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Chromosome number reports in five *Onobrychis* species (*O.* sect. *Onobrychis*, Fabaceae) in Iran

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Abstract

In this study the original mitotic chromosome counts are presented for 5 *Onobrychis* species of *O.* sect. *Onobrychis* in Iran, $2n = 2x = 14$ for *O. persica*, $2n = 4x = 28$ for *O. viciifolia*, $2n = 4x = 28$ for *O. altissima*, $2n = 2x = 14$ for *O. shahpurensis* and $2n = 2x = 14$ for *O. sosnovskyi*. The basic chromosome numbers of all studied taxa are consistent with the proposed base number of $x = 7$. In addition, the meiotic chromosome number of $2n = 4x = 28$ for *O. viciifolia* and *O. altissima* and of $2n = 2x = 14$ for *O. sosnovskyi* and *O. persica* are reported here. This study is the first report on the chromosome counts of *O. persica* and *O. shahpurensis*. All studied taxa displayed regular bivalent pairing and chromosome segregation at meiosis. However, some abnormalities were observed in the taxa are discussed.

***Keywords*:** chromosome number, Fabaceae, meiotic behavior, mitosis, *Onobrychis*, Iran

Introduction

Onobrychis Miller comprises of about 170 species under 12 higher taxa mainly distributed in southwest Asia, the Mediterranean region, temperate Europe and Asia, a few of which are cultivated as fodder or as ornamentals (Lock and Simpson, 1991; Yakovlev et al., 1996; Mabberley, 1997). Boissier (1872) subdivided the genus *Onobrychis* into two sections, *Euonobrychis* Bunge and *Sisyrosema* Bunge, based on characters of indumentums and corolla. He accepted 24 species within these 2 sections. In 'Flora Iranica' (Rechinger, 1984) 54 species from Iran were treated under 8 sections, *Dendrobrychis*, *Lophobrychis*, *Onobrychis*, *Laxiflorae*, *Anthyllium*, *Afghanicae*, *Heliobrychis* and *Hymenobrychis*. The taxonomy of the genus continues to be subject of much confusion, mainly because of the different approaches to species delimitation, resulting in varying numbers of recognized species (Boissier, 1872; Sirjaev, 1925; Hedge, 1970; Ball, 1978; Duman and Vural, 1990; Aktoklu, 2001). Recently, some new taxa of the genus have been described from Iran (Ranjbar et al., 2004, 2007; Ranjbar, 2009; Ranjbar et al., 2009a, 2009b, 2010a, 2010b, 2010c, 2010d, 2010e, 2010f, 2011).

Most of the cytological studies in the genus have concentrated on the chromosome count (Baltisberger, 1991; Karshibaev, 1992; Slavivk et al., 1993), with little work focused on detailed karyological criteria for taxonomic purposes (Khatoon et al., 1991; Mesicek and Sojak, 1992). From these reports, it is evident that the chromosome count is known for just over a quarter of the species. Two basic chromosome numbers ($x = 7$ and $x = 8$) and 4 ploidy levels ($2n = 2x = 14$, $2n = 4x = 28$, $2n = 8x = 56$ and $2n = 2x = 16$, $2n = 4x = 32$) are present in the genus (Abou-el-Enain, 2002). The elucidation of the origins of species has been greatly aided in recent years by the ability to make comparisons between putative progenitor species and their derivatives at the molecular level (Crawford, 1990; Avise, 1994). We describe here mitotic chromosome number, and meiotic chromosome number and behavior of 5 *Onobrychis* species of *O.* sect. *Onobrychis* in Iran.

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Materials and Methods

For mitosis, 5 *Onobrychis* species, *O. persica*, *O. viciifolia*, *O. altissima*, *O. shahpurensis* and *O. sosnovskyi*, were collected from different locations in Iran (figure 1) and pods were collected from healthy plants. Voucher specimens were deposited at the Herbarium of the Bu-Ali Sina University (BASU), Hamedan, Iran. 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% colchicine for 3 h and fixed in 3:1 ethanol: glacial acetic acid for 24 h. Root tips were hydrolyzed for 6 min in 1M HCl at 60 °C, washed briefly in dd H_2O and stained in Feulgen's solution for 1-2 h. All permanent slides were made using Venetian turpentine (Wilson, 1945). The slides were observed under an Olympus BX-41 photomicroscope.

Also the chromosome number and meiotic behavior were analyzed in all above mentioned species. 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). Photographs of chromosomes were taken by Olympus BX-41 photomicroscopes at initial magnification of 1000X. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs.

Results

Mitotic chromosome number and ploidy level

Results from the present study showed that *O. viciifolia* and *O. altissima* are tetraploid with the base number of $2n = 4x = 28$ (figures 2A-2F), while *O. shahpurensis*, *O. persica* and *O. sosnovskyi* are diploid with the base number of $2n = 2x = 14$ (figures 2G-2T).

Onobrychis altissima Grossh. in Sc. Papers Applied Sect. Tiflis Bot. Gard. Pt. V. 141 (1929). Iran: East Azerbaijan, 10 km after Varzaghan, Mirzaali Kandi, 2010 m, Ranjbar and Hadadi 14209. Perennial herbs, stems erect or erect-ascending, 50-90 cm tall, corolla pink, 10-13 mm long, dark standard dark and wings short. It is closely related to *O. viciifolia* growing throughout Iran especially in the shape of leaf, stem indumentum, number of leaflets and flowers and also in the length of standard, keel and pod. *O. altissima* is tetraploid with the base number of $2n = 4x = 28$.

Onobrychis viciifolia Scop. Fl. Carniol., ed. 2. 2:76 (1772).
Iran: West Azerbaijan, Orumieh to Oshnavieh, after Sangar, 1650 m, Ranjbar and Hadadi 14214.

Perennial herbs, stems erect or erect-ascending, 50-90 cm tall, corolla pink, 10-13 mm long, standard dark and wings short. *O. viciifolia* is tetraploid with the base number of $2n = 4x = 28$.

Onobrychis sosnovskyi Grossh. in Sc. Papers Applied Sect. Tiflis Bot. Gard. Pt. V. 162 (1926). Iran: East Azerbaijan, Marand and Kharvanak, 1215-1725 m, Ranjbar and Hadadi 14208 and 14212. Perennial plant, (20) 40-60 cm height; stem erect, branched; corolla pink, 9-11 mm long. This species occurs in Turkey, Iran and Caucasus. It is the only long wing species in northwest Iran. This diploid species shows the basic chromosome number of $2n = 2x = 14$ (figures 2G-2J).

Onobrychis persica Sirj. and Rech. f. Repert. Spec. Nov. Regni Veg. 50: 257 (1941).
Iran: Zanjan, Zanjan to Gheidar, 1950 m, Ranjbar and Hadadi 14197. Perennial plant, 20-50 cm height; corolla pink, 8-9 mm long. It is one of the short wing species in the west of Iran that is diploid and shows the basic chromosome number of $2n = 2x = 14$ (figures 2O-2S).

Onobrychis shahpurensis Rech. f. Fl. Iranica [Rechinger] 157: 414 (1984).
Perennial plant, 25-40 cm tall; corolla white, 8.5-9.5 mm long. It is the only short wing species with white flowers in the Flora Iranica grows in west Iran. It is a diploid species with $2n = 2x = 14$ chromosome number (figures 2K-2N).

Meiotic behavior and abnormalities

Chromosome number and meiotic behavior were determined in 16 populations of 5 species. A summary of their cytological features is given in table 2, and the chromosomes are illustrated in figures 3–7. A total of 5130 diakinesis/metaphase I (D/MI), 2395 anaphase I/telophase I (AI/TI), 1350 methaphase II (MII) and 7263 anaphase II/telophase II (AII/MII) cells were analyzed. The meiotic irregularities observed in the *Onobrychis* species studied here included the occurrence of varied degrees of sticky chromosomes, formation of laggards and bridge in anaphase I & II, telophase I and II, cytomixis, cytoplasmic connections, desynapsis in metaphase I and asynchronous nuclei in metaphase II (figures 3-7).

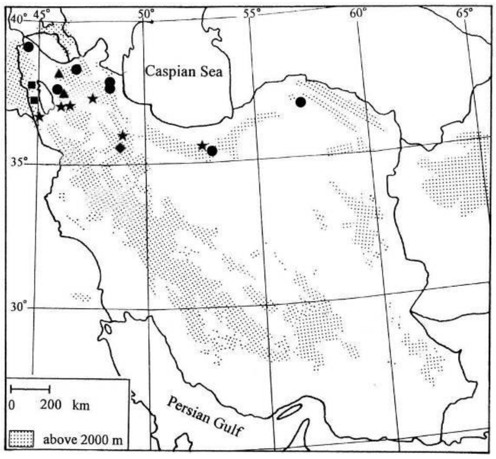

Figure 1. Distribution of *O. viciifolia* (★), *O. altissima* (●), *O. sosnovskyi* (▲), *O. persica* (♦) and *O. shahpurensis* (■) in Iran.

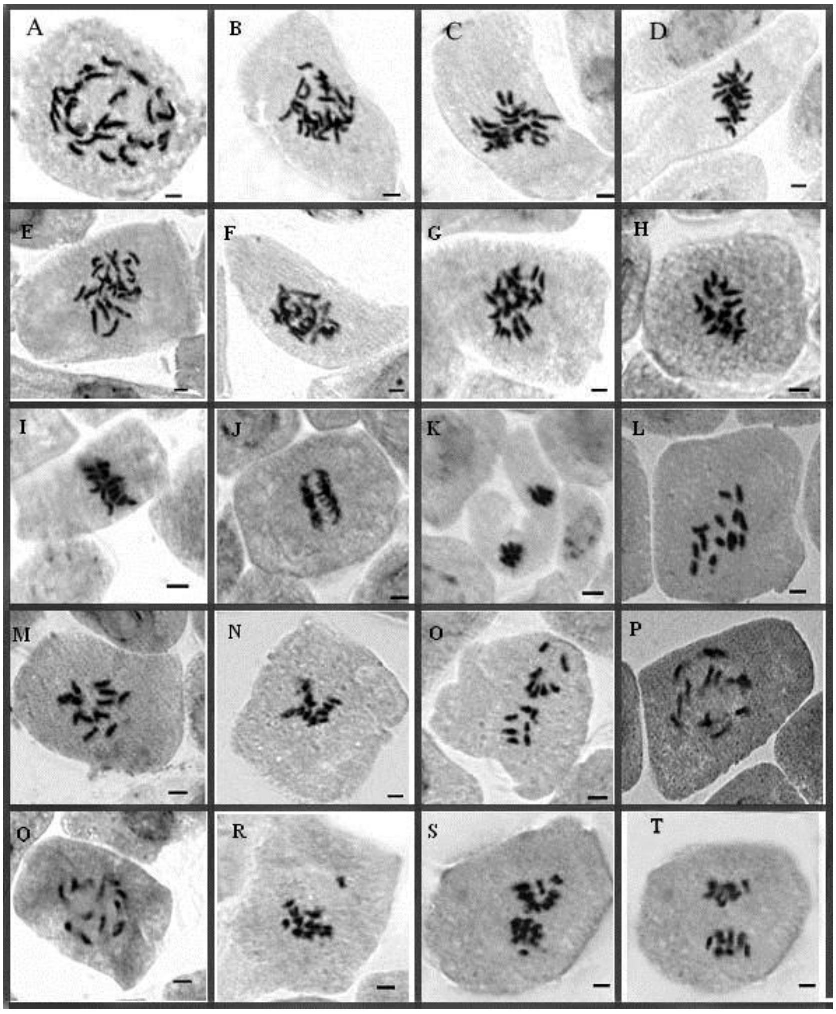

Figure 2. (A − T) Representative mitotic cells in different *Onobrychis* species: (A) Prophase in *O. viciifolia* (14214) (2*n* = 4*x* = 28), (B, C) Prometaphase in *O. viciifolia* (14214), (D) Metaphase in *O. altissima* (14209) (2*n* = 4*x* = 28), (E) Prophase in *O. altissima* (14209), (F) Prometaphase in *O. altissima* (14209), (G) Prometaphase in *O. sosnovskyi* (14208) (2*n* = 2*x* = 14), (H) Prometaphase in *O. sosnovskyi* (14212), (I) Metaphase in *O. sosnovskyi* (14212), (J) Anaphase in *O. sosnovskyi* (14208), (K) Telophase in *O. shahpurensis* (14213) (2*n* = 2*x* = 14). (L, M) Metaphase in *O. shahpurensis* (14213), (N) Anaphase in *O. shahpurensis* (14213), (O) Telophase in *O. persica* (14197) (2*n* = 2*x* = 14), (P) Prophase in *O. persica* (14197), (Q) Prometaphase in *O. persica* (14197), (R) Metaphase in *O. persica* (14197), (S, T) Anaphase in *O. persica* (14197). Scale bars = 3 µm.

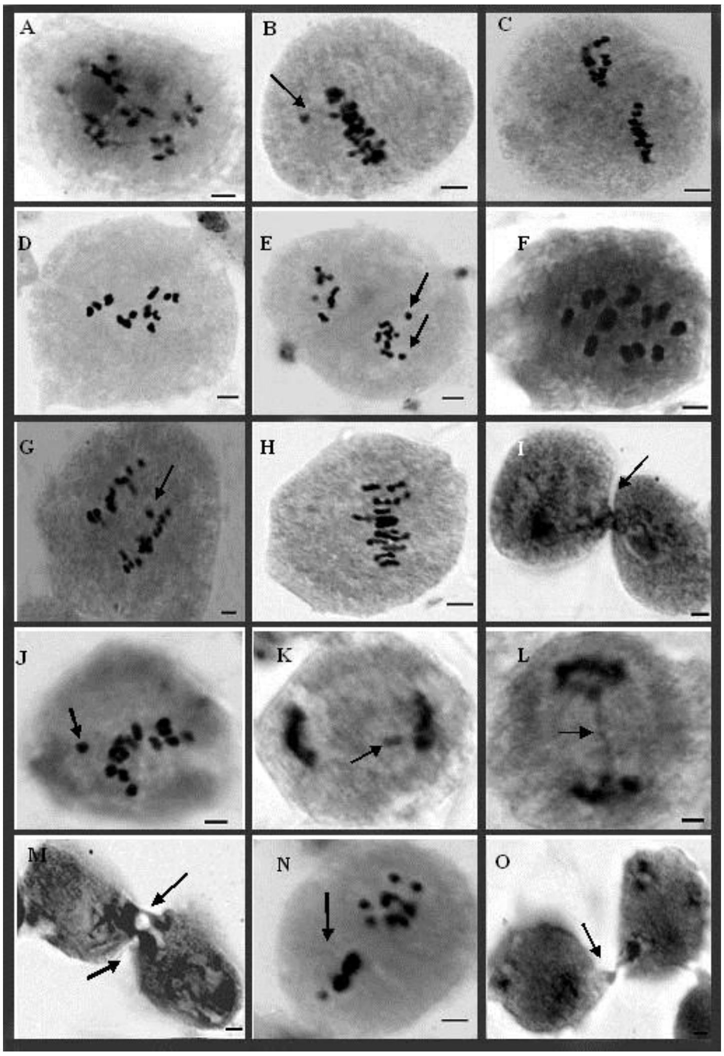

Figure 3. (A − O) Representative meiotic cells in *O. altissima* ($2n = 4x = 28$): (A) Pachytene (14202), (B) Metaphase I showing 14 bivalents and 1 B-chromosome (14202), (C) Metaphase II (14209), (D) Early metaphase I (14204), (E) Telophase I with laggard chromosomes (14204), (F) Early metaphase I (14192), (G) Anaphase I (14192), (H) Early metaphase I (14201), (I) Cytomixis (14537), (J) Fragmented chromosomes in metaphase I (14537), (K) Laggard chromosome in anaphase I (14537), (L) Bridge in telophase I (14537), (M) Cytomixis (14556), (N) Asynchronous nucleus in metaphase II (14556), (O) Cytomixis in telophase II (14556). Scale bars = 3 µm.

Anaphase and telophase laggard chromosomes

In this study only Kaleibar population (14204) of *O. altissima* and two populations of *O. viciifolia* showed formation of laggard chromosomes from anaphase I to telophase II (figures 3E, 3K, 7B, 7E and 7F), while other populations studied here did not form any laggard chromosomes. The highest percentage of AI/TI cells with laggard chromosomes occurred in Taham population (14198) of *O. viciifolia* (table 2).

Chromosome stickiness

Chromosome bridges resulting from stickiness were observed in anaphase I and II as well as telophase I and II stages in *O. viciifolia*, *O. altissima*, *O. shahpurensis* and *O. sosnovskyi* (figures 3L, 4F, 4G, 5F, 6B, 6D, 6F, 6I and table 2).

Desynapsis

A complete desynapsis was observed only in Taham population (14198) of *O. viciifolia* (figure 7H and table 2) and Gheidar population (14197) of *O. persica* (figure 4J and table 2).

Cytomixis

The chromatin/chromosome migration occurred in different directions from early prophase to telophase in some *Onobrychis* species and populations studied (figures 3I, 3M, 3O, 5K, 6B, 7K and table 2).

B-chromosomes

B-chromosomes or accessory chromosomes only was observed (10.64%) in Ardebil population (14202) of *O. altissima* (figure 3B and table 2).

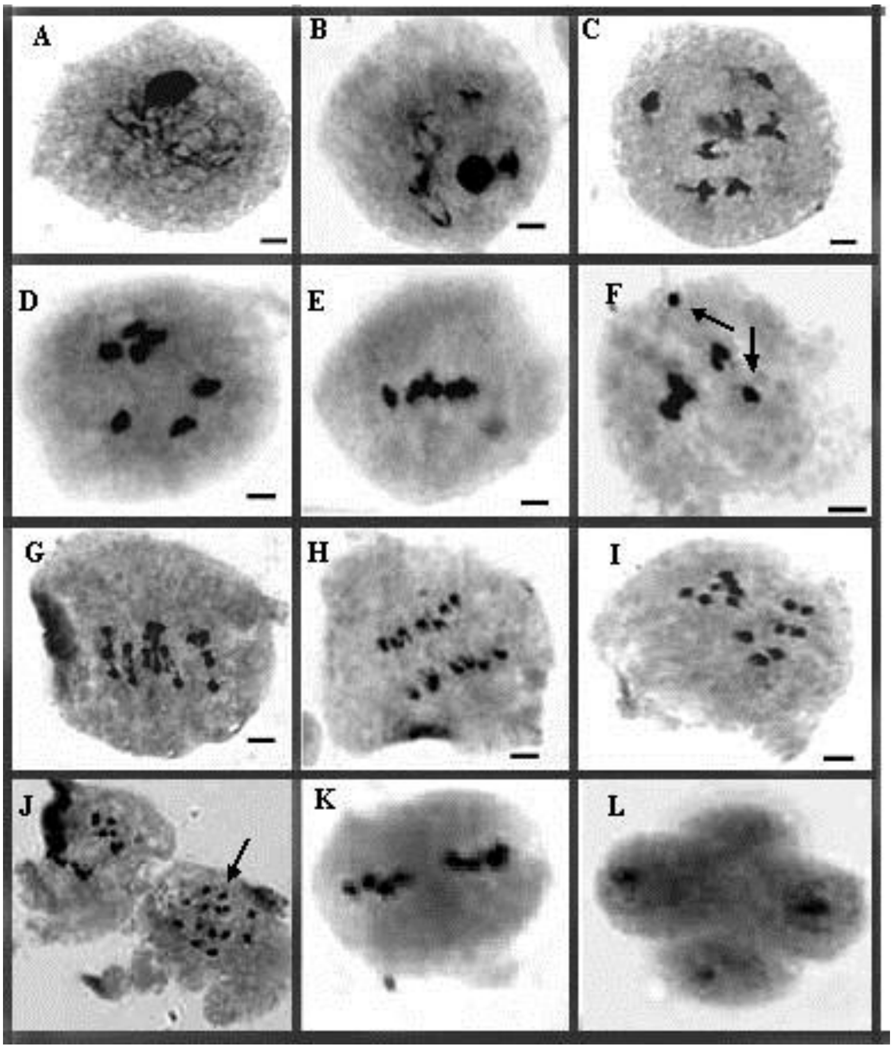

Figure 4. (A − L) Representative meiotic cells in *O. persica* (14197) ($2n = 2x = 14$): (A) Zygotene, (B) Pachytene, (C) Late pachytene, (D) Diakinesis showing 7 bivalents, (E) Metaphase I, (F) Metaphase I with fragmented chromosomes, (G) Early anaphase I, (H) Anaphase I, (I) Telophase I, (J) Anaphase I and desynapsis in metaphase I and 14 monovalents, (K) Metaphase II, (L) Telophase II. Scale bars = 3 µm.

Discussion

Mitotic chromosome number and ploidy level

According to Abou-el-Enain (2002), two basic chromosome numbers ($x = 7$ and $x =8$) and four ploidy levels ($2n = 2x = 14$, $2n = 4x = 28$, $2n = 8x =56$, $2n = 2x = 16$ and $2n = 4x = 32$) are present in the genus *Onobrychis*. Results from the present study showed that *O. viciifolia* and *O. altissima* are tetraploid with the base number of $2n = 4x = 28$, while *O. shahpurensis, O. persica* and *O. sosnovskyi* are diploid with the base number of $2n = 2x = 14$. *O. altissima* and *O. viciifolia* behave as monocarpic perennials in their natural habitats. The comparative biology of *O. viciifolia* and *O. altissima* has led to the hypothesis that these two species have a progenitor-derivative relationship with the former species having differentiated from the latter. However, *O. viciifolia* differs from it by having small teeth on the crest of pod and wings shorter than 3 mm. One ploidy level ($2n =4x = 28$) for *O. viciifolia*, and two ploidy levels ($2n = 2x = 14$ and $2n = 4x = 28$) for *O. altissima* have been perviously reported (Takhtajan, 1990).

Meiotic behavior and abnormalities

The meiotic irregularities observed in the studied *Onobrychis* species showed variation and included the occurrence of varied degrees of sticky chromosomes, formation of laggards and bridge in anaphase I and II, telophase I and II, cytomixis, cytoplasmic connections, desynapsis in metaphase I and asynchronous nuclei in metaphase II. Such irregularities have been also reported previously for *O. viciifolia* and *O. altissima* of this section and also in *O. chorassanica* of *O.* sect. *Hymenobrychis* (Ranjbar et al., 2009a, 2010b, 2010c, 2010d, 2011).

Anaphase and telophase laggard chromosomes

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 unbalanced chromosome numbers (Mansuelli et al., 1995). Non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers (Nicklas and Ward, 1994). It has been suggested that infertility in polyploids is not solely due to the production of aneuploid gametes formed by improper segregation of chromosomes during anaphase/telophase stages, the genetic factors may also bring about pollen sterility as evidenced in different tetraploid species (Hazarika and Rees, 1967; Pagliarini, 1990, 2000; Baptista-Giacomelli et al., 2000).

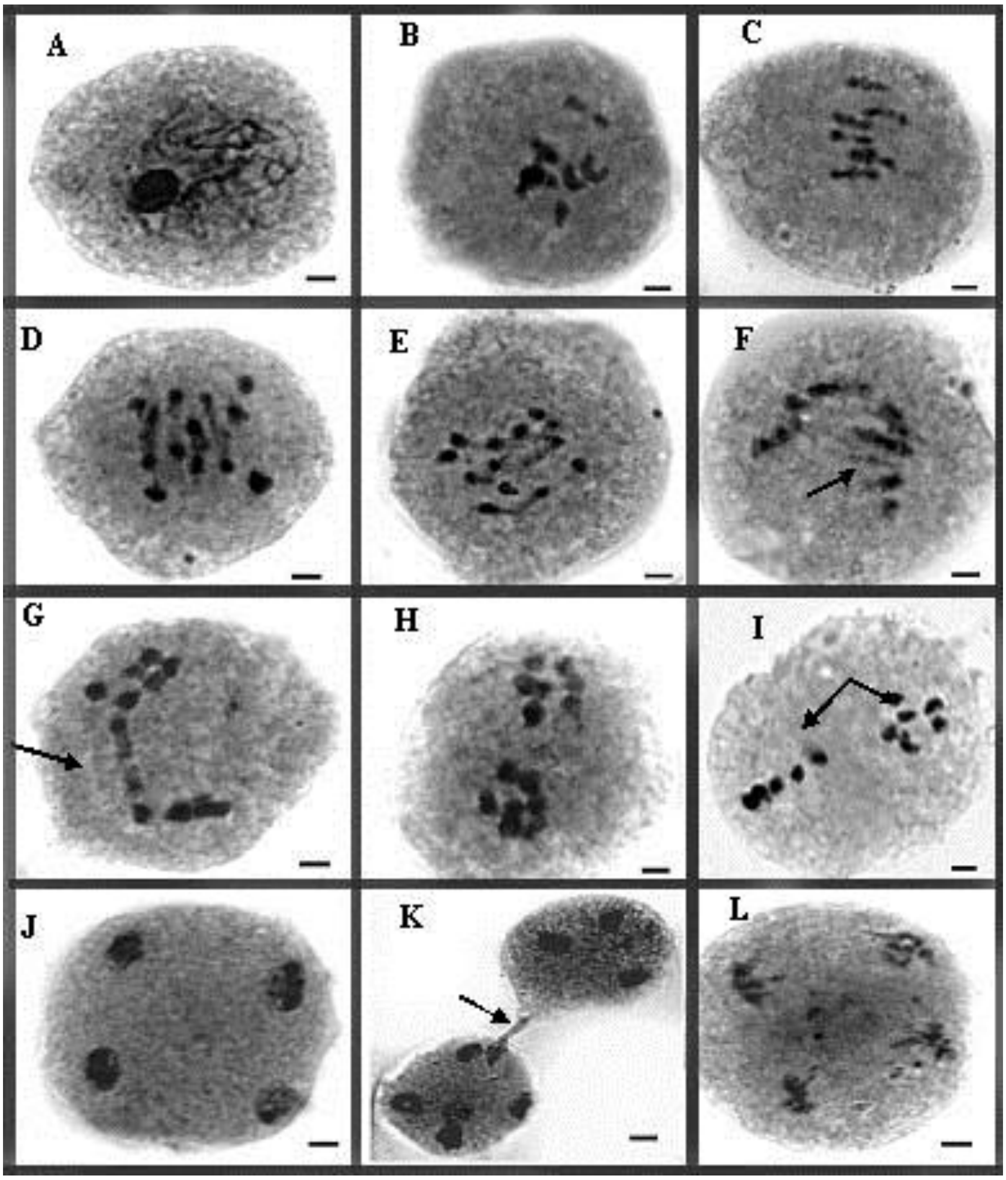

Figure 5. (A − K) Representative meiotic cells in *O. shahpurensis* (19182) ($2n = 2x = 14$): (A) Zygotene, (B) Diakinesis showing 7 bivalents, (C) Metaphase I, (D) Early anaphase I, (E) Anaphase I, (F) Bridge in anaphase I, (G) Bridge in late anaphase I, (H) Telophase I, (I) Anaphase II, (J) Telophase II, (K) Cytomixis in telophase II and pentapolar cells. Scale bars = 3 µm.

Table 1. Taxa and acronyms examined in this study.

Species	Voucher No.	Locality	Alt. (m)	Collector name
O. viciifolia	BASU 14214	West Azerbaijan: Orumieh to Oshnavieh, after Sangar	1650	Ranjbar & Hadadi
O. viciifolia	BASU 14198	Zanjan: Taham	2000	Ranjbar & Hadadi
O. viciifolia	BASU 14529	East Azerbaijan: Hashtrood to Maraghe, 70 km to Maraghe	1529	Ranjbar & Hadadi
O. viciifolia	BASU 14544	Tehran: Polur to Firuzkuh, 5 km after railway	1571	Ranjbar & Hadadi
O. viciifolia	BASU 14527	East Azerbaijan: Hashtrood to Maragheh, 80 km to Maragheh	1617	Ranjbar & Hadadi
O. shahpurensis	BASU 14213	West Azerbaijan: Salmas to Orumieh, Ghooshchi neck	1870	Ranjbar & Hadadi
O. shahpurensis	BASU 19182	West Azerbaijan: Salmas to Kozerash, Kozerash	2225	Ranjbar & Hadadi
O. sosnovskyi	BASU 14208	East Azerbaijan: Kharvanak	1215	Ranjbar & Hadadi
O. sosnovskyi	BASU 14212	East Azerbaijan: 16 km to Marand	1725	Ranjbar & Hadadi
O. altissima	BASU 14209	East Azerbaijan: 10 km after Varzaghan, Mirzaali Kandi	2010	Ranjbar & Hadadi
O. altissima	BASU 14202	Ardebil: Ardebil	1540	Ranjbar & Hadadi
O. altissima	BASU 14201	Ardebil: Saein neck	1770	Ranjbar & Hadadi
O. altissima	BASU 14204	East Azerbaijan: Kaleibar	1650	Ranjbar & Hadadi
O. altissima	BASU 14537	East Azerbaijan: Khoy, Ghotor road, 2 km to Darekhan	1357	Ranjbar & Hadadi
O. altissima	BASU 14556	Khorasan: Bojnourd to Esfarayen, Assadly neck	1800	Ranjbar & Hadadi
O. persica	BASU 14197	Zanjan: Gheidar	1950	Ranjbar & Hadadi

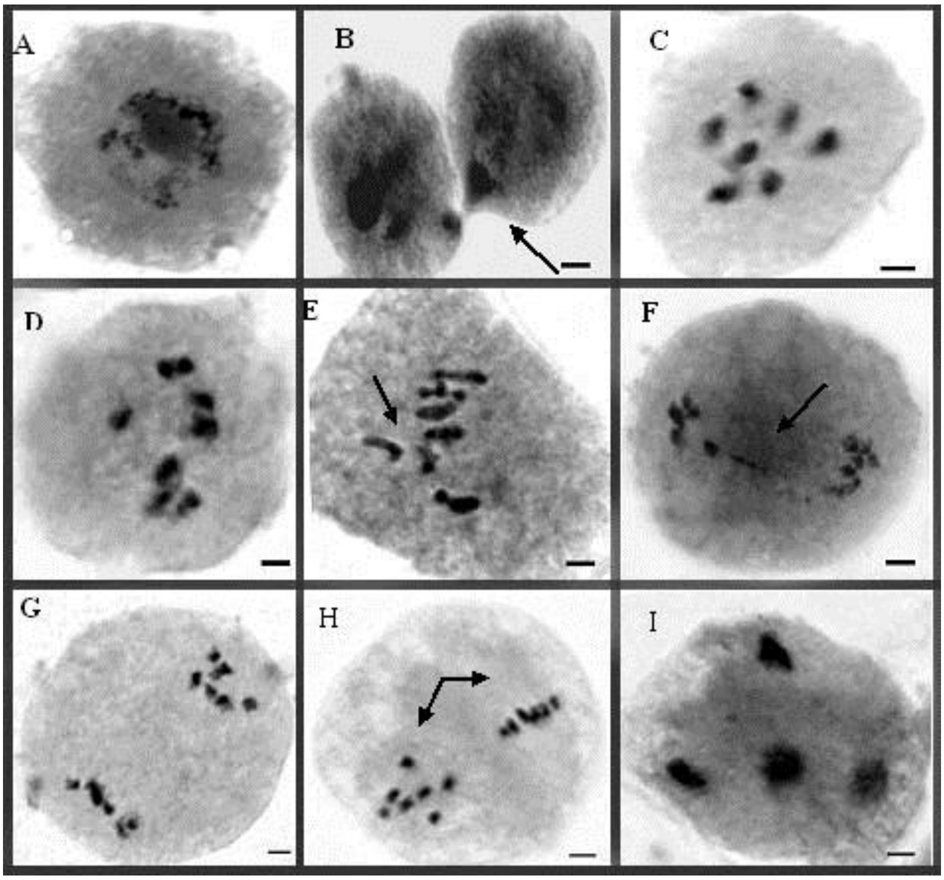

Figure 6. (A − I) Representative meiotic cells in *O. sosnovskyi* ($2n = 24x = 14$): (A) Zygotene (14208), (B) Cytomixis in zygotene (14208), (C) Diakinesis (14208), (D) Diakinesis showing 7 bivalents and (14212), (E) Metaphase I with fragmented chromosome (14212), (F) Bridge in anaphase I (14212), (G) Telophase I (14212), (H) Asynchronous nucleus in metaphase II, (I) Telophase II. Scale bars = 3 µm.

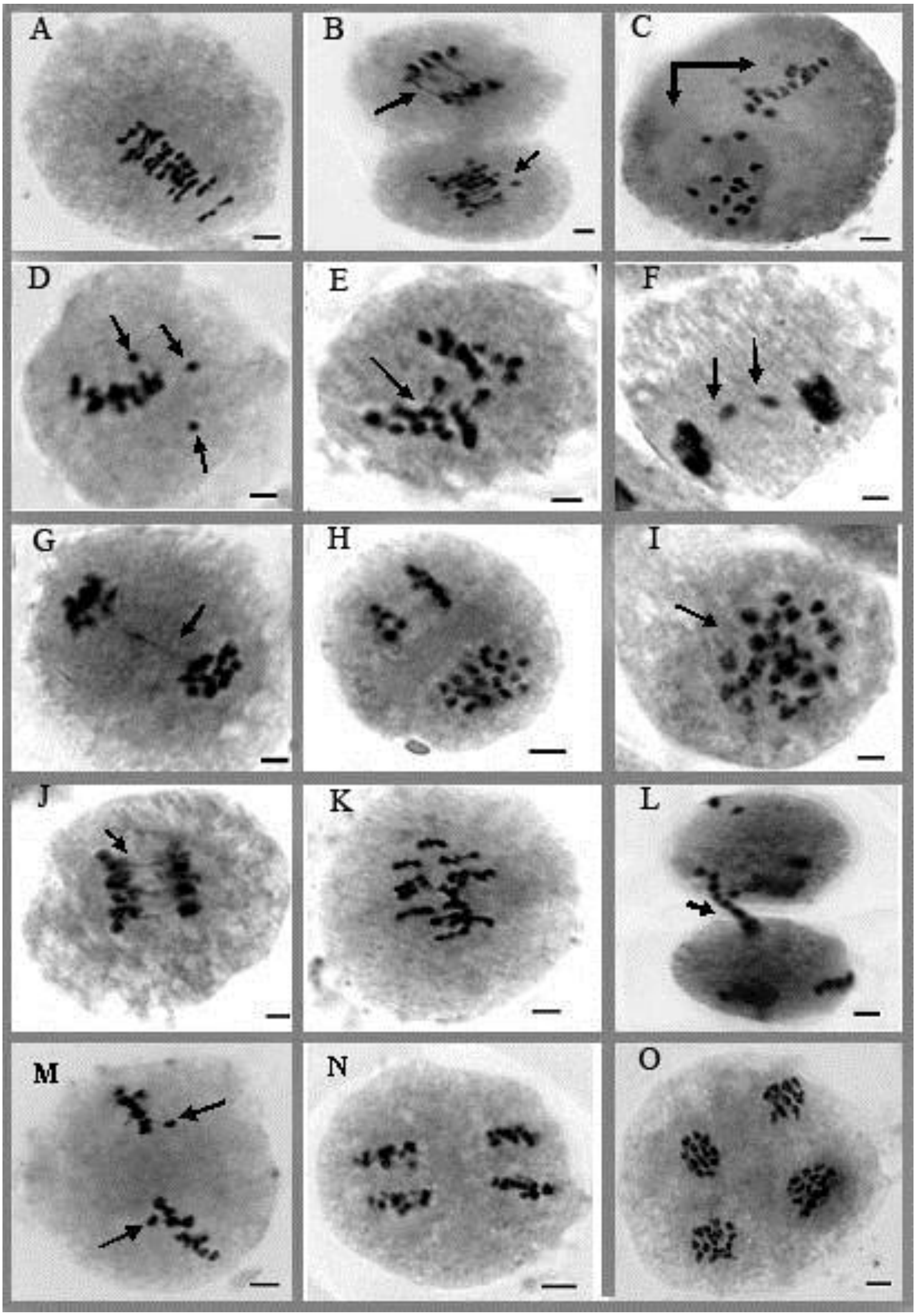

Figure 7. (A − O) Representative meiotic cells in *O. viciifolia* ($2n = 4x = 28$): (A) Early metaphase I (14198), (B) Bridge and laggard anaphase I (14198), (C) Asynchronous nucleus in metaphase I (14198), (D) Fragmented chromosomes metaphase I (14529), (E) Laggard chromosomes in anaphase I (14529), (F) Laggard chromosomes in telophase I (14529), (G) Bridge in telophase I (14529), (H) Asynchronous nucleus in metaphase II (14544), (I) Desynapsis (14544), (J) Bridge in anaphase I (14544), (K) Metaphase I (14527), (L) Cytomixis and laggard chromosomes in metaphase II (14527), (M) Fragmented chromosome in metaphase I (14527), (N) Anaphase II, (O) Telophase II (14527). Scale bars = 3 µm.

Table 2. Meiotic beheavior in different populations of *O viciifolia*, *O. altissima*, *O. shahpurensis*, *O. sosnovskyi*, and *O. persica*.

Taxa	sos 14208	sos 14212	shp 14182	alt 14202	alt 14201	alt 14204	alt 14209	Alt 14537	alt 14556	alt 14527	vic 14554	vic 14529	vic 14214	vic 14199	vic 14198	per 14197
% Z/P	42.3	26.24	52.7	9.88	17.25	33.14	54.72	66.95	30.37	52.15	70.83	3.27	31.85	9.07	66.9	54.49
% Cytomixis/Cytoplasmic connection	3.33	0	0	0	3.1	0	1.19	5.01	4.87	5.17	2.45	4.52	13.78	0	5.25	2.96
% D/M I	30.12	20.28	18.9	25.97	28.18	13.31	1.31	19.52	4.9	10.45	18.4	14.73	18.12	4.47	12.91	21.03
% Cytomixis/Cytoplasmic connection	3.62	0	0	6.84	12.96	0.65	0	7.48	9.46	7.09	8.44	11.76	4.52	0	4.77	3.77
% Fragmented / Forward chromosome	2.12	0	0.81	0	0	0	0	2.26	2.36	0	0.67	9.47	2.5	0	0	3.94
	0	0	0	10.64	0	0	0	0	0	0	0	0	0	0	0	0
0%0 A I/T I	3.16	9.16	6.95	9.2	22.07	2.91	8.3	8.41	10.51	3.71	3.91	3.61	11.19	7.03	8.4	3.95
% Cytomixis/Cytoplasmic connection	0	0	0	0	7.31	0	0	10.52	17.67	0.09	0	13.33	20.6	12.7	6.21	1.6
% Laggard chromosome	2.12	0	43	0	0	7.69	0	3.67	2.67	5.45	15.82	10.11	0.26	5.45	17.51	2.12
% DS	1.1	0	0	0	0	0	0	0	0	0	1.12	0	0	0	0	1.06
% P II	5.45	0	7.89	17.88	1.28	6.47	10.88	11.5	9.32	10.79	0.62	6.98	10.56	14.7	1.8	4.59
% Cytomixis/Cytoplasmic connection	26.2	0	0.83	5.43	8.33	0	1.6	15.76	20.56	11.8	0	17.93	19.68	8.69	21.05	29.3
% M II	1.58	2.22	0.66	2.61	4.39	2.69	5.28	3.8	13.55	5.53	0.06	0.62	5.01	3.7	0.47	1.69
% Cytomixis	0	0	0	7.31	0	0	0	3.48	7.92	3.65	0	15.38	16.8	7.6	0	2.04
% Laggard chromosome	0	0	0	0	0	0.2	0	0	1.97	7.31	0	0	16	0	0	0
% Asynchronous nucleus	35.7	4	0	26.8	17.14	0	9.09	2.32	10.06	9.75	0	0	0	0	0.6	14.28
% A II/T II	17.84	49.97	13.5	34.43	26.79	26.79	19.49	8.277	26.1	17.34	6.21	9.58	24.13	61.38	9.49	28.83
% Cytomixis/Cytoplasmic connection	6.32	2.07	0	1.6	10.8	10.8	3.18	12.29	8.56	12.48	0	1.25	18.19	2.08	0	5.7
% Laggard chromosome	0.84	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Ploidy	2	2	2	4	4	4	4	4	4	4	4	4	4	4	4	2

Abbreviations: Z/P = Zygotene/Pachytene; D/MI = Diakinesis/Metaphase I; *n* = Chromosome number; AI/TI = Anaphase I/Telophase I; PII = Prophase II; MII = Metaphase II; AII/TII = Anaphase II/Telophase II.

Chromosome stickiness

Sticky chromosomes were observed from early stages of prophase till the final stages of meiosis in some populations studied. The thickness of bridges observed and the number of chromosomes involved in their formation varied among different meiocytes and the species studied. Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000).

Desynapsis

Desynapsis occurs either due to the action of recessive ds genes in a homozygous situation or early chiasma terminalisation which may lead to the formation of meiocytes with double normal chromosome number. In several cases such univalents may have difficulty during anaphase I movement and become lagged therefore producing aneuploid gametes causing reduction ion pollen fertility of plants. However, they may skip the first anaphase and form restitution nucleus resulting in the formation of unreduced gametes as reported in some other species (Veilleux, 1985; Sheidai et al., 2006, 2007).

Cytomixis

The chromatin/chromosome migration occurred in different directions from early prophase to telophase in the *Onobrychis* species and populations studied (figures 3I, 3M, 3O, 5K, 6B, 7K and table 2).

B-chromosomes

B-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, although they could arrange themselves along with the A-chromosomes on the equatorial plane of the spindle and move to the poles during anaphase. In some cases they occurred as laggard chromosomes. The significance of B-chromosomes is to be found in their widespread occurrence in hundreds of flowering plants, and also in gymnosperms and in some lower forms such as ferns, bryophytes and fungi (Jones and Rees, 1982).

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