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Karyological study on endemic *Astragalus stereocalyx* Bornm. (Milk-vetch) in Turkey

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Abstract

Chromosome number and karyotype of *Astragalus stereocalyx* Bornm. (Milk-vetch) in the section *Stereocalyx* Bornm. distributing naturally in Turkey were analyzed in detail. This species is endemic to Turkey and growing in Eskişehir and Konya provinces. Squash preparation method was used for chromosome study in this species. The somatic chromosome number was determined as $2n = 16$ in *A. stereocalyx*. The basic chromosome number was observed as $x = 8$. The chromosome number and morphology of *A. stereocalyx* belonging to the genus *Astragalus* was investigated using Image Analysis System. The karyotype formula of this species consists of three median chromosome pairs and five submedian chromosome pairs. The somatic chromosome length between 1.07-2.23 µm. Total haploid chromosomes length was measured as 11.73 µm. The karyogram and ideogram were drawn based on centromeric index and arranged in the decreasing size order.

Key words: Astragalus stereocalyx, Karyotype, Leguminosae, Turkey

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Türkiye'deki endemik *Astragalus stereocalyx* Bornm. (Geven) türünde karyolojik çalışma

Özet

Türkiye'de doğal olarak yetişen *Stereocalyx* Bornm. seksiyonunda yer alan *Astragalus stereocalyx* Bornm. (Geven) türünde kromozom sayı ve karyotipi detaylı olarak analiz edildi. Bu tür Türkiye için endemiktir, Eskişehir ve Konya illerinde yetişmektedir. Bu türün kromozom çalışmasında ezme yayma preparasyon yöntemi kullanıldı. *A. stereocalyx* türünde somatik kromozom sayısı $2n = 16$ olarak belirlendi. Temel kromozom sayısı $x = 8$ şeklinde gözlendi. *Astragalus* cinsine ait olan *A. stereocalyx*'in kromozom sayı ve morfolojisi Görüntü Analiz Sistemi kullanılarak araştırıldı. Türün karyotip formülü üç median ve beş submedian kromozom tiplerinden oluşmaktadır. Somatik kromozom boyu 1.07-2.23 µm arasındadır. Toplam haploid kromozom uzunluğu 11.73 µm olarak ölçüldü. Karyogram ve idiyogramı sentromerik indeks ve büyükten küçüğe doğru azalan oranda çizildi.

Anahtar kelimeler: Astragalus stereocalyx, Karyotip, Leguminosae, Türkiye

1. Introduction

Astragalus L. (Milk-vetch) is one of the largest genera of vascular plants in Eurasia, with an estimated number of 3000 species. Many species are local endemics, while relatively few are widespread, distributed mainly in the Northern Hemisphere, Central Asia, and Western North America (Açık et al., 2004; Podlech, 1986). It is also the largest genus in Turkey, where it is represented by nearly 456 species in 62 sections (Chamberlain and Matthews, 1970; Davis et al., 1988; Dural et al., 2007; Duran and Aytaç, 2005). Generally, the genus *Astragalus* is represented by the highest number of species adapted in especially the steppe environment of high mountains in Irano-Turanian phytogeographic

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region of Turkey which is one of the centers of diversity of the genus, and it contains 210 endemic taxa with a rate of endemism about 47% (Chamberlain and Matthews, 1970; Duman and Akan, 2003; Ghahremaninejad and Behçet, 2003; Podlech, 1999).

Astragalus roots from various species have been used in the traditional Chinese medicine, as immunostimulants, hepatoprotectors, antiperspirants, diuretics, etc., and for the treatment of nephritis, diabetes, leukemia, and cancer (Karagöz et al., 2007; Tang, 1992).

The reported chromosome numbers are $2n$ = 16, 32, 48 and 64 in the genus *Astragalus*. The karyological knowledge of *Astragalus* consists of chromosome counts of more than fifty species (Akan and Aytaç 2004; Aytaç, 1997; Ekici and Aytaç, 2001; Ekici et al., 2005; Hamzaoğlu, 2003; Kandemir et al., 1996; Martin et al., 2008; Spellenberg, 1976). These reports indicate the existence of only one basic chromosome number (x = 8) in the genus. Although there were a lot of cytological studies on the genus *Astragalus*, only a few karyotype had been made on this genus. The lack information about the karyomorphology of the genus is probably due to the small length of its chromosomes.

In this study, we report somatic chromosome number and karyotype of *Astragalus stereocalyx* belonging to section *Stereocalyx* Bornm.

2. Materials and methods

Voucher specimen has been deposited at the herbaria of Selçuk University, Faculty of Education, Konya in 2007. Karyotype was made on somatic metaphases using Image System Analysis. Root meristems from germinating seeds collected in the wild were used. Root tips were pretreated with α-monobromonaphthalene at 4°C for 16 h. Root tips were fixed with Carnoy for 24 h at 4°C. Before staining, the material was hydrolyzed with 1N HCl for 13 minutes at room temperature. The chromosomes were stained with 2% acetic orcein and mounted in 45% acetic acid. Permanent slides were made by using the standard liquid nitrogen method. Photographs were taken through BX50 Olympus microscope. The ideogram was prepared with measurements taken on enlarged micrographs of five well spread metaphase plates. The classification of chromosomes, the length of long and short arm, arm ratio, centromeric index and relative chromosomal length were measured by Software Image Analysis (Bs200Pro). Chromosomes were classified using the nomenclature of Levan et al. (1964).

3. Result and Discussion

3.1. Section: Stereocalyx

Scapose herbaceous perennial with woody caudices. Leaves imparipinnate; leaflets simple-hairy; stipules free. Inflorescence a raceme. Flowers ebracteolate. Calyx globose, woody. Standard glabrous. Legume included in calyx, stipitate, unilocular. Monotypic (Figure 1).

Figure 1. *Astragalus stereocalyx*

3.2. Astragalus stereocalyx

Type: Turkey. B3 Eskişehir: Phrygia (Anatolia): in region subalpine montis Sultan-dagh ditionis oppidi Akscheher, in jugo Teke-dagh, 1600-1700 m, 25.vi.1889, *Bornmüller* 4409 sub nomine *A. nidus avis* Bornm. sp. nov. (W, K).

Plants scapose perennial with woody caudices. Leaves 4-25 cm; leaflets elliptic, acute, mucronate, glabrous or very sparsely simple-pilose; stipules 5-20 mm, narrowly triangular. Flowers in 2-3-flowered racemes. Bracts 5 mm, ovate, glabrous, sometimes ciliate. Calyx 12-20 mm, ±globose, woody and shining, very hard; teeth 3-5 mm, hairy. Corolla white, keel violet-tipped; standard 24-40 mm. Legume c. 7 x 2-3 mm, included in calyx, stipitate, ovoid-oblong, glabrous or with hairy. Fl. 6. Mountains, woods, open forest. 1600-1800 m. Endemic; Ir.-Tur. element.

3.3. Examined specimen

B3 Eskişehir: Sündiken Mountains, Çatacık Forest Enterprise, near fire-tower, open forest, 1750 m, 27.06.2007, A.Duran 7733 (Figure 2).

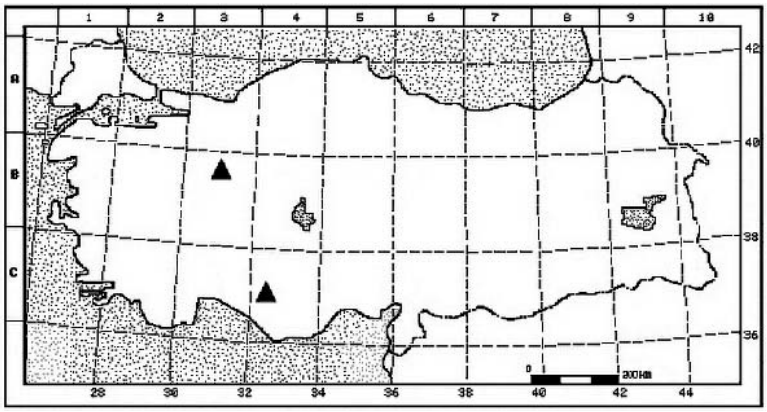

Figure 2. The distribution map (▲) of *Astragalus stereocalyx* in Turkey

3.4. Karyotype Analysis

The chromosome number of *Astragalus stereocalyx* was determined to be 2*n* = 16. This species was determined 2*n* = 2*x* = 16 = 3m+5sm with a basic chromosome number of *x* = 8 (Figure 3). The karyotype consisted of three median chromosomes and five submedian chromosomes. The somatic chromosomes were gradual from 1.07 µm to 2.23 µm. Total haploid chromosome length was 11.73 µm (Table 1). Karyogram of the best metaphases and ideogram of this species were arranged in decreasing length (Figures 4-5). The chromosome number and morphologies were reported for the first time in this study, respectively.

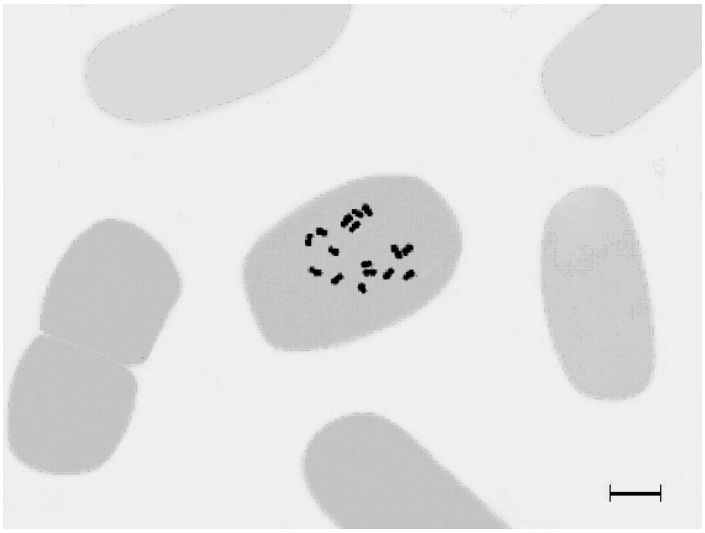

Figure 3. Mitotic metaphase chromosomes in *Astragalus stereocalyx*. Bar: 5 µm

The section of *Dasyphyllium* Bunge (*Astragalus*) has submedian and median chromosomes. Total lengths of mitotic chromosomes varying between 6.95-3.03 µm in these taxa (Aytaç, 1997). The chromosome number was reported to be 2*n* = 16 similar to the findings in our study. However, chromosome lengths of *Astragalus stereocalyx* were found smaller (1.07-2.23 µm) than *Dasyphyllium* section of *Astragalus* taxa.

Table 1. Measurements (µm) of somatic chromosomes in *Astragalus stereocalyx* (*m = median, **sm = submedian)

Chromosome Pair no.	Chromosome arms (µm)		Total length (µm)	Arm ratio (L/S)	Relative length (%)	Centromeric index	Chromosome type
	Long arm (L)	Short arm (S)					
1	1.38	0.85	2.23	1.62	19.07	7.28	m*
2	1.19	0.51	1.70	2.34	14.53	4.35	sm**
3	1.10	0.47	1.57	2.35	13.42	4.01	sm
4	0.91	0.50	1.41	1.82	12.02	4.26	sm
5	0.79	0.51	1.30	1.55	11.08	4.35	m
6	0.81	0.47	1.28	1.73	10.82	3.96	sm
7	0.69	0.47	1.16	1.47	9.89	4.01	m
8	0.73	0.34	1.07	2.12	9.16	2.94	sm

Figure 4. Karyogram of *Astragalus stereocalyx*

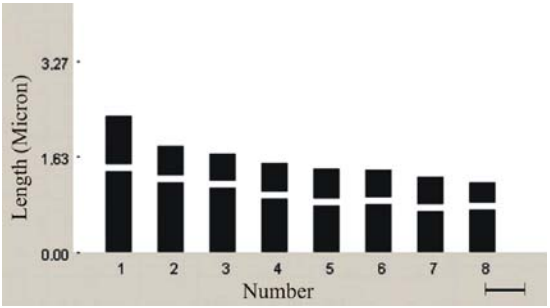

Figure 5. Ideogram of *Astragalus stereocalyx*

Ekici et al., (2005) reported that chromosome morphology of *Astragalus ovalis* Boiss. & Balansa from the section *Ammodendron* Bunge are comprised of median (8m) chromosomes. Somatic chromosome number of *A. ovalis* 2*n* = 16. Total chromosome lengths varying between 1.11 µm and 1.63 µm. Karyotype formula of *Astragalus stereocalyx* (3m+5sm) were different from *A. ovalis.* Also, chromosome lengths of *A. stereocalyx* were found longer (1.07-2.23 µm) than *A. ovalis.* Similar chromosome numbers (2*n* = 16) were reported for other *Astragalus* taxa in Turkey namely; *A. antalyensis* A.Duran & Podlech, *A. nezaketae* A.Duran & Aytaç, *A. cariensis* Boiss. and *A. schizopterus* Boiss. (Martin et al., 2008). However mixoploid cells (4*x* = 32) were reported in *A. schizopterus* and *A. antalyensis*. *A. antalyensis* species had one double, *A. nezaketae* species had two double satellite chromosomes. Neither satellite chromosome nor polyploidy were observed in *A. stereocalyx*. Martin et al., (2008) emphasized that the karyotype of *A. antalyensis* and *A. nezaketae* are comprised of median and submedian chromosomes, the karyotype of *A. cariensis* is comprised of median, submedian and subterminal chromosomes and the karyotype of *A. schizopterus* is comprised of median chromosomes (Martin et al., 2008). However, the karyotype of *A. stereocalyx* 2*n* = 2*x* = 16 = 3m+5sm. In this research, it is determined that the total length of chromosomes varies between 1.07-2.23 µm in *A. stereocalyx.* However the karyotypic data show that *A. stereocalyx* must be considered karyotypically very different from other *Astragalus* taxa as mentioned above. The somatic chromosome numbers of some taxa in different genus were also reported (Madadi et al., 2009; Duran et al., 2010; Kandemir, 2010).

In this study, the chromosome number and karyomorphology of *Astragalus stereocalyx* were determined for the first time. We hope that this study will contribute to the future karyological studies about the genus *Astragalus*.

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