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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 trifoliate 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 (Širjaev 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'ee 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.

Several cytological investigations have been conducted on approximately a hundred *Trigonella* species (Agarwal and Gupta 1983; Ahmad *et al*. 1999; Astanova 1981; Aykut *et al*. 2009; Bal 1990; Bidak and Amin 1996; Darlington and Wylie 1955; Dundas *et al*. 2006; Ghosh 1980; Kumari and Bir 1990; Ladizinsky and Vosa 1986; Martin *et al*. 2006, 2008, 2010, 2011a, 2011b; Pavlova 1996; Ranjbar *et al*. 2011a, 2011b, 2015; Singh and Roy 1970; Singh and Singh 1976; Tutin and Heywood 1964; Yilmaz *et al*. 2009). The reported chromosome numbers of the genus *Trigonella* are 2n=14, 16, 18, 24, 28, 30, 32, 42, 44, 46 and 48.

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.

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

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

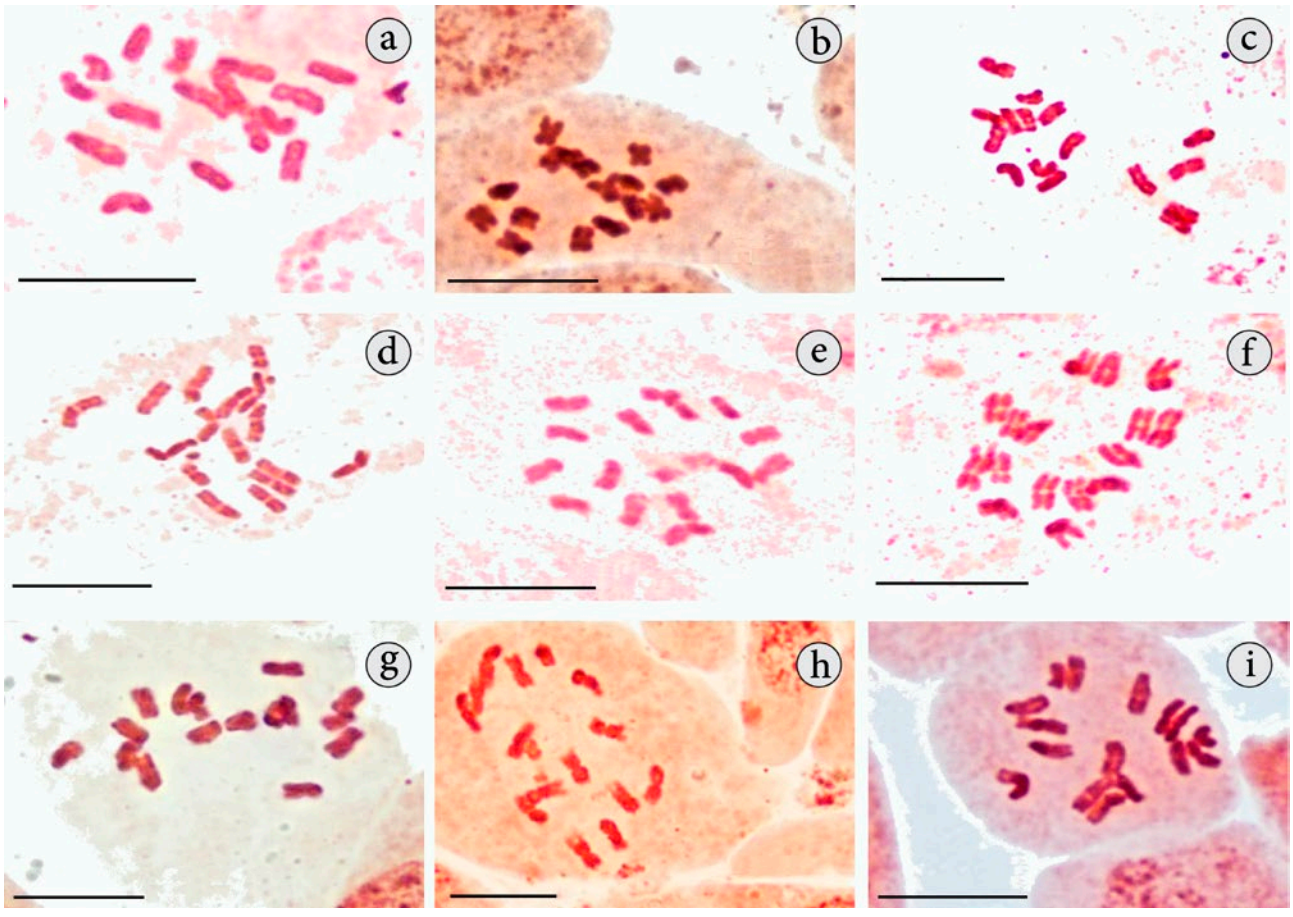

Figure 1. Photographs of somatic metaphase chromosomes of nine populations belonging to six species of the genus *Trigonella* collected from northeast of Iran. (a, b) *T. binaloudensis* from two locality; (c) *T. heratensis*; (d) *T. lasiocarpa*; (e) *T. stipitata;* (f, g, h) *T. subenervis* from three 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.

Species	2n	X	HCL (μm)	CL μm)	AR	RL%	CI	Karyotype formulae	Karyotypic asymmetry (Stebbins)
*T. binaloudensis** (1)	16	8	27.73	2.75-4.83	1.06-1.88	4.96-8.70	0.35-0.49	14m+2sm	1A
*T. binaloudensis** (2)	16	8	24.03	2.15-3.72	1.03-1.82	4.48-7.74	0.35-0.49	14m+2sm	1A
T. heratensis	16	8	22.98	2.46-3.40	1.02-1.69	5.36-7.39	0.37-0.50	16m	1A
*T. lasiocarpa**	16	8	28.90	2.41-4.44	1.09-1.51	4.17-7.68	0.40-0.48	16m	1A
*T. stipitata**	16	8	19.17	1.92-3.13	1.02-1.44	5.00-8.15	0.41-0.49	16m	1A
*T. subenervis** (1)	16	8	19.87	1.94-3.00	1.01-1.94	4.88-7.54	0.34-0.50	14m+2sm	1A
*T. subenervis** (2)	16	8	20.68	2.03-3.04	1.01-1.52	4.91-7.34	0.40-0.50	16m	1A
*T. subenervis** (3)	16	8	28.04	2.67-4.18	1.01-1.44	4.75-7.45	0.41-0.50	16m	1A
*T. torbatejamensis**	16	8	26.77	2.42-4.50	1.05-2.21	4.51-8.41	0.31-0.49	14m+2sm	2A

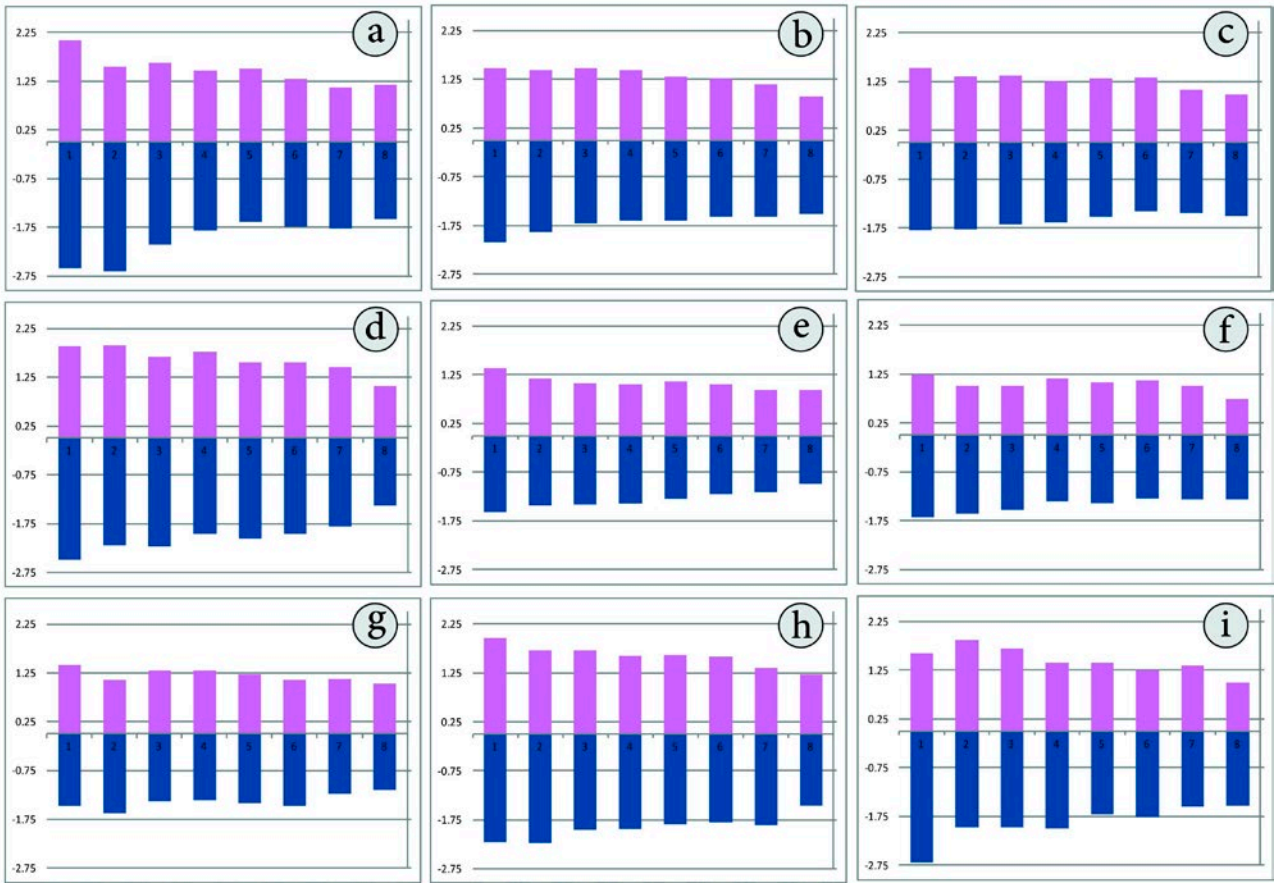

Figure 2. Haploid ideograms of *Trigonella* taxa. (a, b) *T. binaloudensis* from two locality; (c) *T. heratensis*; (d) *T. lasiocarpa*; (e) *T. stipitata;* (f, g, h) *T. subenervis* from three locality; (i) *T. torbatejamensis*. in all of studied taxa 2n=16.

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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