A karyological study of some endemic *Trigonella* species (Fabaceae) in Iran

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**Abstract.** Karyotypes of nine populations belonging to six endemic species of the genus *Trigonella* (Trifolieae/Fabaceae) studied in this survey. All studied species are perennial and recorded only from Northeast of Iran. Excluding the karyotype of *Trigonella subenervis*, which was previously reported, all of the other species studied here (six species) for the first time. Our results present that all assessed genotypes are diploid with 2n=2x=16 and the chromosomal basis of x=8. In addition to the chromosome counts, length of long and short arms of the chromosome and their ratios analyzed and presented in this study.

**Keywords.** Chromosome number, Cytogenetic, Karyotype, Khorassan, Trifolieae, *Trigonella*.

**INTRODUCTION**

The genus *Trigonella* L. is a member of the tribe Trifolieae of the family Fabaceae, with about 135 species worldwide, most of which distributed in the dry regions around the Mediterranean region extended to West Asia, and naturalized in North America. Only two species being present in South Australia (Mabberley 1997).

*Trigonella* consists of annual or perennial herbs with pinnately trifoliolate leaves, a campanulate or tubular calyx with two large and three small equal lobes, diadelphous stamens, uniform anthers, terminal stigma and ovary with numerous ovules (Sirj 1928-1932; Hutchinson 1964; Dangi et al. 2016). According to Rechinger (1984), the genus represented by 58 species in to Flora Iranica area. This number has increased to about 66 species as a result of recent researches (Hamzeh’e 2000; Janighorban 2004; Badrzadeh and Ghafarzadeh-Namazi 2009; Ranjbar, Karamian, and Hajmoradi 2012; Ranjbar and Hajmoradi 2012, 2015, 2016). Of which, 48 taxa (15 endemics (32%)) accommodated in 12 sections growing in Iran; 14 of those are perennial species of *Trigonella sect. Ellipticae* (Boiss.) Sirj.

In Flora Iranica account (Rechinger 1984), section *Ellipticae* is represented with seven perennial species in Iran. The characteristics of the section...
Ellipticae are: perennial species with entire or dentate stipules, calyx campanulate, petals yellow or rarely white with violet veins, sometimes completely dark violet, standard without interlocking projection, fruit a legume which is different in shape and size, elliptic or lanceolate to oblong, beakless, generally transversely veined, wingless or with thin wing and smooth seeds.


To contribute to the karyological study of the genus, we carried out a karyological study on some perennial endemic species collected from different regions in East and Northeast of Iran. This study aimed to verify the chromosome numbers of some endemic Trigonella species recently reported in Iran. In this contribution, we report the somatic chromosome numbers of six taxa (nine populations), that five species are determined for the first time.

**MATERIAL AND METHODS**

The chromosome number were analyzed in nine population of Trigonella. The nomenclature of taxa, collection data, and vouchers are given in Table 1. The mitotic chromosome numbers were studied in three populations of T. subenervis Rech. f., two populations of T. binaloudensis and one population from each of T. lasiocarpa, T. stipitata, T. heratensis and T. Torbatejamensis. Seed materials were collected between the years of 2016 and 2018 from natural habitats. Voucher specimens were deposited at the Ferdowsi University of Mashhad Herbarium (FUMH), Iran.

For karyological observation, to accelerate germination, the seed surfaces were abraded with emery paper. Seeds were sown on wet filter paper in Petri dishes at room temperature. The seeds germinated after 2-3 days. One cm fresh root tip cells were used to study the somatic chromosomes. The root tips pretreated in an ice-water mixture for 24 hours. Afterward, they fixed in Carnoy’s fixative (ethanol: acetic acid 3:1) for 24 h at 4 °C (Fukui and Nakayama 1996). The root tips were washed in distilled water to remove the fixative, then hydrolyzed in 1N HCl for 13–15 minutes at room temperature, and finally stained with 2% aceto-orcein for two hours.

The slides were created using the squash method. The prepared slides were slightly heated under an alcohol frame for 1-2 s before observation. Photographs of chromosomes were taken using a Nikon Eclipse Ni-u (Tokyo, Japan) photomicroscope equipped with Nikon Ds-Fi3 digital camera. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation, and from photomicrographs. To ensure for chromosome number, a minimum of five cells, at somatic metaphase, observed (Figure 1). Karyotypic analyses were conducted on IdeoKar software (version 1.2) to calculate karyotypic parameters and generate ideograms (Mirzaghaderi and Marzangi 2015). Karyotype formulae and nomenclature followed Levan et al. (1964), and karyotype asymmetry followed Stebbins (1971).

**Table 1.** Iranian endemic species of Trigonella analyzed in this study, their locations, and voucher specimens data.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Location</th>
<th>Collection Date</th>
<th>Elevation (m)</th>
<th>Herbarium number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. binaloudensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>population 1</td>
<td>SW Chenaran, Ferizi towards Binaloud mountains</td>
<td>2016/05/30</td>
<td>1730</td>
<td>46443</td>
</tr>
<tr>
<td>population 2</td>
<td>NW Sabzevar, W Jalambadan, in Mnts. near Chromite mine</td>
<td>2018/05/29</td>
<td>1770</td>
<td>46323</td>
</tr>
<tr>
<td><em>T. heratensis</em></td>
<td>S Fariman, between Torbate-Heydariyeh &amp; Fariman</td>
<td>2016/06/06</td>
<td>1685</td>
<td>45959</td>
</tr>
<tr>
<td><em>T. lasiocarpa</em></td>
<td>NE Birjand, Now-Qand towards Bidesk</td>
<td>2016/05/24</td>
<td>2320</td>
<td>46442</td>
</tr>
<tr>
<td><em>T. stipitata</em></td>
<td>N Mashhad, E Chenaran, Mian-Marq</td>
<td>2016/05/25</td>
<td>1420</td>
<td>25771</td>
</tr>
<tr>
<td><em>T. subenervis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>population 1</td>
<td>N Torbate-Heydariyeh, Khomari pass</td>
<td>2016/05/23</td>
<td>1851</td>
<td>46441</td>
</tr>
<tr>
<td>population 2</td>
<td>N Kashmar, NE hills of Chalpu village</td>
<td>2017/06/06</td>
<td>1879</td>
<td>46446</td>
</tr>
<tr>
<td>population 3</td>
<td>N Shirvan, 12 km from Lowjalli toward Namanlu village</td>
<td>2017/06/24</td>
<td>1820</td>
<td>45844</td>
</tr>
<tr>
<td><em>T. torbatejamensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE Torbate-Jam, between Saleh-Abad &amp; Gush-Laqar,</td>
<td>2016/05/04</td>
<td>750</td>
<td>45958</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Our investigations comprised nine populations belonging to 6 species of the genus *Trigonella*, of which data for five species reported here for the first time. The chromosomes of these taxa at mitotic metaphase shown in Figure 1. Detailed karyotypic parameters, formulae, and asymmetry are summarized in Table 2. In this study, the basic chromosome number of all taxa is x=8, and all of them are diploid.

*Trigonella binaloudensis* Ranjbar & Karamian

Population 1 (SW Chenaran):

The chromosome number was 2n=2x=16 (Figure 1e1). Haploid chromosome length was 27.73 μm. Chromosome length varied from 2.75 to 4.83 μm, and arm ratio from 1.06 to 1.88. The chromosome complement at mitotic metaphase consisted of 14 median region and two submedian region chromosomes. Karyotypic asymmetry was 1A.

Population 2 (NW Sabzevar):

The chromosome number was 2n=2x=16 (Figure 1e2). Haploid chromosome length was 24.03 μm. Chromosome length varied from 2.15 to 3.72 μm, and arm ratio from 1.030 to 1.82. The chromosome complement at mitotic metaphase consisted of 14 median region and two submedian region chromosomes. Karyotypic asymmetry was 1A.

*Trigonella heratensis* Rech.f

The chromosome number of *T. heratensis* was 2n=2x=16 (Figure 1c). Haploid chromosome length was 22.98 μm. Chromosome length varied from 2.46 to 3.40 μm.

Figure 1. Photographs of somatic metaphase chromosomes of nine populations belonging to six species of the genus *Trigonella* collected from northeast of Iran. (a, b) *T. binaloudensis* from two locality; (c) *T. heratensis*; (d) *T. lasiocarpa*; (e) *T. stipitata*; (f, g, h) *T. subenervis* from three locality; (i) *T. torbatejamensis*. Scale bars = 10 μm.
μm, and arm ratio from 1.02 to 1.69. The chromosome complement at mitotic metaphase consisted of 16 median region chromosomes, and karyotypic asymmetry was 1A.

**Trigonella lasiocarpa Ranjbar & Z.Hajmoradi**

The chromosome number of *T. lasiocarpa* was 2n=2x=16 (Figure 1a). Haploid chromosome length was 28.9 μm. Chromosome length varied from 2.44 to 4.44 μm, and arm ratio from 1.09 to 1.51. The chromosome complement at mitotic metaphase consisted of 16 median region chromosomes, and karyotypic asymmetry was 1A.

**Trigonella stipitata Ranjbar & Joharchi**

The chromosome number of *T. stipitata* was 2n=2x=16 (Figure 1b). Haploid chromosome length was 19.17 μm. Chromosome length varied from 1.92 to 3.13 μm, and arm ratio from 1.02 to 1.44. The chromosome complement at mitotic metaphase consisted of 16 median region chromosomes, and karyotypic asymmetry was 1A.

**Trigonella subenervis Rech.f**

Population 1 (N Torbate-Heydariyeh):

The chromosome number was 2n=2x=16 (Figure 1f1). Haploid chromosome length was 19.87 μm. Chromosome length varied from 1.94 to 3.00 μm, and arm ratio from 1.01 to 1.94. The chromosome complement at mitotic metaphase consisted of 14 median region and two submedian region chromosomes. Karyotypic asymmetry was 1A.

Population 2 (N Kashmar):

The chromosome number was 2n=2x=16 (Figure 1f3). Haploid chromosome length was 20.68 μm. Chromosome length varied from 2.03 to 3.04 μm, and arm ratio from 1.01 to 1.52. The chromosome complement at mitotic metaphase consisted of 16 median region chromosomes, and Karyotypic asymmetry was 1A.

Population 3 (N Shirvan):

The chromosome number was 2n=2x=16 (Figure 1f3). Haploid chromosome length was 28.04 μm. Chromosome length varied from 2.67 to 4.18 μm, and arm ratio from 1.01 to 1.44. The chromosome complement at mitotic metaphase consisted of 16 median region chromosomes, and Karyotypic asymmetry was 1A.

**Trigonella torbatejamensis Ranjbar**

The chromosome number of *T. torbatejamensis* was 2n=2x=16 (Figure 1d). Haploid chromosome length was 26.77 μm. Chromosome length varied from 2.42 to 4.50 μm, and arm ratio from 1.05 to 2.21. The chromosome complement at mitotic metaphase consisted of 14 median region and two submedian region chromosomes. Karyotypic asymmetry was 2A.

**DISCUSSION**

All taxa in the present study showed the same basic chromosome number x=8 and same polyploidy level, which is congruent with those previously reported by Ranjbar *et al.* (2016) in *Trigonella subenervis* and six other species of the section *Ellipticae*. This section comprises most of the perennial species of the genus *Trigonella* and widely distributed in Iran, Afghanistan and Middle Asia.

### Table 2. Somatic chromosome numbers and Karyotypes of nine *Trigonella* taxa. HCL: haploid chromosome length, Cl: chromosome length, AR: arm ratio(L/S), RL%: relative length of the chromosome, CI: centromeric index.

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>X</th>
<th>HCL (μm)</th>
<th>CL (μm)</th>
<th>AR</th>
<th>RL%</th>
<th>CI</th>
<th>Karyotype formulae</th>
<th>Karyotypic asymmetry (Stebbins)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. binaloudensis</em> (1)</td>
<td>16</td>
<td>8</td>
<td>27.73</td>
<td>2.75-4.83</td>
<td>1.06-1.88</td>
<td>4.96-8.70</td>
<td>0.35-0.49</td>
<td>14m+2sm</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. binaloudensis</em> (2)</td>
<td>16</td>
<td>8</td>
<td>24.03</td>
<td>2.15-3.72</td>
<td>1.03-1.82</td>
<td>4.48-7.74</td>
<td>0.35-0.49</td>
<td>14m+2sm</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. heratensis</em></td>
<td>16</td>
<td>8</td>
<td>22.98</td>
<td>2.46-3.40</td>
<td>1.02-1.69</td>
<td>5.36-7.39</td>
<td>0.37-0.50</td>
<td>16m</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. lasiocarpa</em></td>
<td>16</td>
<td>8</td>
<td>28.90</td>
<td>2.41-4.44</td>
<td>1.09-1.51</td>
<td>4.17-7.68</td>
<td>0.40-0.48</td>
<td>16m</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. stipitata</em></td>
<td>16</td>
<td>8</td>
<td>19.17</td>
<td>1.92-3.13</td>
<td>1.02-1.44</td>
<td>5.00-8.15</td>
<td>0.41-0.49</td>
<td>16m</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. subenervis</em> (1)</td>
<td>16</td>
<td>8</td>
<td>19.87</td>
<td>1.94-3.00</td>
<td>1.01-1.94</td>
<td>4.88-7.54</td>
<td>0.34-0.50</td>
<td>14m+2sm</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. subenervis</em> (2)</td>
<td>16</td>
<td>8</td>
<td>20.68</td>
<td>2.03-3.04</td>
<td>1.01-1.52</td>
<td>4.91-7.34</td>
<td>0.40-0.50</td>
<td>16m</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. subenervis</em> (3)</td>
<td>16</td>
<td>8</td>
<td>28.04</td>
<td>2.67-4.18</td>
<td>1.01-1.44</td>
<td>4.75-7.45</td>
<td>0.41-0.50</td>
<td>16m</td>
<td>1A</td>
</tr>
<tr>
<td><em>T. torbatejamensis</em></td>
<td>16</td>
<td>8</td>
<td>26.77</td>
<td>2.42-4.50</td>
<td>1.05-2.21</td>
<td>4.51-8.41</td>
<td>0.31-0.49</td>
<td>14m+2sm</td>
<td>2A</td>
</tr>
</tbody>
</table>
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All of the studied taxa in this study, analyzed karyotypically for the first time, covering chromosome length, karyotype formulae, and asymmetry (Table and Figure 2). In term of karyotypic parameters, Our results support the results reported by Riasat (2015) about *Trigonella elliptica*, a perennial species from section *Ellipticae*. In later study, the karyotype formulae reported as 14m+2sm, 16m, 8m+8sm, and 12m+4sm in different genotypes. We found the same variations in karyotype formulae and asymmetry among different species and populations that are shown in Table 2.

Karyotypic asymmetry in most of the studied taxa, was as A1 but in *T. torbatejamensis* which is A2. Karyotype formulae in most of the specimens were as 16m and in four specimens (*T. torbatejamensis*, two populations of *T. binaloudensis* and one population of *T. subenervis*) was as 14m+2sm. The incongruences may result from variations among different populations and chromosome preparation treatments. It seems that chromosome variation and evolution of *Trigonella* species need more comparative cytological studies based on more collections from different populations.

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Figure 2. Haploid ideograms of *Trigonella* taxa. (a, b) *T. binaloudensis* from two locality; (c) *T. heratensis*; (d) *T. lasiocarpa*; (e) *T. stipitata*; (f, g, h) *T. subenervis* from three locality; (i) *T. torbatejamensis*. in all of studied taxa 2n=16.


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