Meiotic Behavior and Chromosome Number of 5 Species of the *Astragalus* sect. *Megalocystis* Bunge (Fabaceae) in Iran

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Summary Meiotic behavior of 7 Iranian populations of 5 *Astragalus* species from the section *Megalocystis*, *A. submitis* Boiss. & Hohen., *A. raddei* Basil., *A. halicacabus* Lam., *A. ebenoides* Boiss. and *A. fuhsii* Freyn & Sint. is presented. All species are diploid and possess $2n=2x=16$ chromosome number, consistent with the proposed base number of $x=8$ from ICPN. Although the taxa displayed regular bivalent pairing and chromosome segregation at meiosis, some meiotic abnormalities observed include a varied degree of fragmented chromosomes in metaphase I; cytomixis in metaphase I; asynchronous nucleus in metaphase II and cytomixis in telophase II.

Key words *Astragalus*, Iran, *Megalocystis*, Meiotic behavior.

Astragalus L. (Fabaceae) is the most diverse genus in southwest Asia (*ca.* 1000 spp.). With more than 840 species, it is the largest genus in the flora of Iran and the most problematic group in the legume systematic (Lock and Simpson 1991, Yakovlev *et al.* 1996, Ranjbar and Maassoumi 1998, Ranjbar and Karamian 2002, 2003a, 2004, Ranjbar *et al.* 2005, 2007a, 2007b, Ranjbar 2007a, 2007b). In addition to its economic importance, its great diversity has also attracted much interest in ecological as well as systematic studies. *Astragalus* sect. *Megalocystis*, with about 40 species, is a rather large section within the genus. The first species from *A. sect. Megalocystis* was introduced as *A. Anthylloidei* by Lamark (1783). Bunge (1868–69) introduced a new section, namely *Megalocystis* Bunge from the *Astragalus* group, with inflated fruiting calyx from the subgenus *Calycophysa*; he was unaware that this had already been done by Candolle (1825) as *Anthylloidei*. The validity of this taxon was returned to its former level in Podlech's typification (1990) system. But this name is invalid, because the term section was wrongly used for *Anthylloidei* serie by Podlech (1990) (contrary to code articles 4.1, 5, 33.4, Podlech personal communication 2009). However, the name *Megalocystis* Bunge is validated here by reference to the previously and effectively published Latin diagnosis (Bunge 1868–69).

The species of this section are cushion-forming plants, mostly with paripinnate leaves and inflated fruiting calyces covered with white or black and white hairs (Figs. 1–3). They are distributed in several Southwest Asian countries, but Iran, with 24 species, is the centre of diversity of this section. However, some diversity of the species is found also in Turkey (5 species) and Afghanistan (4 species). As one of the most heterogeneous and difficult groups of spiny *Astragali*, the section has been revised several times as a whole (Bunge 1868–69, Boissier 1872, Rechinger *et al.* 1958, Tietz and Zarre 1994) and for the area of Flora Iranica (Podlech and Zarre 2001).

The genus *Astragalus* L. is one of the most complex genera of the Astragaleae tribe (Ranjbar and Karamian 2003b). Most of the cytological studies in the tribe have concentrated on the chromosome count (Aryavand 1983, Maassoumi 1987, Maassoumi 1989, Bader and Sherif 2007,

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Sheidai and Jalilian 2008, Sheidai *et al.* 1996, 2000). The basic chromosome number ($x=8$) and 2 ploidy levels ($2n=2x=16$, $2n=4x=32$) are present in the genus in the old world. Studies on the impact of karyotypic data on the interspecific and phylogenetic relationships and also on meiotic behavior in the genus are still limited. Also, little is known about the nature of genetic variability in diploid species and the taxonomic relationships of the different taxa in the genus. In this study, we describe the meiotic behavior of 7 Iranian populations of 5 *Astragalus* species from *A. sect. Megalocystis*.

Materials and methods

This study is mainly based on field observations during several excursions in Iran and on herbarium materials. The field studies were carried out in different parts of Iran, primarily in north and northeastern Iran (Fig. 4). Plants of several populations were collected and archived using conventional herbarium methods. All vouchers have been preserved in BASU with some in other herbaria. Also, several sheets have been examined for each species from the following herbaria: W, WU, TARI, FUMH, BASU, Herbarium of Esfahan University and Herbarium of Research Centers of Natural Resources and Animal Affairs of Mashhad, Isfahan, Kashan, Tabriz, Semnan, Shiraz, Kerman and Zahedan. We studied different Iranian populations of 5 *Astragalus* species from *Astragalus sect. Megalocystis*: *A. submitis* Boiss. & Hohen. (2 populations), *A. raddei* Basil. (2 populations), *A. halicacabus* Lam. (1 population), *A. ebenoides* Boiss. (1 population) and *A. fuhsii* Freyn & Sint. (1 population).

Chromosome number and meiotic behavior were studied in 5 species of *A. sect. Megalocystis*. For each species, 15 flower buds from at least 5 plants at an appropriate stage of development were fixed in 96% ethanol, chloroform and propionic acid (6 : 3 : 2) 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 slides were made permanent by the Venetian turpentine. Photographs of chromosomes were taken on an Olympus BX-41 photomicroscope at initial magnification of 1000 X. Chromosome counts was made from well-spread metaphases in intact cells, by direct observation and from photomicrographs. Voucher specimens are kept at BASU, Hamedan, Iran (Table 1).

Results and discussion

Different meiotic stages were found in anthers within the same flower in studied species (Figs. 5–31). A total of 564 prophase (31.1%), 428 diakinesis/metaphases I (D/MI) (23.6%), 394 anaphase I/telophase I (AI/TI) (21.7%), 416 anaphase II/telophase II (AII/MII) (22.9%) cells in *A. submitis* of Abali (Figs. 5–8), a total of 940 prophase (27.55%), 526 diakinesis/metaphases I (D/MI) (15.42%), 1161 anaphase I/telophase I (AI/TI) (34.03%), 21 metaphase II (MII) (0.6%), 764 anaphase II/telophase II (AII/MII) (22.39%) cells in *A. submitis* of Avaj. A total of 885 prophase (25.45%), 1369 diakinesis/metaphases I (D/MI) (39.41%), 250 anaphase I/telophase I (AI/TI) (7.12%), 10 metaphase II (MII) (0.3%), 961 anaphase II/telophase II (AII/MII) (27.72%) cells in *A. fuhsii* (Fig. 9). A total of 626 prophase (41.3%), 336 diakinesis/metaphases I (D/MI) (22.2%), 247 anaphase I/telophase I (AI/TI) (16.3%), 297 anaphase II/telophase II (AII/MII) (19.6%) cells in *A. raddei* of Shirvan (Fig. 10) and a total of 469 prophase (24%), 135 diakinesis/metaphases I (D/MI) (6.91%), 442 anaphase I/telophase I (AI/TI) (22.62%), 16 metaphase II (MII) (0.82%), 892 anaphase II/telophase II (AII/MII) (45.65%) cells in *A. raddei* of Beshghardash (Fig. 11). A total of 647 prophase (41.36%), 61 diakinesis/metaphases I (D/MI) (3.9%), 190 anaphase I/telophase I (AI/TI) (12.15%), 10 metaphase II (MII) (0.64%), 656 anaphase II/telophase II (AII/MII) (41.96%) cells in *A. ebenoides* (Fig. 12), and a total of 69 prophase (9.1%), 268 diakinesis/metaphases I (D/MI) (35.36%), 183 anaphase I/telophase I (AI/TI)

Figs. 1–4. 1=*A. submitis* habit with inflated fruiting calyces. 2=Details of inflorescences and calyx covered with black and white hairs in *A. submitis*. 3=Details of inflorescence, bract and bractole in *A. fuhsii*. 4=Distribution map of *A. submitis* of Abali (open triangular), *A. submitis* of Avaj (filled triangular), *A. fuhsii* (open circle), *A. raddei* of Shirvan (open star), *A. raddei* of Beshghardash (filled star), *A. ebenoides* (filled rectangular) and *A. halicacabus* (filled circle) in Iran.

Table 1. Taxa studied and acronyms

Taxa	Locality	Altitude (m)	Voucher specimen
<i>A. submitis</i>	Tehran: Abali to Mobarak abad	2150	15308 (BASU)
<i>A. submitis</i>	Hamedan: After neck Avaj	1747	15309 (BASU)
<i>A. raddei</i>	Khorasan: Shirvan after Lojeli	1710	15310 (BASU)
<i>A. raddei</i>	Khorasan: Beshghardash	1155	15311 (BASU)
<i>A. fuhsii</i>	Khorasan: Chenaran	1870	15312 (BASU)
<i>A. halicacabus</i>	Zanjan: Bifurcate Shirinbolagh	1713	15313 (BASU)
<i>A. ebenoides</i>	Hamedan: After Abegarm	1485	15314 (BASU)

(24.14%), 12 metaphase II (MII) (1.58%), 253 anaphase II/telophase II (AII/MII) (33.38%) cells in *A. halicacabus* (Fig. 13) were analyzed. The meiotic irregularities included the occurrence of varied degree of binuclear in prophase; anucleus in prophase; desynapsis in diakinesis/metaphase I; fragmented chromosomes in metaphase I; sticky chromosome in metaphase I; cytotoxicity in

Figs. 5–13. Different stages of meiosis in *A. submitis*, *A. raddei*, *A. fuhsii*, *A. ebenoides* and *A. halicacabus*. 5=Diakinisis with ring bivalents in *A. submitis* of Abali. 6=Telophase I in *A. submitis* of Abali. 7=Late anaphase II in *A. submitis* of Abali. 8=Telophase II in *A. submitis* of Abali. 9=Prophase in *A. fuhsii*. 10=Diakinesis in *A. raddei* of Shirvan. 11=Metaphase I in *A. raddei* of Beshghardash. 12=Diakinesis in *A. ebenoides*. 13=Metaphase I in *A. halicacabus*. Scale bar=3 μ m.

diakinesis/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, Figs. 14–31).

The highest score of binuclear expressed in 0.03% Z/P in *A. submitis* of Avaj, 0.06% Z/P in *A. ebenoides*, 0.13% Z/P in *A. halicacabus* (Fig. 25). The highest score of anucleus expressed in 2.75% Z/P in *A. submitis* of Avaj, 0.05% Z/P in *A. raddei* of Beshghardash, 0.7% Z/P in *A. ebenoides* (Fig. 21). The highest score of desynapsis expressed only in 0.13% D/MI cells of *A. halicacabus*.

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

Meiotic characters	<i>A. raddei</i> 10	<i>A. submiss</i> 50	<i>A. fuhsii</i>	<i>A.</i> <i>ebenoides</i>	<i>A. submiss</i> 47	<i>A.</i> <i>halicacabus</i>	<i>A. raddei</i> 55
Total cell number	1506	1806	3465	1564	3412	758	1954
P	626	564	885	647	940	69	469
% P	41.3	31.1	25.45	41.36	27.55	9.1	24
% Binucleus	0	0	0	0.06	0.03	0.13	0
% Cytoplasmic channel	5.41	6.05	0.84	13.39	3.05	0.13	3.53
D/MI	336	428	1369	61	526	268	135
% D/MI	22.2	23.6	39.41	3.9	15.42	35.36	6.91
% Cytoplasmic channel	3.5	2.47	0.6	0.06	0.64	0	0.51
% Sticky and laggard chromosomes	0	0	0	0.06	0.76	0	0.05
% Anucleus	0	0	0	0.7	2.75	0	0.05
% B chromosome	0	0	0.55	0.38	1.61	0.79	0.42
% Cytomixis	0	0	0.03	0	0	0	0
% Ring bivalents	0	0	0	0.51	0.9	0.13	0
% Fragmented chromosome	4.42	8.81	0.17	0.38	1.87	0.39	0.25
AI/TI	247	394	250	190	1161	183	442
% AI/TI	16.3	21.7	7.12	12.15	34.03	24.14	22.62
% Cytomixis	0	0	0	0	0.08	0	0
% Cytoplasmic channel	1.91	21.7	0.06	0.06	2.37	0	1.38
% Laggard chromosome	0	0	0.08	0.51	0.7	0.13	0
% Forward chromosome	0	0	0	0.32	0.58	0	0
% Bridge	0	0	0.03	0	0.35	0	0
% Micronucleus	0	0	0	0	0.08	0	0.11
% Asynchronous nucleus	0	0	0	0.83	0.2	0.26	0.61
MII	0	0	10	10	21	12	16
% MII	0	0	0.3	0.64	0.6	1.58	0.82
% Fragmented chromosome	0.33	0.49	0	0	0	0	0
% Cytomixis	0	0	0	0	0	0	0
% Cytoplasmic channel	0	0	0	0	0	0	0.05
AII/TII	297	416	961	656	764	253	892
% AII/TII	19.6	22.9	27.72	41.94	22.39	33.38	45.65
% Micronucleus	0	0	0	0.25	0.08	0	0
% Laggard chromosome	0	0	0	0.06	0.06	0.39	0
% Cytoplasmic channel	0.72	2.14	0.52	1.34	0.49	0	0.46
% Tripolar	0	0	0.03	0	0.2	0.39	0
% Hexapolar	0	0	0	0	0	1.18	0
% Pentapolar	0	0	0	0.06	0	0	0
% Forward chromosome	0	0	0	0.13	0	0	0
% Cytomixis	0	0	0.03	0	0.08	0	0
% Bridge	0	0	0	0	0.08	0.13	0
<i>n</i>	8	8	16	8	8	16	8

Abbreviations: P=prophase, D/MI=diakinesis/metaphase I, AI/TI=anaphase I/telophase I, MII=metaphase II, AII/TII=anaphase II/telophase II, *n*=Chromosome number.

Fragmented chromosomes for being unable to orient at the metaphase plate were observed during metaphase I or metaphase II. In *A. submiss* of Abali 8.81% metaphase I, in *A. submiss* of Avaj 49% metaphase II, in *A. fuhsii* 87% metaphase, in *A. raddei* of Shirvan 0.17% metaphase I, in *A. raddei* of Beshghardash 4.42% metaphase I, 0.33% metaphase II, in *A. ebenoides* 0.25% metaphase I and in *A. halicacabus* 0.39% metaphase I cells were observed (Fig. 15).

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

Figs. 14–22. Meiotic behavior in *A. submitis* and *A. fuhsii*. 14=B-chromosome in *A. submitis* of Abali. 15=B-chromosome and fragmented chromosomes in *A. submitis* of Abali. 16=Cytoplasmic channels in *A. submitis* of Abali. 17=Forward chromosomes in *A. submitis* of Avaj. 18=Laggard and bridge in *A. submitis* of Avaj. 19=Bridge in telophase I in *A. submitis* of Avaj. 20=Bridge in telophase II in *A. submitis* of Avaj. 21=Anucleus in *A. fuhsii*. 22=Metaphase I with univalent precocious ascension in *A. fuhsii*. Scale bar=3 μ m.

Houben 2003). The highest percent of B-chromosomes was observed in 1.61% metaphase I of *A. submitis* of Avaj, 0.55% metaphase I in *A. fuhsii*, 0.42% metaphase I in *A. raddei* of Beshghardash, 0.38% metaphase I in *A. ebenoides*, 0.79% metaphase I of *A. halicacabus* (Figs. 14, 15, 27). The occurrence of B-chromosome in *A. submitis* and *A. ebenoides* is reported here for the first time.

The highest score for laggard chromosomes expressed in 0.7% AI/TI and 0.06% AII/TII in *A. submitis* of Avaj, 0.08% AI/TI in *A. fuhsii* 0.51% AI/TI, 0.06% AII/TII, 0.13% AI/TI, 0.39% AII/TII in *A. ebenoides*. 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

Figs. 23–31. Meiotic behavior in *A. submitis*, *A. raddei*, *A. ebenoides* and *A. halicacabus*. 23=Polypolar in *A. submitis*. 24=Tripolar in *A. submitis*. 25=Binucleus in *A. raddei* of Shirvan. 26=Asynchronous nucleus in telophase I in *A. raddei* of Shirvan. 27=B-chromosome in *A. raddei* of Beshghardash. 28=Micronucleus in *A. ebenoides*. 29=Sticky chromosome by laggard in *A. ebenoides*. 30=Tripolar and bridge in *A. halicacabus*. 31=Polypolar in *A. halicacabus*. Scale bar=3 μ m.

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 unbalanced chromosome numbers (Mansuelli *et al.* 1995), such as aneuploids (Defani-Scoarize *et al.* 1995) (Fig. 18). The highest score of forward chromosome expressed in 0.58% AI/TI in *A. submitis* Avaj and 0.32% AI/TI, 0.13% AII/TII in *A. ebenoides* (Fig. 17). Chromosome bridges resulting from stickiness were the highest score of chromosome bridges expressed in 0.35% AI/TI and 0.08% AII/TII in *A. submitis* of Avaj, 0.03% AI/TI in *A. fuhsii* and 0.13% AII/TII in *A. halicacabus*. The thickness of bridges observed and the number of chromosomes involved in their formation varied among different meiocytes. Genetic as well as environmental factors have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao 1996) (Figs. 18, 19, 20, 30).

The highest score of asynchronous nucleus expressed in 0.2% AI/TI in *A. submitis* of Avaj, 0.61% AI/TI in *A. raddei* of Beshghardash, and 0.83% AI/TI, and 0.19% AII/TII in *A. ebenoides* and 0.26% AI/TI in *A. halicacabus* (Fig. 26). Micronucleus is another abnormality that was found in 0.08% AI/TI, 0.08% AII/TII in *A. submitis* of Avaj, 0.25% AII/TII in *A. ebenoides* that produced micronuclei during meiosis was eliminated from microspores as microcytes. The micronucleus reached the microspore wall and formed a kind of bud, separated from the microspore. The eliminated microcytes gave origin to small and sterile pollen grains (Baptists-Giacomoelli *et al.* 2000) (Fig. 28). 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). It was found in 0.08% AI/TI in *A. submitis* of Avaj and 0.03% D/MI, 0.03% AII/TII in *A. fuchsii* (Table 2, Fig. 16). 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. The plasmodesmata become completely obstructed by the deposition of callose, but in some cases they still persist during meiosis and increase in size forming conspicuous inter-meiocytic connections or cytoplasmic channels that permit the transfer of chromosomes. Chromosome migration may also occur through cell wall dissolution among the neighboring meiocytes and forming syncyte (Falistocco *et al.* 1995). Cytomixis is not considered to be of great evolutionary importance, but it may lead to production of aneuploid plants, or result 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.

Observations on chromosome morphology and size made in this study are an insight into what will be expected at meiosis. The distribution of several meiotic abnormalities observed include a varied degree of fragmented chromosomes in metaphase I; cytomixis in metaphase I; asynchronous nucleus in metaphase II and cytomixis in telophase II, which is likely to result in many structural changes and rearrangements at meiosis, and which could then lead to speciation. Akpabio and Olorode (1988) observed a similar trend in *Crotalaria*, another leguminous genus. Much larger numbers of species and accessions from South, Central and West Iran, and especially of the other section from the *Astragalus* group with inflated fruiting, must be analyzed cytologically. Detailed DNA studies and the use of genome in situ hybridization are also essential to obtain a clearer picture of *Astragalus* species relationship and evolution.

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