; KADEREIT and JEFFREY 2007). The infrageneric classification of *Artemisia* is also an unresolved problem. Some authors have proposed different series of sections and subsections in *Artemisia* (KOROBKOV 1981; LING 1991a, b; LING 1995a, b; SHISHKIN and BOBROV 1995), but a global treatment of the entire genus at these levels has not yet been achieved. Many species of *Artemisia* which are a paraphyletic genus are largely similar (BREMER 1994). These similarities make it difficult to taxonomical classification of the genus. During the taxonomic revision of the genus *Artemisia* L., KURŞAT (2010) and CIVELEK *et al.* (2010) have observed that there were differences between the classification of subgenus *Dracunculus* in the Flora of the USSR and Flora of Turkey. In the present study, we have followed the nomenclature in the Flora of the USSR adopted by SHISHKIN and BOBROV (1995).

Karyotype parameters of two taxa studied could not be obtained due to difficulties in their germination and the lack of enough metaphase plates (*A. campestris* var. *campestris* and *A. campestris* var. *araratica*). Mitotic metaphase chromosomes of four taxa belonging to subgenus *Dracunculus* of *Artemisia* are displayed in Fig.1 and haploid idiogram and karyograms of *A. campestris* var. *marschalliana* and *A. scoparia* are present in Fig. 2.

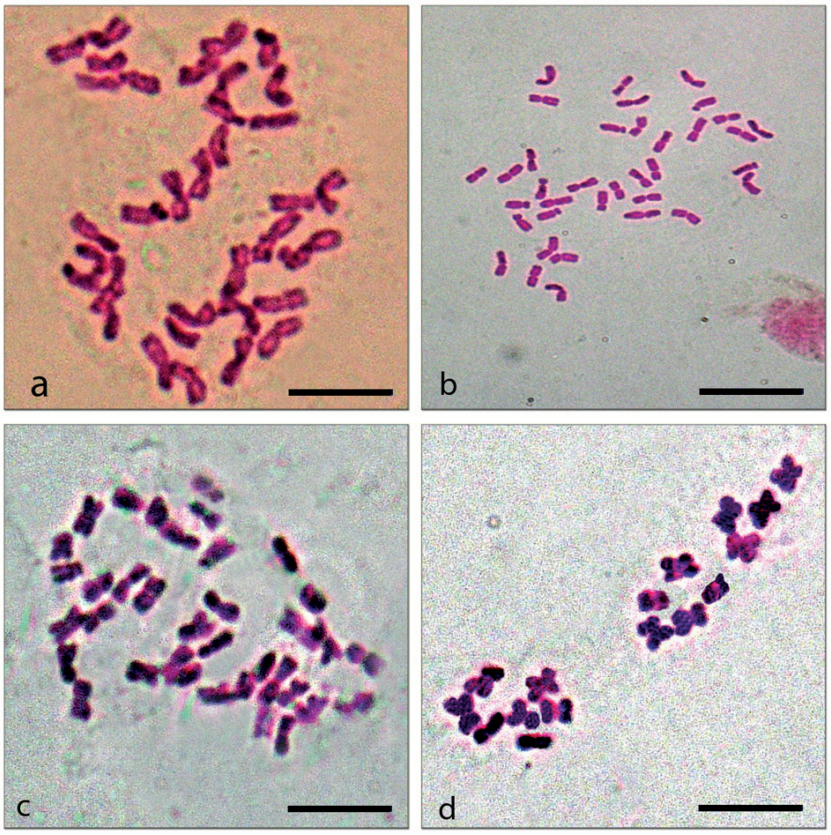

Fig. 1 — Mitotic metaphase chromosomes of four taxa belong to subgenus *Dracunculus* of *Artemisia*. **a:** *A. campestris* var. *campestris* (2n = 4x = 36), **b:** *A. campestris* var. *marschalliana* (2n = 4x = 36), **c:** *A. campestris* var. *araratica* (2n = 4x = 36), **d:** *A. scoparia* (2n = 2x = 16). Karyotype parameters of two taxa studied could not be obtained due to difficulties in their germination and the lack of enough metaphase plates. Scale bars in all figures = 10µm.

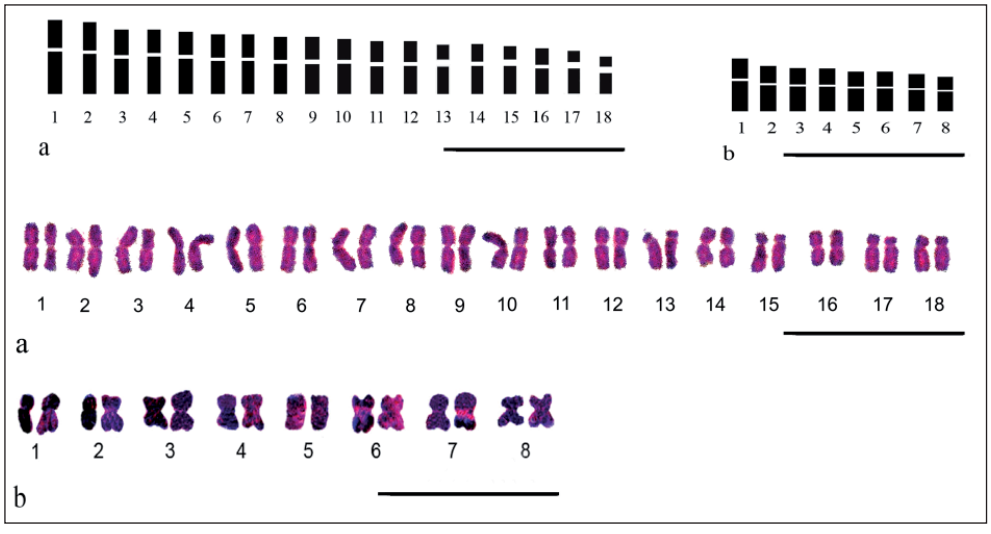

Fig. 2 — Haploid idiogram and karyograms of *A. campestris* var. *marschalliana* (a) and *A. scoparia* (b). Scale bars in all figures = 10µm.

***A. campestris* L.** – In the Flora of Turkey and the East Aegean Islands, *A. campestris*, *A. marschalliana* and *A. araratica* are classified into three species separately (DAVIS 1975), while these species are considered as varieties of *A. campestris* in Flora of the USSR (var. *campestris*, var. *marschalliana* (Spreng.) Poljak. and var. *araratica* (Novopokr.) Poljak.). Recently, KURŞAT (2010) and CIVELEK *et al.* (2010) asserted that *A. campestris, A. araratica, A. marschalliana* were quite similar taxa and misidentified in Flora of Turkey. Therefore, we approved the selection in the Flora of the USSR adopted by SHISHKIN and BOBROV (1995). Chromosome numbers of these three varieties belong to *A. campestris* were determined as 2n = 4x = 36.

A. campestris* L. var. *campestris – 2n = 4x = 36 (Fig. 1a). Turkey, B4 Ankara: Polatlı Highway, 27 km from the Polatlı, three kilometers from the Temelli, roadside, 843m. a.s.l., N 39º 43.997, E 32º 23.860, M. Kurşat 1096, 23.x.2007.

According to our data, chromosome number of this taxon is 2n = 4x = 36 and this count is the first report of the chromosome number of this taxon in Turkey. This count is accordance with many other reports on material from other areas (WATANABE 2010 and references therein; D'ANDREA *et al.* 2003; BENNETT and LEITCH 2005; KREITCHITZ and VALLÈS 2007). However, the taxon have evaluated as a species or subspecies by many of these authors. According to **both our** research and that of others, ploidy level of the taxon is based on x = 9 that is common in Asteraceae.

***A. campestris* L. var. *marschalliana* (Spreng.) Poljak.** – 2n = 4x = 36 (Fig. 1b). Turkey, B9 Bitlis: between Tatvan - Ahlat, exit of Tatvan (Sorgun), 8 km after Tatvan, roadside, vicinity of Military zone, 1715 m. a.s.l., N 38° 32.486, E 42° 21.303, Ş. Civelek, M. Kurşat 1047,19.11.2007.

Chromosome number of *A. campestris* L. var. *marschalliana* was identified as 2n = 4x = 36. To our knowledge, this count confirms previous reports of the tetraploid level in this taxon. In two populations that was collected from Armenia and Iran, chromosome number of *A. marschalli-*

TABLE 1 — Morphometric karyotype data of *A. campestris* L. var. *marschalliana* (2n=4x=36). Total chromosome length (TCL), long arm length (L), short arm length (S), arm ratio (AR=L/S), centromeric index (CI=100 x S/TCL), relative length (RL= TCL / total haploid length x 100).

Chromosome pair	TCL (µm)	L (µm)	S (µm)	AR (µm)	CI (µm)	RL (µm)	Chromosome types
1	2.29	1.23	1.06	1.19	45.60	3.68	m
2	2.14	1.27	0.87	1.40	41.50	3.43	m
3	2.03	1.14	0.89	1.29	43.56	3.26	m
4	1.96	1.15	0.81	1.43	41.00	3.15	m
5	1.91	1.06	0.85	1.24	44.50	3.06	m
6	1.86	1.11	0.75	1.44	40.86	2.98	m
7	1.81	1.05	0.76	1.35	42.54	2.90	m
8	1.78	1.02	0.76	1.31	43.25	2.86	m
9	1.74	1.02	0.72	1.41	41.37	2.79	m
10	1.69	0.97	0.72	1.38	42.01	2.71	m
11	1.64	0.97	0.67	1.37	42.07	2.63	m
12	1.61	0.97	0.64	1.51	39.75	2.58	m
13	1.59	1.09	0.50	2.18	31.44	2.55	sm
14	1.56	0.97	0.59	1.62	38.06	2.50	m
15	1.51	1.01	0.50	2.02	33.11	2.42	sm
16	1.45	0.88	0.57	1.52	39.58	2.33	m
17	1.37	0.88	0.49	1.79	35.76	2.20	sm
18	1.17	0.80	0.37	2.16	31.62	1.88	sm

Karyotype formula of *A. campestris* L. var. *marschalliana* is 28m + 8Sm. sm (submetacentric), m (metacentric). Chromosome types are given according to Levan & al. (1964) classification system.

ana was counted as 2n = 36 (TORRELL *et al*. 2001). In one population that was collected from Russia, the individuals with chromosome number 2n = 18 was also identified (PELLICER *et al*. 2007a). However, the taxon has been also evaluated as a species in all previous studies. After all, these studies support that the basic chromosome number is x = 9 in this variety.

The metaphase complement of this variety consists of 28 metacentric and 8 submetacentric chromosomes. Total chromosome length of this taxon was 1.17-2.29 µm**,** its relative length was 1.88-3.68 µm and its arm ratio was 1.19-2.18 µm (Table 1). Haploid idiogram and karyograms of var. *marschalliana* were indicated in Fig. 2a. This work is the first detailed report of morphometric karyotype data of var. *marschalliana*.

***A. campestris* L. var. *araratica* (Novopokr.) Poljak.** – 2n = 4x = 36 (Fig. 1c). Turkey, B6 Malatya: Doğanşehir, Dedeyazı Village, Location of Çanakcı, steppeland, 1495 m. a.s.l., N 38° 11.942, E 37° 50.622, M. Kurşat 1084, 20.x.2007.

In the present work, chromosome number of var. *araratica* was determined as 2n = 4x = 36. According to our literature research, this is the first report of the chromosome number in this taxon. However, karyotype of the taxon could not be investigated due to difficulties in their germination and the lack of enough metaphase plates.

To understand relationship between the taxa of *A. campestris* complex are needed to more detailed karyological studies. A fine molecular phylogenetic research may confirm this classification and the relationships among the taxa of the group.

***A. scoparia* Waldst. and Kit** – 2n = 2x = 16 (Fig. 1d). Turkey, B9 Muş: Malazgirt, between Malazgirt- Aktuzla, vicinity of Nuretin Village, 1620 m. a.s.l., N 39º 14.210, E 42º 25.223, M. Kurşat 1115, 26.xı.2007.

Chromosome number of *A. scoparia* was determined as 2n = 2x = 16. Many chromosome counts have been published for this widespread Eurasian species (WATANABE 2010 and references therein). Most of them agree with our data and indicate diploid level as 2n = 16 (MENDELAK and SCHWEIZER 1986; KAPUSTINA *et al*. 2001; D'ANDREA *et al*. 2003; RABIEI *et al*. 2003; ROTREKLOVÁ *et al.* 2004; BOJŇANSKÝ and FARGAŠOVÁ 2007; WATANABE 2010 and references therein), only a few of them are different from our counts and reported as 2n = 18 (YAN *et al*. 1989; CHEHREGANI and MEHANFAR 2008; ATRI *et al.* 2009) and as 2n = 36 (KAWATANI and OHNO 1964). Two populations with different chromosome number 2n = 2x = 16 and 18 were found in Hamedan, Iran (CHEHREGANI *et al.* 2010). Descending dysploidy is confirmed in *A. scoparia* according to these results. VALLÈS and ŠILJAK-YAKOVLEV (1997) found evidence that the dysploidy in the genus *Artemisia* descends from x = 9 to x = 8, as a result of a chromosomal fusion. It was supposed that the dysploidy may take place in *Artemisia* during the spread and evolution of this genus, which probably contributes to its adaptation to different ecological factors (ZHAO et al. 2009).

Although there are many literature related to the determination of chromosome number of this species, its morphometric karyotype data are very scarce. The karyotype formula of the species was given as 12m + 4Sm by WANG

TABLE 2 — Morphometric karyotype data of *A. scoparia* (2n=2x=16). Total chromosome length (TCL), long arm length (L), short arm length (S), arm ratio (AR=L/S), centromeric index (CI=100 x S/TCL), relative length (RL= TCL / total haploid length x 100).

Chromosome pair	TCL (µm)	L (µm)	S (µm)	AR (µm)	CI (µm)	RL (µm)	Chromosome types
1	1.71	0.98	0.73	1.34	42.69	7.42	m
2	1.60	0.91	0.69	1.31	43.12	6.95	m
3	1.53	0.89	0.64	1.39	41.83	6.64	m
4	1.46	0.82	0.64	1.28	43.83	6.34	m
5	1.38	0.77	0.61	1.26	44.20	5.99	m
6	1.34	0.80	0.54	1.48	40.29	5.82	m
7	1.28	0.70	0.58	1.20	45.31	5.56	m
8	1.21	0.67	0.54	1.24	44.62	5.25	m

Karyotype formula of *A. scoparia* is 16m. Metacentric (m). Chromosome types are given according to Levan & al. (1964) classification system.

(2000). In the present work, the karyotype formula of *A. scoparia* was determined as 16m and its detailed karyotype analyses are presented in Table 2. Haploid idiogram and karyograms of *A. scoparia* were summarized in Fig. 2b.

This is a work centered on the subgenus *Dracunculus* of genus *Artemisia*, and it is an approach toward a better understanding of what kind of karyological characters are taking place at subgeneric level. The karyological characters may be essential for drawing significant conclusions on the relative closeness and distance of the various taxa. However, they are not enough to draw any definitive taxonomic conclusion and to resolve taxonomic conflicts in the genus. Detailed karyotype analyses, including the use of chromosome banding techniques, combined with molecular studies required to establish the subgeneric natural classification system of *Artemisia*. Our present data in here will throw some more light on the karyological knowledge of the subgenus.

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