Plant Ecology and Evolution 143 (2): 170–175, 2010
doi:10.5091/plecevo.2010.431

REGULAR PAPER

Meiotic chromosome number and behaviour of *Onobrychis avajensis* (Fabaceae): a new species from western Iran

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Background and aims – The present study is focused on the cytogenetic and morphological criteria allowing to distinguish a new taxon from *Onobrychis* sect. *Heliobrychis*. This section is the largest section in *O.* subg. *Sisyrosema* represented with 21 species in Iran. The new species belongs to the *O.* subsect. *Boissierianae* characterized by perennial plants with well-developed stems and *O. andalanica* group with uniformely yellow corolla.
Methods – The morphological features and meiotic chromosome number and behaviour were studied in *O. avajensis* Ranjbar.
Key results – The novelty *Onobrychis avajensis* Ranjbar, endemic to Iran, is described and illustrated from two collections from a single locality between Avaj and Abgram in Qazvin Province in the west Zagros. It is closely related to *O. andalanica* Bornm. but differs from it in a few morphological characters. In addition, meiotic chromosome number and behaviour were studied in *O. avajensis*. This report is the first cytogenetic analysis of this taxon. *O. avajensis* is a diploid plant and possesses $2n = 2x = 16$ chromosomes, consistent with the proposed base number of $x = 8$. The general meiotic behaviour of the species was regular, with bivalent pairing and normal chromosome segregation at meiosis. Meiotic abnormalities were observed included a varying degree of sticky chromosomes with laggards, precocious division of centromeres in metaphase I, bridges in anaphase I and multipolar cells in telophase II.
Conclusion – Morphological features support the separation of *Onobrychis avajensis* Ranjbar as a new species.

Key words – chromosome number, Fabaceae, *Heliobrychis*, Iran, meiosis, new species, *Onobrychis*.

INTRODUCTION

The genus *Onobrychis* (Fabaceae, Hedysareae) comprises nearly 130 species and is mainly distributed in the north temperate regions, but centres of diversity are found in the eastern Mediterranean area and western Asia. *Onobrychis* includes annual or perennial, mostly caulescent herbs (rarely spiny shrubs), which have an indumentum with simple hairs or are glabrous. A few taxa of the genus such as *O. viciifolia* and *O. altissima* are cultivated as fodder or for ornamental value (Lock & Simpson 1991, Yakovlev et al. 1996, Mabberley 1997, Ranjbar et al. 2009b). The taxonomy of the genus is still confused, mainly because of the different approaches to species delimitation, resulting in varying numbers of recognized species (Boissier 1872, Sirjaev 1925, Hedge 1970, Ball 1978, Rechinger 1984, Duman & Vural 1990, Aktoklu 2001). Most of the cytological studies in the genus have focused on chromosome counting (Baltisberger 1991, Karshibaev 1992, Slavivk et al. 1993), with few works focusing on detailed karyological and meiotic behaviour criteria for taxonomic purposes (Khatoon et al. 1991, Mesicek & Sojak 1992). From these reports, it is evident that the chromosome number is known for just over a quarter of the species. Two basic chromosome numbers ($x = 7$ and $x = 8$) and four 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). Recently several new taxa have been described in *Onobrychis* and *Hedysarum*, both of the tribe Hedysareae, from Iran (Ranjbar et al. 2004, 2006, 2007a, 2007b, Ranjbar 2009, Ranjbar et al. 2009a, 2009b, 2010a, 2010b).

The present study is focused on the cytogenetic and morphological criteria allowing to distinguish a new taxon from *Onobrychis* sect. *Heliobrychis*. This section is the largest section in *O.* subg. *Sisyrosema* represented with 21 species in Iran. The new species belongs to the *O.* subsect. *Boissierianae* (characterized by perennial plants with well-developed stems) and *O. andalanica* group (characterized by a uniformely yellow corolla). Investigations on living material and herbarium specimens, suggest that its populations,

exclusive to the Avaj submountains, are especially close to *O. andalanica*. However, several differences in morphology allow us to treat it as a new species.

MATERIALS AND METHODS

Morphology

This study is mainly based on the herbarium material and the field observations during excursions in western, northwestern and central Iran. Plants from different populations were collected and archived using conventional herbarium methods; vouchers have been preserved in BASU. Also several other sheets have been examined for each species from the following herbaria: W, WU, TARI, FUMH, BASU, Herbarium of Isfahan University, Herbarium of Research Centres of Natural Resources and Animal Affairs of Mashhad, Isfahan, Kashan, Tabriz, Semnan, Shiraz, Kerman and Zahedan.

Cytogenetics

For our cytogenetic study, fifteen flower buds from at least five plants were fixed in modified Carnoy's solution in ethyl alcohol (96%), chloroform and propionic acid (6:3:2) for 24 h at room temperature and then stored in 70% ethyl alcohol at 4°C until used (Fukui & Nakayama 1996). Anthers were squashed and stained with 2% (w/v) acetocarmine. All slides were made permanent by the Venetian turpentine (Wilson 1945). Photographs of chromosomes were taken on an Olympus BX-41 photomicroscope at an initial magnification of 1000 ×. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs.

RESULTS AND DISCUSSION

Morphology

Onobrychis avajensis Ranjbar, **sp. nov.**

Affinis *O. andalanica* Bornm. sed ab illa differt plantis ad 35 cm (versus ad 70 in *O. andalanica*) cm altis, pedunculis 4–12 (vs. c. 25 in *O. andalanica*) cm longis, foliis ad 10, petiolo 1–4(–8) (vs. ad 15 cm longis, petiolo 10–15 cm longo in *O. andalanica*), ellipticis ad lanceolatis (vs. ad ovatis in *O. andalanica*), foliolis 17–25 × 5–7 (vs. 30–40 × 15–20 in *O. andalanica*) mm, bracteis 3–3.5 (vs. 4–5 in *O. andalanica*) mm longis, calyce 6–9 (vs. c. 9 in *O. andalanica*) mm longo, vexillum 11.5–13.5 (vs. 22–23 in *O. andalanica*) mm longum, alis 4.5–5 (vs. c. 11 in *O. andalanica*) mm longis, carina 7.5–9 (vs. c. 20 in *O. andalanica*) mm longa, legumen setis purpureis (vs. albis in *O. andalanica*). – Type: Iran, W Iran, Qazvin Province, 10–15 km from Avaj to Abgarm, alt. 1500 m, 18 May 2007, *Ranjbar* 8271 (holo-: BASU; iso-: TARI).

Perennial plant, erect to ascending, with thick woody rootstock, up to 35 cm tall, branched at the base; hairs white appressed to subappressed on vegetative parts. Stipules thinly free, herbaceous to chartaceous, 6–7.5 mm long, 2–3 mm wide, triangular, acuminate, densely covered by appressed silvery-pilose hairs. Leaves: petiole 1–4(–8) cm long; blades 6–17 cm long; rachides remote, slender to thickened, straight or curved-ascending, densely covered by appressed hairs, 0.5–1.5 mm long; petiole 1–4(–8) cm long, leaves with 3–4 pairs of leaflets, 17–25 mm long, 5–7 mm wide, elliptic to lanceolate or narrowly rhombic-ovate, rounded at base, rarely truncate, acute to obtuse at apex, rather densely covered by hairs on both sides, greyish-green or upper side loosely covered by hairs. Inflorescence narrow, oblong before anthesis, many-flowered, elongating in fruit; 4–12 cm long. bracts papery, yellowish, 2–3.5 mm long, 1–1.5 mm wide, oblong-lanceolate, acute to acuminate, densely covered by appressed hairs. Flowers: pedicel 1.5–2 mm long; bracteoles narrowly linear, 1–1.5 mm long; calyx greenish, 6–9 mm long, densely covered by pubescent or appressed hairs, 0.5 to 1 mm long; teeth 3–6 mm long, linear or subulate; corolla greenish-cream, thinly brown to yellowish when drying; standard 11–15 × 10.5–13 mm; wings and keel shorter than standard; limb 2–3 mm long, 1.5–2 mm wide, oblong, acuminate at tip, claw filiform, c. 2 mm long; keel longer than wings, 7.5–9 mm long; limb 4–5 mm long, 5–6 mm wide; claw 3–4 mm long; filaments 11–13 mm long, the free portion 2–4 mm long. Pod with a stipe up to 4 mm long, semiorbicular, spreading to pendent 10–12 mm long, 2.5–3 mm at thickest part, 7–8 mm wide, densely pubescent and foveolate on disc, covered by purple setae, 3.5–4 mm long. Table 1, figs 1 & 2 in comparison with *O. andalanica* Bornm.

Distribution and phytogeography – The type material of *O. andalanica* was available to us in Vienna. *O. avajensis* is found between Avaj and Abgram, near Hamedan in Qazvin Province in western Iran (35°76'04"N 49°28'27"E). It is most closely related to *O. andalanica*, having few leaves, and comparable shape, structure and colour of flowers and pods. These features assign the two to *O.* sect. *Heliobrychis*. However, *O. avajensis* differs from *O. andalanica* mainly by having shorter and narrower leaflets, by the colour of the setae on pod discs, and by its shorter keel, wing and standard. A morphological comparison between the two species is presented in table 1.

Table 1 – Diagnostic morphological characters of *Onobrychis andalanica* and *O. avajensis*.

	O. andalanica	***O. avajensis***
Plant height	up to 70 cm	up to 35 cm
Leaf length	up to 15 cm	up to 10 cm
Petiole length	10–15 cm	1–4(8) cm
Leaflet shape	nearly ovate	elliptic to lanceolate
Leaflet size	30–40 × 15–20 mm	17–25 × 5–7 mm
Peduncle length	c. 25 cm	4–12 cm
Bract length	4–5 mm	3–3.5 mm
Standard length	22–23 mm	11.5–13.5 mm
Keel length	c. 20 mm	7.5–9 mm
Wing length	c. 11 mm	4.5–5 mm
Colour of setae on pod disc	white	purple

Figure 1 – *Onobrychis avajensis*: A, habit with details of flower and pod; B, calyx; C, standard; D, keel; E, wings; F, androecium; G, pistil; H, pod. From *Ranjbar* 8271 (BASU). Scale bar: A = 2 cm and B–H = 1 cm. Drawn by M. Hezarkhani.

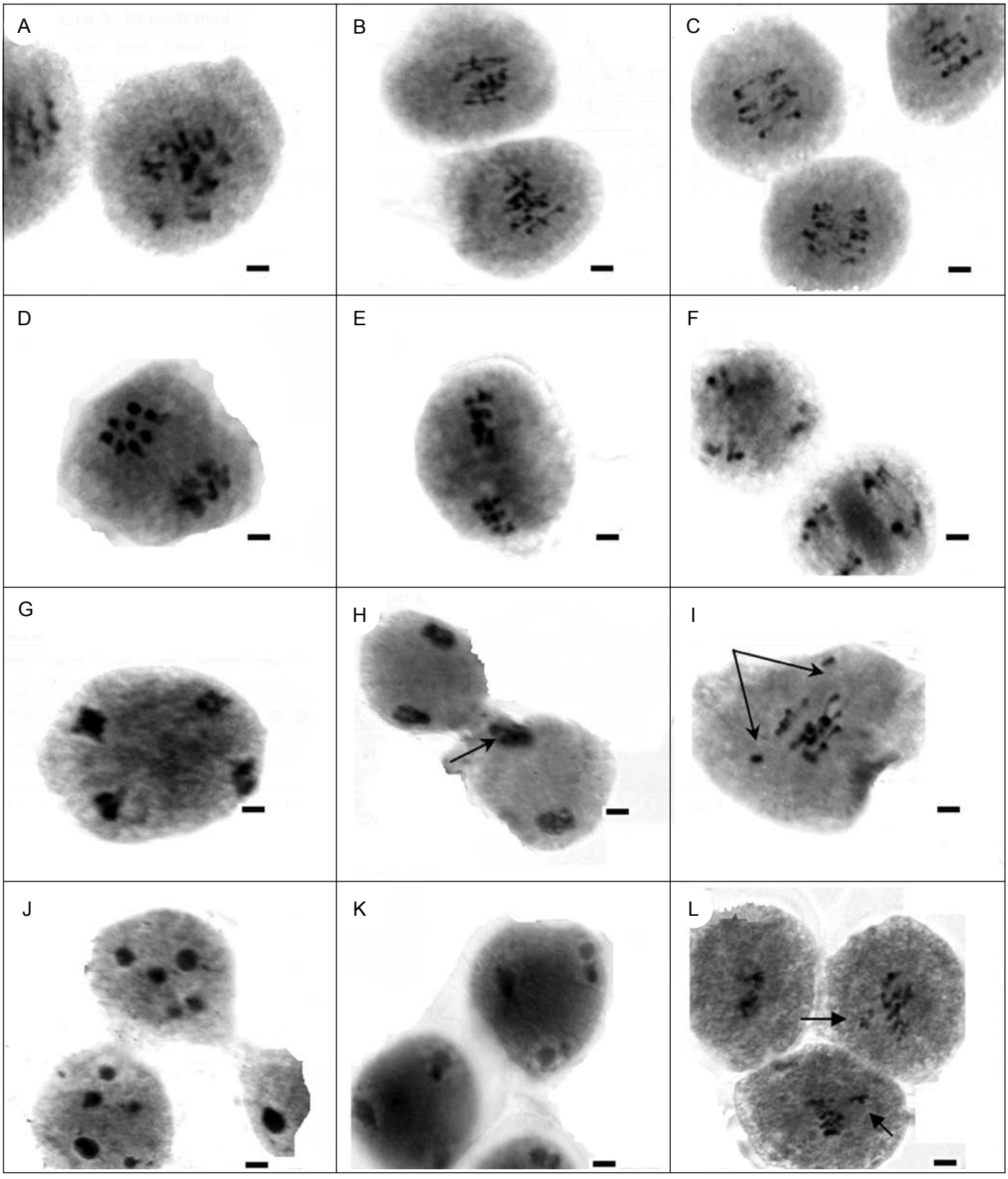

Figure 2 – Representative meiotic cells in *Onobrychis avajensis*: A, diakinesis; B, metaphase I; C, anaphase I; D, telophase I; E, metaphase II; F, anaphase II; G, telophase II; H, cytomixis; I, precocious division of centromeres; J, pentapolar cells; K, tripolar cells; L, laggard chromosome in metaphase I. Scale bar = 3 μm.

Key to species of the *Onobrychis andalanica* Bornm. group in Iran

1. Plants completely glabrous.....*O. szovitsii*
1. Plants densely covered by hairs.....2
2. Leaflets predominantly simple, pod densely hirtulous and foveolate on the disc..... *O. aurea*
2. Leaflets predominantly compound, pod densely hirtulous and disc setose.....3
3. Plants up to 70 cm long, leaflets nearly ovate, 30–40 mm × 15–20 mm; peduncles up to 25 cm long; standard 22–23 mm, keel c. 20 mm and wings c. 11 mm long, disc of pod densely white-setose..... .....*O. andalanica*
3. Plants up to 35 cm long, leaflets elliptic to lanceolate, 17–25 mm × 5–7 mm; peduncles 4–12 cm long; standard 11.5–13.5 mm, keel 7.5 mm and wings 4.5–5 mm long; disc of pod densely covered by purple setae.....*O. avajensis*

Additional specimen studied – **Iran**: Qazvin, c. 110 km to Hamedan, between Avaj and Abgarm, alt. 2100 m, 24 Jun. 2004, *Ranjbar* 6211 (BASU).

Etymology – The species is named after the village Avaj, in Qazvin Province.

Cytogenetics

The meiotic irregularities observed in *O. avajensis* include: chromosome stickiness and precocious division of centromeres in D/MI; chromosome bridges resulting from stickiness, cytomixis in AI/TI and TII, tripolar and pentapolar cells in AII/TII (fig. 2). A wide range of meiotic stages were found in anthers within the same flower in *O. avajensis*. A total of 301 diakinesis/metaphases I (D/MI) (34.48%), 301 anaphase I/telophase I (AI/TI) (34.48%), and 193 anaphase II/telophase II (AII/MII) (22.1%) cells were analysed. The D/MI cells were usually regular with predominant bivalent (II) pairing. Varying degrees of sticky chromosomes with laggards were found in 1.3% of diakinesis cells (fig. 2E–G). Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause chromosome stickiness (Pagliarini 2000). Precocious division of centromeres is another abnormality that was found in 0.33% of metaphase I cells (fig. 2I). Chromosome bridges resulting from stickiness were observed in 5.31% of anaphase I cells (fig. 2K & L). 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 & Rao 1996). The phenomenon of cytomixis consists in the migration of chromosome between meiocytes through cytoplasmic connection. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered a mechanism of evolutionary significance (Ghaffari 2006). This phenomenon occurred in 0.66% of AI/TI cells and in 2.59% of AII/TII cells. The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during metaphase. Any distortion or breakage in the spindle may result in random sub-grouping of the chromosome (Nimala & Rao 1996). Tripolar and pentapolar cells were observed in 2.07% and 6.21% of telophase II, respectively (fig. 2L & O), may lead to the formation of abnormal tetrads and infertile pollen grains.

The results showed the equal basic chromosome number of $x = 8$ in all species of *O*. sect. *Heliobrychis* (Abou-el-Enain 2002, Ghaffari et al. 2005, Ranjbar et al. 2009b). In general, speciation within this section has occurred at the diploid level. Nearly all members of this section are diploid ($2n = 2x = 16$), whereas those of *O*. sect. *Onobrychis* are diploid or tetraploid with $2n = 2x = 14$, $2n = 2x = 16$, $2n = 4x = 28$ and $2n = 4x = 32$ chromosomes (Ranjbar et al. 2009b). The members of *O*. sect. *Hymenobrychis* are diploid with $2n = 2x = 14$, $2n = 2x = 16$ chromosomes (Ranjbar et al. 2010c). The results of the present study increase our knowledge about chromosome number and morphological evidence in the genus *Onobrychis*, especially in *O*. sect. *Heliobrychis* by establishing relationships between cytogenetic criteria and taxonomic delimitation.

ACKNOWLEDGMENTS

The great help of E. Vitek, B. Wallnofer, and W. Till during the first author's visit to W, WU in Vienna is much appreciated. This research has received financial support from the Bu-Ali Sina University. We would like to thank the Director of the Herbarium of Ferdowsi University of Mashhad (FUMH), and the Herbarium Research Centres of Natural Resources and Animal Affairs of Isfahan, Kashan, Kerman, Mashhad, Semnan, Shiraz, Tabriz and Zahedan for making the herbarium facilities available for our study. We thank two anonymous reviewers for their critical comments in the transferring of the manuscript to the new journal. We also thank Mrs. M. Hezarkhani for preparing the illustrations.

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Manuscript received for *Belgian Journal of Botany* 28 Feb. 2009; accepted in revised version 11 Mar. 2010.

Communicating Editor: Renate Wesselingh.