

CHROMOSOME COUNTS REPORT OF SIX ASTRAGALUS L. (FABACEAE) SPECIES FROM IRAN

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Received 2022. 04. 08; accepted for publication 2022. 06. 07

Jalilian, N., Sadeghian, S., Safari, H., Jalili, A. & Asadi -Corom, F. 2022. 06. 30: Chromosome counts report of six *Astragalus* L. (Fabaceae) species from Iran. *Iran. J. Bot.* 28 (1): 69-75. Tehran.

Somatic chromosome numbers of six endemic and rare species of the genus *Astragalus* were studied. The basic chromosome number for all studied species was $x = 8$. Five of the studied species including *A. assadabadensis*, *A. carduchorum*, *A. kirrindicus*, *A. nervistipulus*, and *A. microcephalus* subsp. *pynocladius* were diploid and *A. trachyacanthos* was tetraploid. The chromosomal parameters of the species were calculated and their idiograms were drawn. The karyotypic formula of the species was different, but in all species most of the chromosomes were metacentric and there were zero to three submetacentric chromosomes. *Astragalus assadabadensis* had the longest chromosomes and *A. trachyacanthos* had the shortest chromosomes. Chromosome counts of three species including *A. carduchorum* from the sect. *Acidodes*, *A. microcephalus* subsp. *pynocladius* and *A. trachyacanthos* from the sect. *Rhacophorus* are reported for the first time.

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Keywords: *Astragalus*; chromosome counts; Fabaceae; idiograms; karyotype; Iran

شمارش کروموزومی شش گونه از جنس *Astragalus* (Fabaceae) از ایران

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عدد کروموزومی ۶ گونه انحصاری و نادر از جنس *Astragalus* در این تحقیق بررسی شد. عدد پایه کروموزومی برای تمام گونه‌ها $x=8$ بود. گونه‌های *A. microcephalus* subsp. *pyncocladus* و *A. nervistipulus*, *A. kirrindicus*, *A. carduchorum*, *A. assadabadensis* دیپلوئید و گونه‌ی *A. trachyacanthos* تتراپلوئید بود. پارامترهای کروموزومی گونه‌ها بررسی شد و ایدیوگرام آنها رسم گردید. فرمول کاریوتیپی گونه‌ها متفاوت بود اما در تمام آنها بیشتر کروموزوم‌ها متاستریک بودند و تعداد صفر تا ۳ کروموزوم ساب متاستریک در فرمول کاریوتیپی وجود داشت. گونه‌ی *A. assadabadensis* بلندترین طول کروموزوم‌ها و گونه‌ی *A. trachyacanthos* کوتاه‌ترین طول کروموزوم‌ها را داشتند. عدد کروموزومی گونه‌ی *A. carduchorum* از بخش *Acidodes*، گونه‌ی *A. microcephalus* subsp. *pyncocladus* و *A. trachyacanthos* از بخش *Rhacophorus* برای اولین بار گزارش می‌گردد.

INTRODUCTION

Astragalus L. (Fabaceae) is the largest genus of vascular plants with approximately 2900 species, which has two main centers of diversity in the world, Eurasia (Old World) and America (New World). Most of the species are distributed in the Old World (ca. 2400 species.), whereas ca. 500 species are restricted to the New World (Zarre & Azani 2013). Iran is known for its high diversity of the genus *Astragalus* which comprises 844 species, of which 620 species are endemic (Maassoumi 2016b). The most common basic chromosome number in *Astragalus* is $x=8$, but $x=11$, 12, 13, 14, 15 and five ploidy levels ($2n=2x=16$, $2n=4x=32$, $2n=6x=48$, $2n=8x=64$ and $2n=12x=96$) have been reported in the world (Spellenberg 1976; Aryavand 1977, 1983; Zhukova, 1983; Parfitt & al. 1985; Maassoumi 1987, 1989; Badr & al., 1996; Sheidai & Gharemaninejad 2008; Sheidai & al. 1996; 2009; Yousefzadeh & al. 2010; Ranjbar & al. 2011; 2015; Rani & al. 2014; Javadi & al. 2019; Martin & al. 2019; Ghaffari 2020; Akhavan & Jalili 2021; Bagheri & al. 2022). In the present study, chromosome number and karyotype of six species of the genus *Astragalus*, mostly endemics to Iran were investigated.

MATERIALS AND METHODS

This study was carried out by using seeds collected from natural habitats in Kermanshah Province. The vouchers are deposited in the herbarium of Kermanshah Agricultural and Natural Resources

Research and Education Centre (RANK: acronym according to Thiers 2021). For the cytological study, root tips were cut about 1-1.5 cm in length, from germinated seeds on wet filter paper in Petri dishes at 22°C temperature. Then they were pretreated with 0.5% saturated α -Bromo naphthalene at 4°C for 3-4 h and fixed in 10% formaldehyde and chromium trioxide (1:1) at 4°C for 16 to 20 h. The fixed root tips were rinsed for 1 h in distilled water and hydrolyzed in NaOH (1 Normal) at 60°C for 20-30 min. The specimens were washed with distilled water and stained with hematoxylin-iron for 1 h at room temperature and squashed in a droplet of 45% acetic acid. The preparations were observed with an optical microscope (BH2 Olympus supplemented Digital color video camera) at a magnification of 2000x. The best metaphase plates were selected and measured by Micromerit 3.3 software (Reeves & Tear 2000). The chromosome morphology was studied based on Levan & al. (1964). Idiograms were depicted for each species. Karyotypic parameters and asymmetry indices were measured as follows: the long arm (LA), short arm (SA), total chromosome length of the haploid complement (TL), arm ratio (AR), and total form percentage (TF%), (Huziware 1962). Also, karyotypic evolution has been determined using the symmetry classes of Stebbins (SC), (Stebbins, 1971). Details regarding the studied materials are presented in Table 1.

Table 1. Karyotype characters of the six studied species. Abbreviations: Chr. No. Chromosome number, TL: Total haploid chromosome length, LA: Long arm, SA: Short arm, AR: Arm ratio, DRL: Difference of relative length, CI: Centromeric index, A₁: Intrachromosome asymmetry index, A₂: Interchromosome asymmetry index, VRC: Value of relative chromatin, TF%: Total form percentage, SC: Symmetry classes of Stebbins and K.F.: Karyotype formula.

Species	Chr. No.	TL	LA	SA	AR	DRL	CI	A ₁	A ₂	VRC	%TF	SC	K.F.
<i>A. assadabadensis</i>	2n=2x=16	4.00	2.43	1.56	1.57	5.48	0.39	0.32	0.15	4.00	39.17	2A	6m+2sm
<i>A. nervistipulus</i>	2n=2x=16	3.85	2.34	1.51	1.80	7.21	0.39	0.34	0.20	3.85	39.22	2A	6m+2sm
<i>A. carduchorum</i>	2n=2x=16	3.94	2.44	1.50	1.64	6.25	0.38	0.37	0.14	3.94	37.99	1A	5m+3sm
<i>A. kirrindicus</i>	2n=2x=16	3.17	1.79	1.37	1.30	5.06	0.43	0.22	0.11	3.17	43.51	1A	8m
<i>A. microcephalus</i> subsp. <i>pyncocladus</i>	2n=2x=16	3.17	1.84	1.33	1.40	3.57	0.42	0.26	0.08	3.17	42.04	1A	7m+1sm
<i>A. trachyacanthos</i>	2n=4x=32	2.57	1.4	1.16	1.33	2.73	0.43	0.22	0.13	2.57	43.31	1A	15m+1sm

RESULTS AND DISCUSSION

In the present study, somatic chromosome numbers and karyotype features of six endemic and rare species of the genus *Astragalus* sections *Acidodes*, *Rhacophorus*, *Hymenostegis*, and *Alopecuroidei* were studied. All six studied taxa had homogeneous karyotypes characterized by the basic chromosome number of $x=8$, which is similar to the most other previously studied species of *Astragalus*. The chromosome numbers for two endemic and one rare taxon of the flora of Iran, including *A. trachyacanthos*, *A. microcephalus* subsp. *pyncocladus* and *A. carduchorum* are reported for the first time. The mean length of the long arm of the chromosome (LA) varied from 1.33 µm in *A. trachyacanthos* to 2.44 µm in *A. carduchorum*. The average length of the short arm of the chromosome (SA) ranged from 1.05 µm in *A. trachyacanthos* to 1.56 µm in *A. assadabadensis*. The mean length of the total haploid chromosome (TL) varied from 2.38 µm in *A. trachyacanthos* to 4 µm in *A. assadabadensis* (Table 1). The average values of chromosome arm ratio (AR) ranged from 1.30 in *A. kirrindicus* to 1.80 in *A. nervistipulus*. Five of the studied taxa including *A. carduchorum* ($2n=2x=16$), *A. microcephalus* subsp. *pyncocladus* ($2n=2x=16$), *A. assadabadensis* ($2n=2x=16$) and *A. kirrindicus* ($2n=2x=16$) were diploid and *A. trachyacanthos* ($2n=4x=32$) was tetraploid. All the taxa were located in Stebbins classes (SC) A (1A and 2A).

Mitotic chromosome counts for examined species are presented as follows:

Section *Hymenostegis* Bunge

Astragalus assadabadensis F. Ghahrem. & Podlech

Specimen examined: Iran, Kermanshah Province: Sonqor, Khoshyar, 1948-1990 m, 34.845 N, 47.601 E, Jalilian & Nemati 10597 (RANK).

Astragalus assadabadensis is an endemic subshrub species. The distribution range is in the west of Iran in Hamedan and Kermanshah Provinces (Maassoumi 2016a). The results showed that this species is diploid with a chromosome number of $2n=2x=16$, as had been reported for the Assadabad (Hamadan) population by Bagheri & al. (2022). Karyotype in this species consisted of six pairs of metacentric (m) and two submetacentric (sm) chromosomes (Fig. 1A). It was categorized as type 2A (Stebbins 1971). TL (4µm) and SA (1.56 µm) values in this species were the highest among the studied species (Table 1).

Section *Hymenostegis* Bunge

Astragalus nervistipulus Boiss. & Hausskn.

Specimen examined: Iran, Kermanshah Province: Paveh, Khaneqah, 1515 m, 35.034 N, 46.348 E, Jalilian & Nemati 8779 (RANK).

Astragalus nervistipulus is a rare subshrub species reported from Iran and Iraq. Its distribution range is in the northwest and west of Iran (Azerbaijan, Hamedan, Kurdistan, and Kermanshah Provinces) (Maassoumi 2016a). The results showed that this species is diploid and the chromosome number, the karyotypic formula, and Stebbins category (2A) are the same as *A. assadabadensis* with a chromosome number of $2n=2x=16$. (Fig. 1B). Thus, this report confirms the

result of the previous study by Bagheri & al. (2022) on the Sanandaj (Kurdistan) population. DRL (7.21), AR (1.80) and A_2 (0.20) values were the highest among the studied species (Table 1).

Section *Acidodes* Bunge

Astragalus carduchorum Boiss. & Haussk. ex Boiss.
Specimen examined: Iran, Kermanshah Province, Paveh, Saryas, 2785-2928 m., 35.029 N, 46.451 E, Jalilian & Nemati, 10652 (RANK).

Astragalus carduchorum is a rare pulvinate subshrub reported from Iran and Iraq. Its distribution range is in the northwest and west of Iran in Azerbaijan, Kurdistan, and Kermanshah Provinces (Maassoumi 2016a). Its chromosome count is reported here for the first time. The results showed that this species is diploid with a chromosome number of $2n=2x=16$. Karyotype consisted of five pairs of metacentric (m) and three submetacentric (sm) chromosomes (Fig. 1C). It was categorized as type 2A (Stebbins 1971). LA (2.44 μm) and A_1 (0.37) values were the highest, while TF (37.99%) and CI (0.38) values were the lowest among the studied species (Table 1).

Section *Alopecuroidei* DC.

Astragalus kirrindicus Boiss.

Specimen examined: Iran, Kermanshah Province, Sahneh, Bid Sorkh, 1803-1860 m., 34.434 N, 47.828 E, Jalilian & Nemati, 9759 (RANK).

Astragalus kirrindicus is a rare perennial species reported from Iran and Iraq. Its distribution range is in the center and west of Iran (Kurdistan, Ilam, Tehran, Isfahan, Lorestan, Kohgiluyeh and Boyer-Ahmad, Chaharmahal, va Bakhtiari Provinces (Maassoumi 2003). The results showed that this species is diploid with a chromosome number of $2n=2x=16$. Karyotype consisted of eight pairs of metacentric (m) chromosomes (Fig. 1D). The karyotype tended to be symmetrical and classified as type 1A (Stebbins 1971). Similar results were obtained by Hesamzadeh & al. (2009) on the population of Khorram Abad in Lorestan province.

Section *Rhacophorus* Bge.

Astragalus microcephalus subsp. *pyncocladus* (Boiss. & Hausskn.) Sirj.

Specimen examined: Iran, Kermanshah Province, 15 km from Paveh to Ravansar, 1789-1880 m., 34.954 N, 46.448 E, Jalilian & Nemati 9222 (RANK).

Astragalus microcephalus is represented by two subspecies in the flora of Iran (Massoumi, 2016a), *Astragalus microcephalus* subsp. *pyncocladus* is an endemic pulvinate subshrub species, with a distribution range in the northwest and west Iran in Azerbaijan, Kurdistan, Lorestan, and Hamedan provinces (Maassoumi, 2016a). Its chromosome count is reported here for the first time. The results showed that this species is diploid with a chromosome number of $2n=2x=16$. The karyotypic formula was $7m + 1sm$ (Fig. 1E). The karyotype was symmetrical and classified as Stebbins 1A symmetry (Table 1). DRL (3.57) and A_2 (0.08) values were the lowest among the studied species (Table 1).

Chromosome number of *A. microcephalus* subsp. *microcephalus* had previously been reported as tetraploid ($2n=4x=32$) for Ghazvin & Orumiyeh populations (Sheidai & al. 2009) and Caucasian populations (Magulaev 1989), while, it had been reported for eastern Mediterranean populations as diploid ($2n=2x=16$), (Cartier, 1979).

Section *Rhacophorus* Bge.

Astragalus trachyacanthos Fisch.

Specimen examined: Iran, Kermanshah Province, Paveh, Qori Qaleh, 1689-1780 m., 34.905 N, 46.498 E, Jalilian & Nemati 10502 (RANK).

Astragalus trachyacanthos is an endemic pulvinate subshrub, with a distribution range in the central and west of Iran in Kermanshah, Hamedan, Lorestan, Isfahan, Chaharmahal va Bakhtiari, and Tehran Provinces (Massoumi, 2016a). Its chromosome count is reported here for the first time. The results showed that this species is tetraploid with a chromosome number of $2n=4x=32$. The karyotypic formula was $15m + 1sm$ (Fig. 1F). It was categorized as type 1A (Stebbins 1971). TL (2.57 μm), LA (1.4 μm), SA (1.16 μm), A_1 (0.22), VRC (2.57), and DRL (2.73) values were the lowest, and CI (0.43) was the highest value among studied species (Table 1).

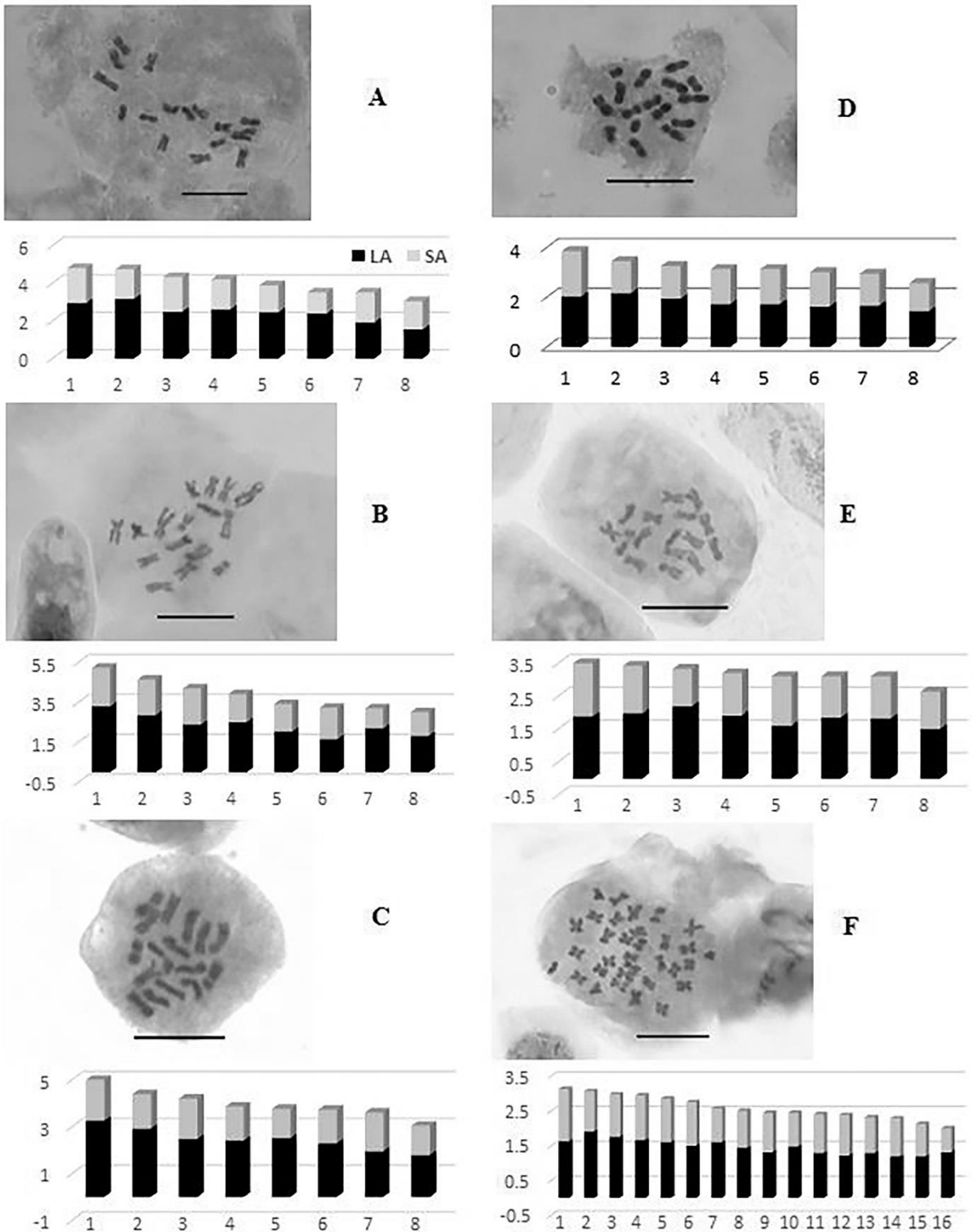


Fig. 1. Somatic metaphases and idiograms of, A, *Astragalus assadabadensis* (2n=2x=16); B, *A. nervistipulus* (2n=2x=16); C, *A. carduchorum* (2n=2x=16); D, *A. kirrindicus* (2n=2x=16); E, *A. microcephalus* subsp. *pycnocladus* (2n=2x=16); F, *A. trachyacanthos* (2n=4x=32). Scale bar = 10 μ m.

ACKNOWLEDGEMENT

The authors are grateful to the authorities of the Research Institute of Forests and Rangelands (RIFR) for financial support.

REFERENCES

- Akhavan Roofigar, A. & Jalili, A. 2021: Chromosome numbers report of five endemic *Astragalus* L. (Fabaceae) species of Iran. -Iran. J. Bot. 27 (2): 177-181.
- Aryavand, A. 1977: IOPB chromosome number reports LVII. -Taxon 26: 443-452.
- Aryavand, A. 1983: IOPB Chromosome Number Reports LXXX. -Taxon 32: 504-511.
- Badr, A., Hamoud, M., El-Rabey, H. 1996: Chromosomal studies in the Egyptian flora V. Chromosomal relationship in the genus *Astragalus* L. (Fabaceae) and their taxonomic inferences. -Cytologia 61 (1):105-111.
- Bagheri, A., Akhavan Roofigar, A., Nemati, Z. & Blattner, F. R. 2022: Genome size and chromosome number evaluation of *Astragalus* L. sect. *Hymenostegis* Bunge (Fabaceae).-Plants. 11.435.
- Cartier D., 1979 -Premieres prospections caryologiques du genre *Astragalus* L. dans la flore du bassin mediterraneen oriental. Revue de Cytologie et de Biologie Végétales, le Botaniste, 2: 169-181.
- Ghaffari, S. M. 2020: Index to plant chromosome number of Iran. Research Institute of Forests and Rangeland. Tehran, Iran. 336 pp.
- Hesamzadeh Hejazi, S.M. & Ziaei Nasab, M. 2009: Color chromosome atlas of Legumes collected in the natural resource's gene bank of Iran. Research Institute of Forests and Rangelands, Tehran, Iran, 103pp.
- Huziware, Y. 1962: Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. -Am. J. Bot. 49: 116-119.
- Javadi, H., Salehi Shanijani, P. & Safavi, S.R. 2019: Chromosome counts and karyomorphology of some species of *Astragalus* (Fabaceae) from Iran. -Chromosome Science. 22 (1-4): 3-12.
- Levan, A., Fredgra, K., Sandberg, A. A. 1964: Nomenclature for centromeric position on chromosomes. -Hereditas. 52: 201-220.
- Maassoumi, A. A. 1987: Notes on the genus *Astragalus* in Iran I, cytotoxic studies on some species. -Iran J. Bot. 3 (2): 117-128.
- Maassoumi, A. A. 1989: Notes on the genus *Astragalus* in Iran IV, cytotoxic studies on some species. -Iran J. Bot. 4 (2): 153-163.
- Maassoumi, A. A. 2003: Flora of Iran No.43, *Astragalus* I. Tehran: Research Institute of Forests and Rangeland. 384 pp.
- Maassoumi, A. A. 2016a: Flora of Iran No. 85, *Astragalus* II. Tehran: Research Institute of Forests and Rangeland. 504 pp.
- Maassoumi, A. A. 2016b: The role of *Astragalus* in ecosystem equilibrium, Iran Nature, 1 (1): 47-41.
- Magulaev, A. J. 1980: Chromosome numbers of some Fabaceae in North Caucasus. -Botany Zhurnal 65: 836-843.
- Magulaev, A. J. 1989: Chromosome numbers in some *Astragalus* (Fabaceae) species of the Caucasus flora. Botaničeskij Žurnal (Moscow & Leningrad). 74: 1519-1521.
- Martin, E., Icyer Dogan, G., Karaman Erkul, S. & Eroglu, H. E. 2019: Karyotype analyses of 25 Turkish taxa of *Astragalus* from the sections *Macrophyllum*, *Hymenostegis*, *Hymenocoleus*, and *Anthylloidei* (Fabaceae). -Turk. J. Bot. 43: 232-242.
- Parfitt, B. D., Baker, M. A. & Gallagher, M. L. 1985: Chromosome Number Reports LXXXVI -Taxon 34: 159-164.
- Rani, S., Kumari, S., Gupta, R. C. & Chahota, R. K. 2014: Cytological studies of Angiosperms (174 species) from District Kangra, Himachal Pradesh (India). -Plant Syst. Evol. 300: 851-862.
- Ranjbar, M., Karamian, R. & Nouri, S. 2011: Diploid-tetraploid mixoploidy in a new species of *Astragalus* (Fabaceae) from Iran. -Ann. Bot. Fennici. 48: 343-351.

- Ranjbar, M. & Mahmoudian, B. 2015: An overview on cytogenetics of the genus *Astragalus* subgenus *Hypoglottis* (Fabaceae). -Caryologia 68 (2): 109-124.
- Reeves, A., Tear, J. 2000: Micro measure software. Colorado State University, from <http://www.colostate.edu/Depts/Biology/MicroMeasure>
- Romero-Zarco, C. 1986: A new method for estimating karyotype asymmetry. -Taxon. 35 (3): 526-530.
- Sheidai, M., Maassoumi, A. A. & Pakravan, M. 1996: Karyotypes of some *Astragalus* taxa (sect. *Xiphidium* BGE) from Iran. -The nucleus 39: 111-113
- Sheidai, M. & Gharemaninejad, F. 2008: New chromosome number and karyotype analysis in four *Astragalus* L. (Fabaceae) species. -Iran. J. Bot. 15 (1): 21-26.
- Sheidai, M., Zarre, S., & Ismaeilzadeh, J. 2009: New chromosome number reports in tragacanthic *Astragalus* species. -Caryologia 62 (1): 30-36.
- Spellenberg, R. 1976: Chromosome numbers and their cytotaxonomic significance for North American *Astragalus* (Fabaceae). -Taxon 25: 463-476.
- Stebbins, G.L. 1971: Chromosomal Evolution in Higher Plants; Edward Arnold: London, 216p.
- Yousefzadeh, K., Houshmand, S. & Zamani Dadane, G. 2010: Karyotype analysis of *Astragalus effusus* Bunge (Fabaceae). -Caryologia 63: 257-261.
- Zarre, S., & Azani, N. 2013: Perspectives in taxonomy and phylogeny of the genus *Astragalus* (Fabaceae): a review. Progress in Biological Sciences, 3, 16.
- Zhukova, P. G. 1983: Chromosome numbers of some species of the family Fabaceae from North-East Asia. -Botaniceskij Žurnal (Moscow & Leningrad) 68 (7): 925-932.