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

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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).

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 Vene- talk...

**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).
The species is named after the location Yasuj, Iran.

Perennial herb, 32–50 cm. Caudex branched, ± sparsely hairy 0.2–0.4 mm. Stipules herba -ceous, 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
2. Pod without wing ....................................................... 6
3. Pod wings 1–2 mm long; pod stipe 1–3 mm long .......... .................. *T. latialata*
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. 2. Localities of Trigonella yasujensis (●) and T. elliptica (■) in Iran.

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 (Table 1).
(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*.

<table>
<thead>
<tr>
<th></th>
<th>T. yasujensis</th>
<th>T. elliptica</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peduncle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of parenchyma tissue</td>
<td>spongy</td>
<td>palisade and spongy</td>
</tr>
<tr>
<td>Number of parenchyma layers</td>
<td>2–4</td>
<td>2–3</td>
</tr>
<tr>
<td>Number of collenchyma layers</td>
<td>1–2</td>
<td>0–1</td>
</tr>
<tr>
<td>Number of sclerenchyma layers</td>
<td>3–4</td>
<td>2–3</td>
</tr>
<tr>
<td>Number of vascular bundles</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Transverse section outline</td>
<td>elliptic</td>
<td>elliptic</td>
</tr>
<tr>
<td>Crystal</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td><strong>Stem</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of parenchyma tissue</td>
<td>spongy</td>
<td>spongy</td>
</tr>
<tr>
<td>Number of parenchyma layers</td>
<td>2–3</td>
<td>1–2</td>
</tr>
<tr>
<td>Number of collenchyma layers</td>
<td>4–5</td>
<td>0–3</td>
</tr>
<tr>
<td>Number of sclerenchyma layers</td>
<td>2–3</td>
<td>2–3</td>
</tr>
<tr>
<td>Number of vascular bundles</td>
<td>20</td>
<td>15–16</td>
</tr>
<tr>
<td>Transverse section outline</td>
<td>pentagonal</td>
<td>sinuate</td>
</tr>
<tr>
<td>Crystal</td>
<td>present</td>
<td>present</td>
</tr>
</tbody>
</table>

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 meio-
cytes. 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 fea-
tures (cf. Tables 1–3). The cytogenetic study
showed that the basic chromosome number of x
= 8 for T. yasujensis is similar to the other spe-
cies 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 mem-
ers of the section are diploid (2n = 2x = 16),
whereas annuals are diploid, tetraploid or hexa-
ploid with 2n = 2x = 16, 2n = 4x = 32 and 2n
= 6x = 48 chromosome numbers (Martin et al.

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References

Agarwal, K. & Gupta, P. K. 1983: Cytological studies in the
and activity of rRNA genes on fenugreek (Trigo-
nella foenum-graecum L.) chromosomes by fluorescent
Al-habori, M., Al-aghbari, A. M. & Al-mamary, M. 1998:
Effects of fenugreek seeds and its extracts on plasma
lipid profile: a study on rabbits. — Phytother. Res. 12:
572–575.
Aykut, Y., Martin, E., Ünal, F. & Akan, H. 2009: Karyologi-
cal study on six Trigonella L. species (Leguminosae) in
Turkey. — Caryologia 62: 89–94.
(Fabaceae) in the northwest Himalaya. — J. Econ.
Taxon. Bot. 5: 11–16.
Belling, J. 1923. Microscopical methods used in examining
chromosomes in iron-acetocarmine. — Am. Nat. 57:
Bena, G. 2001: Molecular phylogeny supports the morpho-
logically based taxonomic transfer of the “medicagoid”
Trigonella species to the genus Medicago L. — Plant
Bhatti, M., Khan, M., Ahmad, B., Jamshaid, M. & Ahmad,
W. 1996: Antibacterial activity of Trigonella foenum-
Boissier, E. 1872: Trigonella. — In: Boissier, E. (eds.),
Lugundi.
Brookes, B. & Small, E. 1988: Enhanced floral analysis by
low temperature scanning electron microscopy. — Scan-
ning Microsc. 2: 247–256.
Chopra, R. N., Nayyaarand, S. L. & Chopra, I. E. 1956: Gloss-
ary of Indian medicinal plants. — CSIR, New Delhi.
Clarke, G. C. S. & Kupicha, F. L. S. 1976: The relationships
of the genus Cicer L. (Leguminosae): the evidence from
pollen morphology. — Bot. J. Linn. Soc. 72: 35–44.
Dangi, R. S., Lagu, M. D., Choudhary, L. B., Ranjekar, P. K.
& Gupta, V. S. 2004: Assessment of genetic diversity in
Trigonella foenum-graecum and Trigonella caerulea
4: 13–23.
Diez, M. I. & Ferguson, L. K. 1994: The pollen morphology
of the tribes Loteae and Coronilleae, Lotus L. and related
genera (Papilionoideae: Leguminosae). — Rev. Palaeo-
bot. Palynol. 81: 233–255.
of meiotic chromosome number and karyotype analysis
of an accession of Trigonella balansae (Leguminosae).
54: 561–564.
Fahn, A. 1990: Plant anatomy, 4th ed. — New York, Perga-
mon Press.
Ferguson, I. K. 1990: The significance of some pollen mor-
phology characters of the tribe Amorpheae and the genus
Ferguson, I. K. & Skvarla, J. I. 1981: The pollen morphology
of the subfamily Papilionoideae (Leguminosae). — In:
Polhill, R. M. & Raven, P. H. (eds.), Advances in legume
Ferguson, I. K. & Stirton, C. H. 1993: Pollen morphology of
the genera Panarea and Bowdichia from Leguminosae
Ghaftari, S. M. 2006: Occurrence of diploid and polyploidy
microspores in Sorghum bicolor (Poaceae) is the result