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Karyotype analysis of *Astragalus stenosemioides* (*Fabaceae*)*

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Astragalus stenosemioides (*Fabaceae*)'in karyotip analizi

Abstract: In this research the metaphase chromosome number and karyotype of *Astragalus stenosemioides* species growing naturally in Turkey are studied. The study revealed that the chromosome number of *A. stenosemioides* is 2*n* = 16. The basic chromosome number of species is determined as *x* = 8. The karyotype formula of *A. stenosemioides* is 8m. The total length of the somatic chromosomes of *A. stenosemioides* ranges between 1.96 - 3.38 µm. The total haploid chromosome length of *A. stenosemioides* is determined as 21.36 µm. In addition, the karyotype asymmetry of the species was evaluated by different methods; Stebbins classification, TF%, AsK%, Syi and Rec, A1, A2, DI, A, and AI.

Key words: *Astragalus, Fabaceae,* karyotype, Türkiye

Özet: Bu çalışmada Türkiye'de doğal olarak yetişen *Astragalus stenosemioides* türünün metafaz kromozom sayısı ve karyotipi incelenmiştir. Çalışmada *A. stenosemioides*'in kromozom sayısının 2*n* = 16 olduğu tespit edilmiştir. Türün temel kromozom sayısı *x* = 8 olarak belirlenmiştir. *A. stenosemioides*'in karyotip formülü 8m'dir. *Astragalus stenosemioides*'in somatik kromozomlarının toplam uzunluğu 1.96 - 3.38 µm arasında değişmektedir. *A. stenosemioides*'in toplam haploid kromozom uzunluğu 21.36 µm olarak belirlenmiştir Ayrıca türün karyotip asimetrisi; Stebbins sınıflandırması, %TF, %AsK, Syi ve Rec, A1, A2, DI, A ve AI gibi farklı yöntemlerle değerlendirilmiştir.

Anahtar Kelimeler: *Astragalus, Fabaceae,* karyotip, Türkiye

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1. Introduction

Astragalus L. (Fabaceae), is one of the largest genera of vascular plants in the world, with an estimated number of 3000 species. Many species are narrow endemics (Maassoumi, 1998). It is also the largest genus in Turkey, where it is represented by nearly 484 taxa including 203 endemic in 63 sections (Hamzaoğlu, 2020; Karaman Erkul et al., 2021). In Turkey, the genus *Astragalus* is represented best in the steppe environment of high mountains. In the Irano-Turanian phytogeographic region of Turkey, which is one of the centers of diversity of the genus (Karaman Erkul et al., 2014).

Astragalus stenosemioides Bornm. Ex D.F.Chamb.& V.A. Matthews included in the *Hololeuce* Bunge section is very local endemic species growing only Erciyes Mountain in Kayseri and at a low population density (Fig. 1, Table 1).

Cytological investigations which were carried out on taxa of the genus *Astragalus* showed that chromosome numbers in these taxa were found to be 2*n* = 14, 16, 22, 24, 32, 48 and 64 (Aryavand, 1977; Dawe and Murray, 1979; Magulaev, 1980; Astanova, 1981; Spellenberg, 1981; Hung, 1984; Mu and Shue, 1985; Maassoumi, 1987; Ward and Spellenberg, 1988; Magulaev, 1989; Maassoumi, 1989; Liston, 1990; Daviña and Gómez-Sosa, 1993; Kandemir et al., 1996; Aytaç, 1997; Murray and Kelso, 1997; Gervais et al., 1999; Yan et al., 2000; Nazarova, 2004; Martin et al., 2008; Sheidai et al., 2009; Sheidai and Gharemani-Nejad, 2009; Kazem et al., 2010; Borjian et al., 2012; Ranjbar et al., 2012; Martin et al., 2019). Ekici et al. (2005) investigated that chromosome morphology of *Astragalus ovalis* Boiss. & Balansa from the section *Ammodendron* Bunge. Ekici and Çelik (2005) examined autoecological and morphological features of *A. stenosemioides*, but there is no study on the chromosome morphology of the species.

In the present study, the chromosome number and karyotype of *A. stenosemioides* has been studied. We hope that this study will contribute to future karyological studies of the genus *Astragalus*.

2. Material and Method

The karyotype was carried out on root tips. The root-tip meristems were provided from seed by germinating them on wet filter paper in petri dishes at room temperature. Firstly root tips pretreated for 16 h in α-monobromonaphthalene at 4°C, fixed in 3:1 absolute alcohol/glacial acetic acid, then the root tips were hydrolyzed with 1 N HCl for 12 min at room temperature and stained with 2% aceto-orcein for 3 h at room temperature. Stained root tips were squashed in a drop of 45% acetic acid and permanent slides were made by mounting in Depex. For karyotype analysis the photographs enlarged 10×100 were taken using a microscope with a camera attachment. The karyotypes were measured by Software Image Analyses (Bs200ProP) loaded on a

*A part of this study was presented as an abstract at International Symposium on Biodiversity and Edible Wild Species Congress, 2017.

Figure 1. The habitat of *Astragalus stenosemioides.*

personal computer. Ideograms of these taxa were arranged in decreasing length. The karyotype asymmetry of the species has been determined by different methods: Stebbins classification (Stebbins, 1971), TF% (Huziwara, 1962), AsK% (Arano, 1963), Rec and Syi index (Greilhuber and Speta, 1976), A1 and A2 (Zarco, 1986), A (Watanabe et al., 1999), DI (Lavania and Srivastava, 1999), and AI (Arano and Saito, 1980).

Table 1. Collection data of *Astragalus stenosemioides* species examined here from karyological point of view.

Species	Locality
A. stenosemioides	B5 Kayseri: Erciyes Mount, above Tekir plateau, 2316 m, 38º 32' 533" N, 035º 31' 105" E, 17.06.2016, *B. Atasagun 1078* (ERCH).

3. Results

Karyotype analysis of *Astragalus stenosemioides* was analyzed in detail. The chromosome number of *A. stenosemioides* was determined to be 2*n* = 16 in the karyomorphological study (Fig. 2,3). The smallest chromosome has a length of 1.96 µm and the largest has a length of 3.38 µm. Total haploid chromosomes length was measured as 21.36 µm. The karyotype formula of this species consists of eight median chromosome. Chromosome arm ratio is ranging from 1.06 to 1.39. Centromeric index from 3.86-7.66 and relative length from 9.18-15.81 (Fig. 2,3, Table 2). The karyotype asymmetry is as follows: Stebbins index 1A, TF% 44.85, AsK% 55.15, Syi 80.95, Rec 78.99, A1 0.19, A2 0.18, DI 8, A 0.11, AI 3.79.

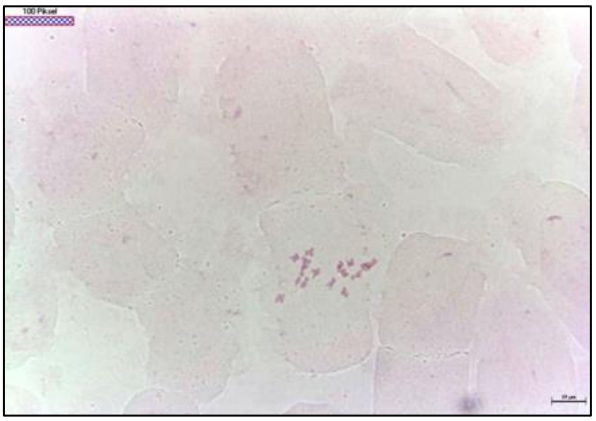

Figure 2. Metaphase chromosomes of *Astragalus stenosemioides.* Scale bar: 10 µm.

Table 2. Measurements (µm) of somatic chromosomes in *Astragalus stenosemioides* (m = median)

Chromosome	Chromosome arms (µm)		Total length (µm)	Arm ratio (L/S)	Relative length (%)	Centromeric index	Chromosome type
Pair no.	Long arm (L)	Short arm (S)					
1	1.74	1.64	3.38	1.06	15.81	7.66	m
2	1.74	1.31	3.05	1.32	14.31	6.16	m
3	1.69	1.27	2.96	1.33	13.84	5.95	m
4	1.48	1.33	2.81	1.12	13.16	6.21	m
5	1.56	1.12	2.68	1.39	12.53	5.25	m
6	1.27	1.15	2.42	1.11	11.36	5.39	m
7	1.16	0.94	2.1	1.24	9.81	4.38	m
8	1.14	0.82	1.96	1.38	9.18	3.86	m

4. Discussions

This study reports the diploid chromosome number and karyotype of *Astragalus stenosemioides.* Martin et al. (2019) reported that taxa in four different sections (*Macrophyllium* Bunge, *Hymenostegis* Bunge, *Hymenocoleus* Bunge and *Anthylloidei* DC.) of the genus *Astragalus* naturally grown in Turkey were examined in terms of cytogenetics. Somatic chromosome numbers have been reported as in section *Macrophyllum*: *Astragalus oleaefolius* DC., *A. dipodurus* Bunge, *A. cephalotes* Banks & Sol. and *A. yukselii* Karaman & Aytaç 2*n* = 16, *A. longifolius* Lam. 2*n* = 32 and *A. isauricus* Hub.-Mur. & Matthews 2*n* = 48, in section *Hymenostegis*; *Astragalus zohrabi* Bunge, *A. sosnowskyi* Grossh., *A. velenovskyi* Nabelek, *A. trifoliastrum* Hub.-Mur. & Matthews and *A. uraniolimneus* 2*n* = 16 Boiss., *A. hymenocystis* Fisch. & C.A.Mey., 2*n* = 18, *A. lagopoides* Lam., *A. hirticalyx* Boiss. & Kotschy, *A. gueruenensis* Podlech, *A. ciloensis* Podlech 2*n* = 48, in section *Hymenocoleus*; *A. vaginans* DC. 2*n* = 48 and in section *Anthylloidei*; *Astragalus szowitsii* Fisch. & Mey., *A. ermineus* Matthews, *A. zederbaueri* Stadlmann, *A. anthylloides* Lam., *A. halicacabus* Lam., *A. chardinii* Boiss.and *A. wagneri* Bunge2*n* = 16, *A. surugensis* Boiss. & Hausskn. 2*n* = 48.

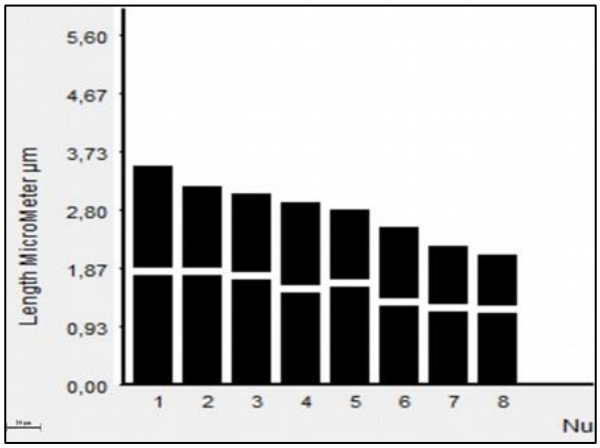

Figure 3. Ideogram of *Astragalus stenosemioides* Scale bar: 10 µm.

The chromosome number of *Astragalus stenosemioides* was determined as 2*n* = 16 in this study. In terms of chromosome number, it overlaps with *Astragalus oleaefolius*, *A. dipodurus*, *A. cephalotes*, *A. yukselii*, *A. zohrabi*, *A. sosnowskyi*, *A. velenovskyi*, *A. trifoliastrum*, *A. uraniolimneus*, *A. szowitsii*, *A. ermineus*, *A. zederbaueri*, *A. anthylloides*, *A. halicacabus*, *A. chardinii* and *A. wagneri*.

When compared in terms of karyological measurement results, the karyotype formula consists of 8m types in *Astragalus stenosemioides*. Species with the same karyotype formula; *A. oleaefolius*, *A. dipodurus*, *A. cephalotes*, *A. yukselii*, *A. sosnowskyi*, *A. velenovskyi*, *A. uraniolimneus*, *A. szowitsii*, *A. ermineus*, *A. zederbaueri*, *A. anthylloides*, *A. halicacabus*, *A. chardinii* and *A. wagneri*, while other species have different karyotype formulas.

Ekici et al. (2005) stated that the chromosome of *A. ovalis* were consisted of median (8m) chromosomes. The somatic chromosome number of *Astragalus ovalis* was determined as 2*n* = 16. Total chromosome lengths range from 1.11 µm to 1.63 µm. The karyotype formula of *A. stenosemioides* (8m) was the same as *A. ovalis*. In addition, chromosome lengths of *A.stenosemioides* were found to be longer than *A. ovalis*.

Although it has the same chromosome number and karyotype formula, it is the closest *Astragalus halicacabus* species in terms of chromosome measurement results. While the average chromosome lengths were between 1.96 µm and 3.38 µm in *Astragalus stenosemioides*; 1.89 and 3.49 µm in *A. halicacabus* species. In terms of total haploid chromosome length, it is 21.36 µm in *A. stenosemioides*, while it is 21.28 µm in *A. halicacabus* (Martin et al., 2019).

As in the *Astragalus stenosemioides* species, Atasagun et al. (2018) reported that the somatic chromosome number of *A. argaeus* was as 2*n* = 16 and it was stated that it had a different karyotype formula. However, we can state that they are different from each other in terms of chromosome measurement results. While the karyotype formula was 8m in *A. stenosemioides*, it was reported as 3m+5sm in *A. argaeus*. While the average chromosome lengths were between 1.96 µm and 3.38 µm in *A. stenosemioides*; 1.76 and 3.14 µm in *A. argaeus*, in terms of total haploid chromosome length; It is 21.36 µm in *A. stenosemioides* and 19.44 µm in *A. argaeus* (Atasagun et al., 2018).

The *Hololeuce* section includes 15 taxa in Turkey (Ekici & Ekim, 2004). In this section, two species (*Astragalus incertus* Ledeb. and *Astragalus sibthorpianus* Boiss.) were examined in terms of cytogenetics. It has been stated that *A. incertus* has 2n=4x=32 tetraploid, while *A. sibthorpianus* has 2n=16 chromosomes (Cartier, 1979; Magulaev, 1980). In this study, it was determined that *A. stenosemioides* was diploid and there was no polyploidy in the squash preparation method.

Since the genus *Astragalus* has a large number of taxa in the world, there are chromosomal studies on different taxa of the genus. However, these chromosome studies are usually on the determination of the somatic chromosome number.

The number of taxa in which detailed chromosome measurements of the genus are made is quite low. In karyotype studies, metacentric and submetacentric and very rare subtelocentric chromosome pairs are generally observed in taxa belonging to the genus. At the same time, we can talk about polyploidy, a folding of the basic chromosome numbers in different taxa of the genus. No polyploidy was found in the studied species. Rarely, it is observed in some taxa of the genus in different situations such as satellites and B chromosomes. However, no difference was observed in the taxa in our study as a result of repeated squash preparation method. If we evaluate in terms of chromosome measurement results; we can say that the chromosomes do not show a very large obvious measurement difference in the different taxa of the genus so far.

In this study, the karyotype asymmetry of *Astragalus stenosemioides* were determined for the first time. We hope that this study will contribute to the future karyological studies about the genus *Astragalus*.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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