

Chromosome number reports in *Astragalus* sect. *Onobrychoidei* (Fabaceae) from Iran

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Abstract

In this study, original mitotic chromosome counts have been presented for 10 populations belonging to 6 species of *Astragalus* sect. *Onobrychoidei*: *A. aduncus*, *A. arguricus*, *A. cancellatus*, *A. lilacinus* and *A. vegetus*. All taxa were diploid and possessed $2n = 2x = 16$ chromosome number, consistent with the proposed base number of $x = 8$. In addition, meiotic studies revealed chromosome number of $2n = 2x = 16$ for *A. aduncus*21 and *A. brevidens* and also $2n = 4x = 32$ for *A. vegetus*99. Although this taxon displayed regular bivalent pairing and chromosome segregation at meiosis, some abnormalities were observed.

Key words: *Astragalus*, Chromosome number, Fabaceae, Meiotic behavior, Mitosis, Iran

Introduction

Astragalus L. (Gavan in Persian) is probably the largest genus of flowering plants, containing up to 3000 species (Polhill, 1981; Lock and Simpson, 1991; Yakovlev *et al.*, 1996; Ranjbar and Karamian, 2002, 2003; Bagheri *et al.*, 2011). Iran is one of the largest centers of diversity for this genus, with approximately 700 species and an endemic rate of 57% (Ghahreman *et al.*, 2002; Podlech, 2001). *Astragalus* sect. *Onobrychoidei* DC. with more than 80 species is a rather large section within genus. The section was revised for the flora of the former Soviet Union (Gontscharov, 1946) and for certain areas, e.g. the flora of Iran (Rechinger *et al.*, 1958), the flora of Turkey (Chamberlain and Matthews, 1970), and the flora of Iraq (Townsend and Guest, 1974). In the course of the work on *A.* sect. *Onobrychoidei* in Iran have been investigated (Rechinger *et al.*, 1958; Ranjbar and Maassoumi, 1998; Podlech and Sytin, 2002).

Most of the cytological studies in the genus have concentrated on the chromosome count (Aryavand, 1983; Maassoumi, 1987, 1989; Sheidai *et al.*, 1996, 2000; Ghaffari, 2006; Akhavan and Saeidi, 2010; Faramarzi and Saeidi, 2011; Jalilian and Rahiminejad, 2011; Ranjbar and Mahmoudian, 2013). The basic chromosome number ($x = 8$) and four ploidy levels ($2n = 2x = 16$, $2n = 4x = 32$, $2n = 6x = 48$, $2n = 8x = 64$ and $2n = 12x = 96$) are present in the genus. The present study reports that there are chromosome abnormalities in

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A. sect. Onobrychoidei. It also shows the existence of polyploidy in *A. vegetus*99.

Materials and Methods

For mitosis, materials of 10 populations belonging to 5 species of *Astragalus* sect. *Onobrychoidei* were collected from different localities in Iran (Figure 1), in 2000 through 2008 and pods were separated from healthy plants. Voucher specimens were deposited at the Herbarium of the Bu-Ali Sina University (BASU), Hamedan, Iran (Table 1). Then, pods were left to dry at room temperature, and seeds obtained from dry pods and kept at 4 °C until used. Young root tips were obtained from seeds germinated in Petri dishes pretreated with 0.05% colchicines for 3 h and fixed in 3 : 1 ethanol: glacial acetic acid for 24 h. Root tips were hydrolyzed for 6 minutes in 1 M HCl at 60 °C, washed briefly in dd H₂O and stained in Feulgen's solution for 1-2 h. All permanent slides were made using Venetian turpentine (Wilson, 1945). The slides were examined under an Olympus BX-41 photomicroscope.

Also chromosome number and meiotic behavior were analyzed in three species of *A. vegetus* and *A. brevidens* and *A. aduncus*. 15 flower buds from at least 5 plants at an appropriate stage of development were fixed in Piennr's fluid containing ethanol (96%), chloroform and propionic acid, 6 : 3: 2 (v/v/v), for 24 h at room temperature and then stored in 70% alcohol at 4 °C until used. Anthers were squashed and stained with 2% acetocarmine. All permanent slides were made using Venetian turpentine (Wilson, 1945).

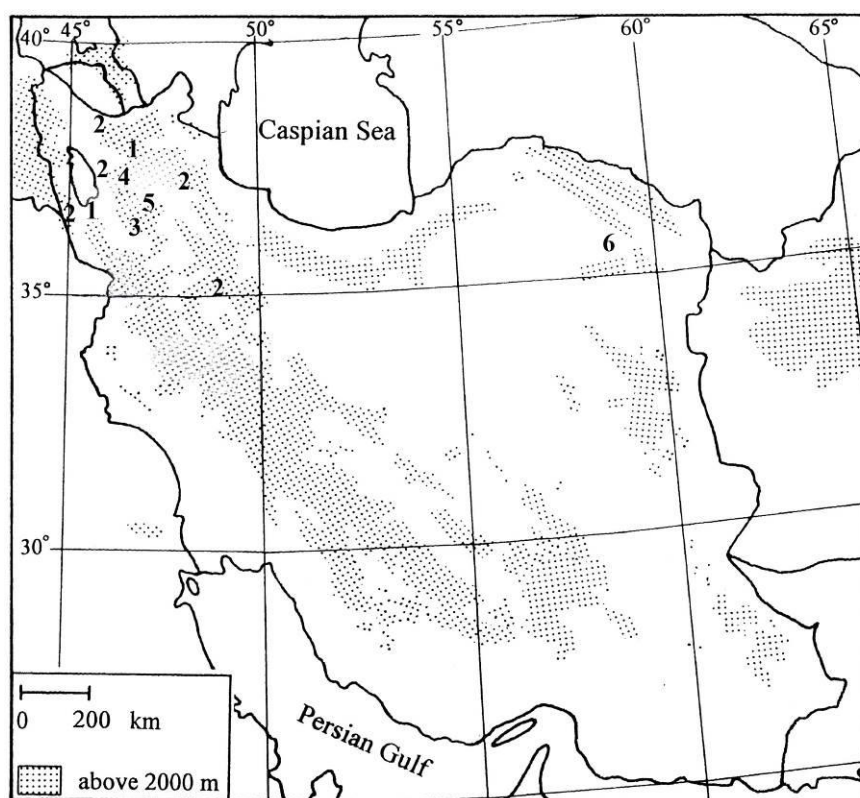


Figure 1. Distribution map of *Astragalus cancellatus* (1), *A. aduncus* (2), *A. arguricus* (3), *A. vegetus* (4), *A. lilacinus* (5), and *A. brevidens* (6)

Table 1. Taxa studied and acronyms

Taxa	Locality	Altitude (m)	Voucher specimen	Coordinate
<i>A. cancellatus</i>	East Azerbaijan: Misho Dagh	1830	4933	45°47'5.54"E 38°19'9.27"N
<i>A. cancellatus</i>	East Azerbaijan: Kaleybar	1215	15751	47°2'14.70"E 38°52'4.13"N
<i>A. aduncus</i>	West Azerbaijan: 25 km to Urmieh	1300	4953	45°7'53.48"E 37°34'51.35"N
<i>A. aduncus</i>	East Azerbaijan: Kandavan	2010	15822	47°54'48.37"E 38°7'27.16"N
<i>A. aduncus</i>	West Azerbaijan: Oshnaviyeh	2150	4949	45°5'54.00"E 37°2'23.00"N
<i>A. aduncus</i>	East Azerbaijan: 25 km to Marand	1300	5498	45°50'36.53"E 38°20'36.58"N
<i>A. aduncus</i>	Hamedan: Tuyserkan	2000-2200	7821	48°26'49.00"E 34°32'53.00"N
<i>A. vegetus</i>	East Azerbaijan: Varzaqan	1415	15798	46°39'16.00"E 38°30'35.00"N
<i>A. arguricus</i>	East Azerbaijan: Misho Dagh	1830	4947	45°47'5.54"E 38°19'9.27"N
<i>A. lilacinus</i>	East Azerbaijan: Bostan Abad to Sarab	1660	15451	46°49'59.99"E 37°51'0.01"N
<i>A. brevidence</i>	Khorasan: Ghochan	1412	18424	58°30'42.94"E 37°6'22.90"N
<i>A. vegetus</i>	Hamedan: Toyserkan	1294-1370	18799	8°26'49.00"E 34°32'53.00"N
<i>A. aduncus</i>	Qazvin: Abgarm	1540	18321	49°17'13.00"E 35°45'26.00"N

Results and discussion

Mitosis

1. *Astragalus cancellatus* Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 102 in clave [et l.c. 15(1): 178. 1869].

Iran. West Azerbaijan: Misho Dagh, 1830 m, *Ranjbar 4933* (BASU). East Azerbaijan: Kaleybar, 1215 m, *Ranjbar 15751* (BASU).

The basic chromosome number of $2n = 2x = 16$ is reported for *A. cancellatus*. According to our literature review, this is the first chromosome count for this taxon (Figures 1-2).

2. *Astragalus aduncus* Willd. 1802, Sp. Pl. 3: 1269.

Iran. West Azerbaijan: Urmieh, 1300 m, *Ranjbar 4953* (BASU); Oshnaviyeh, 2150 m, *Ranjbar 4949* (BASU). East Azerbaijan: Kandavan, 2010 m, *Ranjbar 15822* (BASU); 25 km to Marand, 1300 m, *Ranjbar 5498* (BASU). Hamedan: Tuyserkan, 2000-2200 m, *Ranjbar 7821* (BASU).

In this study, chromosome count for this taxon reported with $2n = 2x = 16$ (Figures 1-2).

3. *Astragalus arguricus* Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 103 in clave [et l.c. 15(1): 181. 1869].

Iran. West Azerbaijan: Misho Dagh, 1830 m, *Ranjbar 4947* (BASU).

Morphologically, it is a variable species, normally found in open fields and along roadsides, and flourishes in spring. Chromosome count of $2n = 2x = 16$ (Figures 1-2).

4. *Astragalus vegetus* Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 102 in clave [et l.c. 15(1): 181. 1869].

Iran. East Azerbaijan: Varzaqan, 1415 m, *Ranjbar 15798* (BASU).

For this species, chromosome count of $2n = 2x = 16$ (Figures 1-2) is the first report.

5. *Astragalus lilacinus* Boiss., Diagn. Pl. Orient., ser. 1, 9: 42. 1849.

Iran. East Azerbaijan: Bostan Abad to Sarab, 1660 m, *Ranjbar 15451* (BASU).

For this species, chromosome count of $2n = 2x = 16$ is the first report (Figures 1-2).

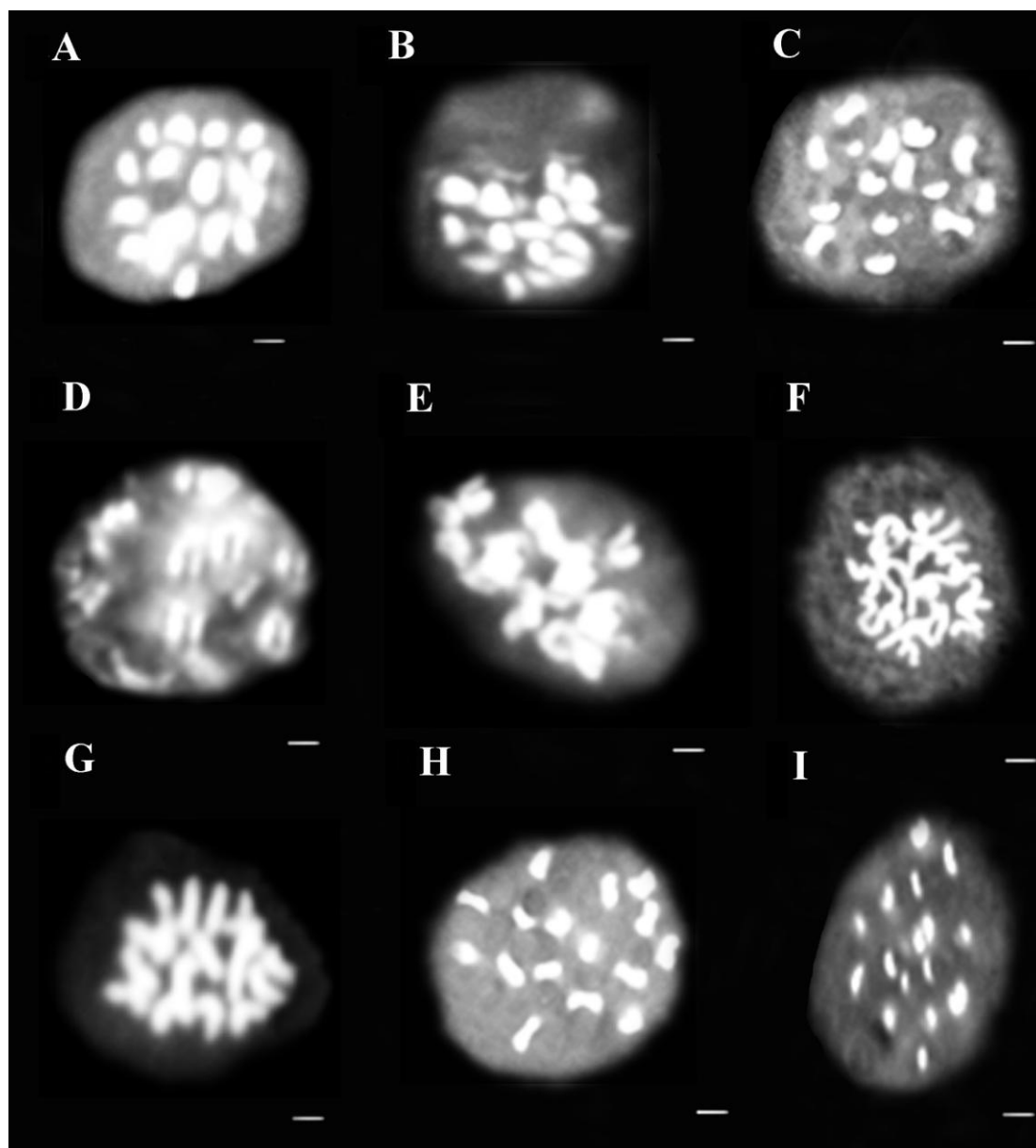


Figure 2. Mitosis in *Astragalus cancellatus* (15751) species studied: A. Prophase. Mitosis in *A. cancellatus* (4933) species studied: B. Prophase. Mitosis in *A. aduncus* (5498) species studied: C. Prophase. Mitosis in *A. aduncus* (15822) species studied: D and E. Prophase. Mitosis in *A. aduncus* (4953) species studied: F. Prophase. Mitosis in *A. aduncus* (7821) species studied: G. Prophase. Mitosis in *A. arguricus* (4947) species studied: H. Prophase. Mitosis in *A. vegetus* (15798) species studied: I. Prophase

Meiotic

The cytological data for the examined taxa is summarized in Table 2 and Figures 3-4. Chromosome numbers based on the basic number of $x = 8$ are found in the majority of the studied taxa, a diploid number of $2n = 2x = 16$ is recorded in the majority taxa, whereas a tetraploid number ($2n = 4x = 32$) is recorded in only one sample of *A. vegetus*⁹⁹. Ledingham (1960) found that the *Astragalus* 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 . This report was substantiated by Ledingham and Rever (1963). The chromosome number of *Astragalus*, based on $x = 8$ have been reported in the vast majority of the old world in *Astragalus* genus while counts based on base numbers $x = 7$ or $x = 6$ have been encountered in only a few species (Maassoumi, 1987; Badr *et al.*, 1996; Malallah *et al.*, 2001; Badr and Sharawy, 2007). The preponderance of *Astragalus* species with a basic number of $x = 8$ led Badr *et al.* (1996) to conclude that it is the primary basic number of *Astragalus* species. They claimed that the $x = 7$ and $x = 6$ numbers have been derived from $x = 8$ by aneuploid loss of chromosomes. Polyploidy in this species have been reported by Badr and Sharawy (2007). They determined the chromosome number in a karyological study on 24 species of Egyptian *Astragalus* and found different ploidy levels such as diploid ($2n = 16, 14$ and 12), triploid ($2n = 24$), tetraploid ($2n = 32, 30$ and 28), pentaploid ($2n = 30$), hexaploid ($2n = 48$) and octaploid ($2n = 64$) and Sheidai *et al.* (2009) have reported $2n = 16, 32$ and 48 , in some species of Iranian *Astragalus*, indicating the role of polyploidy in the evolution of this genus. 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 $n = 8$. Recent molecular systematic studies (Liston, 1992a, 1992b; Sanderson and Doyle, 1993; Wojciechowski *et al.*, 1993; Wojciechowski *et al.*, 1999), indicating the use of cytological data in studying the phylogenetic relationship of *Astragalus* species (Hu *et al.*, 2008). Chromosome migration may also occur through cell wall dissolution among the neighboring meiocytes and forming syncyte (Falistocco *et al.*, 1995). The distribution of several meiotic abnormalities observed include a various degree of fragmented chromosomes; cytomixis; asynchronous nucleus; laggard chromosome; B chromosome and unequal distribution chromosome, which is likely to result in many structural changes and rearrangements at meiosis, and which could then lead to speciation. In this study, a total of 754 prophase (55.2%), 135 diakinesis/metaphases I (D/MI) (9.89%), 199 anaphase I/telophase I (AI/TI) (14.57%), 45 metaphase II (MII) (3.29%), 232 anaphase II/telophase II (AII/TII) (16.1%) cells in *A. brevidence* of Ghochan (Figures 3: A-C). A total of 697 prophase (48.63%), 185 diakinesis/metaphases I (D/MI) (12.9%), 425 anaphase I/telophase I (AI/TI) (29.6%), 22 metaphase II (MII) (1.53%), 104 telophase II (TII) (7.25%) cells in *A. aduncus*²¹ of Abgarm of Qazvin (Figures 3: D-H). A total of 372 prophase (33.60%), 133 diakinesis/metaphases I (D/MI) (12.01%), 208 anaphase I/telophase I (AI/TI) (18.7%), 98 metaphase II (MII) (8.85%), 296 anaphase II/telophase II (AII/MII) (26.73%) cells in *A. vegetus*⁹⁹ of Toyserkan of Hamedan (Figure 3: I-L).

Table 2. Number of pollen mother cells (PMCs) analyzed and percentage of PMCs meiotic behavior

Meiotic characters	<i>A. brevidence</i>	<i>A. aduncus</i> ²¹	<i>A. vegetus</i> ⁹⁹
Total cell number	1365	1433	1107
P	754	697	372
%P	55.2	48.63	33.60
%Cytomixis	2.38	0.03	0
D/MI	135	185	133
%D/MI	9.89	12.9	12.01
%Laggard chromosome	0.05	0.06	0.41

Meiotic characters	<i>A. brevidense</i>	<i>A. aduncus21</i>	<i>A. vegetus99</i>
%B chromosome	1.71	1.23	1.06
%Fragmented chromosome	0	0.03	0
AI/TI	199	425	208
%AI/TI	14.57	29.6	18.7
%Cytomixis	0	0	0.05
%B chromosome	0.03	0	0
%Cytoplasmic channel	0	0	0.13
%Laggard chromosome	0	1.01	0.02
%Unequal distribution chromosome	0	1.2	0
MII	45	22	98
%MII	3.29	1.53	8.85
AII/TII	232	104	296
%AII/TII	16.1	7.25	26.73
%Tripolar	0	0	0.02
%Pentapolar	0	0	0.04
N	8	8	32

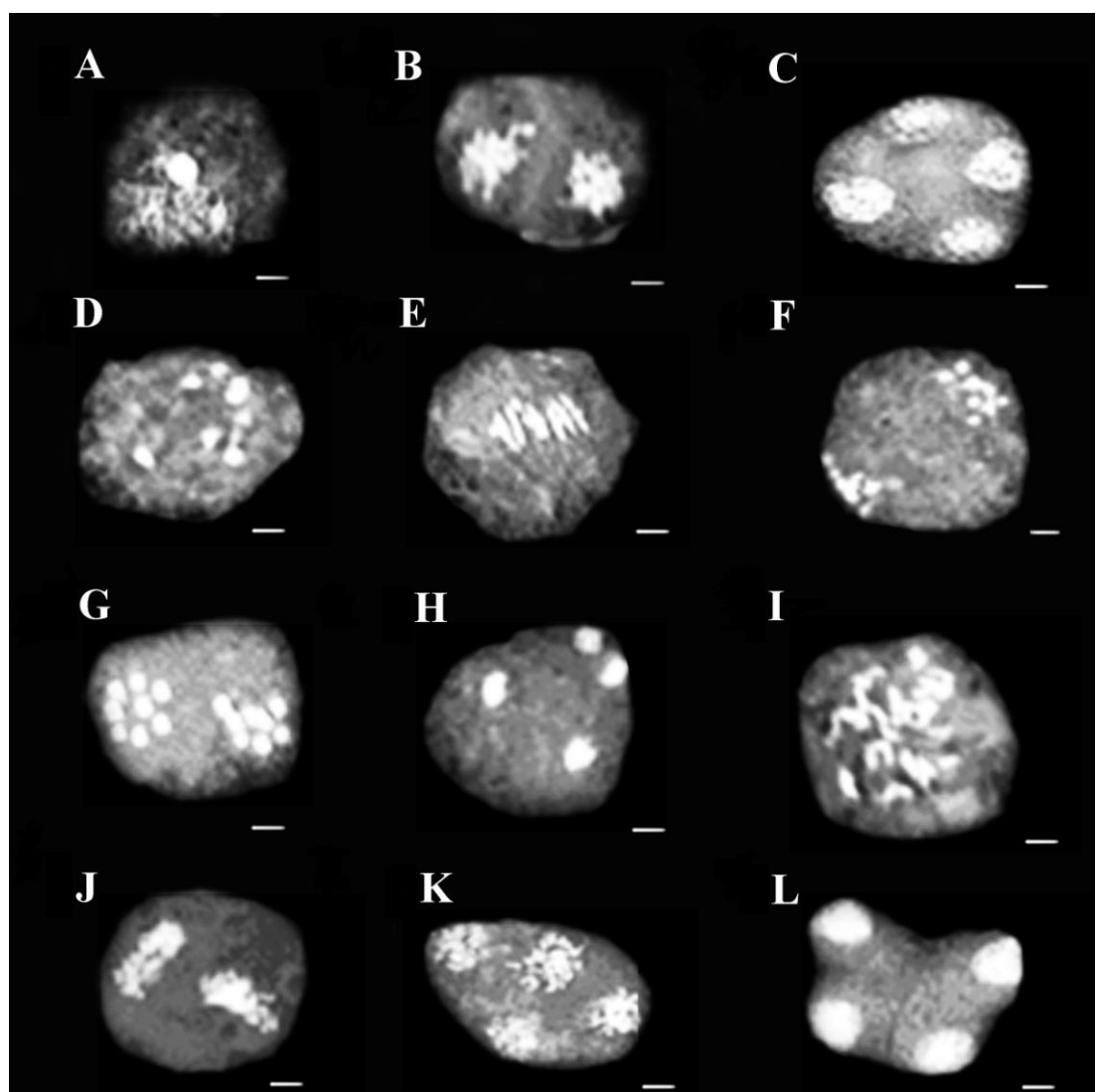


Figure 3. Different stages of meiosis in *Astragalus brevidense* with $2n = 2x = 16$: A. Porophase; B. Telophase; I and C. Telophase II. Different stages of meiosis in *A. aduncus21* with $2n = 2x = 16$: D. Diakinesis; E. Metaphase; I and F. Anaphase; I and G. Telophase; I and H. Anaphase II. Different stages of meiosis in *A. vegetus99* with $2n = 4x = 32$: I. Diakinesis; J. Metaphase II; K. Telophase II; L. Tetrad in telophase II

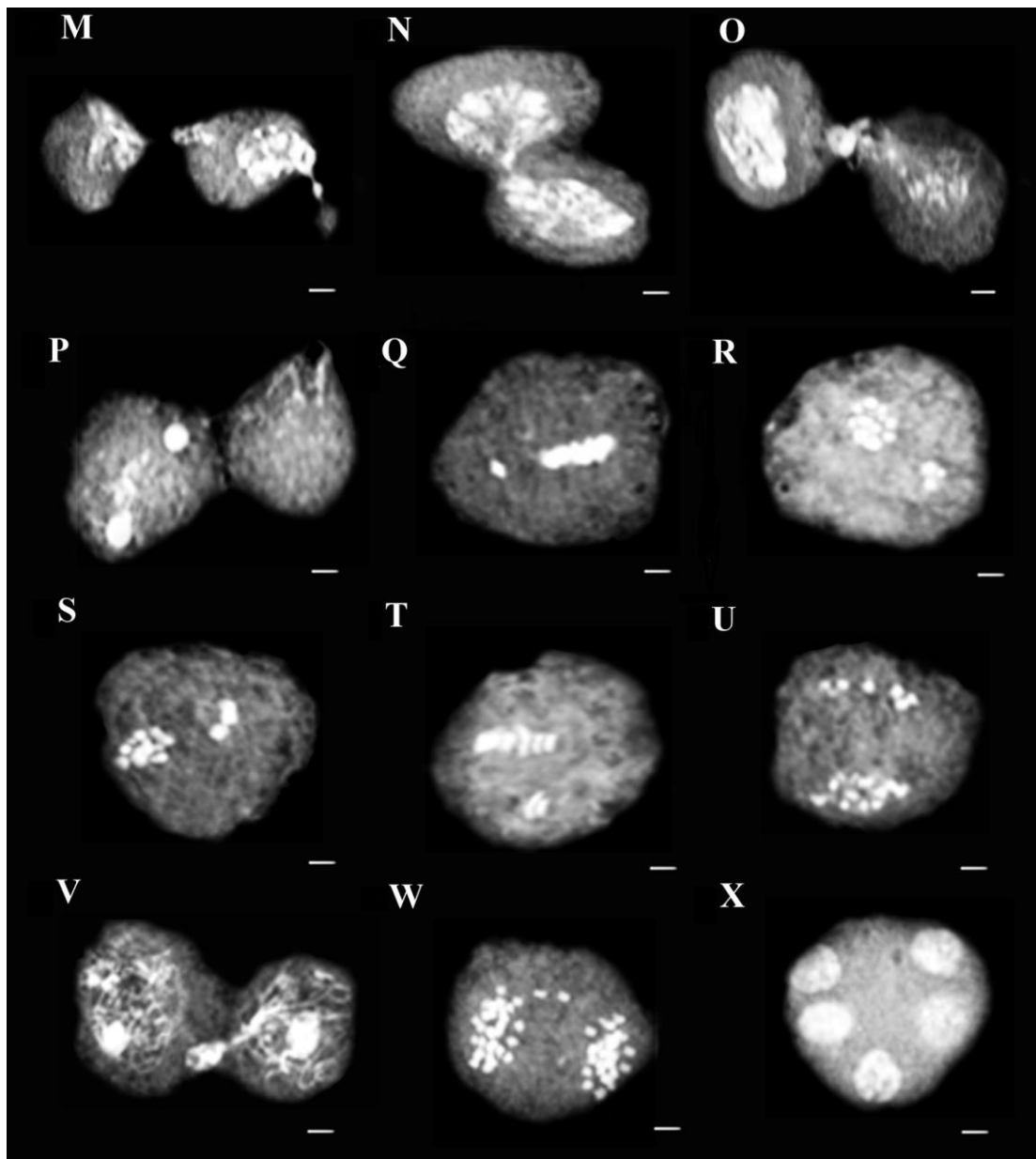


Figure 4. Meiotic behavior in *Astragalus brevidence* with $2n = 2x = 16$: M, N and O. Cytomixis in prophase I. Meiotic behavior in *A. aduncus*21 with $2n = 2x = 16$: P. Cytomixis in prophase I; Q. B chromosome in methaphase I; R and S. Unequal distribution chromosome in telophase I; T. Fragmented chromosome in methaphase I; U. Unequal distribution chromosome in telophase I. Meiotic behavior in *A. vegetus*99 with $2n = 4x = 32$: V. Cytomixis in prophase I; W. laggard chromosome in anaphase I; X. Pentapolar.

The meiotic irregularities included the occurrence of various degree of binuclear in prophase; anucleus in prophase; desynapsis in diakinesis/metaphase I; fragmented chromosomes in metaphase I; sticky chromosome in anaphase I; cytomixis indiakinesis/metaphase I, anaphase I/telophase I, anaphase II/telophase II; cytoplasmic channel in prophase, diakinesis/metaphase I, anaphase I/telophase I, metaphase II, anaphase II/telophase II; B Chromosome in diakinesis/metaphase I; micronucleus in anaphase I/telophase I, anaphase II/telophase II; laggard chromosome in anaphase I/telophase I, anaphase II/telophase II; forward chromosome in anaphase I/telophase I, anaphase II/telophase II; bridge in anaphase I/telophase I, anaphase II/telophase II; tripolar and

polypolar in anaphase II/telophase II; asynchronous nucleus in telophase I; some abnormalities in meiotic behavior of the taxa are described (Table 2, Figure 4: M-X). The phenomenon of cytomixis consists of the migration of chromosome between meiocytes through cytoplasmic channel. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered as a mechanism of evolutionary significance (Ghaffari, 2006). Cytomixis is not considered to be of great evolutionary importance, but it may lead to production of aneuploid plants, or results in the production of unreduced gametes, as reported in several grass species (Falistocco *et al.*, 1995). Unreduced gamete formation is of evolutionary importance as it can lead to the production of plants with higher ploidy levels. It was found in 2.38% P in *A. brevidens* of Ghochan, 0.03% P in *A. aduncus*21, 0.05% AI/TI in *A. vegetus*99 of Toyserkan (Table 2, Figure 4). Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic channels originated from the pre-existing system of plasmodesmata formed within the tissues of the anther. It was found in 0.13% AI/TI in *A. vegetus*99 of Toyserkan.

According to Nicklas and Ward (1994), non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers. Pagliarini (1990) reported that laggards may result from late chiasma terminalization. Ascending chromosomes are the result of precocious migration and, according to Utsunomiya *et al.* (2002), generally consist of univalent chromosomes formed during late prophase stages by precocious chiasma terminalization in early metaphase I or may even result from low chiasma frequency or from the presence of asynaptic or desynaptic genes (Pagliarini, 2000). Laggards and non-oriented chromosomes may produce micronuclei, if they fail to reach the poles in time to be included in the main telophase nucleus (Koduru and Rao, 1981; Utsunomiya *et al.*, 2002), leading to the formation of micro-pollen and probably to gametes with an unbalanced chromosome numbers (Mansuelli *et al.*, 1995), such as aneuploids (Defani-Scoarize *et al.*, 1995). The highest score for laggard chromosomes expressed in 0.5% D/MI in *A. brevidens* of Ghochan, 0.06% D/MI, 1.01% AI/TI in *A. aduncus*21 and 0.41% D/MI, 0.02% AI/TI in *A. vegetus*99 of Toyserkan.

B chromosomes or accessory chromosomes that occur in addition to the standard or A chromosomes in some of the plants are smaller than other chromosomes and do not form any association with them. B chromosomes, when present in high numbers affect negatively the growth and vigor of the plants, while in low numbers may benefit the plant possessing them (Jones and Houben, 2003). The highest percent of B chromosomes were observed in 1.71% D/MI and 0.03% AI/TI, in *A. brevidens* of Ghochan, 1.23 % D/MI in *A. aduncus*21 of Abgarm, 1.06% D/MI in *A. vegetus* 99 of Toyserkan.

Fragmented chromosomes, because of being unable to orient at the metaphase plate were observed during diakinesis and metaphase I and II. The highest percent of fragmented chromosomes were observed in 0.03% D/MI in *A. aduncus*21 of Abgarm.

A considerable number of cells showed the unequal distribution of chromosomes that might be attributed to abnormalities in spindle formation causing unequal distribution of chromosomes. The highest percent of unequal distribution chromosome 1.2% AI/TI in *A. aduncus*21 of Abgarm.

In conclusion, original mitotic chromosome counts were presented for 10 populations belonging to 6 species of *Astragalus* sect. *Onobrychoidei*: *A. cancellatus*, *A. aduncus*, *A. arguricus*, *A. vegetus* and *A. lilacinus*. All taxa were diploid and possessed $2n = 2x = 16$ chromosome number, consistent with the proposed base number of $x = 8$. In addition, meiotic chromosome number of $2n = 2x = 16$ for *A. aduncus*21 and *A. brevidens* $2n = 4x = 32$ for *A. vegetus*99. All studied taxa displayed regular bivalent pairing and chromosome segregation at

meiosis. However, some abnormalities which were observed in the taxa are discussed. 3905 cells were examined in this study. Although chromosomes of the investigated species showed regular behavior during meiosis, some abnormalities were observed as laggard chromosomes in diakinesis/metaphase I; anaphase I/telophase I; B chromosomes in diakinesis/metaphase I; anaphase I/telophase I; fragmented chromosomes in diakinesis/metaphase I; unequal distribution chromosome in telophase I; multipolar cells in telophase II; channels in the cytoplasmic anaphase I/telophase I; cytomixis phenomenon in prophase I and anaphase I/telophase I.

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گزارش عدد کروموزومی از جنس *Astragalus* بخش *Onobrychoide* از تیره Fabaceae در ایران

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چکیده

در مطالعه حاضر، گزارش عدد کروموزومی میتوزی برای ۱۰ جمعیت متعلق به شش گونه از جنس *Astragalus* بخش *Onobrychoidei* شامل: *A. aduncus*، *A. arguricus*، *A. cancellatus*، *A. lilacinus* و *A. vegetus* ارائه شده است. تمام گونه‌های مطالعه شده دیپلوئید هستند و عدد $2n=2x=16$ را نشان می‌دهند که با عدد کروموزومی پایه پیش‌بینی شده $x=8$ مطابقت دارد. علاوه بر این، مطالعات میوز تعداد کروموزوم $2n=2x=16$ را برای *A. aduncus* و *A. brevidens* همچنین $2n=4x=32$ را برای *A. vegetus* نشان داد. اگرچه جفت‌شدگی بی‌والان‌ها و تفکیک کروموزوم‌ها در این گونه عادی است، لیکن برخی ناهنجاری‌ها در طی میوز در این گونه مشاهده می‌شود.
واژه‌های کلیدی: *Astragalus*، عدد کروموزومی، Fabaceae، رفتار میوز، میتوز، ایران