

CHROMOSOME NUMBERS REPORT OF FIVE ENDEMIC *ASTRAGALUS* L. (FABACEAE) SPECIES OF IRAN

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Chromosome counts of five species of the genus *Astragalus* including *A. campylanthus* and *A. susianus* from sect. *Campylanthus*; *A. callistachys*, *A. fragiferus*, and *A. microphysa* from sect. *Microphysa*, are reported for the first time. Three of the studied species including *A. campylanthus* ($2n=2x=16$), *A. susianus* ($2n=2x=16$), and *A. microphysa* ($2n=2x=16$) were diploid and 2 species including *A. callistachys* ($2n=4x=32$) and *A. fragiferus* ($2n=4x=32$) were tetraploid. A basic chromosome number of $x=8$ was confirmed for investigated species. Karyotypic analyses of all species are presented.

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گزارش اعداد کروموزومی برای پنج گونه انحصاری *Astragalus* (Fabaceae) از ایران
آزاده اخوان روفیگر: استادیار پژوهش، بخش تحقیقات منابع طبیعی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان اصفهان، سازمان تحقیقات، آموزش و ترویج کشاورزی، اصفهان، ایران
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عدد کروموزومی پنج گونه از جنس گون شامل گونه‌های *A. campylanthus* و *A. susianus* از بخش *Campylanthus* و *A. callistachys* و *A. fragiferus* و *A. microphysa* از بخش *Microphysa* برای اولین بار گزارش می‌شوند. سه گونه شامل گونه‌های *A. campylanthus* ($2n=2x=16$)، *A. susianus* ($2n=2x=16$) و *A. microphysa* ($2n=2x=16$) دیپلوئید و دو گونه شامل گونه‌های *A. callistachys* ($2n=4x=32$) و *A. fragiferus* ($2n=4x=32$) تتراپلوئید بودند. عدد پایه کروموزومی $x=8$ برای تمام گونه‌های مطالعه شده مورد تأیید قرار گرفت. تحلیل کاربوتایی تمام گونه‌ها ارائه می‌شوند.

INTRODUCTION

The flora of Iran is rich in the number of endemic taxa. Chromosomal studies have not been performed on many endemic species in Iran. *Astragalus* is the most prominent genus in Iran according to the number of species (Maassoumi 1998). Information on the karyology of *Astragalus* species is meager and the ploidy level of the species is not well documented.

According to the IPCN (Index to Plant Chromosome Numbers, www.tropicos.org/Project/ IPCN), IFCI (The Iran Flora Chromosomal index, http://ifci.rifr-ac.ir/), as well as Plant Chromosome Book of Iran (Ghahremaninejad & al. 2013) and Index to plant chromosome number of Iran (Ghaffari 2020), the chromosome numbers for most species of the genus *Astragalus* are not reported yet. In relation to the

number of species (more than 3000 spp.) worldwide, there are a few detailed studies, of *Astragalus*. However, there are reports of chromosome counts of some taxa in the genus (Borjian & al. 2012; Javadi & al. 2019; Yousefzadeh & al. 2010; Maassoumi 1987, 1989; Martin & al. 2008; Martin & al. 2019; Rani & al. 2014; Ranjbar & Mahmoudian 2015; Ranjbar & al. 2011; Sheidai & Gharemaninejad 2008; Sheidai & al. 2009; Ghaffari 2020). Somatic chromosome number and karyotype information provide valuable characters in delimiting species and in distinguishing some closely related taxa. This study was aimed to determine chromosome number and provide general information on karyotype characteristics of selected endemic species of the genus *Astragalus* occurring in Iran. These species include *A. campylanthus* Boiss. and *A. susianus* Boiss. from sect. *Campylanthus*, *A. callistachys* Buhse, *A. fragiferus* Bunge and *A. microphysa* Boiss. from sect. *Microphysa*. The chromosome numbers for these species have not been reported yet, so here we report them for the first time.

MATERIALS AND METHODS

This study was carried out by using seeds collected from natural habitats in Isfahan province. Herbarium vouchers are housed in the herbarium of Isfahan Agricultural and Natural Resources Research and Education Center (SFAHAN). The seeds were grown in the lab. The chromosome slides were prepared from the root tips meristem. Briefly, roots were pretreated in a 0.01% aqueous solution of colchicines for 3-4 h. Then they were fixed in two steps at room temperature in Carnoy's solution (1:3 v/v of glacial acetic acid and absolute ethanol), first for about 3 h, and after that for about 12 h; then the root tips were washed three times in distilled water. Hydrolysis was done in 1 Normal HCl at 60 °C for 14 min. Fixed root tips were kept in 2% aceto-orcein for 3 h and incubated for 10-15 minutes in cellulase-pectinase enzyme solution at 37°C. The stained roots were squashed in 45% acetic acid under a stereomicroscope and the best metaphase plates were photographed under an Olympus (BX40) microscope.

Karyotypes were prepared and chromosome pairs were classified according to Levan & al. (1964) and Stebbins (1971). The chromosomes were arranged according to their length. The long arm (p), short arm (q), and total chromosome length (CL) were measured. Idiograms were drawn for each species and general information on karyotype characteristics such as total form percentage (TF%) (Huziwara 1962), percentage karyotype asymmetry index (As K%) (Arano 1963), index of karyotype symmetry (Syi) (Greilhuber & Speta 1976) was evaluated.

RESULTS

All taxa studied in this study are endemic species for the Iranian flora. The karyotype features as well as chromosome counts for five species of sections *Campylanthus* and *Microphysa* are provided here. The mean length of chromosome long arm (p) varied from 1.06 µm in *A. susianus* to 1.58 µm in *A. microphysa*. The average length of chromosome short arm (q) ranged from 0.78 µm in *A. susianus* to 1.11 µm in *A. microphysa*. Length of chromosome (CL) varied from 1.58 µm in *A. susianus* to 2.71 µm in *A. microphysa* (table 1).

Section *Campylanthus*

Astragalus campylanthus Boiss.

Specimen examined: Isfahan province: Fereidunshahr, Tangeh Kolang Mt., 33° 00' 13" N, 50° 37' 02" E, 2450 m, 30 May 2017, Akhavan & Bagheri 16531 (SFAHAN!).

Distribution: Chaharmahal va Bakhtiari, Fars, Isfahan, Kerman, and Tehran provinces.

The results showed that this species is diploid with a chromosome number of $2n=2x=16$ and asymmetric karyotype with seven metacentric and a submetacentric chromosomes (fig. 1A, table 1). TF (40%) and Syi (67%) values in this species were the lowest among studied species and AsK (60%) values were the highest among studied species (table 1). The chromosome number and karyotype features of this species are presented here for the first time.

Astragalus susianus Boiss.

Specimen examined: Isfahan province, Damaneh to Khansar, 33° 04' 22" N, 50° 29' 28" E, 2450 m, 20 June 2017, Akhavan & Bagheri 16507 (SFAHAN!).

Distribution: Chaharmahal va Bakhtiari, Fars, Isfahan and Kohgiluyeh va Boyer-Ahmad provinces.

This species is diploid with $2n=2x=16$. The chromosomes were metacentric (M, m) (table 1). Ideogram is depicted for this species (fig. 1B). The length of chromosomes in this species is shorter than in other species. S (45%) value was the lowest among the studied species. According to available data, this is the first chromosome count reported for this species.

Section *Microphysa*

Astragalus callistachys Buhse

Specimen examined: Isfahan province, Chadegan, Gardane Sorkh, 32° 46' 53" N, 50° 50' 43" E, 2280 m, 20 June 2017, Akhavan & Bagheri 16441 (SFAHAN!).

Distribution: Fars, Isfahan, Kerman, Tehran, and Yazd provinces.

Chromosome number in this species was $2n=4x=32$. The karyotype was formed of metacentric (2M+14m) chromosomes (fig. 1C). TF (45%) and Syi (84%) values in this species were the highest among the studied species (table 1).

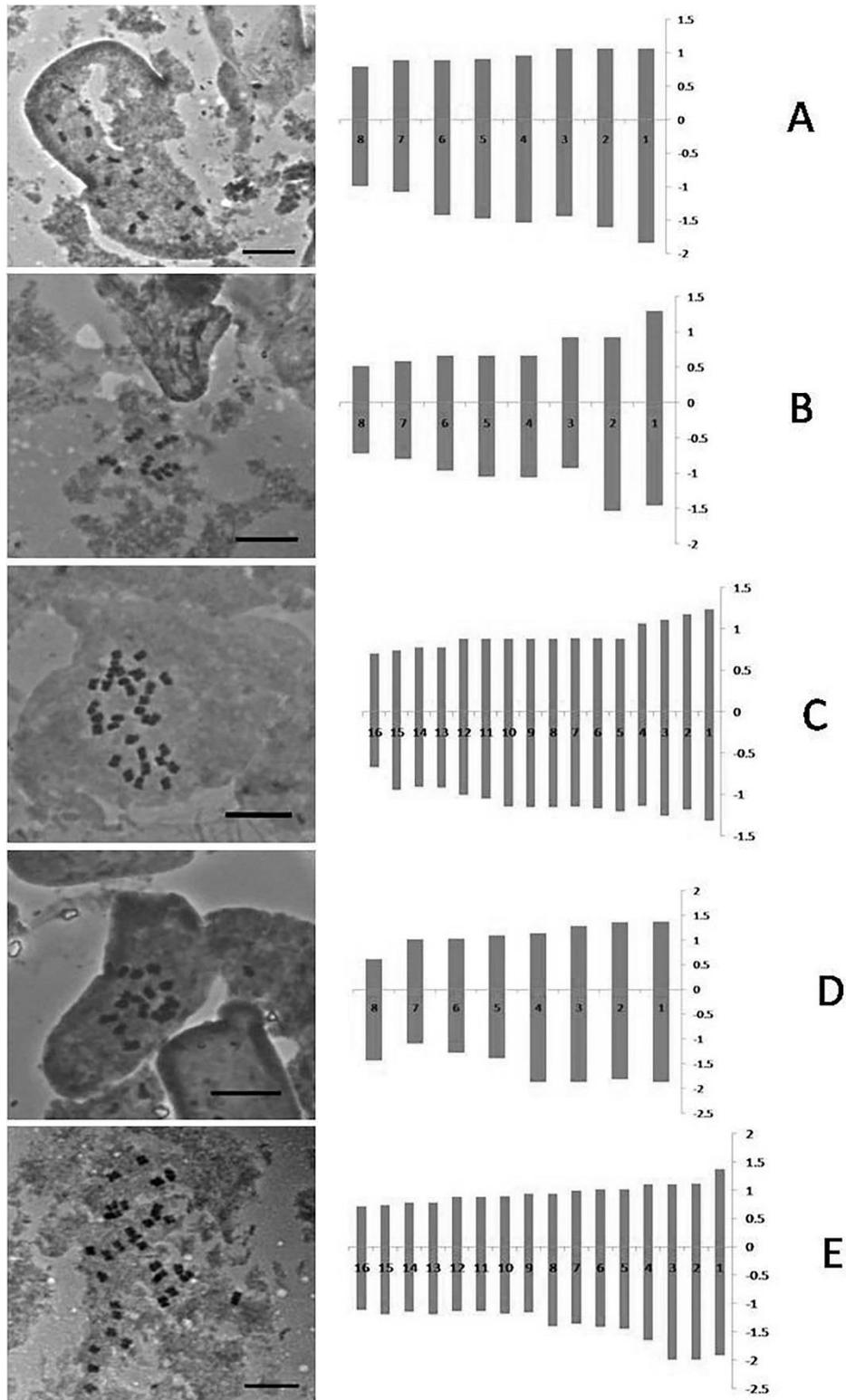


Fig. 1. Somatic metaphases and idiograms in A, *Astragalus campylanthus* ($2n=2x=16$); B, *A. susianus* ($2n=2x=16$); C, *A. callistachys* ($2n=4x=32$); D, *A. microphysa* ($2n=2x=16$); E, *A. fragiferus* ($2n=4x=32$). Scale Bar: 10 μ m.

***Astragalus microphysa* Boiss.**

Specimen examined: Isfahan province, Fereidunshahr, Soroshjan, 32° 54' 45" N, 50° 11' 40" E, 2365 m, 4 Aug 2018, Akhavan & Feyzi 16725 (SFAHAN!).

Distribution: Chaharmahal va Bakhtiari, Fars, Isfahan, Kerman, Lorestan, Tehran, and Yazd provinces.

Karyological analysis of specimens showed a diploid chromosome number of $2n=2x=16$ with seven metacentric and one submetacentric chromosome (fig. 1D). The length of chromosomes in this species is longer than the other studied species. Also, the S value (63%) was the highest among the studied species (table 1).

***Astragalus fragiferus* Bunge**

Specimen examined: Isfahan province, Fereidunshahr, Nehzatabad, 32° 56' 44" N, 50° 06' 34" E, 2780 m, 30 May 2017, Akhavan & Bagheri 16420 (SFAHAN!).

Distribution: Chaharmahal va Bakhtiari, Isfahan, Kerman, and Kohgiluyeh va Boyer-Ahmad provinces.

The chromosome counts for this species is $2n=4x=32$ with 14 metacentric and two submetacentric chromosome pairs (fig. 1E). TF (40%) value in this species and *A. campylanthus* was the lowest among the studied species (table 1). The chromosome number of this species is presented here for the first time, also $n=8$ for this species previously has been reported (Ghaffari 2020).

Table 1. Chromosome counts and karyomorphological parameters of the five examined *Astragalus* species. Abbreviations: CL, chromosome length; q, long arm; p, short arm; TF%, total form percentage; S%, symmetry index; AsK%, Arano index of karyotype asymmetry; Syi%, index of karyotype symmetry; KF, karyotype formula.

Species	chromosome number	CL	q	p	TF%	S%	Ask%	Syi%	KF
<i>A. campylanthus</i>	$2n = 16$	0.49	0.22	0.27	64	63	54	86	8m
<i>A. susianus</i>	$2n = 16$	0.61	0.28	0.33	45	49	55	83	1M+7m
<i>A. callistachys</i>	$2n = 32$	0.47	0.2	0.27	42	60	58	73	1M+5m+2sm
<i>A. microphysa</i>	$2n = 16$	0.94	0.39	0.55	31	65	68	46	7m+1sm
<i>A. fragiferus</i>	$2n = 32$	0.84	0.31	0.53	30	68	69	44	1M+3m+4sm

DISCUSSION

In the present study, new chromosomal data and karyotype features are reported for five species of the genus *Astragalus* sections *Campylanthus* and *Microphysa*. Karyotype analyses were carried out in detail for the first time in all species. All five studied species have homogeneous karyotypes characterized by the basic chromosome number of $x=8$ which is similar to most other studied species of *Astragalus*. The karyotypes are somewhat symmetrical composing mainly of metacentric (M and m) and submetacentric (sm) chromosomes. TF% has a perfect negative correlation with AsK%, and a perfect positive correlation with the Syi index. The Syi index has shown a perfect negative correlation with AsK%. The most symmetrical karyotype is *A. callistachys* based on TF% and Syi index values. Also, *A. susianus* based on S% value has a symmetrical karyotype. karyotype formulae of two later species showed metacentric (M & m) chromosomes that supported their symmetric features. While the most asymmetrical one is *A. campylanthus*

according to TF%, Syi%, and Ask%. *A. microphysa* and *A. fragiferus* based on S% and TF% respectively are asymmetric species among studied species. These taxa have submetacentric chromosomes (sm) in their karyotype formulae. Generally, karyotype parameters indicate the close relationship among studied species. The variations observed in the data obtained were not significant enough to distinguish some closely related taxa. Because no species from these two sections were studied, we were unable to have a comparison between the sections. Also, we were unable to have a judgment about the sectional specificity of the karyotype because these types of data for many species of these sections are still missing.

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