NEW CHROMOSOME NUMBER AND KARYOTYPE ANALYSIS IN FOUR ASTRAGALUS L. (FABACEAE) SPECIES

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Received 20.10.2008. Accepted for publication 25.11.2008

Sheidai, M. & Gharemani-nejad, F. 2009 06 30: New chromosome number and karyotype analysis in four *Astragalus* L. (*Fabaceae*) species. *–Iran. J. Bot. 15(1): 21-26.* Tehran.

Karyotype studies were performed in four *Astragalus* species of *A. cornu-carpae*, *A. mucronifolius*, *A. talimansurensis* from the section *Leucocersis* and *A. crysostachys* from the section *Hymenostegis*. All four species showed 2n = 2x = 16 chromosome number and had metacentric and sub-metacentric chromosomes. The size of chromosomes varied from 0.75 μm in *A. talimansurensis* to 4.00 μm in *A. cornu-carpae*. The species studied differed significantly in the size of their chromosomes and in karyotype formulae indicating the role of quantitative change in the chromatin (DNA) material as well as chromosomes structural changes in the species diversification. The chromosome numbers of all four species studied are new to science. PCA ordination of the species showed their karyotype distinctness and that such data are of no use in the sectional delimitation in *Astragalus*.

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Key words. Astragalus, Fabaceae, karyotype, Iran.

گزارش جدید کروموزومی و مطا لعه کاریوتیپی چهار گونه گون (Astragalus L.) در ایران

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خصوصیات کاریوتیپی چهار گونه گون (*Astragalus*) با نامهای *A. cornu-carpae*، *A. mucronifolius* و *A. talimansurensis* از بخشه *Leucocersis* و گونه *A. crysostachys* از بخشه *Hymenostegis*. مطالعه شدند. گونه ها وجود 2n = 2x = 16 را نشان دادند که تمامی گزارش جدید می‌باشند. کروموزومها از نوع متا و ساب متا بودند و اندازه آنها از ۰/۷۵ μm در *A. talimansurensis* تا 4 μm در *A. cornu-carpae* متغیر بود. گونه‌های مطالعه شده اختلاف معنی داری را در اندازه کروموزومها نشان دادند که بیانگر تغییرات کمی در ماده کروموزومی (DNA) گونه‌ها در فرایند گونه‌زایی می‌باشد. تفاوت در فرمول کاریوتیپی این گونه‌ها وجود داشت که نشاندهنده وقوع تغییرات ساختمانی کروموزومها است. گروه‌بندی گونه‌ها با استفاده از تجزیه به مولفه‌های اصلی (PCA) تمایز کاریوتیپی گونه‌ها را نشان داد و اینکه داده‌های کروموزومی برای تفکیک بخشه‌های گون مفید نمی‌باشند.

Introduction

The genus *Astragalus* L. (*Fabaceae*) is the largest genus of vascular plants on Earth with about 2500 mostly perennial species (Mabberley 1997) distributed primarily around the northern hemisphere and South America. Many *Astragalus* species are narrow endemics, often found in marginal habitats or to those requiring edaphic specializations, while relatively few are widespread. *Astragalus* is especially diverse in south-west Asia (ca. 1000-1,500 spp.), the Sino-Himalayan region (500 spp.), western North America (ca. 400-450 spp.) and along the Andes in South America (ca. 100 spp.). *Astragalus* is also diverse in Mediterranean climatic regions along the Pacific coasts of North and South America, and in southern Europe and northern Africa (Polhill 1981, Mabberley 1997).

A number of *Astragalus* species from south-west and south-central Asia (e.g., *A. gummifer* Lab., Iran) are the source of "gum tragacanth" - a substance tapped from roots or stems with hydrophilic and colloidal properties valuable in ice creams, lotions, pharmaceuticals. A few species are edible (*A. canadensis* L., N. America) or have medicinal uses, and some are used for livestock forage (*A. cicer* L., USA).

The old world *Astragalus* taxa are comprised of 89 sections out of which, 15 sections belong to thorny *Astragalus* (Zarre 1999).

An extensive number of cytological studies have been performed in *Astragalus* throughout the world including Iran (Spellenberg 1976, Aryavand 1977, Cartier 1979, Magulaev 1980, Löve & Löve 1982, Zhukova 1983, Parfitt et al. 1985, Maassoumi 1986, 1987, Magulaev 1989, Badr et al. 1996, Sheidai et al. 1996, Javadi 2006), reporting the basic chromosome numbers of x = 7, 8, 11-15 and diploid, tetraploid, hexaploid and octaploid levels for the genus. The present study reports somatic chromosome number of four species and presents details of karyotype in three *Astragalus* species for the first time.

Materials and methods

Plant material

Somatic chromosome number of four *Astragalus* species was determined. The species studied are: 1- *A. cornu-carpae* Serj & Rech. f. (Isfahan: Muteh Protected area, 2 km from the main road to Muteh, 1000 m, Mozaffarian & Karimi 37818), 2- *A. mucronifolius* Boiss. (Baluchistan: Taftan region, between Choo-Kalaki, Janabad and Kharestan, 2200 m, Mozaffarian 52973, 3- *A. talimansurensis* Serj & Rech. f., from the section *Leucocersis* Bunge (Khuzestan: Between Behbahan and Pole Kheirabad, 300 m, Mozaffarian 63273 and 4- *A. crysostachys* Boiss., from the section *Hymenostegis* (Kohgilouyeh and Boirahmad: Yasuj, Sisakht, near Kukhdan, 29 52 19 N; 50 28 30 E, 2500 m, Ghahremaninejad 112. The voucher specimens are deposited in TARI.

Cytological studies

For karyotypic studies freshly grown root tips were collected from the seeds of at least ten randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (1–2 hrs.). Squash technique was used for cytological studies and karyotypic details were studied in at least 5 well-prepared metaphase plates as reported earlier (Sheidai & Rashid 2007).

The chromosomes were identified according to Levan et al. (1964), karyotype symmetry was determined according to Stebbins (1971), while other karyotypic parameters like total form percentage (TF %), as well as coefficient of variation (CV) of the chromosome size were determined (Sheidai & Jalilian 2008).

Statistical analyses

The analysis of variance (ANOVA) and the least significant difference test (LSD) were performed to reveal significant differences in the size of chromosomes among the populations of each species as well as among species with similar somatic chromosome numbers in each section and also among the 3 sections studied (Sheidai & Rashid 2007).

Pearson coefficient of correlation was determined among karyotype parameters among the species to study the pattern of their relationships (Sheidai et al. 1996). In order to group the species studied based on similarity in their karyotypic features, UPGMA (Unweighted Paired Group with Arithmetic Average) and Neighbor Joining (NJ) clustering methods as well as ordination based on principal component analysis (PCA) were performed. NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) was used for statistical analyses.

Results and discussion

Details of karyotype analyses in the *Astragalus* species studied are presented in Tables 1 & 2 and Figs. 1-3. All four species studied showed 2n = 2x = 16 which are new to science.

The basic chromosome number reported for the old world thorny *Astragalus* species (both from Iran and outside) is x = 8 and only diploid and tetraploid levels have been reported (Magulaev 1980). Therefore 2n = 2x = 16 observed in the species studied is in accordance with previous results.

The species studied showed metacentric (M & m) and sub-metacentric (sm) chromosomes in their karyotype. The size of chromosomes varied from 0.75 μm in *A. talimansurensis* to 4.00 μm in *A. cornu-carpae*. The highest value of total haploid chromosome length occurred in *A. cornu-carpae* (41.61μm) while the lowest value occurred in *A. talimansurensis* (14.99μm). Similarly the highest mean chromosome length (5.20μm) occurred in *A. cornu-carpae*, while the lowest value o the same (1.87μm) occurred in *A. talimansurensis*. ANOVA test followed by LSD test revealed significant difference for the size of chromosomes among the species studied indicating that a significant quantitative change in the chromatin material (DNA) has occurred during the species diversification.

The highest value of coefficient of variation (CV%) for the size of the chromosomes (18.30) was observed in *A. cornu-carpae* while the lowest CV (14.18) occurred in *A. talimansurensis*. A higher CV shows a higher size variation among the chromosomes.

Astragalus species studied differed in their karyotype formulae indicating changes in the form of their chromosomes possibly due to structural changes like inversion, translocation, etc., which is considered

Table 1. Karyotype features of *Astragalus* species studied.

Species	2n	Ploidy	TL	L	S	L/S	X	CV	TF	KF	ST
A. cornu-carpae	16	2x	41.61	4.00	1.75	2.28	5.20	18.30	44.00	8 m	1A
A. mucronifolius	16	2x	34.00	3.30	1.30	2.54	4.25	17.33	44.41	1M + 7m	1A
A. talimansurensis	16	2x	14.99	1.37	0.75	1.83	1.87	14.18	44.16	2M + 6m	1A
A. chrysostachys	16	2X	32.04	3.20	0.87	3.68	4.00	17.39	40.17	1M +56m+ 2sm	2A

TL = Total haploid chromosome length (μm), L = Size of the longest chromosome (μm), S = Size of the shortest chromosome (μm), L/S = Ratio of the longest chromosome/ shortest chromosome (μm), X = Mean chromosome length (μm), CV = Coefficient of variation, TF = Total form percentage, KF = Karyotype formulae and ST = Stebbins' class.

Table 2. Pearson coefficient of correlation among karyotype parameters.

		TL	L	S	LS	X	CV	TF	ST
TL	Pearson Correlation	1.000	.998**	.846	.371	1.000**	.989*	-.094	.082
	Sig. (2-tailed)	.	.002	.154	.629	.000	.011	.906	.918
	N	4	4	4	4	4	4	4	4
L	Pearson Correlation	.998**	1.000	.812	.426	.998**	.996**	-.149	.138
	Sig. (2-tailed)	.002	.	.188	.574	.002	.004	.851	.862
	N	4	4	4	4	4	4	4	4
S	Pearson Correlation	.846	.812	1.000	-.181	.846	.758	.410	-.436
	Sig. (2-tailed)	.154	.188	.	.819	.154	.242	.590	.564
	N	4	4	4	4	4	4	4	4
LS	Pearson Correlation	.371	.426	-.181	1.000	.370	.503	-.910	.928
	Sig. (2-tailed)	.629	.574	.819	.	.630	.497	.090	.072
	N	4	4	4	4	4	4	4	4
X	Pearson Correlation	1.000**	.998**	.846	.370	1.000	.989*	-.093	.081
	Sig. (2-tailed)	.000	.002	.154	.630	.	.011	.907	.919
	N	4	4	4	4	4	4	4	4
CV	Pearson Correlation	.989*	.996**	.758	.503	.989*	1.000	-.226	.218
	Sig. (2-tailed)	.011	.004	.242	.497	.011	.	.774	.782
	N	4	4	4	4	4	4	4	4
TF	Pearson Correlation	-.094	-.149	.410	-.910	-.093	-.226	1.000	-.996**
	Sig. (2-tailed)	.906	.851	.590	.090	.907	.774	.	.004
	N	4	4	4	4	4	4	4	4
ST	Pearson Correlation	.082	.138	-.436	.928	.081	.218	-.996**	1.000
	Sig. (2-tailed)	.918	.862	.564	.072	.919	.782	.004	.
	N	4	4	4	4	4	4	4	4

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

TL = Total haploid chromosome length (μm), L = Size of the longest chromosome (μm), S = Size of the shortest chromosome (μm), L/S = Ratio of the longest chromosome/ shortest chromosome (μm), X = Mean chromosome length (μm), CV = Coefficient of variation, TF = Total form percentage, KF = Karyotype formulae and ST = Stebbins, class.

as a qualitative change in the genome (Sheidai & Rashid 2007).

All four *Astragalus* species studied showed high values of TF% (>40) and were placed in 1A and 2A classes of Stebbins system (Table 1), indicating the presence of symmetrical karyotypes in them.

Pearson correlation determined among the karyotype features (Table 2) shows that total haploid chromosome length, size of the longest chromosome, the mean chromosome length and CV are positively correlated ($r = >0.99$, $p < 0.01$). This indicates that increase in the total chromosome size and the mean chromosome value is mainly accompanied by increase in the size of longest chromosome of the karyotype complement which also increase the size difference (CV) among the chromosomes and bring about more

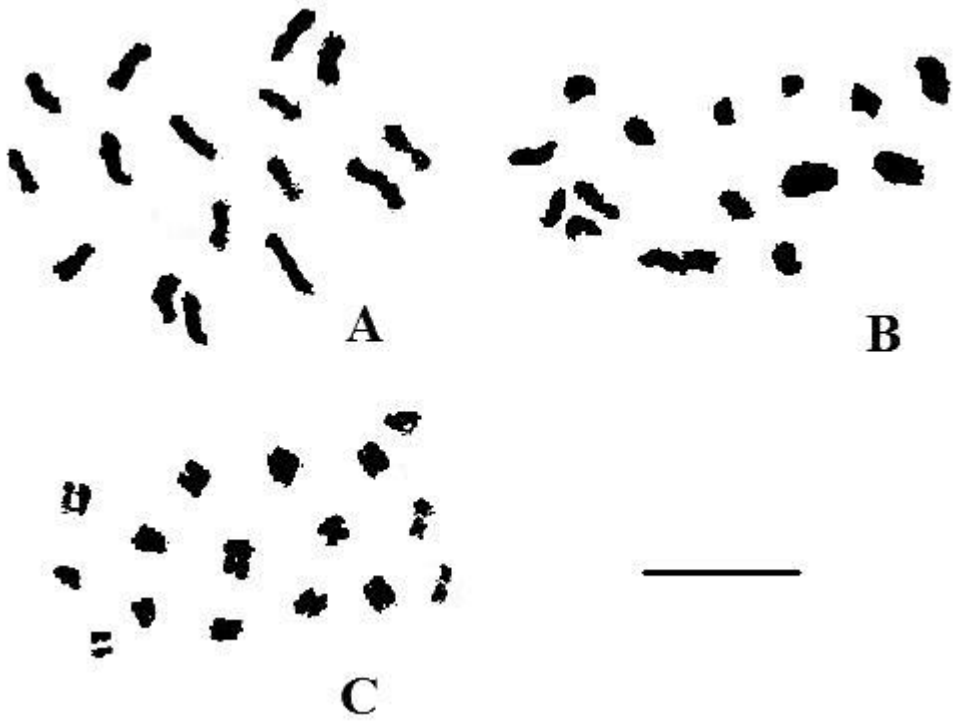

Fig. 1. Representative somatic metaphase cells in *Astragalus* species. A = *A. chrysostachys*, B = *A. cornu-carpae* and C = *A. talimansurensis*. Scale bar = 10 μm.

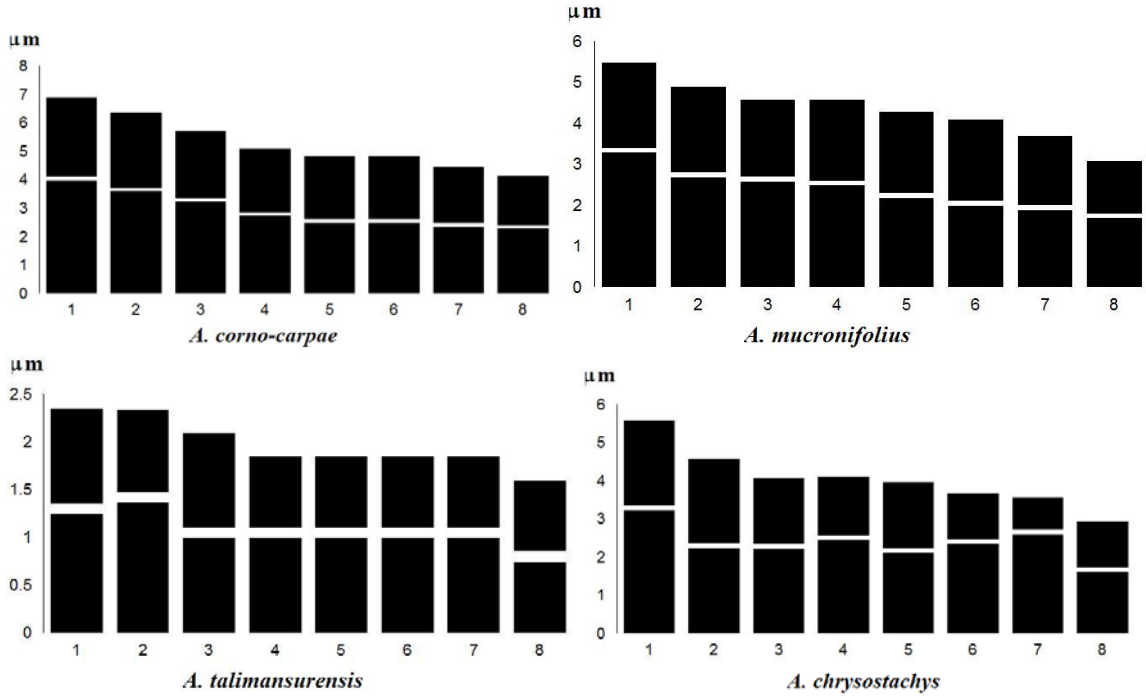

Fig 2. Idiograms of *Astragalus* species studied.

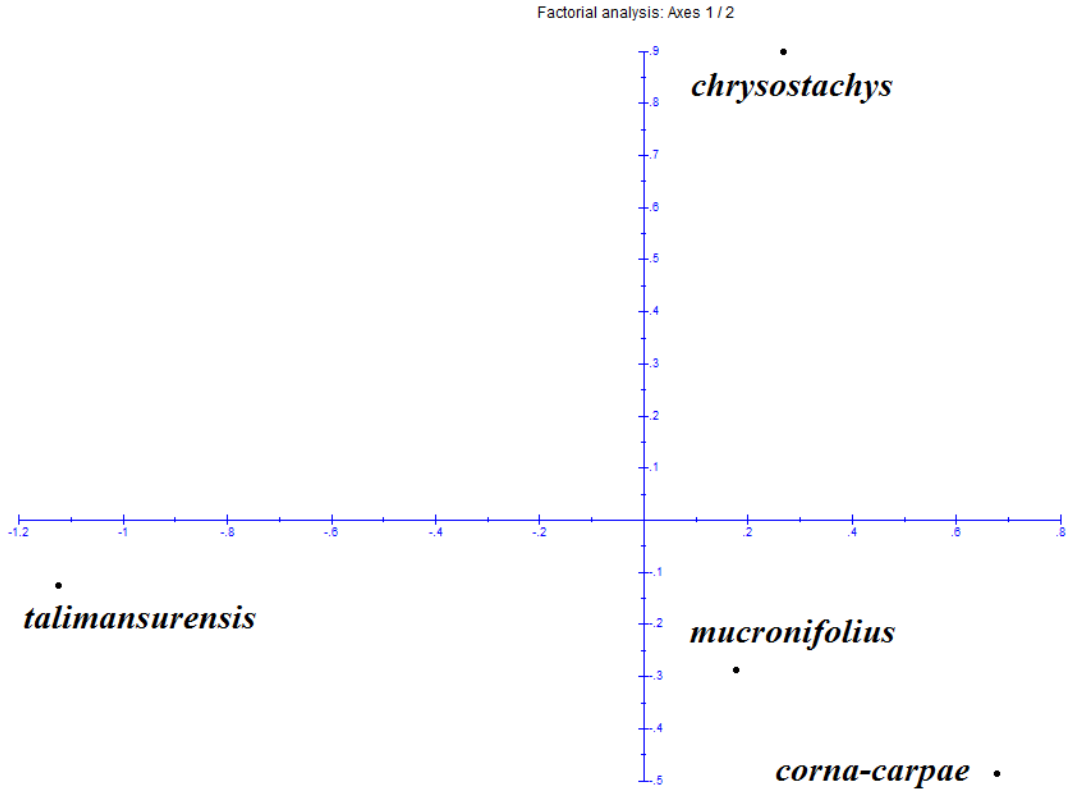

Fig. 3. PCA ordination of *Astragalus* species.

variability among the chromosomes of each complement. Moreover a significant negative correlation is observed between TF and Stebbins' class which is obvious, with higher TF value and the karyotype becomes more symmetrical; therefore more primitive class of Stebbins' is expected.

It is interesting to note that despite the homogeneity of morphological and ecological tendencies within the Astragalean clade, chromosome number, exhibits skewed taxonomic and biogeographic distribution (HU et al. 2008). Eleven of its twelve genera are euploids based on n = 8. Only *Astragalus* exhibits aneuploidy, and it does so consistently only in the New World (though sporadically elsewhere). Some 95% of all Eurasian *Astragalus* species have euploid numbers based on n = 8, whereas 94% of all New World *Astragalus* have aneuploid numbers in a series from n = 11-15 (Spellenberg 1976, Wojciechowski et al. 1993, Wojciechowski et al. 1999, HU et al. 2008).

While aneuploidy is rare in Eurasia (22 species), euploidy is rare in North America (12 species) and completely absent from South America (Wojciechowski et al. 1993, Wojciechowski et al. 1999, HU et al. 2008). Moreover, the most common number in the New World, n = 11, is absent in Eurasia, and the most common aneuploid number in Eurasia, n = 14, is the rarest in the New World (HU et al. 2008). These data have lead to the hypothesis that the New World aneuploid species of *Astragalus* form a monophyletic group (Spellenberg 1976). Recent molecular systematic studies (Liston 1992a, b, Sanderson & Doyle 1993, Wojciechowski et al. 1993, Wojciechowski et al. 1999) supported the monophyly of the New World aneuploid species (termed "Neo-Astragalus), indicating the use of cytological data in studying the phylogenetic relationship of *Astragalus* species (Hu et al. 2008).

Different clustering and ordination of the *Astragalus* species showed similar results, indicating karyotype distinctness of the species studied (Fig. 3). Two species of *A. cornu-carpae* and *A. mucronifolius* show more karyotype similarity and are placed close to each other, while two species of *A. talimansurensis* and *A. crysostachys* are placed far from the others due to their karyotype differences. The results also indicates that karyotype features are of less use in the sectional

delimitation of the species studied as the three species of *A. cornu-carpae*, *A. mucronifolius* and *A. talimansurensis*from the section *Leucocersis* have not been placed in a single group close to each other and are distributed in different clusters or groups.

References

Aryavand, A. 1977: IOPB chromosome number reports LVII. -Taxon 26: 443-452.

Badr, A., Hamoud, M.A. and El-Rabey, H.A. 1996: Chromosomal studies in the Egyptian flora V. Chromosomal relationships in the genus Astragalus L. (Fabaceae). -Cytologia 60: 105-111

Cartier, D. 1979: Premieres prospections caryologiques du genre Astragalus L. dans la flore du basin mediterraneen oriental. -Revue de Cytologie et de Biologie Végétales, le Botaniste 2: 169-181.

Hu, J.M., Sanderson, M. J. and Wojciechowski, M.F. 2008: Website for the largest genus of vascular plants, Astragalus. http://loco.biosci.arizona.edu/astragalus/astragalus home.htm

Javadi, H. 2006: Study of kryotype in three Astragalus species. -Pajouhesh & Sazandegi 73: 131-135.

Levan, A., Fredga, K. and Sandberg, A. 1964: Nomenclature for centromeric position on chromosomes. -Hereditas 52, 201–220.

Liston, A. 1992a: Variation in the chloroplast genes rpoC1 and rpoC2 of the genus Astragalus (Fabaceae): evidence from restriction site mapping of a PCR-amplified fragment. -American Journal of Boany 79: 953-961.

Liston, A. 1992b: Isozyme systematics of Astragalus sect. Leptocarpi subsect. Californici (Fabaceae). -Systematic Botany 17: 367-379.

Löve, Á. and Löve, D. 1982: IOPB chromosome number reports LXXV. -Taxon 31 (2): 344-360.

Maassoumi, A.A. 1986: Astragalus L., Vol. 1, Annuals Publication, Technical Publication 47. -Research Institute of Forests and Rangelands, Tehran.

Maassoumi, A.A. 1987: Notes on the genus Astragalus in Iran I, cytotaxonomic studies on some species. -Iranian Journal of Botany 3: 117-128.

Mabberley, D.J. 1997: The Plant-Book, A Portable Dictionary of the Vascular Plants, 2nd edition. -Cambridge University Press.

Magulaev, A.J. 1980: Chromosome numbers of some Fabaceae in North Caucasus. - Botany Zurnal 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

Parfitt, B.D., Baker, M.A. and Gallagher, M.L. 1985: Chromosome Number Reports LXXXVI. -Taxon, 34: 159-164 .

Polhill, R.M. 1981: Carmichaelieae. In: Polhill, R. M. and Raven, P.H. (eds.), Advances in Legume Systematics, part 1, 364-366. -Royal Botanical Gardens, Kew, U. K.

Sanderson, M.J. and Doyle, J.J. 1993: Phylogenetic relationships in North American Astragalus (Fabaceae) based on chloroplast DNA restriction site variation. -Systematic Botany 18: 395-408.

Sheidai, M., Maasoumii, A.R. and Pakravan, M. 1996: Karyotypes of some Astragalus taxa (Sec. Xiphidium Bge) from Iran. -Nucleus 39 (3): 111-113.

Sheidai, M. and Rashid, S., 2007: Cytogenetic study of some Hordeum L. species in Iran. -Acta Biol. Zseged. 51: 107-112.

Sheidai, M. and Jalilian, N. 2008: Karyotypic studies in some species and populations of the genus Lotus L. in Iran. -Acta Botanica Croatica 67: 45-52.

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.

Wojciechowski, M.F., Sanderson, M.J., Baldwin, B.G. and Donoghue, M.J. 1993: Monophyly of anueploid Astragalus (Fabaceae): Evidence from nuclear ribosomal DNA-ITS sequences. -American Journal of Botany 80:711-722.

Wojciechowski, M.F., Sanderson, M.J. and Hu, J.M. 1999: Evidence on the monophyly of Astragalus (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA trnL intron data. -Systematic Botany 24: 409-437.

Zarre, M.S. 1999: Systematic revision of Astragalus sect. Adiaspastus, sect. Macrophyllium and sect. Pterophorus (Fabaceae). -Englera 18: 1-219.

Zhukova, P.G. 1983: Chromosome numbers of some species of the family Fabaceae from North-East Asia. -Botaniceskjij Žurnal (Moscow & Leningrad) 68 (7): 925-932.