Cytotaxonomic Study of *Astragalus* Sect. *Megalocystis* (Fabaceae) in Iran

Massoud Ranjbar*, and Samane Jahanian

Department of Biology, Herbarium Division, Bu-Ali Sina University,
P.O. Box 65175/4161, Hamedan, Iran

Received December 26, 2012; accepted April 28, 2013

Summary The meiotic chromosome number and behavior of 12 Iranian populations belonging to six *Astragalus* species, *A. submitis*, *A. raddei*, *A. crassispinus*, *A. ebenoides*, *A. szovitsii* and *A. lalesarensis* of *A.* sect. *Megalocystis*, are presented here. All taxa studied 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, included varied degrees of fragmented chromosomes, cytomixis, asynchronous nucleus, laggard, sticky and forward chromosomes, desynapsis and unequal polars.

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

Astragalus L. is a large genus belonging to the tribe Astragaleae of Papilionoideae in the family Fabaceae. In terms of species number, *Astragalus* may be the largest genus of vascular plants, represented by a total of ca. 2500 taxa (Lock and Simpson 1991, Yakovlev *et al.* 1996, Mabberley 1997, Ranjbar and Karamian 2003). *Astragalus* sect. *Megalocystis*, with about 40 species is a rather large section within the genus. 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. They are distributed in several southwest Asian countries, but Iran with about 24 species, is the centre of the diversity of the section.

Most of the cytological studies in the tribe have concentrated on chromosome counts (Maassoumi 1987, Maassoumi 1989, Ranjbar *et al.* 2009a, b, 2010a, b, c, d, e). The basic chromosome number ($x=8$) in two ploidy levels ($2n=2x=16$, $2n=4x=32$) are present in the genus in the Old World. Cytogenetic analyses have been widely used as important tools to study the hybridization process (Rieseberg and Carney 1998, Soltis and Soltis, 2009). The determination of chromosome number in plants is a critical step to detect mechanisms that make feasible abrupt speciation, such as hybridization, polyploidy, aneuploidy and dysploidy (Briggs and Walters 1997, Guerra 2008). Moreover, the knowledge of chromosome numbers and morphology, and also of their meiotic behavior may help to differentiate between allopolyploidy or autopolyploidy considering the differences or similarities between the genomes involved in the hybrid (Stebbins 1971, Guerra 2008). In this paper, meiotic chromosome number and behavior of 12 Iranian populations belonging to six *Astragalus* species of *A.* sect. *Megalocystis* are reported for the first time.

Materials and methods

Morphology

This study is mainly based on field observations during several excursions in Iran and on her-

* Corresponding author, e-mail: ranjbar@basu.ac.ir

DOI: 10.1508/cytologia.78.181

Table 1. Taxa studied and acronyms.

Taxa	Locality	Altitude (m)	Voucher specimen	Abrivation
<i>A. crassispinus</i> *	Khorasan: Fariman toward Torbate-Jam, Kalate Bozorg village	1420	BASU 22982	cra82
<i>A. ebenoides</i> *	Hamedan: Hamadan toward Ghorveh, before Veynesar village	1830	BASU 24733	ebe33
<i>A. ebenoides</i>	Hamedan: After Abegarm	1485	BASU 15314	ebe14
<i>A. ebenoides</i> *	Azerbaijan Gharbi: Bukan toward Miandoab	1280	BASU 25022	ebe22
<i>A. ebenoides</i> *	Azerbaijan Gharbi: 15 km after Bukan, toward Mahabad	1500	BASU 24736	ebe36
<i>A. ebenoides</i> *	Azerbaijan Gharbi: Mahabad	1320	BASU 24732	ebe32
<i>A. fuhsii</i>	Khorasan: Chenaran	1870	BASU 15312	fuh12
<i>A. halicacabus</i>	Zanjan: Shirinbolagh bifurcate	1713	BASU 15313	hal13
<i>A. lalesarensis</i> *	Kerman: Negar toward Baft, Ghalee Asgar village	2528	BASU 22926	lal26
<i>A. raddei</i>	Khorasan: Beshghardash	1155	BASU 15311	rad11
<i>A. raddei</i>	Khorasan: Shirvan after Lojeli	1710	BASU 15310	rad10
<i>A. raddei</i> *	Bojnord: Arkan village	1453	BASU 27932	rad32
<i>A. raddei</i>	Bojnord: Arkan village	1453	BASU 21101	rad01
<i>A. raddei</i> *	Bojnord: Arkan village	1453	BASU 27933	rad33
<i>A. submitis</i>	Tehran: Abali to Mobarak abad	2150	BASU 15308	sub08
<i>A. submitis</i>	Hamedan: After neck Avaj	1747	BASU 15309	sub09
<i>A. submitis</i> *	Alborz: 4 km after Taleghan	1742	BASU 21560	sub60
<i>A. szovitsii</i>	Azerbaijan Sharghi: before Zonuz	1435	BASU 24735	szo35
<i>A. szovitsii</i>	Azerbaijan Gharbi: Jolfa toward Soufian, 76 km to Soufian	1400	BASU 22032	szo32

* New records.

barium materials. The field studies were carried out in different parts of Iran (Figure 25). Plants of several populations were collected and archived using conventional herbarium methods. All vouchers have been preserved in BASU. We studied different Iranian populations of six *Astragalus* species from *A. sect. Megalocystis*, namely *A. submitis* (1 population), *A. lalesarensis* (1 population), *A. raddei* (3 populations), *A. crassispinus* (1 population), *A. ebenoides* (4 populations) and *A. szovitsii* (2 populations). The specimens studied morphologically are listed in Table 1 and used as operational taxonomic units (OTUs). A numerical taxonomic analysis on different individuals of these populations was carried out based on 63 quantitative/qualitative characters related to vegetative and reproductive organs. A list of morphological characters studied here is presented in Table 2. Data were entered into computerized spreadsheet program, Microsoft Excel version 7. The spreadsheet was later transformed into a file format suitable for phenetic analysis. Principal coordinates analysis (PCO) was carried out using MVSP software version 3.2 (Kovach 1985–2002), with a matrix of standardized data. For PCO, an Average Distance Matrix of standardized data was obtained.

Cytogenetics

Meiotic chromosome number and behavior were studied in six species of *A. sect. Megalocystis*. For each species, 15 flower buds from at least five 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 taken on an Olympus BX-41 photomicroscope at initial magnification of 1000 X. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs. Voucher specimens are kept at BASU, Hamedan, Iran (Table 1). All data obtained from cytogenetic study were analyzed by MVSP software and the relationships between different populations were examined.

Table 2. Morphological characters and character state matrix in different populations of *A. submitis*, *A. raddei*, *A. crassispinus*, *A. ebenoides*, *A. szovitsii*, *A. lalesarensis* and *A. brevipilus*.

Morphological characters	szo30	cras82	ebe33	ebe22	ebe32	ebe36	szo35	sub60	rad01
Plant height (cm)	13.7	17.5	19	22.75	25.6	24	15	34	27.5
Leaf length (cm)	7.7	8.75	8.5	16.16	10	12.35	7.75	8.3	6.65
Leaf shape (paripinnate=1, imparipinnate=0)	1	0	1	1	1	1	1	1	0
Petiole length (mm)	2.5	1.75	4.25	5	5.25	5.5	4.75	3	2.3
Number of leaflet pairs	15	14	18	22	12	18	15.5	23	12
Hair length on lower surface (mm)	0.7	1.8	0.5	0.4	0.5	0.6	0.7	0.25	0.65
Hair density lower surface (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	3	3	3	3
Hair state on lower surface (appressed=0, spreading=1)	0	0	0	0	0	0	0	1	1
Leaflet shape	4	4	0	0	0	0	0	0	4
Leaflet length (mm)	2.5	5.5	5	6	8	2.1	5	2.75	4.75
Leaflet width (mm)	1.5	2.75	1.8	2.5	2	0.5	2	2.5	2
Hair length on upper surface (mm)	0.7	0.75	0.5	0.4	0.45	0.5	0.7	0.25	0.55
Hair state on upper surface (appressed=0, spreading=1)	0	0	0	0	0	0	0	1	1
Hair density on upper surface (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	3	3	3	3
Leaflet mucronate length (mm)	0.1	0.35	1	0.3	0.1	1	0	0.3	0.5
Stipule length (mm)	5	9	8	6	11	10	6	11	12
Stipule width (mm)	2	2	1.7	2	4	1.5	2	6	3
Stipule texture (membranous=1, foliate=0, chartaceous=2)	1	2	1	1	0	1	1	0	2
Hair length on stipule (mm)	0.75	35	0.75	0.5	0.75	0.85	0.9	0.2	0.9
Hair density of stipule (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	4	4	4	4	3	3	4	3
Hair state of stipule (appressed=0, spreading=1)	1	1	1	1	1	1	1	1	1
Hair color of stipule (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	0	0	0	0	0	0
Peduncle length (cm)	8.5	6.75	16	21.25	13.75	17.5	9.5	33	19
Hair length on peduncle	0.5	1.3	0.85	0.3	0.65	0.65	0.75	0.3	0.6
Hair density on peduncle (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	3	3	3	3
Hair color of peduncle (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	0	0	0	0	3	3
Hair state of peduncle (appressed=0, spreading=1)	0	0	1	0	0	0	0	1	1
Bract length (mm)	3.5	7.5	5	5.5	8	7	6	3	8
Bract width (mm)	1	3.75	2	2	3	2.2	2.3	2	1.9
Hair length on bract (mm)	0.7	0.675	0.75	0.45	0.45	0.6	0.85	0.55	0.65
Hair state on bract (appressed=0, spreading=1)	1	0	1	1	1	1	1	1	1
Hair density on bract (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	3	3	3	3
Hair color on bract (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	0	0	0	0	2	3
Bract length (mm)	2	6	4	3	5	4	5	3.5	5.4
Bract width (mm)	0.5	1.1	0.7	0.5	0.6	0.3	1	1	0.5
Hair length on bracteole (mm)	0.5	1.05	0.75	0.75	0.75	0.5	0.95	0.45	0.7
Hair state on bracteole (appressed=0, spreading=1)	0	0	1	1	1	1	1	1	1
Hair density on bracteole (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	3	3	3	3
Hair length of pedicel (mm)	0.7	0	0.7	0.5	0.6	0.55	0.5	0.5	0.5
Hair color of pedicel (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	5	0	0	0	0	0	2	4

Table 2. Continued.

Morphological characters	szo30	cras82	ebe33	ebe22	ebe32	ebe36	szo35	sub60	rad01
Calyx length (mm)	17	18	12.6	11.7	14	14.2	13.5	13.5	15.25
Calyx tube length (mm)	14	11	9	8	9.5	10	9	12.5	11.5
Calyx width (mm)	10	11	11	8.5	13.5	10.5	9.5	15	1.45
Calyx teeth length (mm)	3	7	3.6	3.7	4.5	4.2	4.5	1.5	3.75
Hair length on calyx (mm)	2.5	2.9	1.25	1.2	1.5	1.25	1.8	0.5	2.05
Hair state on calyx (appressed=0, spreading=1)	1	0	1	1	1	1	1	1	1
Hair density of calyx (sparse=1, loose=2, dense=3, ciliate at the margin=4)	2	3	3	3	3	3	3	3	3
Hair color of calyx (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	0	0	0	0	2	0
Standard length (mm)	15	16	11.5	12	14.5	12.3	15.5	26	16
Standard width (mm)	5	7.5	5	4	6.6	4.3	7	11	6
Keel length (mm)	13	14	11.8	12	13	13.5	15	16	15.3
Keel width (mm)	1.5	3	2.5	2.3	2.7	2.5	2.7	4	2.8
Keel claw length (mm)	10	10	8	8.5	9	10	11	11	11
Wing length (mm)	15	14.8	11.6	8.5	13.4	10.7	15.3	19.5	14.3
Wing width (mm)	2.5	3	2.8	2.3	2.8	2.2	3	4	2.6
Wing claw length (mm)	9	9.3	8	5	9.2	9	10	13	9.5
Wing angulate length (mm)	1.5	2	1	1.2	1	1	1	2.2	0.5
Ovary stipe	1.5	1	1.6	1.5	2	2	1.5	0	0.5

Morphological characters	rad32	rad33	lal26	sub08	fuh12	bre13	rad11	ebe14	rad10	sub09
Plant height (cm)	33.2	35.5	17	45	35	30.8	20.5	27.5	16	34
Leaf length (cm)	8	10	8.6	16.1	27	17	9.85	7.9	6.8	14
Leaf shape (paripinnate=1, imparipinnate=0)	0	0	1	1	1	1	1	1	0	1
Petiole length (mm)	2.5	4.5	3.5	6.3	7.5	5	3.25	2.65	2.18	7
Number of leaflet pairs	13	13	15	28	14.5	15	16	16.5	14	28.3
Hair length on lower surface (mm)	0.95	0.65	0.35	0.8	0	0.2	0.7	0.45	0.5	0.9
Hair density lower surface (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	2	0	3	3	3	3	3
Hair state on lower surface (appressed=0, spreading=1)	1	1	1	1	2	0	1	1	0	0
Leaflet shape	4	4	1	2	4	1	4	4	1	1
Leaflet length (mm)	4.5	0.6	3	1.9	11.8	7	6.5	3	5.4	5
Leaflet width (mm)	3	2.1	2	1.4	5	5	3.5	1.75	1.6	2.5
Hair length on upper surface (mm)	0.9	0.65	0.85	0.45	0	0.2	0.5	0.4	0.25	0.7
Hair state on upper surface (appressed=0, spreading=1)	1	1	1	1	2	0	1	1	0	0
Hair density on upper surface (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	1	0	3	3	3	3	3
Leaflet mucronate length (mm)	0.7	0.5	0.45	0.1	0.5	0	0.2	0.3	0.3	0.3
Stipule length (mm)	11	15	9	4.8	11	5	11	7	6.5	16
Stipule width (mm)	4	3	1.5	1.6	5	1	4	2.5	1.2	4
Stipule texture (membranous=1, foliate=0, chartaceae=2)	2	2	1	1	1	1	1	1	0	1
Hair length on stipule (mm)	1.15	0.9	0.5	0.55	0.6	0.75	1	0.7	0.25	0.5
Hair color of stipule (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	0	0	0	0	0	4	0
Peduncle length (cm)	21.5	26	10	5	27.5	17	0.05	13	13.7	21
Hair length on peduncle	0.9	0.65	0.3	0.65	0.35		0.5	1.4	0.15	0.7
Hair density on peduncle (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	2	1	2	3	3	3	2
Hair color of peduncle (white=0, black=1, more white=2, more black=3, equal white and black=4)	3	3	0	3	0	0	0	0	2	0

Table 2. Continued.

Morphological characters	rad32	rad33	lal26	sub08	fuh12	bre13	rad11	ebe14	rad10	sub09
Bract width (mm)	2	2.5	2.8	0.7	2	0.5	2	3	0.5	1
Hair length on bract (mm)	1	0.8	0.3	0.7	0.35	0.35	0.25	0.6	0.35	0.7
Hair state on bract (appressed=0, spreading=1)	1	1	1	1	0	0	1	1	1	1
Hair density on bract (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	3	3	3	3	3
Hair color on bract (white=0, black=1, more white=2, more black=3, equal white and black=4)	3	3	3	2	4	3	0	0	2	2
Bract length (mm)	5	6	4.75	3	0	2	5	4	2.1	2
Bract width (mm)	0.6	1	0.5	0.5	0	0.5	0.5	0.7	0.5	0.5
Hair length on bracteole (mm)	1.25	1	0.4	0.25	0	0.35	1.1	0.75	1	0.5
Hair state on bracteole (appressed=0, spreading=1)	1	1	0	1	2	1	1	1	1	1
Hair density on bracteole (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	0	3	3	3	3	3
Hair color on bracteole (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	4	5	2	0	0	0	2
Pedicle length (mm)	1.6	1.3	0	1.1	0	1	0	1.7	0.9	1
Hair length of pedicel (mm)	1.1	0.65	0	0.8	0	1	0	0.65	0.2	0.5
Hair color of pedicel (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	5	2	5	2	5	0	0	2
Hair density of pedicel (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	0	2	0	3	0	3	3	3
Hair state of pedicel (appressed=0, spreading=1)	1	1	2	1	2	1	2	1	1	1
Calyx length (mm)	14.4	12	15.2	12.2	25	9	16	13	12.4	12.75
Calyx tube length (mm)	10.2	9	12.2	10.2	20	8	11.5	9	10.9	10
Calyx width (mm)	13	13	15	7.9	14	16	9.5	12	9.5	12.5
Calyx teeth length (mm)	4.2	3	3	2	4.5	1	4.5	4	2	2
Hair length on calyx (mm)	1.9	1.4	0.55	0.85	0.35	0.75	1.25	2	1.25	1.5
Hair state on calyx (appressed=0, spreading=1)	1	1	1	1	0	1	1	1	1	1
Hair density of calyx (sparse=1, loose=2, dense=3, ciliate at the margin=4)	3	3	3	3	3	2	3	3	3	3
Hair color of calyx (white=0, black=1, more white=2, more black=3, equal white and black=4)	0	0	0	3	4	3	0	0	0	2
Standard length (mm)	14.5	15	2.1	16.4	30	14	15	12.2	13.4	20
Standard width (mm)	5.3	6	8.5	6.6	15	5	6.25	5.5	6.2	8.5
Keel length (mm)	17	16	18	15.8	27	14	15	11.5	15.4	18.5
Keel width (mm)	3	2.8	3.2	2.5	7	2	3	3	2.6	3
Keel claw length (mm)	13	10	13	10.5	13	9	14	8.5	10.4	12
Wing length (mm)	16.4	16.5	24	16.6	28	15	15.5	13	15.8	20
Wing width (mm)	2.4	3	2.7	2.3	6	2	2.5	3	2.7	3.5
Wing claw length (mm)	12	12	14	10.8	13	9	9.5	9	10.6	12
Wing angulate length (mm)	0.6	0.5	1.2	1.3	2	1.5	0.7	1	0.8	1
Ovary stipe	0.5	0.5	2.2	1.5	3	0	0.8	2	0.6	1.3
Wing angulate length (mm)	0.6	0.5	1.2	1.3	2	1.5	0.7	1	0.8	
Ovary stipe	0.5	0.5	2.2	1.5	3	0	0.8	2	0.6	

Table 3. Number of pollen mother cells analyzed and percentage of PMCs.

Meiotic characters	sub60	ebe33	lal26	ebe22	rad32	rad01	ead33	ebe36	ebe32
Total cell number	687	295	1712	343	528	898	825	241	848
D/MI	32	239	504	134	31	210	57	57	362
% D/MI	4.65	81.01	29.43	39.35	5.87	23.38	6.9	23.65	42.68
% Sticky chromosome	0	0	5.95	0	0	2.85	0	0	0
% B-chromosome	9.37	0	3.96	0	0	2.38	5.26	3.5	0
% Cytomixis	0	6.27	0	0	12.9	0	0	0	2.48
% Ring bivalent	0	1.67	2.38	14.81	0	0	0	0	0
% Fragmented chromosome	18.75	5.02	48.8	7.4	25.8	5.01	36.84	52.63	51.93
AI/TI	35	6	192	110	154	550	341	93	114
% AI/TI	5.09	2.03	11.21	32.06	29.16	61.24	41.3	38.58	13.44
% Cytomixis	0	0	0.52	0	0	0	0	0	0
% Laggard chromosome	0	0	5.2	0	15.58	1.81	1.49	2.15	1.75
% Forward chromosome	5.71	0	0	0	1.29	0	0	4.3	1.75
% Bridge	0	0	1.04	9.09	1.29	0	0.87	2.15	0
% Micronucleus	0	0	1.56	0	4.54	0	0	2.15	5.26
% Asynchronous nucleus	51.42	0	13.54	9.09	0	0	0	8.6	0
MII	12	6	8	13	7	31	27	5	22
% MII	1.74	2.03	0.46	3.79	1.32	3.45	3.27	2.07	2.59
% Fragmented chromosome	0	0	0	0	28.75	16.12	0	0	18.18
AII/TII	608	44	1008	85	336	107	400	86	350
% AII/TII	88.5	14.91	58.87	24.78	63.63	11.91	48.48	35.68	41.27
% Micronucleus	0.49	0	0	0	0	0	3	2.32	0
% Laggard chromosome	0	11.36	1.19	0	0.59	0	0	2.32	0
% Tripolar	0	2.27	0	0	0	0	0	0	0
% Forward chromosome	0	0	0	0	6.54	0	0	0	0
% Cytomixis	0.82	0	0	0	0	0	0	0	0
% Bridge	0	0	0	0	0	1.48	0	1.16	0

Meiotic characters	szo30	szo35	cras82	rad10	sub50	fuh12	ebe14	sub47	bre13	rad11
Total cell number	746	677	285	1506	1806	3465	1564	3412	758	1954
D/MI	212	252	51	336	428	1369	61	526	268	135
% D/MI	28.41	37.22	17.89	22.2	23.6	39.41	3.9	15.42	35.36	6.91
% Sticky chromosomes	0	0	0	0	0	0	0.06	0.76	0	0.05
% B-chromosome	0	0	0	0	0	0.55	0.38	1.61	0.79	0.42
% Cytomixis	0	0	0	0	0	0.03	0	0	0	0
% Ring bivalents	5.66	0	0	0	0	0	0.51	0.9	0.3	0
% Fragmented chromosome	18.86	28.57	5.88	4.42	8.81	0.17	0.38	1.87	0.39	0.25
AI/TI	233	180	192	247	394	250	190	1161	183	442
% AI/TI	31.23	26.58	67.36	16.3	21.7	7.12	12.15	34.03	24.14	2.62
% Cytomixis	0	0	0	0	0	0	0	0.08	0	0
% Laggard chromosome	4.29	0	0	0	0	0.08	0.51	0.7	0.13	0
% Forward chromosome	4.29	0	0	0	0	0	0.32	0.58	0	0
% Bridge	0	0	0	0	0	0.03	0	0.35	0	0
% Micronucleus	0	0	0	0	0	0	0	0.08	0	0.11
% Asynchronous nucleus	25.75	3.33	15.62	0	0	0	0.83	0.2	0.26	0.61
MII	21	4	3	0	0	10	10	21	12	16
% MII	2.81	0.59	1.05	0	0	0.3	0.64	0.6	1.58	0.82
% Fragmented chromosome	47.61	0	0	0.33	0.49	0	0	0	0	0
AII/TII	280	241	39	297	416	961	656	764	253	892
% AII/TII	37.53	35.59	13.68	19.6	22.9	27.72	41.94	22.39	33.38	45.65
% Micronucleus	1.42	0	0	0	0	0	0.25	0.08	0	0
% Laggard chromosome	0	2.9	0	0	0	0	0.06	0.06	0.39	0
% Tripolar	0	0	7.69	0	0	0.03	0	0.2	0.39	0
% Forward chromosome	0	0	0	0	0	0	0.13	0	0	0
% Cytomixis	3.92	0	0	0	0	0.03	0	0.08	0	0
% Bridge	0	0	0	0	0	0	0	0.08	0.13	0

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.

Results

Cytogenetics

Different meiotic stages were found in anthers within the same flower in studied species (Table 3, Figs. 1–24). A total of 687 cells, 32 diakinesis/metaphase I (D/MI) (4.65%), 35 anaphase I/telophase I (AI/TI) (5.09%), 12 metaphase II (MII) (1/74%) and 608 anaphase II/telophase II (AII/TII) (88/50%) cells in *A. submitis* were observed (Figures 2 and 8). A total of 1712 cells, 504 diakinesis/metaphases I (D/MI) (29.43%), 192 anaphase I/telophase I (AI/TI) (11.21%), 8 meta-

Figs. 1–12. Representative meiotic cells in *A. crassispinus*, *A. submitis* and *A. lalesarensis*. 1=Diakinesis in *A. crassispinus*, 2=Telophase II in *A. submitis*, 3=Metaphase I in *A. lalesarensis*, 4=Asynchronous nucleus in *A. submitis*, 5=Anaphase I in *A. lalesarensis*, 6=Binucleus in *A. crassispinus*, 7=Cytomixis in *A. lalesarensis*, 8=Telophase I in *A. submitis*, 9=Fragmented chromosome in *A. crassispinus*, 10=Forward chromosome in *A. submitis*, 11=Asynchronous nucleus & laggard chromosome in *A. lalesarensis*, 12=fragmented chromosome in *A. submitis*. Scale bar=3 μ m.

Figs. 13–24. Representative meiotic cells in *A. szovitsii*, *A. ebenoides* and *A. raddei*. 13=Ring bivalents in *A. ebenoides* of Hamedan, 14=Bridge and sticky chromosomes in *A. ebenoides* of Bukan, 15=Unequal polars and laggard in *A. szovitsii* of Zonuz, 16=Sticky chromosomes in *A. raddei* 01, 17=Laggard chromosome in *A. ebenoides* of Mahabad, 18=Telophase I in *A. szovitsii* of Jolfa, 19=Metaphase II in *A. raddei* 32, 20=Cytomixis and fragmented chromosome in *A. raddei* 32, 21=Sticky and fragmented chromosomes in *A. raddei* 01, 22=Micronucleus and laggard in *A. ebenoides* 36, 23=Micronucleus in *A. szovitsii* of Jolfa, 24=Prophase in *A. raddei* 33. Scale bar=3 μ m.

phase II (MII) (0.46%) and 1008 anaphase II/telophase II (AII/TII) (58.87%) cells in *A. lalesarensis* were analyzed (Figs. 3 and 5). A total of 295 cells, 239 diakinesis/metaphase I (D/MI) (81.01%), 6 anaphase I/telophase I (AI/TI) (2.03%), 6 metaphase II (MII) (2.03%) and 44 anaphase II/telophase II (AII/TII) (14.91%) cells in *A. ebenoides* of Hamedan; a total of 343 cells, 135 diakinesis/metaphase I (D/MI) (39.35%), 110 anaphase I/telophase I (AI/TI) (32.06%), 13 metaphase II (MII) (3.79%) and 85 anaphase II/telophase II (AII/MII) (24.78%) cells in *A. ebenoides* of Bukan, and a total of 528 cells, 31 diakinesis/metaphase I (D/MI) (5.87%), 154 anaphase I/telophase I (AI/TI) (29.16%), 7 metaphase II (MII) (1.32%) and 336 anaphase II/telophase II (AII/MII)

(63.63%) cells in *A. raddei* 32 (Fig. 19); a total of 898 cells, 210 diakinesis/metaphase I (D/MI) (23.38%), 550 anaphase I/telophase I (AI/TI) (61.24%), 31 metaphase II (MII) (3.45%) and 107 anaphase II/telophase II (AII/MII) (11.91%) cells in *A. raddei* 01 were observed. A total of 825 cells, 57 diakinesis/metaphase I (D/MI) (6.90%), 341 anaphase I/telophase I (AI/TI) (41.33%), 27 metaphase II (MII) (3.27%) and 400 anaphase II/telophase II (AII/MII) (48.48%) cells in *A. raddei* 33 (Figure 24); a total of 241 cells, 57 diakinesis/metaphase I (D/MI) (23.65%), 93 anaphase I/telophase I (AI/TI) (38.58%), 5 metaphase II (MII) (2.07%) and 86 anaphase II/telophase II (AII/TII) (35.68%) cells in *A. ebenoides* 36, and a total of 848 cells, 362 diakinesis/metaphase I (D/MI) (42.68%), 114 anaphase I/telophase I (AI/TI) (13.44%), 22 metaphase II (MII) (2.59%) and 350 anaphase II/telophase II (AII/TII) (41.27%) cells in *A. ebenoides* of Mahabad were analyzed. A total of 746 cells, 212 diakinesis/metaphase I (D/MI) (28.41%), 233 anaphase I/telophase I (AI/TI) (31.23%), 21 metaphase II (2.81) (MII) and 280 anaphase II/telophase II (AII/MII) (37.53%) cells in *A. szovitsii* of Jolfa (Fig. 18); a total of 677 cells, 252 diakinesis/metaphase I (D/MI) (37.22%), 180 anaphase I/telophase I (AI/TI) (26.58%), 4 metaphase II (MII) (0.59%), 241 anaphase II/telophase II (AII/MII) (35.59%) cells in *A. szovitsii* of Zonuz, and a total of 285 cells, 51 diakinesis/metaphase I (D/MI) (17.89%), 192 anaphase I/telophase I (AI/TI) (67.36%), 3 metaphase II (MII) (1.05%) and 39 anaphase II/telophase II (AII/MII) (13.68%) cells in *A. crassispinus* were analyzed.

The meiotic irregularities included binuclear in prophase; unequal polars in TI; fragmented chromosomes in D/MI and MII; sticky chromosomes in D/MI, AI/TI, MII and AII/TII; cytomixis in D/MI, AI/TI and AII/TII; B-chromosomes in D/MI; micronucleus in AI/TI and AII/TII; laggard chromosomes in AI/TI and AII/TII; forward chromosomes in AI/TI and AII/TII; bridges in AI/TI and AII/TII; tripolar cells in AII/TII; and asynchronous nucleus in TI. Some abnormalities in meiotic behaviors of the taxa are described in details below (Table 3, Figs. 1–24). Fragmented chromosomes, being unable to orient at the metaphase plate, were observed during D/MI in all the taxa, MII in *A. raddei* 32, *A. raddei* 01 and *A. ebenoides* of Mahabad and *A. szovitsii* of Jolfa (Figs. 9, 12, 20 and 21). 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 percentage of B-chromosomes was observed in 9.37% diakinesis/metaphase I of *A. submitis*, 5.26% of *A. raddei* 33, 3.96% of *A. lalesarensis*, 3.50% of *A. ebenoides* 36, and 2.38% of *A. raddei* 01. The highest score for laggard chromosomes expressed in 15.58% AI/TI of *A. raddei* 32 and 11.36% AII/TII of *A. ebenoides* of Hamedan, 5.20% AI/TI of *A. lalesarensis*, 1.81% of *A. raddei* 01, 1.46% of *A. raddei* 33, 2.15% of *A. ebenoides* 36, 1.75% of *A. ebenoides* of Mahabad, 1.19% AII/TII of *A. lalesarensis*, 0.59% of *A. raddei* 32, 2.32% of *A. ebenoides* 36, 2.90% of *A. szovitsii* of Zonuz (Figs. 11, 15, 17 and 22). According to Nicklas and Ward (1994), non-oriented bivalents may be related to the 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 (Fig. 18) 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 unbalanced chromosome numbers (Mansuelli *et al.* 1995), such as aneuploids (Defani-Scoarize *et al.* 1995). The highest score of forward chromosomes expressed in 5.71% AI/TI cells of *A. submitis*, 6.54% AII/TII cells of *A. raddei* 32, 1.29% AI/TI cells of *A. raddei* 32, 4.30% of *A. ebenoides* 36, 1.75% of *A. ebenoides* of Mahabad and 4.29% of *A. szovitsii* of Jolfa (Fig. 10). Chromosome bridges, resulting from sticki-

ness were the highest score of chromosome bridges, expressed in 9.09% AI/TI cells of *A. ebenoides* of Bukan, 1.48% AII/TII cells of *A. raddei* 01, 1.04% AI/TI cells of *A. lalesarensis*, 1.29% cells of *A. raddei* 32, 0.87% cells of *A. raddei* 33, 2.15% AII/TII cells of *A. ebenoides* 36 and 1.16% of *A. ebenoides* 36 (Fig. 14). 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 reasons for chromosome stickiness in different plant species (Nirmala and Rao 1996).

The highest score of asynchronous nucleus expressed in 51.42% AI/TI of *A. submitis*, 13.54% AII/TII of *A. lalesarensis*, 9.09% of *A. ebenoides* of Bukan, 8.60% of *A. ebenoides* 36, 25.75% of *A. sovitsii* of Jolfa, 3.33% of *A. szovitsii* of Zonuz and 15.62% of *A. crassispinus* (Figs. 4 and 11). Micronucleus is another abnormality, which was found in 1.56% AI/TI of *A. lalesarensis*, 4.54% of *A. raddei* 32, 2.15% of *A. ebenoides* 36, 5.26% AII/TII of *A. ebenoides* of Mahabad, 0.49% of *A. submitis*, 3% of *A. raddei* 33, 2.32% of *A. ebenoides* 36 and 1.42% of *A. szovitssi* of Jolfa (Figs. 22 and 23). Chromosome elimination is a common phenomenon results from combination of two distinct genomes in hybrids. A kind of this phenomenon is micronuclei elimination which one or more micronuclei approach to microspore wall and form a kind of bud. Subsequently this bud separate from microspore, eliminate as a microcyte and give rise to small, sterile pollen grains (Baptists-Giacomoelli *et al.* 2000). The phenomenon of cytomixis consists of the migration of chromosome between meiocytes through cytoplasmic channels. 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 6.27% D/MI of *A. ebenoides* of Hamedan, 12.90% of *A. raddei* 32, 2.48% AI/TI of *A. ebenoides* of Mahabad of *A. lalesarensis*, 0.52% AII/TII of *A. submitis* and 0.82% of *A. szovitsii* of Jolfa 3.92% (Figs. 7 and 20). Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic channels originating 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-meioytic 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 the 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. Unequal polar is another abnormality that was found in 0.41% AII/TII of *A. szovitsii* of Zonuz (Fig. 15).

Discussion

Morphology

Morphological study showed a high level of intraspecific variation and resulted in seven groups. Populations szo30, szo35, ebe14, ebe32, ebe33, ebe36 and ebe22 were included in group 1 (Figure 25); populations rad10, rad33, rad32 and rad01 in group 2; populations sub09, sub60, sub08 and bre13 in group 3; population fuh12 in group 4; population cra82 in group 5; and population lal26 in group 6, and population rad11 in group 7 (Figure 26). The populations included in group 1 share some characters, such as the largest stipule and bracteole lengths. The populations of group 2 were well distinguished by their largest bracts. Populations with the largest plant and leaf and petiole lengths were included in group 3. Group 4 was separated from the other groups by having the largest keel, wing and standard lengths. Group 5 has taxa with white flowers and group 6 includes plants with the smallest length. Group 7, with only population rad11 was separated from group 2 (with different populations of *A. raddei* possess white hairs) by having black and white

Fig. 25. Distribution of *A. raddei* 32, 01 and 33 (1), *A. crassispinus* (2), *A. lalesarensis* (3), *A. submitis* (4), *A. ebenoides* (5), *A. szovitsii* of Zonuz (6), *A. szovitsii* (7), *A. ebenoides* 32 (8), *A. ebenoides* 36 and *A. ebenoides* 32 (8) in Iran.

Fig. 26. PCO analysis of different populations of *A. sect. Megalocystis* based on morphological characters (abbreviations are as listed in Table 1).

hairs (Fig. 26).

Cytogenetics

The results from cytogenetic study represented seven groups, populations rad33, szo35, ebe22, sub60, ebe36 and szo30 included in group 1; populations ebe33, rad10, ebe14 and rad11 in group 2;

Fig. 27. PCO analysis of different populations of *A. sect. Megalocystis* based on cytogenetic data (abbreviations are as listed in Table 1).

populations sub50, sub47 and bre13 in group 3; population fuh12 in group 4; population cra82 in group 5; population lal26 in group 6; and populations rad01, rad32 and ebe32 in group 7 (Fig. 27). The plants of group 1 showed some meiotic abnormalities, such as asynchronous nucleus, fragmented chromosomes, ring bivalents and bridges in AI/TI. Group 2 was separated because of the highest frequency of laggards in AI/TI and D/MI. The populations, which were included in group 3 represented the highest number of AI/TI cells. Group 4 was separated from the other groups because of the highest number of D/MI cells. Group 5, containing only population cra82, was distinguished by the highest frequency of AI/TI and tripolar cells. The population lal26, forming group 6, showed the highest frequencies of sticky chromosomes and cyomixis in AI/TI cells. All populations belonging to group 7 represented equal amounts of bridges, and forward and laggard chromosomes in AII/TII (Fig. 27). Results obtained from PCO analysis based on cytogenetic data showed inter-population variation as well as morphological characters that resulted in seven groups.

Acknowledgement

The field work in Iran was supported by grants from the Bu-Ali Sina University.

References

- Baptista-Giacomelli, F. R., Pagliarini, M. S., and Almeida, J. L. 2000. Multiple anaphase bridges on meiosis in Brazilian oats (*Avena sativa* L.). *Nucleus* **43**: 58–63.
- Briggs, D., and Walters, S. M. 1997. *Plant variation and evolution*, 3rd ed., Cambridge: Cambridge University Press.
- Defani-Scoarize, M. A., Pagliarini, S. M., and Aguiar, C. G. 1995. Evaluation of meiotic behavior in double-cross maize hybrids and their parents. *Maydica* **40**: 319–324.
- Falisticco, E., Tosti, T., and Falcinelli, M. 1995. Cyto-mixis in pollen mother cells of diploid *Dactylis* one of the origins of 2n gametes. *J. Hered.* **86**: 448–453.
- Ghaffari, S. M. 2006. Occurrence of diploid and polyploidy microspores in *Sorghum bicolor* (Poaceae) is the result of cyto-mixis. *Afr. J. Biotechnol.* **5**: 1450–1453.
- Guerra, M. 2008. Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenet. Genome Res.* **120**: 339–350.
- Jones, N., and Houben, A. 2003. B chromosomes in plant: escapees from the A chromosome genome? *Trends Plant Sci.* **8**:

- 417–423.
- Koduru, P. R. K., and Rao, M. K. 1981. Cytogenetics of synaptic mutants in higher plants. *Theor. Appl. Genet.* **59**: 197–214.
- Kovach, W. 1985–2002. Institute of Earth Studies University College of Wales ABERYSTWYTH (Shareware) MVSP Version 3.2 (1985–2002), Kovach Computing Services Available from: <http://www.kovcomp.com/MVPs/down2.html>.
- Lock, J. M., and Simpson, K. 1991. Legumes of West Asia a check-list. Royal Botanic Gardens Kew, Richmond.
- Maassoumi, A. A. 1987. Notes on the genus *Astragalus* in Iran I, cytotaxonomic studies on some species. *Iran J Bot* **3**: 117–128.
- Maassoumi, A. A. 1989. Notes on the genus *Astragalus* in Iran IV, cytotaxonomic studies on some species. *Iran J Bot* **4**: 153–163.
- Mansuelli, R. W., Mansuelli, R. W., Tanimoto, E. Y., Brown, C., and Comai, L. 1995. Irregular meiosis in a somatic hybrid between *Solanum bulbocastanum* and *S. tuberosum* detected by species-specific PCR markers and cytological analysis. *Theor. Appl. Genet.* **91**: 401–408.
- Nicklas, R. B., and Ward, S. C. 1994. Elements of error correction in mitosis: microtubule capture, release and tension. *J. Cell Biol.* **126**: 1241–1253.
- Nirmala, A., and Rao, P. N. 1996. Genetics of chromosome numerical mosaicism in higher plants. *Nucleus* **39**: 151–175.
- Mabberley, D. J. 1997. The plant book A portable dictionary of the vascular plants 2nd ed. Cambridge University Press, Cambridge.
- Pagliarini, M. S. 1990. Meiotic behavior and pollen fertility in *Aptenia cordifolia* (Aizoaceae). *Caryologia* **43**: 157–162.
- Pagliarini, M. S. 2000. Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. *Genet. Mol. Biol.* **23**: 997–1002.
- Ranjbar, M., and Karamian, R. 2003. Caraganeae, a new tribe with note on the genus *Chesneya* Lindl ex Ende (Fabaceae) from Flora of Iran. *Thaiszia Journal of Botany* **13**: 67–75.
- Ranjbar, M., Karamian, R., and Hadadi, A. 2009a. Biosystematic study of *Onobrychis vicifolia* Scop. and *Onobrychis altissima* Grossh. (Fabaceae) in Iran. *Iran J Bot* **15**: 85–95.
- Ranjbar, M., Hajmoradi, F., and Karamian, R. 2009b. Mitotic study of some species of *Onobrychis* sect. *Hymenobrychis* DC. in Iran. *Iranian Journal of Plant Biology* **1**(1–2): 47–54.
- Ranjbar, M., Karamian, R., and Bayat, S. 2010a. New chromosome counts in five species of *Oxytropis* (Fabaceae) in Iran. *Iran J Bot* **16**(1): 42–48.
- Ranjbar, M., Karamian, R., and Afsari, S. 2010b. Meiotic chromosome number and behaviour of *Onobrychis avajensis* (Fabaceae): A new species from western Iran. *Plant Ecol Evol* **143**: 170–175.
- Ranjbar, M., Karamian, R., and Hadadi, A. 2010c. Cytosystematics of three *Onobrychis* species (Fabaceae) in Iran. *Caryologia* **63**: 237–249.
- Ranjbar, M., Karamian, R., and Hajmoradi, F. 2010d. Chromosome number and meiotic behaviour of two populations of *Onobrychis chorassanica* Bunge (*O.* sect. *Hymenobrychis*) in Iran. *Journal of Cell and Molecular Research* **2**(1): 49–55.
- Ranjbar, M., Karamian, R., and Enayati Akmal, A. 2010e. Meiotic Behavior and Chromosome Number of 5 Species of the *Astragalus* sect. *Megalocystis* Bunge (Fabaceae) in Iran. *Cytologia* **75**(1): 49–58.
- Rieseberg, L. H., and Carney, S. E. 1998. Tansley Review Number 102. Plant hybridization. *New Phytol.* **140**: 599–624.
- Stebbins, G. L. 1971. Chromosomal evolution in higher plants. Edward Arnold, London.
- Soltis, P. S. and Soltis, D. E. 2009. The role of hybridization in plant speciation. *Annu Rev Plant Biol* **60**: 561–588.
- Utsunomiya, K. S., Bione, N. C. P., and Pagliarini, M. S. 2002. How many different kinds of meiotic abnormalities could be found in a unique endogamous maize plant? *Cytologia* **67**: 169–176.
- Yakovlev, G. P., Sytin, A. K., and Roskov, Yu. R. 1996. Legumes of Northern Eurasia a check-list. Royal Botanic Gardens, Kew, Richmond.