KARYOTYPE ANALYSES OF FOUR *ASTRAGALUS* L. (FABACEAE) SPECIES FROM TURKEY

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ABSTRACT

Astragalus L. is a perennial herbaceous genus belonging to the family Fabaceae. Chromosome morphology was defined for the first time in four species of *Astragalus* namely; *A. antalyensis* A.Duran & Podlech, *A. nezaketae* A.Duran & Aytaç, *A. cariensis* Boiss. & *A. schizopterus* Boiss. All species contained diploid chromosome numbers of *2*n = 16. However, polyploid cells (4x = 32) were also observed in *A. schizopterus* and *A. antalyensis*. *Astragalus nezaketae* species had two double satellite chromosomes. Karyotypes of species were made using an Image Analysis System. Chromosome numbers are all first reports, except for that of *A. schizopterus*.

KEY WORDS: *Astragalus*, Leguminosae, Image Analysis System, karyotype, Turkey

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Astragalus L. is one of the largest genera of vascular plants in the world, with an estimated number of 3000 species. Many species are narrow endemics. However, a few are widespread, mainly in the Northern Hemisphere, Central Asia, and Western North America (Podlech, 1986; Maassoumi, 1998; Açık et al., 2004). It is also the largest genus in Turkey, where it is represented by nearly 455 species in 61 sections (Chamberlain and Matthews, 1970; Davis et al., 1988; Duran and Aytaç, 2005). In Turkey, the genus *Astragalus* is represented best in the steppe environment of high mountains (Chamberlain and

Matthews 1970; Podlech, 1999). In the Irano-Turanian phytogeographic region of Turkey, which is one of the centers of diversity of the genus (Ghahremaninejad and Behçet, 2003) 210 endemic taxa occur, which is a rate of endemism of about 47% (Duman and Akan, 2003). The species examined in the present study are economically valuable and all are endemic to Turkey.

Karyological knowledge of *Astragalus* consists of chromosome counts of more than fifty species (Spellenberg, 1976; Cartier, 1976, 1979; Davis et al., 1988; Çobanoğlu and Altan, 1989; Şahin et al., 1990; Tünbel, 1993; Kandemir et al., 1994, 1996; Engin et al., 1994; Civelek et al., 1997; Aytaç, 1997; Ekici and Aytaç, 2001; Wang et al., 2002; Hamzaoğlu, 2003; Ekici et al., 2005). Such reports indicate the existence of only one basic chromosome number (x = 8) in the genus. Although chromosome counts are reported for many species in *Astragalus*, few workers have described karyotypes of its species. The lack of karyological studies in Astragalua is probably due to the small length of the chromosomes.

In this contribution, we report mitotic metaphase chromosome numbers and karyotypes of four species of *Astragalus* belonging to sections *Caprini* DC., *A. antalyensis*; *Incani* DC., *A. nezaketae*; *Proselius* Bunge, *A. cariensis* Boiss. and *A. schizopterus* Boiss. (Chamberlain and Matthews, 1970; Podlech, 1999; Duran and Aytaç, 2005). The systematic importance of the results is discussed when appropriate.

MATERIAL AND METHODS

Plant materials of the genus *Astragalus* namely *A. antalyensis*, *A. nezaketae*, *A. cariensis* and *A. schizopterus* representing three sections namely; *Caprini*, *Incani* and *Proselius* were collected from different localities in Turkey as detailed in Table 1. Voucher specimens have been deposited at the herbaria of Selçuk University. All taxa except A. *schizopterus*, are local endemics (Chamberlain and Matthews, 1970; Podlech, 1999; Duran and Aytaç, 2005).

Karyomorphological observations were made on mitotic metaphase cells of root-tips obtained from germinated seeds. Root tips

were pretreated for 16 h in α-monobromonaphthalene at 4°C and washed with distilled water and finally fixed in Carnoys solution (3:1 absolute ethanol : glacial acetic acid, overnight. The root tips were hydrolysed for 10 min in 1 N HCl at room temperature, washed and stained in 2% (w/v) aceto-orcein for 2 h. Stained root tips were then squashed in a drop of 45% acetic acid and permanent slides were made by mounting in Depex. Chromosome measurements were made in at least five well-spread metaphases, bearing the same chromosome contraction. The total lengths (μm) and the arm ratio values were used for comparisons of the karyotypes. Ideograms were designed by using an Image Analysis System (BsPro200).

RESULTS

Chromosome morphologies, total chromosome lengths, arm ratios and centromeric indices are summarized in Table 2. A chromosome number of 2n = 16 was determined for all species. Results of the study are given below.

Section: *Caprini*

Astragalus antalyensis

Chromosome numbers were determined 2n = 2x = 16 = 7m+1sm with a basic number of x = 8 (Fig. 1). The metaphase karyotype consisted of 7 median chromosomes and 1 submedian chromosome. The species had one double satellite metaphase chromosome. Chromosomes varied from 1.36 μm to 2.82 μm. Total haploid chromosome length was 16.71 μm. The ideogram is given in Fig. 5.

Section: *Incani*

Astragalus nezaketae

The chromosome number of this species was determined as 2n = 2x = 16 = 6m+2sm (Fig. 2) The metaphase karyotype consisted of 6 median chromosomes, and 2 submedian chromosomes. The species had two satellite metaphase chromosomes. Chromosomes size ranged from

2.54 μm to 3.35 μm. Total haploid chromosome length was 23.42 μm. The ideogram is given in Fig. 5.

Section: *Proselius*

Astragalus cariensis Boiss.

The chromosome number of this species was determined as 2n = 2x = 16 = 1m+6sm+1st (Fig. 3). The metaphase karyotype consisted of 1 median chromosome, 6 submedian chromosomes and 1 terminal point chromosome. Chromosome sizes ranged from 3.16 μm to 4.69 μm. Total haploid chromosome length was 33.67 μm. The ideogram is given in Fig. 5.

Astragalus schizopterus Boiss.

The chromosome number of this species was determined as 2n = 2x = 16 = 8m (Fig. 4). The metaphase karyotype consisted of 1 median chromosome, 6 submedian chromosomes and 1 terminal point chromosome. Chromosome sizes ranged from 1.81 μm to 3.97 μm. Total haploid chromosome length was 20.90 μm. The ideogram is given in Fig. 5.

DISCUSSION

The chromosome numbers of four species of the genus *Astragalus* were determined based on an analysis of somatic metaphases. The chromosome number 2n = 2x = 16 was found for all species, establishing a basic chromosome number of x = 8. The total haploid chromosome lengths ranged from 16.71 to 33.69 μm with average chromosome lengths from 1.36 to 4.69 μm. The median (m) and submedian (sm) chromosomes are found to form the main part of chromosome complement, while the terminal point (T) chromosomes were rare or absent. Tetraploidy (2n = 4x = 32) is reported here for the first time in *A. antalyensis* and *A. schizopterus*. SAT chromosomes were analyzed for two species (*A. antalyensis* and *A. nezaketae*).

Somatic chromosome number and morphology of *A. antalyensis* are first reported in this study. Some tetraploid cells (2n =

4x = 32) were observed, in accordance with some previous reports (Çobanoğlu and Altan, 1989; Cartier, 1976, 1979). Diploid chromosome number of 2n = 16 is in line with studies made in various taxa of the *Astragalus* (Cartier, 1976, 1979; Çobanoğlu and Altan, 1989; Şahin et al., 1990; Kandemir et al., 1994, 1996; Engin et al., 1994; Civelek et al., 1997; Aytaç, 1997). However, chromosome lengths were the shortest (1.36 μm) in this species among other species studied. In addition, the highest centromeric index (5.09) was found in this species. This species also contained one double satellite on its mitotic chromosomes, separating it from other species.

Chromosome number (2n = 16) and karotype of *A. nezaketae* are first reported in this study. The species contains two double satellites on its chromosomes. Polyploid cells were not observed. The species contains the same somatic chromosome number (2n = 16) as that of *A. ovabaghensis* from the section *Alopecuroidei* DC. (Akan and Aytaç, 2004).

The other endemic species, *A. cariensis*, also had 2n = 16. The longest chromosome was measured as 4.69 μm. Total haploid chromosome length was 33.67 μm and the arm ratio was 2.33. This species had the lowest (3.93) centromeric index among the species examined. Satellite chromosome or polyploidy were not observed in this species.

The chromosome number of *A. schizopterus*, which is not endemic to Turkey, was reported to be 2n = 16 (Index to Plant Chromosome Numbers; Missouri Botanical Garden, http: //mobot.mobot.org/W3T/Search/ipcn.html) with some tetraploid somatic cells. The karyotype formula was different from yet other taxa which consisted of only median chromosome pairs.

In general, the four *Astragalus* species from three sections did not show variation in chromosome number. However, chromosome morphologies varied significantly. For instance, the karyotype of *A. cariensis* was mainly composed of median, submedian and terminal point chromosome pairs, whereas no terminal point chromosomes were observed in other taxa. Similarly, only median chromosome pairs were found in *A. schizopterus*, this contrasting with yet other taxa.

Astragalus cariensis and *A. schizopterus* belonging to the same section (*Proselius*) presented clearly different karyotypes (1m+6sm+1st and 8m). The size of chromosomes were also different in both species. The lack of polyploidy in *A. cariensis* was another distinctive feature of this species.

Astragalus ovalis Boiss. & Balansa from the section *Ammodendron* Bunge has a karyotype formula of 2n = 16 = 8m (Ekici et al., 2005) with total chromosome lengths varying between 1.11 µm and 1.63 µm (haploid chromosome length: 10.79 µm, arm ratios: 1.04 - 1.31). The karyotype formula of *A. schizopterus* is similar to *A. ovalis.* However, chromosome lengths of *A. schizopterus* were longer (1.81-3.97 µm) than those of *A. ovalis.* This was also the case in arm ratio (1.42). The other variation was the existence of tetraploid cells (2n = 4x = 32) in *A. schizopterus.* Ekici and Aytaç (2001) reported that the diploid chromosome number of *A. dumanii* M.Ekici and Aytaç which belongs to the section *Hololeuce* Bunge were also 2n = 16, in line with our reported chromosome number.

A revision of the section *Dasyphyllium* Bunge of the genus *Astragalus* (Fabaceae) in Turkey has been carried out. Its chromosome number was reported to be (2n) = 16 (Aytaç, 1997), similar to the findings in the present study.

Spellenberg (1976) presented 126 original reports of chromosome numbers for 101 taxa of North American *Astragalus*. His study supports earlier work demonstrating the basic division between Old World species, including their close New World relatives (x = 8, euploidy common), and strictly New World lineages (x = 11, 12, 13, 14, 15, euploidy rare). It is proposed, therefore, that the New World species originated from a tetraploid of 2n = 32 or a hypotetraploid, evolutionary radiation in some lineages concomitant with descending aneuploidy. In other lineages with little or no change in number was recorded (Spellenberg, 1976). In another study Wang et al. (2002) reported that the karyotype of *A. complanatus* was K (2n) = 16 = 10m + 6 sm.

Chromosome numbers of 2n = 16 have been reported for other endemic *Astragalus* species in Turkey namely; *A. panduratus* Bunge, *A. barba-jovis* DC. var. *barba-jovis* and *A. plumosus* Willd. var. *nitens* (Freyn and Bornm) Chamb. and Matthews (Tünbel, 1993) and *A. barba-jovis* DC. var. *barba-jovis* (Kandemir et al., 1996).

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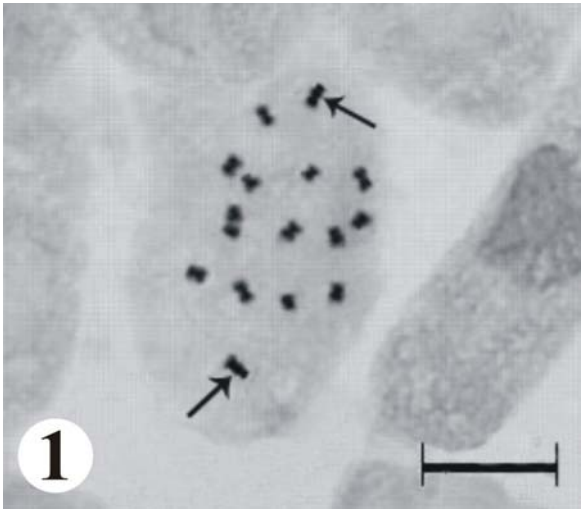
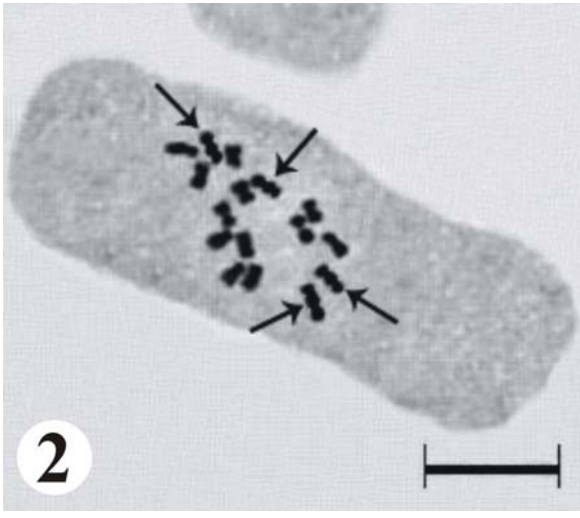
Table 1. The localities, collector and chromosome numbers of *Astragalus* specimens examined.

Taxa	2n	Locality	Vouchers
A. antalyensis	16	C3 Antalya: Akseki Çukurköy Yaylası,Teketaşı mevki, 1900 m, 09.08.2006, eğimli taşlı yamaçlar	A.Duran 7361 and M.Dinç
A. nezaketae	16	B7 Erzincan: Üzümlü, Keşiş Dağı, Merdo'nun kayası mevki, kalker taşlı yerler, 2450 m, 27.07.2006.	M.Dinç 2813 and A.Duran
A. cariensis	16	Muğla: eski Kale Yolu, Yılanlı Dağı, vericiler civarı, 1360 m, 23.07.2006, *P. nigra* ve *Juniperus* açıklığı.	A.Duran 7303 and M.Dinç
A. schizopterus	16	Burdur: Dirmil-Gölhisar, 7. km, 1175 m, 25.07.2006, açık yerler.	A.Duran 7334 and M.Dinç

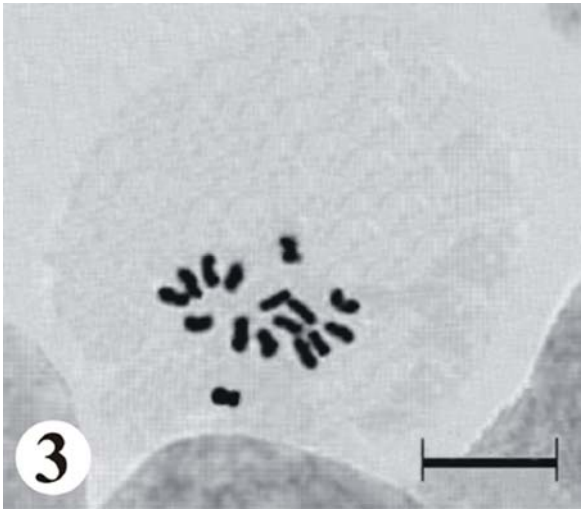
Table 2. Chromosome comparison in the four studied taxa of *Astragalus*. AR: arm ratio; CI: centromeric index; THC: total length of haploid complement; M: median; SM: submedian; T: terminal point.

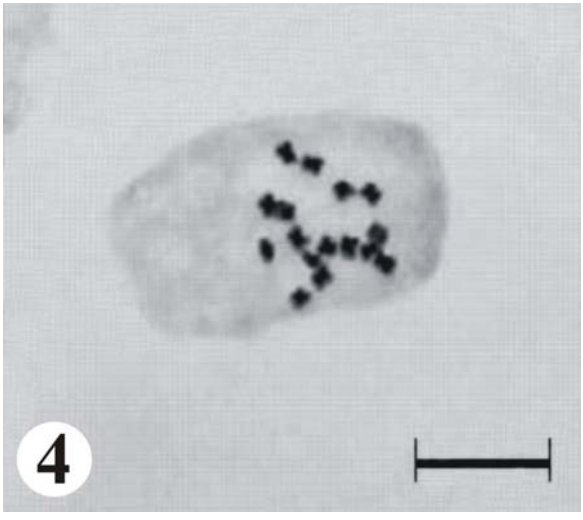
Taxa *Astragalus*	2n	Ploidy level	Chromo-some sizes (µm)	AR	CI	THC (µm)	M	SM	T
antalyensis	16	4x	1.36-2.82	1.49	5.09	16.71	7	1	-
nezaketae	16		2.54-3.35	1.57	4.92	23.42	6	2	-
cariensis	16		3.16-4.69	2.33	3.93	33.67	1	6	1
schizopterus	16	4x	1.81-3.97	1.42	5.18	20.90	8	-	-





Figures 1-2. Somatic metaphase chromosomes in *Astragalus* species. **(**1**)**. *A. antalyensis* (2n = 16), **(**2**)**. *A. nezaketae* (2n = 16). Scale Bar: 10 μm.





Figures 3-4. Somatic metaphase chromosomes in *Astragalus* species. **(3)**. *A. cariensis* (2n = 16), **(4)**. *A. schizopterus* (2n = 16). Scale Bar: 10 µm.

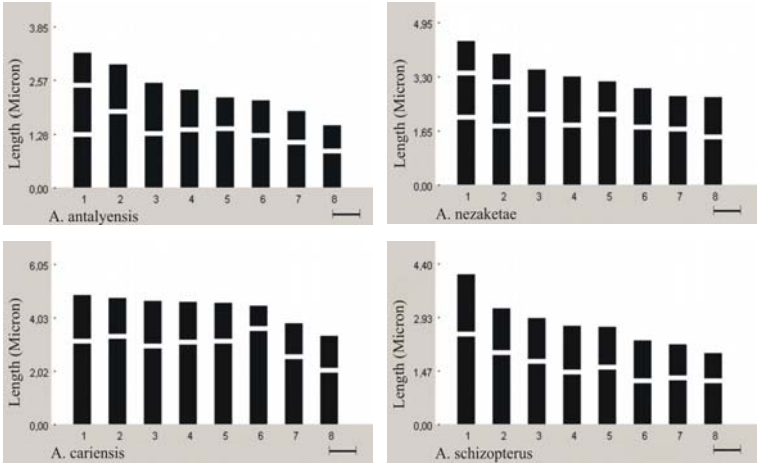


Figure 5. Ideograms in *Astragalus* taxa. Scale bar: 10 µm.