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Karyology of ten Turkish *Trigonella* L. (Leguminosae) species from section *Cylindricae* Boiss.

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Abstract: This study describes the karyotypes of 10 Turkish species of *Trigonella* L. belonging to section *Cylindricae* Boiss. (Leguminosae). Excluding the karyotypes of *T. velutina* Boiss. and of *T. sibthorpii* Boiss., which were previously studied, all of the other 8 species were studied here for the first time. The species of the section were gathered from different localities in Turkey, and it was determined that the somatic chromosome numbers for all species were the same ($2n = 16$). Also, satellite metaphase chromosomes were determined in *T. velutina* and *T. strangulata*.

Key words: Fabaceae, Image Analysis System, karyotype

Türkiye'de *Cylindricae* Boiss. seksiyonundan on *Trigonella* L. (Leguminosae) türünün karyolojisi

Özet: Bu çalışmada, Türkiye'deki *Cylindricae* Boiss. seksiyonundaki on *Trigonella* L. (Leguminosae) türünün karyotipi belirlenmiştir. *Trigonella velutina* Boiss. ve *T. sibthorpii* Boiss. türleri hariç diğer sekiz tür ilk kez çalışılmıştır. Seksiyona ait türler Türkiye'nin farklı lokalitelerinden toplanmış ve somatik kromozom sayıları bütün türlerde aynı ($2n = 16$) elde edilmiştir. Ayrıca, *T. velutina* ve *T. strangulata*'da satellitli metafaz kromozomları tespit edilmiştir.

Anahtar sözcükler: Fabaceae, Görüntü Analiz Sistemi, karyotip

Introduction

Trigonella L. (Leguminosae) includes about 135 species worldwide, and most of the species are distributed in the dry regions around the Mediterranean, western Asia, Europe, northern and southern Africa, and North America, with only 2 species present in southern Australia (Mabberly, 1997).

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Genus *Trigonella* has 50 taxa divided into 13 sections in Turkey (Huber-Morath, 1970). *Trigonella* taxa are localised in different phytogeographical regions in Turkey with 21 endemic species and a 42% endemism rate (Huber-Morath et al., 1970; Akan et al., 2005). After section *Bucerates* Boiss., *Cylindricae* Boiss. is the second largest section of genus *Trigonella* in Turkey. It is mainly distributed in the Irano-Turanian and eastern Mediterranean phytogeographic regions. Only 2 species, *T. kotschyi* Fenzl and *T. cilicica* Hub.-Mor., are endemic to Turkey. The characteristics of section *Cylindricae* are: annual species with stipules entire, keel and wings not joined, legumes deflexed, with distinct beak, sutures indistinct.

According to the literature, some studies have been carried out on the cytogenetics of *Trigonella* species (Darlington & Wylie, 1955; Tutin & Heywood, 1964; Ghosh, 1980; Astanova, 1981; Agarwal & Gupta, 1983; Ladizinsky & Vosa, 1986; Danin & Small, 1989; Bidak & Amin, 1996; Pavlova, 1996; Martin et al., 2006, 2008; Yılmaz et al., 2009).

To contribute to the karyological study of the genus, we carried out a karyological study on species of *Trigonella* belonging to section *Cylindricae* collected from different regions of Turkey.

Materials and methods

Seed materials from Turkey were collected between 2002 and 2006. Vouchers were deposited at the Gazi University Herbarium (GAZI) and the Biology Department of Harran University, Turkey. Root tips were obtained from surface-sterilised seeds germinated for 2 days on petri dishes, pretreated with α-monobromonaphthalene for 16 h at 4 °C, fixed in 3:1 ethanol:glacial acetic acid for 24 h, and stored at 4 °C. They were washed in distilled water to remove the fixative, hydrolysed in 1N HCl for 13 min at room temperature, and stained with 2% aceto-orcein for 2 h. Permanent slides were made with the standard liquid nitrogen method, dried for 24 h at room temperature, and mounted in DePeX. Karyological studies were made from 5 randomly selected chromosomal spreads. The chromosome measurements were calculated with the Bs200Pro Image Analysis System. Chromosomes were classified using the nomenclature of Levan et al. (1964). The karyotype asymmetry index (AI) was calculated according to the formula proposed by Paszko (2006). Idiograms of each species were arranged in order of decreasing length.

Results

In this research, by studying the karyological features, it was determined that the species of section *Cylindricae* of genus *Trigonella* have $2n = 16$ chromosomes.

***Trigonella spruneriana* Boiss.**

C4 Konya: Konya-Akşehir, near Şaharen Village 22.v.2003, 1165 m, roadside, *Akan* 4753 & *Ekici*. Chromosomes 2 and 7 have median centromeres (m-type); chromosomes 1, 3, 4, 5, 6, and 8 have submedian (sm) centromeres (Figure 1). The total haploid chromosome length is 15.29 μm and the chromosome length ranges from 1.57 to 2.29 μm.

***Trigonella sibthorpii* Boiss.**

C3 Antalya: Serik, Kumköy, 22. v. 2002, 5 m, sands, *Akan* 3307 *Aytaç* & *Ekici*; C5 Mersin: Mersin-Viranşehir, 21.v.2002, 10 m, sands, *Akan* 3299 *Aytaç* & *Ekici*.

Antalya population: Chromosomes 1, 3, 4, 5, 6, 7, and 8 have median centromeres (m-type); chromosome 2 has a submedian (sm) centromere (Figure 2). The total haploid chromosome length is 13.15 μm and the chromosome length ranges from 1.23 to 2.15 μm.

***Mersin population*:** Chromosomes 1, 2, 3, 5, 6, 7, and 8 have median centromeres (m-type); chromosome 4 has a submedian (sm) centromere (Figure 3). The total haploid chromosome length is 12.83 μm and the chromosome length ranges from 1.18 to 2.17 μm.

***Trigonella kotschyi* Fenzl**

C4 İçel: İçel-Pozantı, between Ulukışla-Çiftehan, 21.v.2003, stony mountain skirts, *Akan* 4769 & *Ekici*. Chromosomes 1, 3, 4, 6, and 7 have median centromeres (m-type); chromosomes 2, 5, and 8 have submedian (sm) centromeres (Figure 4). The total haploid chromosome length is 16.30 μm and the chromosome length ranges from 1.63 to 2.65 μm.

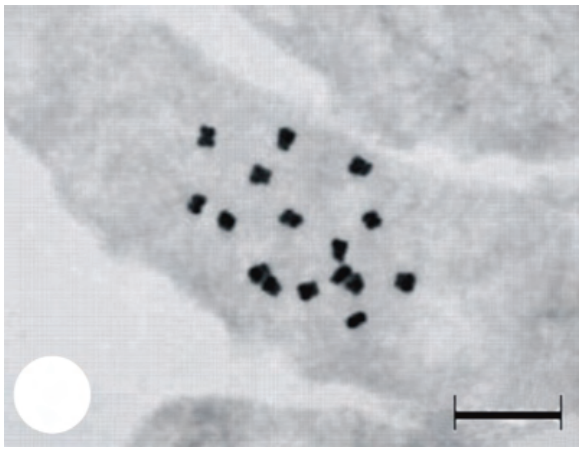

Figure 1. *Trigonella spruneriana* (scale bar: 10 µm).

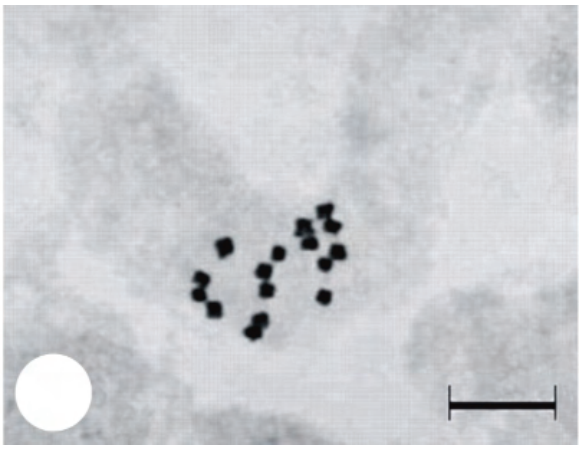

Figure 2. *Trigonella sibthorpii* (Antalya) (scale bar: 10 µm).

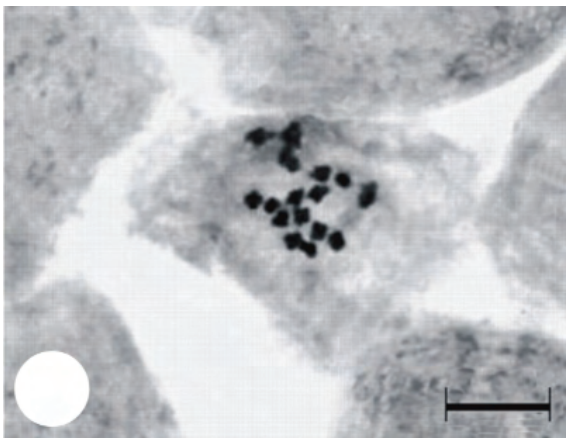

Figure 3. *Trigonella sibthorpii* (Mersin) (scale bar: 10 µm).

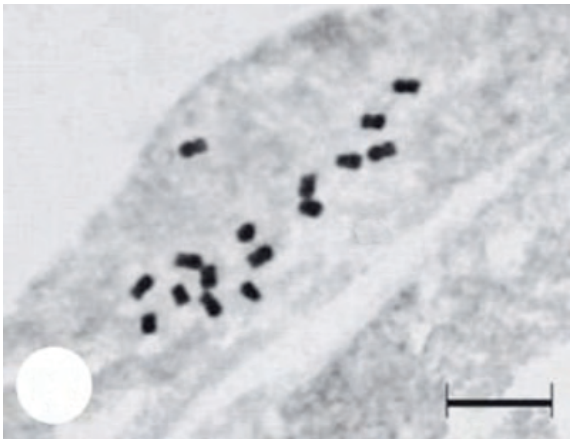

Figure 4. *Trigonella kotschyi* (scale bar: 10 µm).

***Trigonella mesopotamica* Hub.-Mor.**

C7 Şanlıurfa: Şanlıurfa-Suruç, Payamlı, 20.v.2003, 600-750 m, steppe, *Akan* 4577 & *Ekici*. Chromosome 3 is m-type. Chromosomes 1, 2, 4, 5, 6, 7, and 8 have median centromeres (m-type) (Figure 5). The total haploid chromosome length is 15.63 µm and the chromosome length ranges from 1.46 to 2.30 µm.

***Trigonella cylindracea* Desv.**

C3 Antalya: Antalya-Akseki, 10. km, 19.iv.2002, 450 m, roadside, *Akan* 2843 & *Ekici*. Chromosomes 1, 2, 3, 6, and 8 have median centromeres (m-type); chromosomes 4, 5, and 7 have submedian (sm) centromeres (Figure 6). The total haploid chromosome length is 22.01 µm and the chromosome length ranges from 2.23 to 3.52 µm.

***Trigonella cilicica* Hub.-Mor.**

C6 Adana: Şekerpınar-Ulukışla, 1. km, 28.vi.2005, steppe, foots of mountains, *Akan* 5739 & *Ekici*. Chromosomes 1, 4, 5, 7, and 8 have median centromeres (m-type); chromosomes 2, 3, and 6 have submedian (sm) centromeres (Figure 7). The total haploid chromosome length is 20.20 µm and the chromosome length ranges from 1.58 to 3.28 µm.

***Trigonella filipes* Boiss.**

C7 Şanlıurfa: Şanlıurfa-Suruç, 20. km, 18.v.2002, 550-600 m, roadside, *Akan* 3200 & *Ekici*. Chromosomes 1, 2, 3, 5, 6, and 7 have median centromeres (m-type); chromosomes 4 and 8 have submedian (sm) centromeres (Figure 8). The total haploid chromosome length is 10.51 µm and the chromosome length ranges from 1.04 to 1.49 µm.

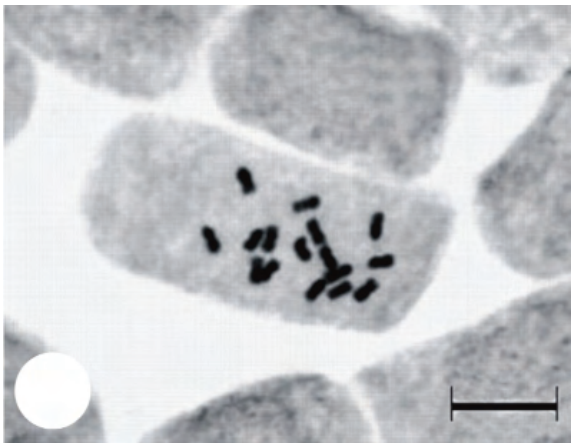

Figure 5. *Trigonella mesopotamica* (scale bar: 10 μm).

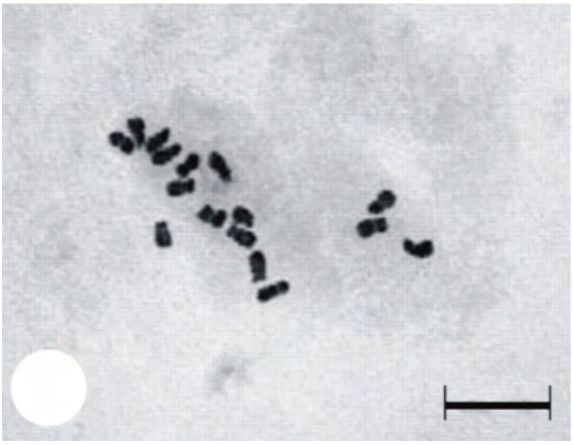

Figure 6. *Trigonella cylindracea* (scale bar: 10 μm).

Figure 7. *Trigonella cilicica* (scale bar: 10 μm).

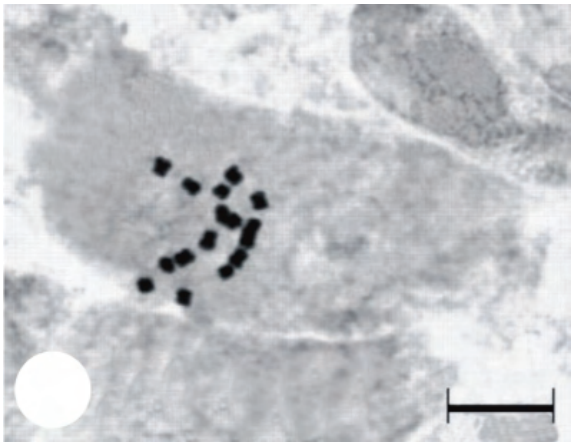

Figure 8. *Trigonella filipes* (scale bar: 10 μm).

***Trigonella velutina* Boiss.**

C4 Karaman: Hadim-Konya, 17.vii.2002, 1350 m, roadside, *Akan* 3790 *Aytaç* & *Ekici*. Chromosomes 1, 2, 3, 4, 5, 7, and 8 have median centromeres (m-type); chromosome 6 has submedian (sm) centromeres (Figure 9). The total haploid chromosome length is 10.23 μm and the chromosome length ranges from 1.03 to 1.41 μm. Chromosome 1 has secondary constrictions.

***Trigonella strangulata* Boiss.**

C5 Mersin: Tarsus-Egemen, 21.v.2002, 870 m, stony places, *Akan* 3289 & *Ekici*; C3 Antalya: Akseki-Manavgat, 7-8 km, 30.vi.2005, 935 m, stony mountain skirts, *Akan* 5758 & *Ekici*.

Antalya population: Chromosomes 1, 2, 4, 5, 7, and 8 have median centromeres (m-type); chromosomes 3 and 6 have submedian (sm) centromeres (Figure 10). The total haploid chromosome length is 10.26 μm and the chromosome length ranges from 1.08 to 1.58 μm.

Mersin population: Chromosomes 1, 2, 3, 4, 5, 6, 7, and 8 have median centromeres (m-type) (Figure 11). The total haploid chromosome length is 14.43 μm and the chromosome length ranges from 1.20 to 2.52 μm. Chromosomes 2 and 3 have secondary constrictions.

***Trigonella smyrnea* Boiss.**

C2 Burdur: Gölhisar-Altınyayla, 12. km, 29.vi.2005, 1275 m, forest, *Akan* 5756 & *Ekici*. Chromosomes 1, 2, 3, 4, 5, 6, 7, and 8 have median centromeres (m-type) (Figure 12). The total haploid chromosome length is 11.25 μm and the chromosome length ranges from 1.03 to 1.80 μm.

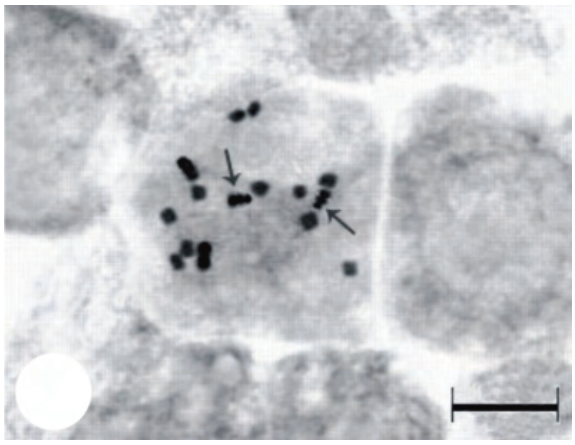

Figure 9. *Trigonella velutina*; arrowheads indicate satellites (scale bar: 10 µm).

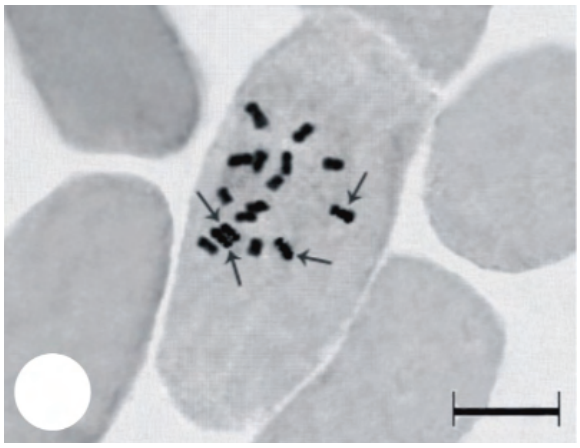

Figure 10. *Trigonella strangulata* (Mersin); arrowheads indicate satellites (scale bar: 10 µm).

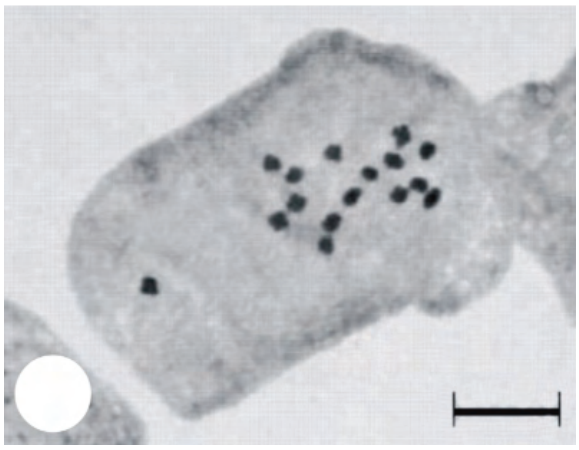

Figure 11. *Trigonella strangulata* (Antalya) (scale bar: 10 µm).

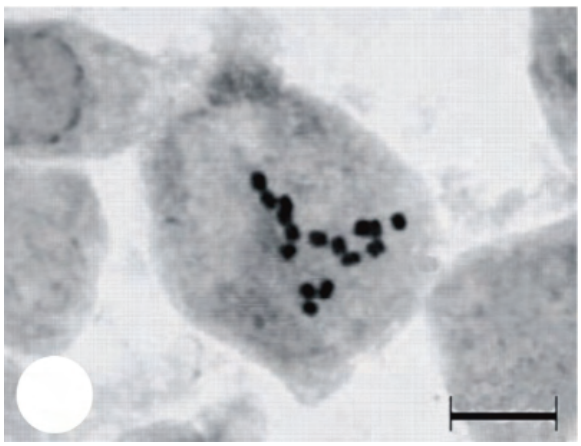

Figure 12. *Trigonella smyrnea* (scale bar: 10 µm).

Idiograms of each species were arranged in order of decreasing length (Figures 13-22).

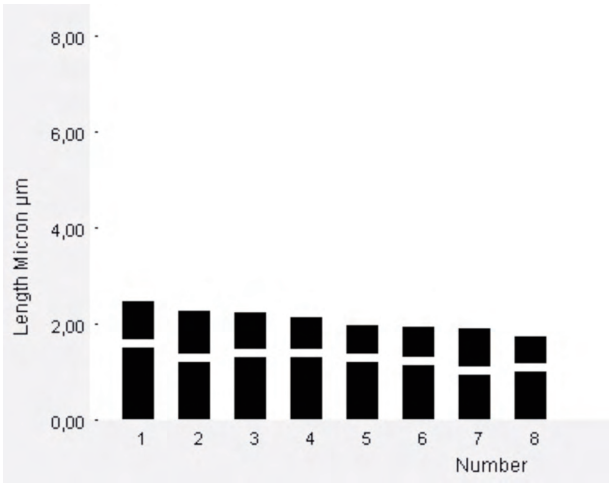

Figure 13. Idiograms of *Trigonella spruneriana*.

Discussion

In the present paper, the chromosome number and chromosome morphology of 8 species are reported for the first time: *Trigonella spruneriana*, *T. kotschyi*, *T. mesopotamica*, *T. cylindracea*, *T. cilicica*, *T. filipes*, *T. strangulata*, and *T. smyrnea*. The somatic chromosome count was $2n = 16$ in all species.

Trigonella velutina and *T. smyrnea* are the species with the shortest chromosome length (1.03 µm) among the species of section *Cylindricae* that were studied. *T. cylindracea* has the longest chromosome

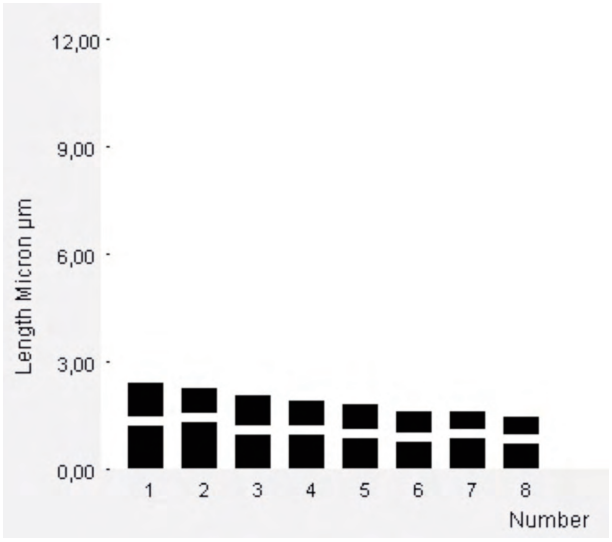

Figure 14a. Idiograms of *Trigonella sibthorpii* (Antalya).

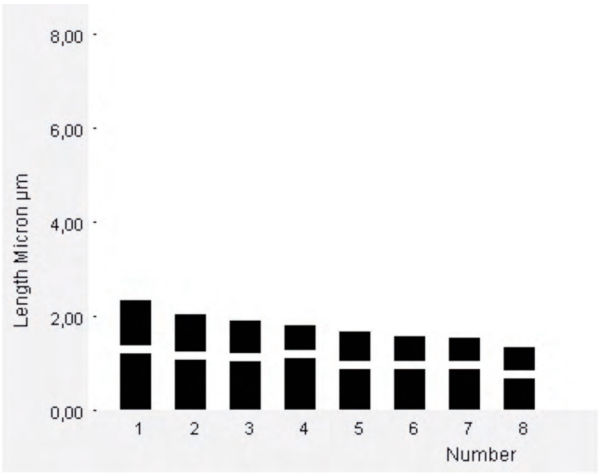

Figure 14b. Idiograms of *Trigonella sibthorpii* (Mersin).

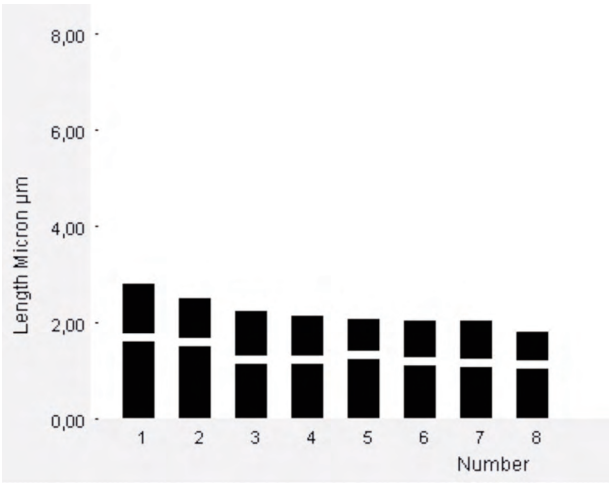

Figure 15. Idiograms of *Trigonella kotschyi*.

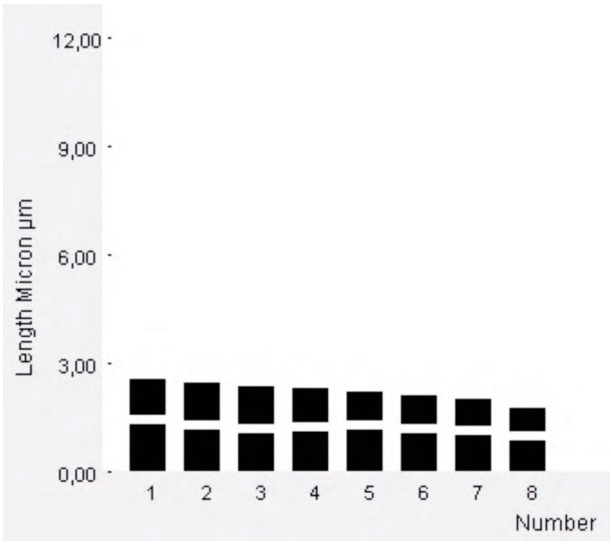

Figure 16. Idiograms of *Trigonella mesopotamica*.

length (3.52 µm) and the longest total haploid chromosome length (22.01 µm) of all the species. *T. velutina* has the shortest total haploid chromosome length at 10.23 µm. Satellite chromosome pairs were observed in 2 species of the section, *T. velutina* and *T. strangulata*.

Cytological investigations conducted on species of genus *Trigonella* showed that chromosome numbers in these taxa were $2n$ = 14, 16, 18, 28, 30, 32, 46, and 48 (Darlington & Wylie, 1955; Tutin & Heywood, 1964; Ghosh, 1980; Astanova, 1981; Agarwal & Gupta, 1983; Ladizinsky & Vosa, 1986; Danin & Small, 1989; Bidak & Amin, 1996; Pavlova, 1996; Martin et al., 2006, 2008; Yılmaz, et al., 2009).

The somatic chromosome number $2n$ = 16 for species *Trigonella velutina* and *T. sibthorpii* Boiss. was reported by Yılmaz et al. (2009) and Danin & Small (1989).

Trigonella spruneriana is one of the very widespread species among the species of the genus in

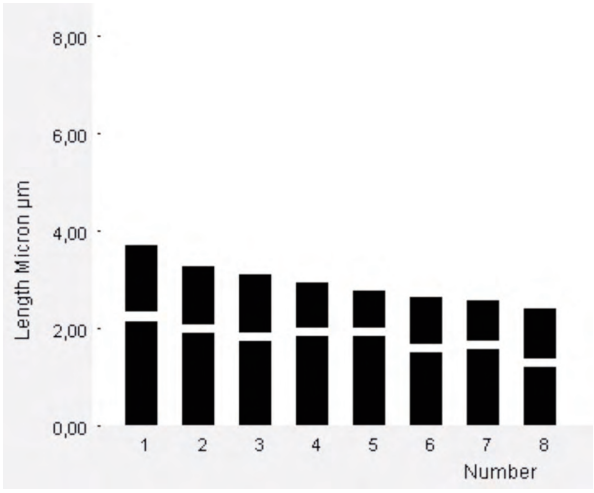

Figure 17. Idiograms of *Trigonella cylindracea*.

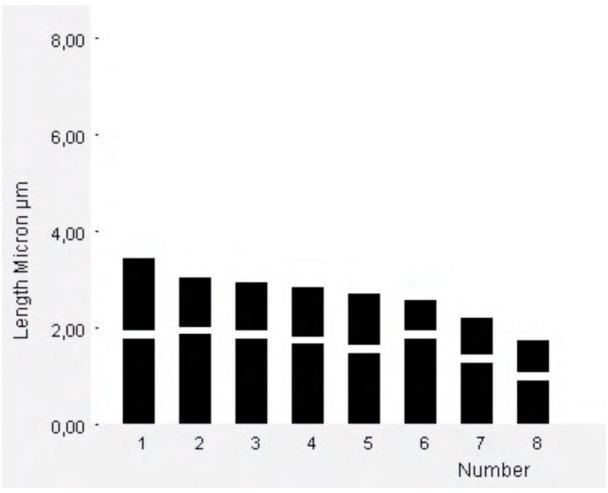

Figure 18. Idiograms of *Trigonella cilicica*.

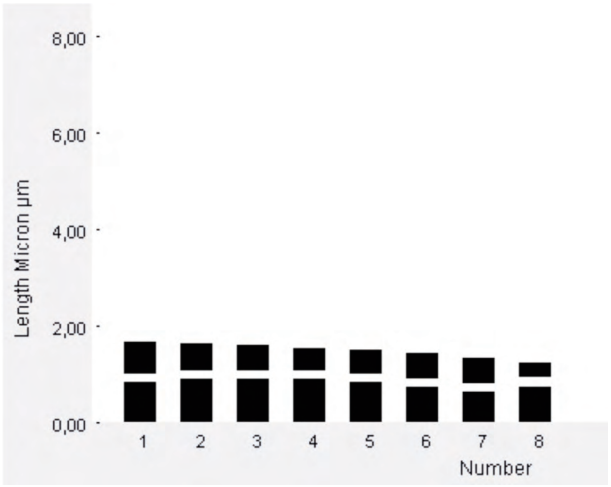

Figure 19. Idiograms of *Trigonella filipes*.

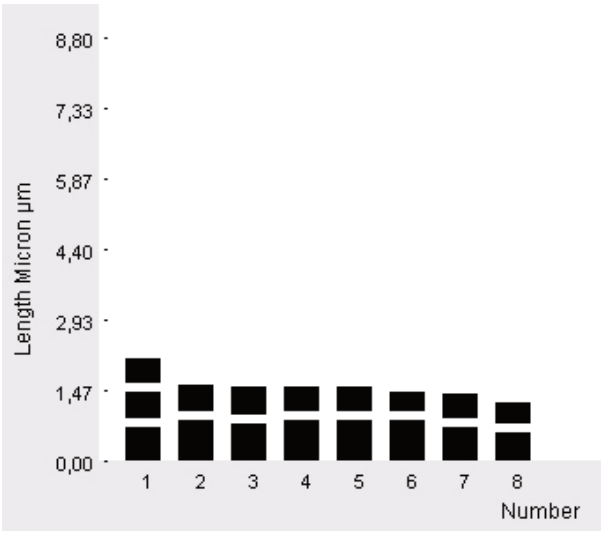

Figure 20. Idiograms of *Trigonella velutina*.

Turkey. In *Flora of Turkey*, *T. spruneriana* is represented by 2 varieties, but *T. spruneriana* Boiss. var. *sibthorpii* (Boiss.) Hub-Mor. was reevaluated as a distinct species. The karyological findings of this study support that var. *sibthorpii* (Boiss.) Hub-Mor. should be reevaluated as a distinct species, with the new name of *T. sibthorpii* Boiss. In this study, *T. sibthorpii* was studied from 2 different localities. The diploid chromosome numbers ($2n$ = 16) were the same in both populations. The metaphase chromosome length of the specimens from Antalya ranged between 1.23 and 2.15 µm, while that from Mersin ranged between 1.18 and 2.17 µm. The karyotype formula, consisting of 7 m + 1 sm, was the same in both populations. In this situation, different chromosome lengths were detected in the same species from different localities.

Trigonella kotschyi is one of the white-flowering species. It is very rare in other parts of the world for the species of *Trigonella* to have white flowers, but in Turkey, 2 species, *T. kotschyi* and *T. cilicica*, have white flowers (Huber-Morath, 1970). Both of them are endemic to Turkey. Both species have a karyotype formula with 5 median and 3 submedian chromosomes, as with most other species.

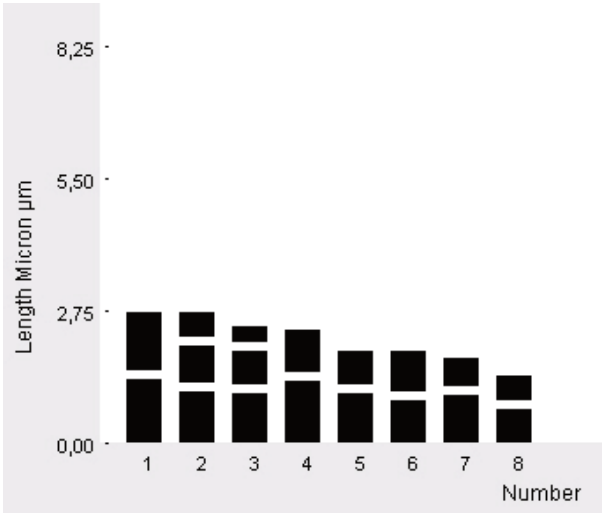

Figure 21a. Idiograms of *Trigonella strangulata* (Mersin).

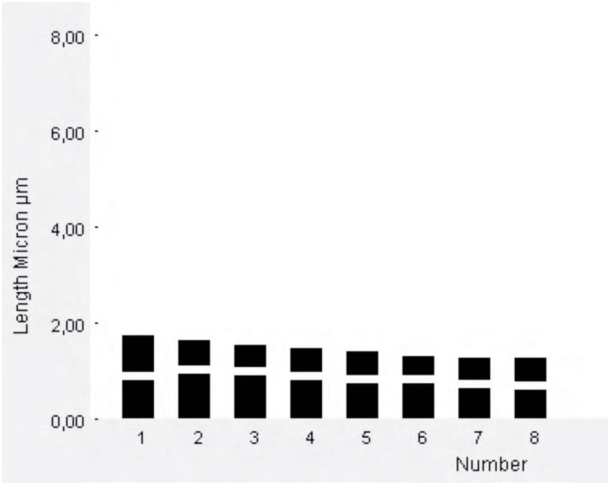

Figure 21b. Idiograms of *Trigonella strangulata* (Antalya).

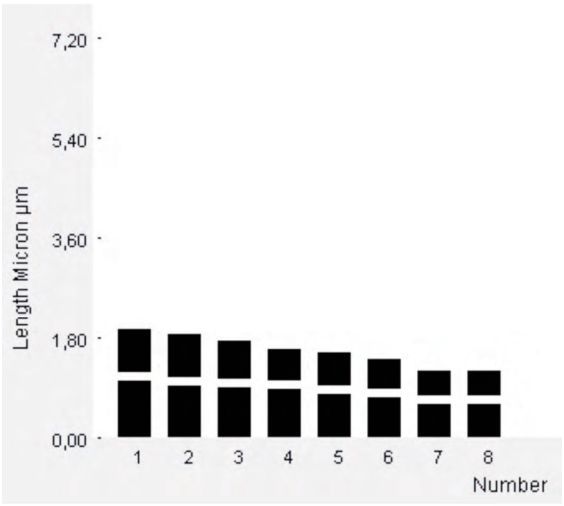

Figure 22. Idiograms of *Trigonella smyrnea*.

Trigonella cylindracea has a distribution in mainly saline and sandy habitats. The chromosome morphology shows some differences from the other species of the section. The karyotype formula consists of 5 median chromosomes and 3 subterminal chromosomes. Among the section's species, it has the longest chromosome length and the longest total haploid chromosome length.

Trigonella cilicica is one of the critically endangered (CR) endemic species of genus *Trigonella* in Turkey. The most closely related species is *T. filipes*. However, *T. cilicica* has white and few flowers, and the length of its peduncle is shorter than in *T. filipes* (Huber-Morath, 1970). *T. cilicica* has the longest arm ratio (2.82 µm) and the shortest relative length (7.82) among section *Cylindricae* species. *T. filipes* has the shortest centromeric index of this section, at 2.76.

Trigonella velutina is one of the widespread species of *Trigonella* in Turkey. It is densely villous and can be easily distinguished morphologically. It has the shortest total haploid chromosome length (10.23 µm) of the section. It is 1 of the 2 species that showed satellite chromosomes, in this case in 1 chromosome. Our determination of $2n = 16$ for this species confirms a previous study on the species from Turkey by Yılmaz et al. (2009). The satellite chromosomes were also previously reported by Yılmaz et al. (2009). In both studies, the collection locality was the same, and our findings support the previous study.

Trigonella strangulata is one of the widespread species of *Trigonella* in Turkey. The legume of the species is strangulated, one of the distinguishing characteristics of the species. It is closely related to *T. smyrnea,* but its legume length is longer than that of *T. smyrnea*, at a maximum of 10 mm (Huber-Morath, 1970). *T. strangulata* specimens from 2 different localities, Mersin and Antalya, were studied. The Mersin karyotype is formed by 8 pairs of median chromosomes, 2 of which have satellites. The Antalya karyotype, meanwhile, is formed by 6 pairs of median chromosomes and 2 pairs of submedian chromosomes. A satellite was not detected in the specimens from Antalya.

Trigonella smyrnea has a narrow distribution in western and southwestern Anatolia, and it is one of the rare endemic flora species of Turkey. It has a very rare natural population, and the threatened (VU) category was suggested for it by *Akan* et al. (2005). Morphologically, it is very close to *T. strangulata*. The karyotype formula of the specimens from Antalya is the same as that of *T. smyrnea* (2*n* = 16). The chromosomes of these 2 species are fairly close to each other. These findings are in agreement with the chromosome numbers given for section *Cylindricae* species in previous research (Darlington & Wylie, 1955; Ladizinsky & Vosa, 1986; Bidak & Amin, 1996; Yılmaz et al., 2009).

Species of genus *Trigonella* and particularly of the cultivated species *T. foenum-graecum* (fenugreek) were known and used for different purposes in ancient times, especially in Greece and Egypt (Petropoulos, 2002). In northern Africa, it has been cultivated around the Saharan oases since very early times (Duke, 1986). Cytogenetic studies of the genus have been limited to chromosome counts and preliminary karyotype descriptions of the cultivated species and a few of the wild related species (Singh & Roy, 1970; Singh & Singh, 1976a; Wanjari, 1976; Ladizinsky & Porath, 1977; Lavania & Sharma, 1980; Agarwal & Gupta, 1983; Ladizinsky & Vosa, 1986). A few euploid and aneuploid cytogenetic stocks, primarily in the form of tetraploids, triploids, and simple primary trisomics, have also been reported (Gopinath, 1974; Singh & Singh, 1976b, 1976c; Raghuvanshi & Singh, 1977). The relatively small number (2*n* = 16) of chromosomes in the chromosome complement of fenugreek makes it a suitable candidate for cytogenetic studies. Fenugreek, an annual legume crop grown mainly for its seeds at present, has the potential to be a high-quality forage crop in western Canada. A cytological survey of germplasm stored at the USDA germplasm center demonstrated a somatic karyotype with a chromosome number of 2*n* = 16. Structural details of 2 pairs of chromosomes (numbers 1 and 2) showed secondary constrictions that were presumed to be the site of rRNA genes. Fluorescent in situ hybridisation (FISH) with a heterologous rRNA gene probe from wheat was utilised to physically map their location for the first time on fenugreek chromosomes (Ahmad et al., 1999). The precise origin of the 2 satellited chromosome pairs in fenugreek is not known, but the involvement of 2 species/cytotype hybridisation could be speculated. This in turn could have been followed by a lack of amphiplasty (suppression of NOR activity), resulting in transcriptional activity of rRNA genes at both of these loci. If this speculative suggestion is at all feasible, then the polyploidisation event during the evolution of *Trigonella* species must have taken 2 different evolutionary lineages, since other *Trigonella* species with fenugreek-like seeds tend to show 2 pairs of satellited chromosomes, while still other species with little or no seed morphology resemblance show only 1 pair of satellited chromosomes (Ahmad et al., 1999). Further research on these lines could prove helpful in understanding evolution in genus *Trigonella*.

Reasat et al. (2003) reported the chromosome numbers and morphologies of *Trigonella elliptica*, *T. foenum-graecum*, *T. spruneriana*, *T. monspeliaca*, *T. uncata*, *T. anguina*, *T. stellata*, and *T. astroites*. All of them were diploid with 2*n* = 16. Their findings are similar to the findings in our study.

In the somatic cells of 2 species, *Trigonella arcuata* C.A.Mey. and *T. procumbens* (Besser) Rchb., 2B chromosomes were reported previously (Martin et al., 2006). The presence of B chromosomes in genus *Trigonella* is unusual, because there is little evidence of this in the literature. The B chromosome was not observed in species of section *Cylindricae*.

The factors endangering living creatures, such as destruction of natural habitats, field clearances, tourism, dams, and irrigation system construction, affects the taxa of *Trigonella*, as well. Therefore, our biological treasures, especially endemic plant species, are endangered. Thus it is an obligation to protect them, ex situ or in situ (Martin et al., 2008).

Karyotype analyses have been conducted in recent years using similar computer programs with different names. The Image Analysis System plays a vital role in the minimisation of errors in karyotype analyses. Moreover, the use of the Image Analysis System, compared to karyotype analyses that are performed using scales and compasses, has 3 chief advantages. First, the preparation of karyotypes takes less time. Second, it is more practical in the measurement of chromosomes. Third, the karyograms and ideograms

are prepared automatically (Martin et al., 2009; Dirmenci et al., 2010; Hamzaoğlu et al., 2010).

The results of the present and previous karyological studies reveal that the chromosome morphologies of different species of section *Cylindricae* differ. The karyotype formulae of species collected from different localities may be different. These karyological diversions may be a result of infrageneric and infraspecific variations, as well as ecological, climatological, geographic, and edaphic variations.

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