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A new species and taxonomic studies in *Trigonella* sect. *Ellipticae* (Fabaceae) in Iran

Massoud Ranjbar*, Roya Karamian & Zahra Hajmoradi

*Department of Biology, Herbarium Division, Bu-Ali Sina University, P.O. Box 65175/4161, Hamedan, Iran (*corresponding author's e-mail: ranjbar@basu.ac.ir)*

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Trigonella yasujensis Ranjbar, Hajmoradi & Karamian *sp. nova* (Fabaceae) of *T.* sect. *Ellipticae* is described and illustrated from a single locality between Lordegan and Yasuj in the Zagros Mountains, Iran. It is closely related to *T. elliptica* but differs from it in a few morphological and anatomical characters. The meiotic chromosome number and behaviour were studied for *T. yasujensis*. It is a diploid species and possesses 2n = 2x = 16 chromosome number, consistent with the proposed base number x = 8. The general meiotic behaviour of the species was regular with normal bivalent pairing and chromosome segregation at meiosis. However, some meiotic abnormalities were observed including various degrees of sticky chromosomes with laggards, asynchronous nuclei, bridges and cytomixis.

Introduction

Trigonella (Fabaceae) with about 100 species is widely distributed throughout the world (Martin *et al*. 2008). In Iran, the genus includes 31 species in 12 sections growing in different phytogeographical regions. There are 12 species endemic to the country (Rechinger 1984). The plants are perennial or annual herbs with pinnately trifoliate leaves, often exhaling an odour and, like other grain legumes, they are important as food and medicine (Chopra *et al*. 1956, Girardon *et al*. 1989, Balodi & Rao 1991, Bhatti *et al*. 1996, Dangi *et al*. 2004, Ranjbar *et al*. 2009).

Eleven of the perennial species endemic to Iran belong to *T*. sect. *Ellipticae*. This section is characterized by glabrous leaves, stems and pods, and yellow or occasionally violet flowers (Boissier 1872, Hedge 1970, Ranjbar *et al*. 2012).

Many studies on different aspects of morphology, cytology and pollen morphology of *Trigonella* were conducted (Boissier 1872, Grossheim, 1945, Hedge 1970, Singh & Roy 1970, Townsend & Guest 1974, Singh & Singh 1976, Small *et al*. 1981, Agarwal & Gupta 1983, Brookes & Small 1988, Small *et al*. 1990, Alhabori *et al*. 1998, Ahmad *et al*. 1999, Pandita *et al*. 1999, Hamzeh'ee 2000, Murakami *et al*. 2000, Kabilan *et al*. 2002, Janighorban 2004, Dundas *et al*. 2006, Ranjbar *et al*. 2009, 2010, 2011a, 2011b, 2012). The mitotic chromosome number of an accession of *T. balansae* from Nisyros (Aegean Sea), an annual pasture legume of Eurasian origin, was first reported by Kamari and Papatsou (1973). Studies on the impact of karyotypic data on the taxonomy and also on meiotic behaviour in the genus are still few. The studies on the pollen grains of the leguminous plants (Clarke & Kupicha 1976, Ferguson & Skvarla 1981, Ferguson 1990, Ferguson & Stirton 1993, Diez & Ferguson 1994, Hughes 1997) dealt mainly with the description of the pollen grains of certain genera or sometimes tribes. Investigations of pollen morphology (Small *et al*. 1981) and pollen–ovule patterns (Small 1986), performed in order to resolve the relationships among and between the genera *Medicago* and *Trigonella*, have always failed to find rigorous diagnostic characters that could be used in distinguishing them (Bena 2001).

The present study is focused on the morphological, cytogenetic, anatomical and pollen morphological criteria for distinguishing a new taxon in *T.* sect. *Ellipticae*. Studies of living material and herbarium specimens suggest that this new species, exclusive to the Yasuj Mountains, is especially close to *T. elliptica*. However, several differences in morphology, pollen morphology and anatomy allow us to treat it as a new, distinct species.

Material and methods

Morphology

The morphological study was mainly based on the herbarium material and the field observations in western, southwestern and central Iran. The collected materials were in vegetative or fruiting phase, and were deposited at BASU, Hamedan, Iran. Also several sheets of herbarium specimens from the following herbaria: FUMH, PR, TARI, W, WU, Herbarium of Esfahan University, Herbarium Research Centre of Natural Resource and Animal Affairs of Tabriz, Mashhad, Esfahan, Shiraz, Kerman and Zahedan, were examined for each taxon.

Pollen morphology

Pollen samples were obtained from herbarium specimens and prepared using the standard method described by Erdtman (1960). They were mounted on unstained glycerin jelly and examined with a Nikon Type-2 microscope. The measurements were based on 25 readings from each specimen. Polar axis (P), equatorial diameter (E), colpus length, colpus width in granule site, colpus width in none-granule site, granule width, and granule length were measured; and the shape index (P/E) was calculated. The terminology used here follows Faegri (1956).

Anatomy

For anatomical study, fresh plant materials were preserved in glycerin–alcohol (1:1) solution, and washed before the analysis with distilled water. Cross sections of the peduncles and stems were prepared by manual cutting. The sections were cleared with sodium hypochlorite, diluted with acetic acid and stained in methylene blue and carmine solutions (Fahn 1990). Nikon Type-2 microscope was used for observing and photographing the structural characters of the tissues. For each species, seven anatomical characters in each of the ten sections were examined.

Cytogenetics

Chromosome number and meiotic behaviour were analyzed in *T. yasujensis*. Fifteen flower buds from at least five plants at an appropriate stage of development were fixed in 96% ethanol–chloroform–propionic acid (6:3:2) for 24 h at room temperature and then stored in 70% ethanol at 4 °C until used. Anthers were squashed and stained with 2% acetocarmine (Belling 1923). All slides were fixed with Venetian turpentine. Photographs of chromosomes were taken under an Olympus BX-41 photomicroscope. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photographs.

Results

Trigonella yasujensis Ranjbar, Hajmoradi & Karamian, *sp. nova* (Fig. 1)

HOLOTYPE: Iran. Lordegan to Yasuj, 65 km to Yasuj, 15 km before Pataveh, 31°15´N, 51°25´E, 1470 m a.s.l., 6 June 2010 *Ranjbar & Hajmoradi 23099* (BASU; isotypes TARI, W, MO).

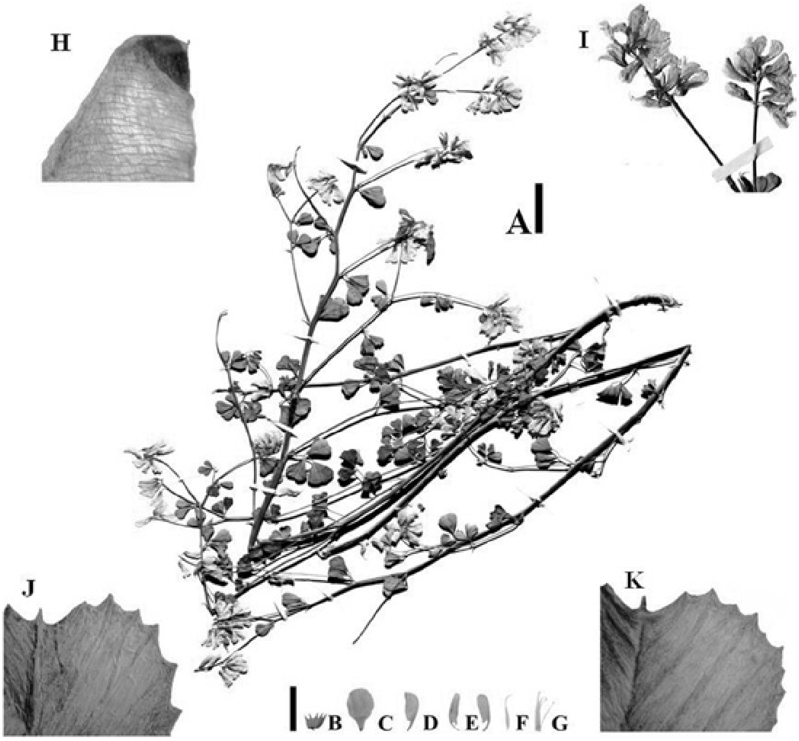

Fig. 1. Holotype of *Trigonella yasujensis.* — **A**: Habit with fruit. — **B**: Calyx. — **C**: Standard. — **D**: Keel. — **E**: Wings. — **F**: Ovary. — **G**: Androecium. — **H**: Glabrous pod. — **I**: Inflorescence with a few flowers. — **J**: Leaflet adaxial surface. — **K**: Leaflet abaxial surface. Scale bars: **A** = 2 cm, **B**–**G** = 1 cm.

ETYMOLOGY: The species is named after the location Yasuj, Iran.

Perennial herb, 32–50 cm. Caudex branched., ± sparsely hairy 0.2–0.4 mm. Stipules herbaceous, 2.5–5 × 0.7–1 mm, adnate to petiole for up to 0.5 mm, behind stem, linear to lanceolate, loosely hairy. Leaves 4–16 mm; petiole 2–14 mm. Leaflets obdeltoid to obcordate, 4–12 × 3–8 mm, truncate or emarginate, sparsely appressed hairy beneath 0.2–0.4 mm, above glabrous; teeth 14–18-tooth, triangular, with a minute mucro 0.3–0.5 mm. Peduncles 2–3.5 cm, erect. Racemes laxly 10–21-flowered, aristate. Bracts hyaline-membranous, 0.3–0.4 × ca. 0.2 mm, triangular, sparse-glabrous. Pedicels 2–3 mm. Calyx ca. 4 mm, tubular, ± sparsely covered with short white appressed hairs 0.2–0.4 mm; teeth filiform to subulate, ca. 1 mm. Corolla yellow. Standard 9–10 × 5–6 mm, elliptic to obovate, angularly narrowed into short cuneate claw, at apex rounded. Wings ca. 9 mm; blades oblong, rounded at apex, ca. 6 × ca. 2 mm, auricle 0.6–1 mm. Keel ca. 9 mm; blades obliquely narrowly triangular-obovate, with straight lower edge and distinctly concave upper edge acutish at apex, 6 × 3 mm; claw ca. 3 mm. Stamen-tube obliquely cut at mouth. Ovary with a stipe ca. 1 mm, glabrous. Pods 12–20 × 4–6 mm, oblong-elliptic, with a slender stipe 1 mm long, at apex obtuse to rounded and recurved beak; valves straw-colored to pale brown, glabrous. Seeds 2–5.

DISTRIBUTION AND PHYTOGEOGRAPHY. *Trigonella elliptica* is common in the Fars and Esfahan Provinces, south to central Iran (Fig. 2). The type material of *T. elliptica* was also available to us in Vienna (Fig. 3). *Trigonella yasujensis* is known from a single locality. It is closely related to *T. elliptica*, but differs from it mainly by having a sparse indumentum or lacking it, many flowers, longer leaves and shorter peduncles (Table 1).

Key to the species of *Trigonella* sect. *Ellipticae* in Iran

1. Pod with wing 2
1. Pod without wing 6
2. Pod wings 1–2 mm long; pod stipe 1–3 mm long .... *T. latialata*

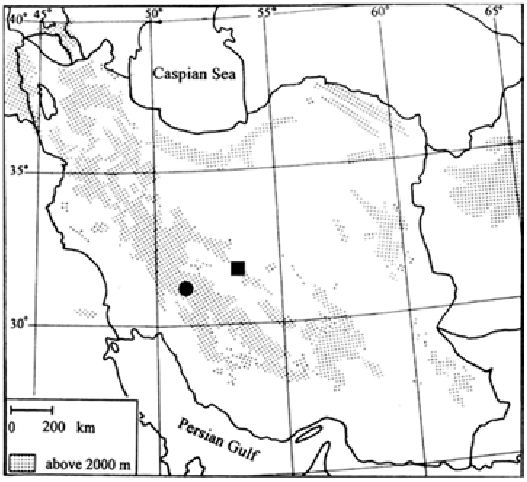

Fig. 2. Localities of *Trigonella yasujensis* (●) and *T. elliptica* (■) in Iran.

2. Pod wings 0.5–1.5 mm long 3
3. Pod wings 1–1.5 mm long; seed number 1–2 .... *T. disperma*
3. Pod wings 0.5–1 mm long; seed number more than 2 ... 4
4. Pods 8–12(15) × 4–5 mm .... *T. tehranica*
4. Pods 20–25 × 4–5.5 mm 5
5. Plant densely pilose; leaflet loosely pilose on upper surface; flowers less than 5 .... *T. elliptica*
5. Plant sparse-glabrous; leaflet glabrous on upper surface; flowers 10–21 .... *T. yasujensis*
6. Flowers 1–3; pods 8 × 3 mm .... *T. subenervis*
6. Flowers 3–7; pods 17–25 × 3–4 mm 7
7. Standard 10–11 mm; pods 17 × 3 mm ..... *T. stenocarpa*
7. Standard 7 mm; pods 20–25 × 3.5–4 mm .... *T. aphaoneura*

Fig. 3. Type of *Trigonella elliptica* (*Aucher-Eloy 4458*, W).

Pollen morphology

The pollen grains in the tribe Trifolieae are very variable in size, shape, number and type of apertures, and even in the exine ornamentation. These variations, especially those of the apertures, form the basis for distinguishing the three pollen types and five subtypes within the tribe (Taia 2004). In *T.* sect. *Ellipticae*, the pollen grains are elliptic to oblong, with often a perforate ornamentation. Some pollen features are shared among some of the species, while others are different among them (Ranjbar *et al*. 2012). *Trigonella yasujensis* is separated from *T. elliptica* (*Hatami*, BASU 14331) well by having larger pollen grains. The ranges of its pollen characters such as colpus width, and granule length and width are different enough for distinguishing the new species (*see* Table 2 and Fig. 4).

Anatomy

There are significant differences between *T. yasujensis* and *T. elliptica* (Table 3, Fig. 5A and C). In *T. yasujensis*, the outline of stem transverse section is pentagonal, and there are more collenchyma layers and vascular bundles than in *T. elliptica*. The outline of the peduncle transverse section is elliptic — similar to *T. elliptica* — but differs by having spongy tissue in the parenchyma layers, thin-walled sclerenchyma fiber cells and by the absence of crystals in the outer layer of the sclerenchyma fibers (Fig. 5B and D).

Cytogenetics

Trigonella yasujensis is a diploid species and possesses 2n = 2x = 16 chromosome number, consistent with the proposed base number x = 8. In addition, some meiotic irregularities were observed in *T. yasujensis* including chromosome stickiness and laggards in diakinesis, metaphases I and metaphases II, D/MI/MII, chromosome bridges resulting from stickiness, cytomixis in anaphase I, anaphase II, diakinesis, metaphases I and prophase II, and an asynchronous nucleus in metaphase II (Fig. 6). All meiotic stages were found in anthers within the same flower in *T. yasujensis*. A total of 630 prophase, 799 diakinesis/metaphases I (D/MI) (33.78%), 248 anaphase I/telophase I (AI/TI) (10.48%), 304 metaphase II

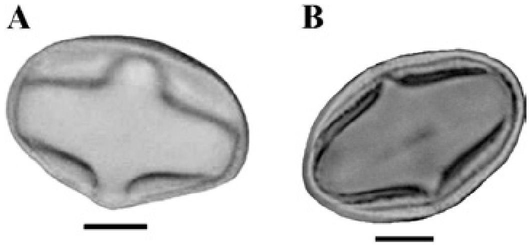

Fig. 4. Pollen light micrographs from polar views in *T. yasujensis* (**A**) and *T. elliptica* (**B**). Scale bars: 6 μm.

Table 1. Diagnostic morphological characters of *Trigonella yasujensis* and *T. elliptica.*

	T. yasujensis	*T. elliptica*
Leaf length (mm)	4–16	3–10
Peduncle length (mm)	2–3.5	3–7
Standard length (mm)	9–10	7–8
Number of flowers	up to 21	3–5(7)
Plant indumentum	sparse-glabrous	dense
Leaflet indumentum in abaxial surface	glabrous	loose
Pod indumentum	glabrous	sparse

Table 2. Pollen morphological characters of *Trigonella yasujensis* and *T. elliptica*. Given are min(mean)max values.

	T. yasujensis	*T. elliptica*
Polar axis (P) (μm)	29(29.45)30	25(27.5)31
Equatorial diameter (E) (μm)	20(22.05)23	18(19.2)22
P/E	1.33	1.43
Colpus length(μm)	18(24.35)26	18(21.3)25
Colpus width in none-granule site (μm)	12(13.6)15	7(8.2)9
Colpus width in granule site (μm)	16(16.55)20	12(13.8)17
Granule width (μm)	4(5.3)6	2(2.5)3
Granule length (μm)	4(5.8)6	2(3.2)4

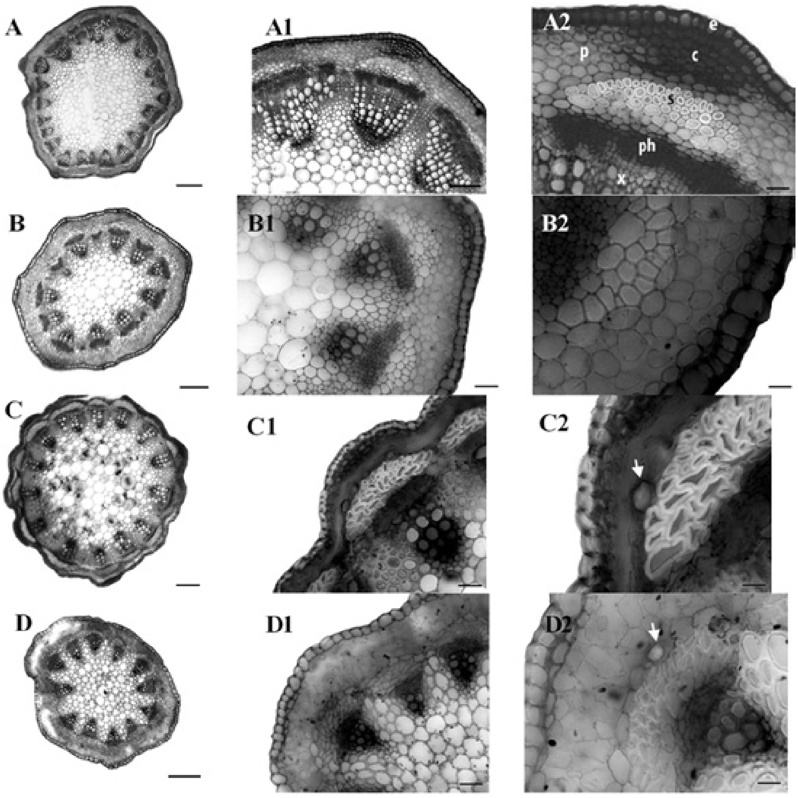

Fig. 5. Stem and peduncle transverse sections of *Trigonella yasujensis* and *T. elliptica*. — **A**: Stem transverse section of *T. yasujensis*. — **B**: Peduncle transverse section of *T. yasujensis*. — **C**: Stem transverse section of *T. elliptica*. — **D**: Peduncle transverse section of *T. elliptica* (arrows show crystals). — e = epidermis; c = collenchyma; p = parenchyma; s = sclerenchyma fiber; ph = phloem; x = xylem. Magnifications in each row from left to right: ×10, ×40 and ×100. Scale bar: 200 μm.

(MII) (12.8%) and 383 anaphase II/telophase II (AII/TII) (16.19%) cells were analyzed. The D/MI cells were usually regular with predominant bivalent (II) pairing. The most frequent abnormality observed in prophase was cytomixis with higher degree in diplotene (90.9%) Cytomixis results from the migration of chromosomes between meiocytes through cytoplasmic connections. Since cytomixis creates variation in the chromosome number of the gametes, it could be

Table 3. Anatomical characters of stem and peduncle in *Trigonella yasujensis* and *T. elliptica*.

	T. yasujensis	*T. elliptica*
Peduncle		
Type of parenchyma tissue	spongy	palisade and spongy
Number of parenchyma layers	2–4	2–3
Number of collenchyma layers	1–2	0–1
Number of sclerenchyma layers	3–4	2–3
Number of vascular bundles	12	12
Transverse section outline	elliptic	elliptic
Crystal	absent	present
Stem		
Type of parenchyma tissue	spongy	spongy
Number of parenchyma layers	2–3	1–2
Number of collenchyma layers	4–5	0–3
Number of sclerenchyma layers	2–3	2–3
Number of vascular bundles	20	15–16
Transverse section outline	pentagonal	sinuate
Crystal	present	present

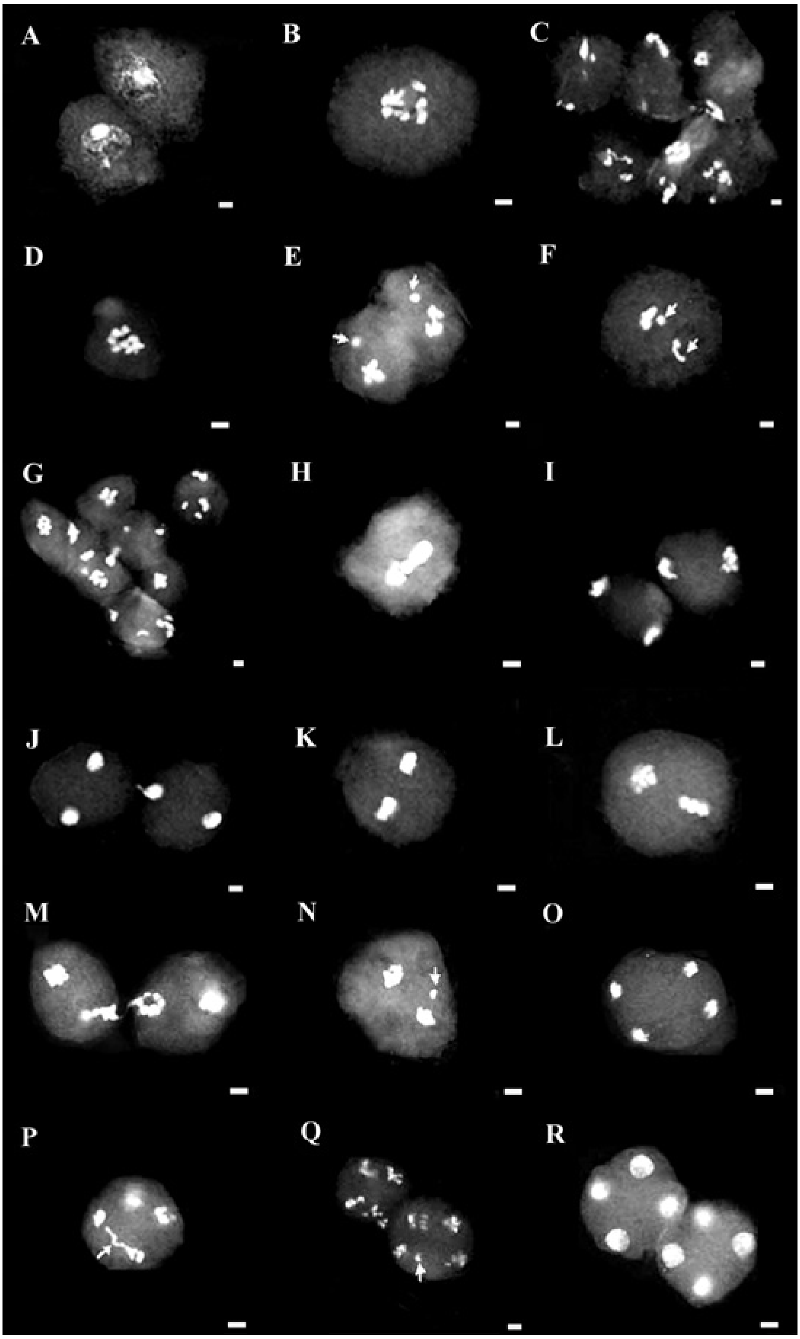

Fig. 6. Representative meiotic cells in *Trigonella yasujensis*. — **A**: Zygotene. — **B**: Diplotene. — **C**: Cytomixis in diplotene. — **D**: Diakinesis with eight bivalents. — **E** and **F**: Laggard and sticky chromosomes in diakinesis. — **G**: Cytomixis in diakinesis. — **H**: Metaphase I. — **I**: Anaphase I. — **J**: Cytomixis in telophase I. — **K**: Metaphase II. — **L**: Asynchronous nuclei in metaphase II. — **M**: Cytomixis in metaphase II. — **N**: Laggard chromosome in metaphase II. — **O**: Anaphase II. — **P**: Bridge in anaphase II. — **Q**: Laggard chromosome in anaphase II. — **R**: Telophase II. Scale bar: 3 μm.

considered a mechanism of evolutionary significance (Ghaffari 2006). Various degrees of sticky chromosomes with laggards and cytomixis were found in 17.1% and 5.2% of diakinesis cells, respectively (Fig. 6E–G). Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause it (Pagliarini 1990). Chromosome bridges resulting from stickiness were observed in 2.5% of anaphase I and 4.5% of anaphase II cells (Fig. 6P). The thickness of the bridges observed and the number of the chromosomes involved in their formation varied among different meiocytes. Genetic as well as environmental factors have been considered the reason for chromosome stickiness in different plant species (Nirmala & Rao 1996). An asynchronous nucleus was found in 3.28% of metaphase II cells (Fig. 6L). Laggard chromosomes were found in 13.6% of anaphase II (Fig. 6Q).

Concluding remarks

Trigonella yasujensis and *T. elliptica* differ in many morphological and palynological features (cf. Tables 1–3). The cytogenetic study showed that the basic chromosome number of x = 8 for *T. yasujensis* is similar to the other species in *T.* sect. *Ellipticae* (Hesamzadeh & Ziaei Nasab 2009). In general, speciation within *T.* sect. *Ellipticae* including perennial species has occurred on the diploid level. Nearly all members of the section are diploid (2n = 2x = 16), whereas annuals are diploid, tetraploid or hexaploid with 2n = 2x = 16, 2n = 4x = 32 and 2n = 6x = 48 chromosome numbers (Martin *et al.* 2008, Aykut *et al.* 2009).

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