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KARYOMORPHOLOGICAL STUDY OF SEVEN SPECIES OF THE GENUS ASTRAGALUS (FABACEAE) FROM IRAN

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Karyomorphological study was carried out on 11 accessions from 6 sections belonging to 7 species of *Astragalus* growing in Iran. Chromosome numbers and karyotypes of two species (*A. cephalanthus* DC. and *A. jacobsii* Podlech) are new records. Two ploidy levels 2*n* = 2x = 16 and 2*n* = 4x = 32 were determined, the chromosomes size varied from 2.51 in *A. cyclophyllos* to 3.79 µm in *A. fasciculifolius* Boiss. in two accessions of *A. cyclophyllos* , a single pair satellite chromosomes were observed.

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Key words: Chromosome number; karyotype; cytogenetic; diversity; Fabaceae

مطالعه کاریومورفولوژیکی هفت گونه از جنس گون در ایران

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مطالعه سیتولوژیکی بر روی ۱۱ نمونه متعلق به ۶ بخش از ۷ گونه Astragalus ایران انجام شد. تعداد کروموزوم‌ها و کاریوتایپ‌های دو گونه (A. cephalanthus DC. و A. jacobsii Podlech) گزارش‌های جدیدی هستند. دو سطح پلوئیدی دی و تتراپلوئیدی 2n = 2x = 16 و 2n = 4x = 32 تعیین و اندازه کروموزوم‌ها از ۲/۵۱ میکرومتر در A. cyclophyllos G. Beck تا ۳/۷۹ میکرومتر در A. ficiculifolius Boiss. متغیر بود. در دو نمونه از گونه A. cyclophyllos، یک جفت ماهواره مشاهده شد.

INTRODUCTION

Astragalus L. (Fabaceae), as the largest genus of the flowering plants, contains an estimated number of 2500-3000 annual and perennial herbaceous or subshruby taxa, grouped in 150-250 sections (Lock & Simpson 1991; ILDIS 2002; Mabberley 1997; Maassoumi 1998). This genus is widely distributed throughout the temperate and arid regions of the world, and is principally located in Asia (1500 species), North America (500 species), South America (150 species) and Europe (120 species), but also on mountains in Africa. However, the center of origin and biodiversity of *Astragalus* is Eurasia, especially the mountainous parts of South-Western and South-Central Asia (Lock & Simpson 1991; Maassoumi 1998). Iran is rich in biodiversity due to its diverse climatic and geographical conditions and with more than 820 published and valid species of *Astragalus* with an endemic rate of 57%, it is one of the most important center of diversity for this genus (Bagheri *&* al. 2011; Ghahremaninejad 2015; Miri & Shamsolshoara 2019; Ranjbar *&* al. 2014).

The first deliberate study on the chromosomes of *Astragalus* was made by Ledingham (1960) who reported the species from the old world have a basic chromosome number of x = 8, while those from the new world have x = 11, 12 and 13 (Badr and Sharawy 2007). Numerous cytological studies have been conducted on *Astragalus* species and the basic chromosome numbers of the genus has been reported as x = 6, 7, 8, 9, 11, 15 (Bozkurt & al. 2019). In addition, five ploidy levels (2*x*, 4*x*, 6*x*, 8*x* and 12*x*) are present in the genus (Ranjbar & al. 2014). The basic number of x = 8 is the primary basic number in *Astragalus*. It seems that x = 7 and x = 6 basic numbers have been derived from x = 8 by aneuploid loss of chromosomes (Badr and Sharawy 2007).

In this study, we explore the chromosome number and karyomorphology of 11 accessions belonging to 7 species of the *Astragalus* L. in Iran.

MATERIALS AND METHODS

Eleven *Astragalus* accessions belonging to 7 species from the collections of Natural Resources Gene Bank of Iran, Research Institute of Forests and Rangelands (RIFR), Tehran, Iran were included in this study (table 1). Voucher specimens have been deposited in the herbarium of Research Institute of Forests and Rangelands (TARI). The seeds were disinfected by 0.2% carboxin thiram fungicide (Vitavax®) for 3 minuts and placed in petri dishes on moist filter paper at 4ºC for two weeks, then they were germinated at 25ºC in a growth chamber under dark conditions. For cytological investigations, 1-1.5 cm long young root tips were pretreated with 1% α-bromonaphtalin solution for 3 h at room temperature and fixed in freshly Lewitsky solution, a mixture of 1:1 chromic acid 1%: formaldehyde 10% for 24 h at 4°C. Root tips were hydrolyzed for 15 min in 1 N HCl at 60°C, washed briefly in ddH_2O and stained in 1% hematoxylin solution for 24 h at room temperature. Squashed preparation was made in a drop of 45% acetic acid. After obtaining the appropriate metaphase, the photographs of at least 10 well-spread chromosomes were taken using digital camera mounted on an Olympus BX41 microscope and chromosomal measurements were made from five mitotic metaphases cells. Based on the measurements of chromosome length and arm ratios, a karyotype formula has been calculated as proposed by Levan & al. (1964). The symmetry class (Sc) of the karyotypes was made according to Stebbins (1971). The karyotype asymmetry was estimated using intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices suggested by Zarco (1986).

RESULTS AND DISCUSSION

The cytological data for the examined accessions is summarized in tables 2 & 3 and their karyotypes and karyogrms are illustrated in fig. 1. Basic chromosome number of x = 8 are found in all studied accessions. The basic chromosome number reported for the old world *Astragalus* species (both from Iran and outside) is x = 8 and di-, tri-, tetra- and hexaploidy levels were observed (Ranjbar & Mahmoudian 2015; Sheidai & al. 2009). In our study, four species were diploid (*A. cephalanthus* DC., *A. curvirostris* Boiss., *A. dactylocarpus* Boiss. and *A. megalotropis* Bunge) and two species (*A. fasciculifolius* Boiss. and *A. jacobsii* Podlech) were tetraploid. In the accessions of *A. cyclophyllos* G. Beck, both di- and tetraploidy were observed. Therefore, both diploid and tetraploid levels occur in a single species which is also present in some other *Astragalus* species such as *A. iranicus* Bunge (Ranjbar & Mahmoudian 2015) and *A. camptoceras* Bunge (Gedik & al. 2019). These reports indicate the role of polyploidy in the evolution of *Astragalus* (Sheidai & al. 2009). As far as we know, the somatic chromosome numbers and karyotypes of two species (*A. cephalanthus* DC. and *A. jacobsii* Podlech) are reported for the first time. Chromosome numbers of the other studied accessions were consistent with previous counts (Badr & Sharawy 2007; Goldblatt & Johnson 2010; Maassoumi 1987; Marhold & Breitwieser 2009; Ranjbar & al. 2011).

The average chromosome length (CL) varies about 1.5 times between accessions (table 2). *A. cyclophyllos* (107732 TARI) exhibit much shorter chromosomes (2.51 µm) and lower total haploid chromosome length (20.71 µm), compared to other accessions. Meanwhile, longest CL (3.79 µm) and TCL (60.68 µm) have been found in *A. fasciculifolius* Boiss. (107733 TARI). Sheidai & Ghahremaninejad (2009), Sheidai & al. (2009) and Martin & al. (2019) reported the chromosome length of *Astragalus* species in the range of 1.87-5.20 µm, 2.20-5.51 µm and 2.00-3.71 µm, respectively. The chromosome length of the examined *Astragalus* accessions are generally medium size. Although the highest mean chromosome length was observed in a tetraploid accession, no significant correlation was found between mean chromosome length and polyploidy (r = 0.05, data not shown). However, Sheidai & al. (2009) reported that with increase in polyploidy level, decrease in the mean chromosome length has occurred (although not significant), which may indicate the loss of DNA during the polyploidy induction and species diversification.

Table 1. Geographical information of the *Astragalus* species, investigated in the present study.

Species	Section	Location	Latitude	Longitude	Altitude (m)	Herbarium code (TARI)
A. cephalanthus DC.	*Microphysa* Bunge	Kerman-Bardsir	N 29º 51' 00''	E 56º 05' 00''	2583	105647
A. curvirostris Boiss.	*Incani* DC.	Zanjan-Mahneshan	N 36º 40' 00''	E 47º 40' 00''	1950	105636
A. cyclophyllos G. Beck	*Incani* DC.	Zanjan-Zanjan	N 36º 40' 44''	E 48º 29' 56''	1638	107732
A. cyclophyllos G. Beck	*Incani* DC.	Kerman-Kerman	N 30º 17' 36''	E 57º 05' 30''	1755	107734
A. cyclophyllos G. Beck	*Incani* DC.	Isfahan-Fardin	N 33º 13' 30''	E 49º 58' 35''	2700	105645
A. cyclophyllos G. Beck	*Incani* DC.	Isfahan-Chadegan	N 32º 43' 00''	E 50º 43' 00''	2060	107735
A. dactylocarpus Boiss.	*Chronopus* Bunge	Yazd-Harat	N 29º 56' 00''	E 54º 05' 00''	1850	105648
A. fasciculifolius Boiss.	*Poterion* Bunge	Khuzestan-Haftkel	N 31º 26' 49''	E 49º 31' 46''	600	107733
A. fasciculifolius Boiss.	*Poterion* Bunge	Hormozgan-Bandar Abbas	N 27º 48' 15''	E 56º 17' 52''	900	105659
A. jacobsii Podlech	*Malacothrix* Bunge	Markazi-Tafresh	N 34º 33' 00''	E 49º 48' 00''	2050	105639
A. megalotropis Bunge	*Alopecuriodei* DC.	Hamedan-Malayer	N 34º 37' 05''	E 48º 43' 20''	2350	105654

Table 2. Karyotypic parameters measured on the 11 accessions of studied *Astragalus* species.

Species	2n	TCL (µm)	CL (µm)	LA (µm)	SA (µm)
A. cephalanthus DC. (105647)	2n=2x=16	28.03	3.50ab**	2.12ab**	1.39ab**
A. curvirostris Boiss. (105636)	2n=2x=16	27.38	3.42ab	2.14ab	1.29 ab
A. cyclophyllos G. Beck *(*107732)	2n=2x=16	20.71	2.51c	1.52c	0.99bc
A. cyclophyllos G. Beck (107734)	2n=4x=32	46.73	2.92 bc	1.78bc	1.14b
A. cyclophyllos G. Beck (105645)	2n=4x=32	58.77	3.36ab	2.10ab	1.31 ab
A. cyclophyllos G. Beck *(*107735)	2n=2x=16	24.82	3.12 bc	1.97b	1.14b
A. dactylocarpus Boiss. (105648)	2n=2x=16	21.27	2.74 bc	1.86 b	0.88c
A. fasciculifolius Boiss. (107733)	2n=4x=32	60.68	3.79a	2.33a	1.46a
A. fasciculifolius Boiss. (105659)	2n=4x=32	46.33	2.89 bc	1.77 bc	1.12b
A. jacobsii Podlech (105639)	2n=4x=32	43.02	2.69 bc	1.67 bc	1.02 bc
A. megalotropis Bunge *(*105654)	2n=2x=16	28.64	3.58ab	2.11ab	1.47a

**: means within a column followed by the same letter are not significantly different according to the Duncan's multiple range test at $p\leq 0.01$.

Table 3. Asymmetrical parameters measured on the 11 accessions of studied *Astragalus* species.

Species	Sc	A_1	A_2	TF%	AR	CI	DRL	KF
A. cephalanthus DC. (105647)	2A	0.32b*	0.16	39.78b**	1.62c**	0.40ab**	5.98abc*	6m+2sm
A. curvirostris Boiss. *(*105636)	2A	0.39ab	0.15	37.27 bc	1.78ab	0.37bc	5.26abc	3m+5sm
A. cyclophyllos G. Beck *(*107732)	1A	0.34b	0.14	39.49 b	1.56c	0.40 ab	4.39bc	5m+3sm
A. cyclophyllos G. Beck *(*107734)	2A	0.34b	0.14	39.03b	1.65c	0.39ab	3.15 bc	12m+4sm
A. cyclophyllos G. Beck *(*105645)	2A	0.36ab	0.17	38.62b	1.75ab	0.38bc	4.74bc	7m+1m^{sat}+8sm
A. cyclophyllos G. Beck *(*107735)	3A	0.39ab	0.18	36.70 bc	1.82ab	0.37bc	6.21ab	3m+1m^{sat}+4sm
A. dactylocarpus Boiss. (105648)	3A	0.49a	0.22	32.37c	2.16a	0.33c	6.63ab	1m+7sm
A. fasciculifolius Boiss. *(*107733)	2B	0.35b	0.23	38.50b	1.67c	0.39bc	4.56bc	9m+7sm
A. fasciculifolius Boiss. *(*105659)	2A	0.34b	0.19	38.81 b	1.60c	0.39bc	4.76bc	12m+4sm
A. jacobsii Podlech *(*105639)	2A	0.38ab	0.12	37.80 bc	1.69bc	0.38bc	2.33c	8m+8sm
A. megalotropis Bunge (105654)	1A	0.29b	0.20	41.07 a	1.50c	0.41a	7.10a	7m+1sm

2n=somatic chromosome number; SC= symmetry classes of Stebbins; A_1= intra-chromosomal index; A_2= inter-chromosomal index; TF%= total form percentage; DRL=Difference of Relative Length; KF=karyotype formula; CI= Centromeric index; TL= total chromosome length; LA= long arm; SA= short arm; AR= arm ratio

* and **: means within a column followed by the same letter are not significantly different according to the Duncan's multiple range test at $p\leq 0.05$ and 0.01, respectively.

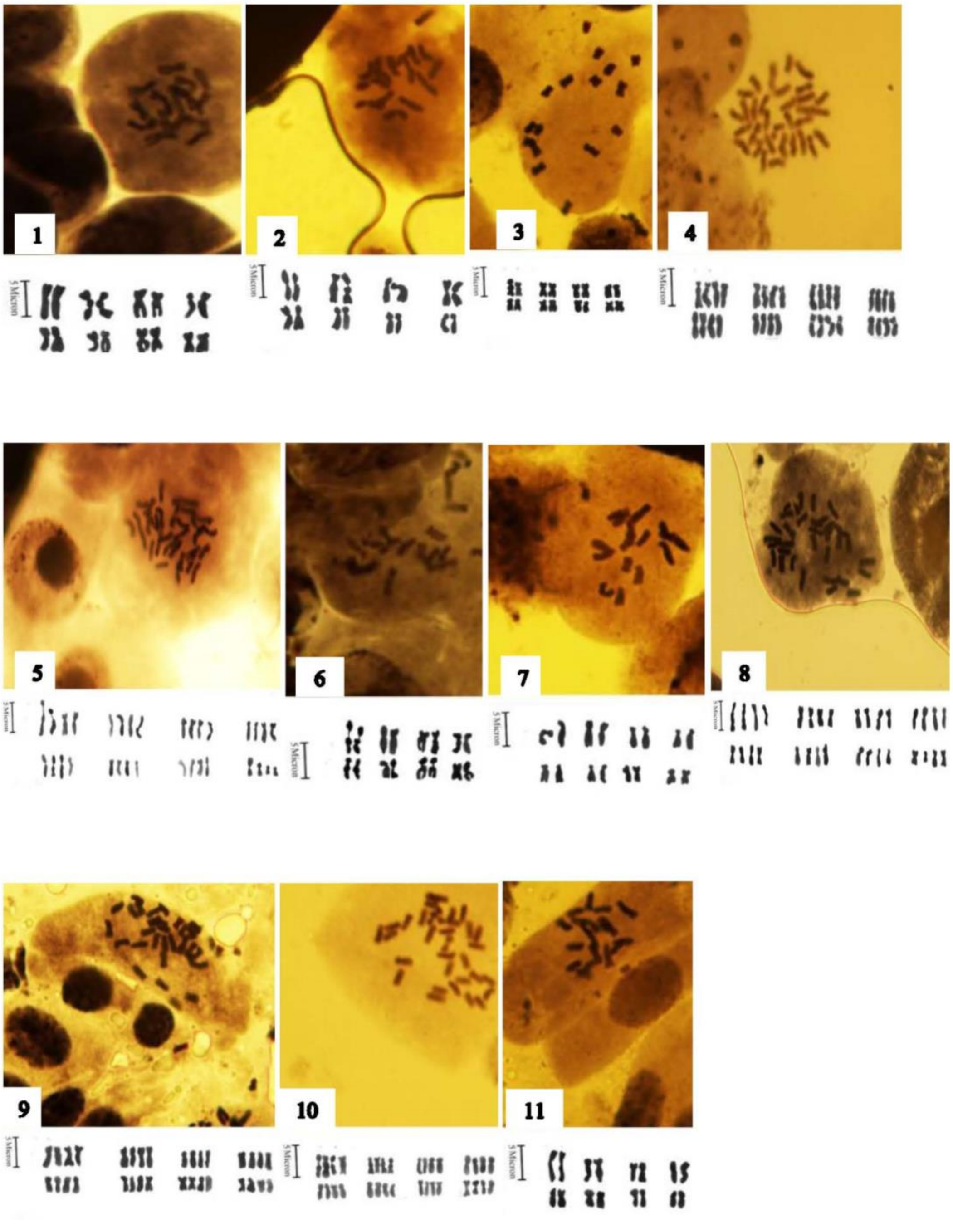

Fig. 1. Karyotypes and karyograms of 11 accessions of *Astragalus* L. 1, *Astragalus cephalanthus* DC. (105647); 2, *A. curvirostris* Boiss. (105636); 3, *A. cyclophyllos* G. Beck (107732); 4, *A. cyclophyllos* G. Beck (107734); 5, *A. cyclophyllos* G. Beck (105645); 6, *A. cyclophyllos* G. Beck (107735); 7, *A. dactylocarpus* Boiss. (105648); 8, *A. fasciculifolius* Boiss. (107733); 9. *A, fasciculifolius* Boiss. (105659); 10, *A. jacobsii* Podlech (105639); 11, *A. megalotropis* Bunge (105654).

In *A. cyclophyllos* (105645 TARI and 107735 TARI) a single pair of satellite on short arm were observed. Previous studies reported that *A. stella* Gouan, *A. victoriae* Podlech & Kirchhoff, *A. densifolius* Lam and *A. uraniolimneus* Boiss. had single satellite chromosome pair (Badr & Sharawy 2007; Bozkurt & al. 2019; Gedik & al. 2019; Martin & al. 2019), and *A. effusus* Bunge had two satellite chromosome pairs (Yousefzadeh & al. 2010). On the other hand, Konichenko & al. (2014) did not observe satellites in the karyotype of *A. sericeocanus* Gontsch. The satellite represents 50-80% on the long arm, depending on the species (Dopchiz & al. 1995). Dopchiz & al. (1995) and Bozkurt & al. (2019) reported species of *Astragalus* possess satellites on the long arm of chromosome, while Badr & Sharawy (2007) and Yousefzadeh & al. (2010) found satellites were always located on the short arms. In *A. cyclophyllos* (105645 TARI), only one of the two pairs of homologous chromosomes has satellites that appear to be allotetraploid. However, more study such as Gimmsa C-bandind or genomic *in situ* hybridization (GISH) methods is required to determine their origin information, that is, whether these tetraploids are allo- or autotetraploids.

Karyotype asymmetry is one of the most important parameters in chromosomal data (Martin & al. 2019). All studied accessions show relatively symmetrical karyotypes, consisting of metacentric and submetacentric chromosomes. Recent studies also confirm similar karyotypes for *Astragalus* (Bozkurt & al. 2019; Gedik & al. 2019; Sheidai & Ghahremaninejad 2009; Sheidai & al. 2009; Yousefzadeh & al. 2010). Some studied *Astragalus* species differed in their karyotype formulae. Even, the populations belong to the same species were divided into different karyotype groups. Similarly, Konichenko & al. (2014) observed that the karyotype formulae of *A. sericeocanus* Gontsch populations were divided into three groups. This indicates that changes in the form of their chromosomes possibly due to structural changes like inversion, translocation, etc., which is considered as a qualitative change in the genome (Sheidai & Ghahremaninejad 2009).

Astragalus megalotropis Bunge showed the most symmetrical karyotype as 41.07, 0.19, 1.50 and 0.41 for TF%, A_1, AR and CI, respectively. Of all the investigated accessions, *A. dactylocarpus* Boiss. presented the most asymmetrical karyotype with a TF% of 32.37%, A_1 index of 0.49, AR of 2.16 and CI of 0.33. The A_2 index ranged from 0.12 (*A. jacobsii* Podlech) to 0.23 (*A. fasciculifolius* Boiss. (107733TARI). Based on the results of interchromosomal asymmetry with the value of lowest A_2 (0.12) and DRL (2.33), *A. jacobsii* Podlech possessed the least asymmetrical karyotype.

Four types of Stebbins symmetry class were found: 1A, 2A, 3A and 2B. The 2B type included *A. fasciculifolius* Boiss. (107733 TARI); 3A type included two accessions (*A. cyclophyllos* (107735 TARI) and *A. dactylocarpus* Boiss. and the remaining 8 accessions belonged to the 1A (2 accessions) and 2A (6 accessions) types. However, Stebbins classification of *Astragalus* species is generally reported in 1A or 2A classes (Sheidai & Ghahremaninejad, 2009; Sheidai & al. 2009), which indicates more asymmetry karyotypes in some accessions studied.

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