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KARYOLOGY OF SEVEN *TRIFOLIUM* L. TAXA GROWING IN TURKEY

YASAR KIRAN[1], AHMET SAHIN[2], ISMAIL TÜRKOĞLU[3], MURAT KURSAT[4], AND IFRAN EMRE[5*]

[1]*Department of Biology, Firat University, 23169, Elazig, Turkey*
[2]*Department of Secondary Science and Mathemathics Education, Erciyes University, 38039, Kayseri, Turkey*
[3]*Department of Secondary Science and Mathemathics Education, Firat University, 23169, Elazig, Turkey*
[4]*Department of Biology, Bitlis Eren University, 13000, Bitlis, Turkey*
[5]*Department of Primary Education, Firat University, 23169, Elazig, Turkey*

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The paper reports karyomorphometric features and somatic chromosome numbers for seven *Trifolium* L. taxa. Three basic chromosome numbers are identified (x=5, 7, 8). *Trifolium speciosum* Willd. and *Trifolium physodes* Stev. ex Bieb. var. *physodes* have somatic chromosome number 2n=16; *Trifolium campestre* Schreb., *Trifolium pratense* L. var. *americanum* Harz and *Trifolium sylvaticum* Gerard ex Lois. have 2n=14; *Trifolium bocconei* Savi has 2n=10; and *Trifolium repens* L. var. *repens* is tetraploid 2n=32. All chromosomes have the centromeres at the median point (M) or median region (m) except for one chromosome of *Trifolium sylvaticum* Gerard ex Lois, which is submetacentric (sm). The only satellite observed was in the genome of *Trifolium bocconei* Savi.

Key words: Chromosome numbers, karyotype, *Trifolium* L.

INTRODUCTION

The Leguminosae are the third largest family of flowering plants, with 727 genera and 19,325 species given (Lewis et al., 2005; Khatoon and Ali, 2006). *Trifolium* L. is one of the largest genera of the Leguminosae, comprising more than 250 species belonging to the tribe *Trifolieae*, of which ten are of considerable agricultural importance (Vizintin et al., 2006; Abberton, 2007). The genus name refers to the distinctive leaves, usually consisting of three leaflets (Salimpour et al., 2008). *Trifolium* originates in the Mediterranean area, Europe and the mountainous regions of Africa and Central, South and North America (Lange and Schifino-Wittman, 2000). The species of *Trifolium* are divided into eight sections: *Lotoidea, Paramesus, Mystillus, Vesicaria, Chronosemium, Trifolium, Tricocephalum* and *Involucrarium* (Lange and Schifino-Wittmann, 2000; Vizintin et al., 2006). All members of *Trifolium* are herbaceous perennials or annuals, frequently prostrate and rarely more than 50 cm in height (Ellison et al., 2006). In the flora of Turkey, *Trifolium* is represented by 118 taxa including 96 species, 22 subspecies and varieties (Davis, 1970; Davis et al., 1988).

Eighty percent of *Trifolium* species are diploid 2n=16, and the basic chromosome number in the majority of examined species is x=8 (Goldblatt, 1981; Zohary and Heler, 1984; Ellison et al., 2006). However, the basic chromosome numbers in *Trifolium* form an aneuploid series of x=5,6 and 7 (Zohary and Heler, 1984; Vizintin et al., 2006). Several studies reported additional chromosome numbers 11, 12 and 13 (Vizintin et al., 2006). Less than 20% of *Trifolium* species are polyploids and show ploidy variation (Ansari et al., 1999; Majumdar et al., 2004). In this study we examined chromosome morphology and determined chromosome number for seven *Trifolium* species growing in Turkey.

MATERIALS AND METHODS

Trifolium specimens and seeds were collected from natural habitats in 2005 and 2006 and the seeds were used for karyological analysis. Voucher

e-mail: iemre@firat.edu.tr

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TABLE 1. Somatic chromosome number, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), asymmetry indexes (A_1, A_2) of Romero Zarco (1986) for the investigated *Trifolium* species

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	TKL (µm)	A_1	A_2
T. repens L.var. *repens*	32	4x	2M+14m	1.17–1.72	22.88	0.18	0.09
T. speciosum Willd.	16	2x	8m	0.99-1.64	9.77	0.22	0.16
T. campestre Schreb.	14	2x	1M+6m	1.13-1.73	9.79	0.15	0.14
T. physodes Stev. ex Bieb. var. *physodes*	16	2x	8m	1.48-2.10	14.19	0.20	0.13
T. pratense L.var. *americanum* Harz	14	2x	1M+6m	1.20-1.79	9.97	0.22	0.14
T. sylvaticum Gerard ex Lois.	14	2x	6m+1sm	1.13-1.81	9.44	0.32	0.17
T. bocconei Savi	10	2x	5m	1.53-2.31	972	0.20	0.17

specimens (voucher numbers 5001–5007) and seeds are stored in the Firat University Herbarium (FUH).

Seeds were germinated at 25°C on moist filter paper in petri dishes. Actively growing root tips 1.5–2 cm in length were excised from the germinating seeds and pretreated with aqueous colchicine (0.05%) for 3 h at room temperature, then fixed with acetic alcohol (1:3) for 24 h and stored in 70% alcohol at 2–4°C. Then the root tips were rinsed in water and hydrolyzed with 1 N HCl for 10–18 min at 60°C and rinsed in running water for a minimum of 3–5 min. Feulgen staining of root tips was carried out for 1–2 h and root tips were put in 1% aceto-orcein for 5–10 min to improve staining. Finally, squash preparations were made in 1% aceto-orcein (Elci, 1982; Bairiganjan and Patnaik, 1989; Sahin, 1993).

Micrographs of good quality metaphase plates were taken using an Olympus BX51 microscope and an Olympus Camedia C-4000 digital camera. The long arm, short arm and total length of each chromosome were measured and the relative lengths, arm ratios, and centromeric indices were determined. Chromosome nomenclature follows Levan et al. (1964). The intra-chromosomal asymmetry index (A_1) was calculated according to the formula given by Romero Zarco (1986), and the inter-chromosomal asymmetry index (A_2) was calculated as the ratio of chromosome length to mean chromosome length.

RESULTS AND DISCUSSION

Somatic chromosome numbers, ploidy level, karyotype formula, chromosome length ranges, total karyotype length (TKL), asymmetry index (A_1, A_2; Romero Zarco, 1986), karyomorphological parameters, metaphase chromosomes and haploid idiograms of the seven *Trifolium* species studied are presented in Tables 1 and 2 and Figures 1–8).

Trifolium repens L. *var. repens* (Tabs. 1, 2; Figs. 1, 8a) B7, Elaziğ, Baskil-Bolucuk village, 1580 m M. Kursat, 2006.

We found that the tetraploid number of the chromosomes for *T. repens var. repens* is 2n=4x=32 (x=8). The karyotype of this species consists of 2 chromosomes with the centromere at the median point (M) and 14 chromosomes with centromeres in the median region (m). No satellite was observed. Our results are compatible with the findings of many other studies (Zohary and Heller, 1984; Taylor, 1987; Schifino-Wittmann and Moraes-Fernandes, 1988; Khatoon and Ali, 1993; Ansari et al., 1999). Some previous studies reported 2n=16, 28 and 30 for *T. repens* (Williams and Verry, 1981; Pandey et al., 1987; Lavrenko et al., 1991).

Trifolium speciosum Willd. (Tabs. 1, 2; Figs. 2, 8b) B7 Elaziğ, Kamislik Mountain, 1260 m, I. Türkoğlu, 2006.

Its chromosome number was found to be 2n=16 (x=8). All chromosomes have the centromere in median regions (m). No satellite was observed. The same chromosome number was noted by El Baba (1980) and Petrova and Kozuharov (1982).

Trifolium campestre Schreb. (Tabs. 1, 2; Figs. 3, 8c) B7 Elaziğ, Kamislik Mountain, 1760 m, I. Türkoğlu, 2006.

The chromosome number was determined as 2n=14 (x=7). The karyotype of this species has 6 chromosomes with the centromere in the median region (m) and one with it at the median point (M). Satellites were not observed in the karyotype of this species. The same chromosome count was given by Lövkvist and Hultgård (1999), Vizintin et al. (2006) and Salimpour et al. (2008).

Trifolium physodes Stev. ex Bieb. var. *physodes* (Tabs. 1, 2; Figs. 4, 8d)
B7 Elaziğ, Baskil-Bolucuk village, 1480 m, M. Kursat, 2006.

The diploid number of the chromosomes was found to be 2n=16 (x=8). All chromosomes are metacentric

TABLE 2. Karyomorphological parameters of *Trifolium* species

Pair No.	RL	AR	CI	Type
T. repens var. *repens*				
I	1.72	1.32	43.02	m
II	1.64	1.56	39.02	m
III	1.56	1.16	46.15	m
IV	1.52	1.37	42.10	m
V	1.51	1.32	43.04	m
VI	1.49	1.29	43.62	m
VII	1.44	1.25	44.44	m
VIII	1.42	1.15	46.47	m
IX	1.41	1.31	43.26	m
X	1.40	1.00	50.00	M
XI	1.37	1.24	44.52	m
XII	1.35	1.36	42.22	m
XIII	1.33	1.18	45.86	m
XIV	1.31	1.14	46.56	m
XV	1.24	1.00	50.00	M
XVI	1.17	1.48	40.17	m
T. physodes var. physodes				
I	2.10	1.33	42.85	m
II	1.98	1.23	44.94	m
III	1.85	1.25	44.32	m
IV	1.80	1.09	47.77	m
V	1.79	1.20	45.25	m
VI	1.67	1.19	45.50	m
VII	1.52	1.41	41.44	m
VIII	1.48	1.34	42.56	m
T. pratense var. *americanum*				
I	1.79	1.35	42.45	m
II	1.55	1.50	40.00	m
III	1.47	1.40	41.49	m
IV	1.40	1.00	50.00	M
V	1.33	1.29	43.60	m
VI	1.23	1.32	43.08	m
VII	1.20	1.22	45.00	m

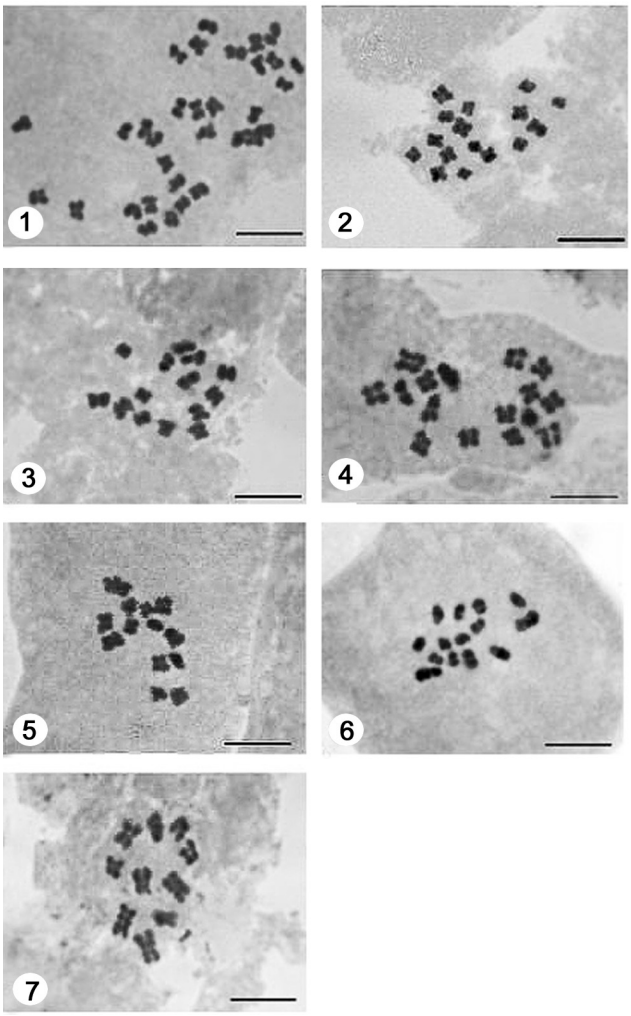
Pair No.	RL	AR	CI	Type
T. speciosum				
I	1.64	1.27	43.90	m
II	1.36	1.26	44.11	m
III	1.24	1.38	41.93	m
IV	1.20	1.22	45.00	m
V	1.15	1.30	43.47	m
VI	1.10	1.29	43.63	m
VII	1.09	1.18	45.87	m
VIII	0.99	1.30	43.43	m
T. campestre				
I	1.73	1.30	43.35	m
II	1.57	1.18	45.85	m
III	1.46	1.11	47.26	m
IV	1.34	1.18	49.25	m
V	1.30	1.00	50.00	M
VI	1.26	1.29	43.65	m
VII	1.13	1.26	44.24	m
T. sylvaticum				
I	1.81	1.58	38.67	m
II	1.47	1.67	37.41	m
III	1.37	1.24	44.52	m
IV	1.26	1.86	34.92	sm
V	1.21	1.46	40.49	m
VI	1.19	1.33	42.85	m
VII	1.13	1.30	43.36	m
T. bocconei				
I	2.31	1.26	44.15	m
II	2.27	1.45	29.07	m*
III	1.91	1.17	46.07	m
IV	1.70	1.32	42.94	m
V	1.53	1.15	46.40	m

*Chromosome with satellite. Abbreviations: RL – relative length; AR – arm ration (L/S); CI – centromeric index (100S/TL); m – median region; sm – submedian region; M – median point. Chromosome pairs are Roman-numbered.

(m) and no satellites were observed in the karyotype. Giri et al. (1981) and Munoz-Rodriguez (1993a) also gave 2n=16 for this taxon.

Trifolium pratense L. var. *americanum* Harz (Tabs. 1, 2; Figs. 5, 8e)
B7 Elaziğ, Kamislik Mountain, 1430 m, I. Türkoğlu, 2006.
The somatic chromosome count was found to be 2n=14 (x=7). The karyotype of this species consists of 6 chromosomes with the centromere in the median region (m) and one with it at the median point (M). Satellites were not observed in the karyotype of this taxon. The present results are in accord those of previous studies (Taylor, 1987; Nikolov, 1991; Muńoz-Rodriguez, 1993b; Vizintin et al., 2006; Abberton, 2007), except for Salimpour et al. (2008) who gave 2n=16 and Armstrong and Cleveland (1970) who gave 2n=28.

Trifolium sylvaticum Gerard ex Lois. (Tabs. 1, 2; Figs. 6, 8f)
B7 Elaziğ, Baskil-Bolucuk village, 1580 m, M. Kursat, 2006.
We found chromosome number 2n=14 (x=7) for this species. Its karyotype consists of 6 chromosomes with the centromere in median regions (m) and one submetacentric chromosome (sm). No satellite was observed. Muñoz-Rodríguez (1993b) noted the same chromosome number for this species.



Figs. 1–7. Metaphase chromosomes of *Trifolium* species. **Fig. 1.** *T. repens* var. *repens* (2n=32). **Fig. 2.** *T. speciosum* (2n=16). **Fig. 3.** *T. campestre* (2n=14). **Fig. 4.** *T. physodes* var. *physodes* (2n=16). **Fig. 5.** *T. pratense* var. *americanum* (2n=14). **Fig. 6.** *T. sylvaticum* (2n=14). **Fig. 7.** *T. bocconei* (2n=10). Bar=10 μm.

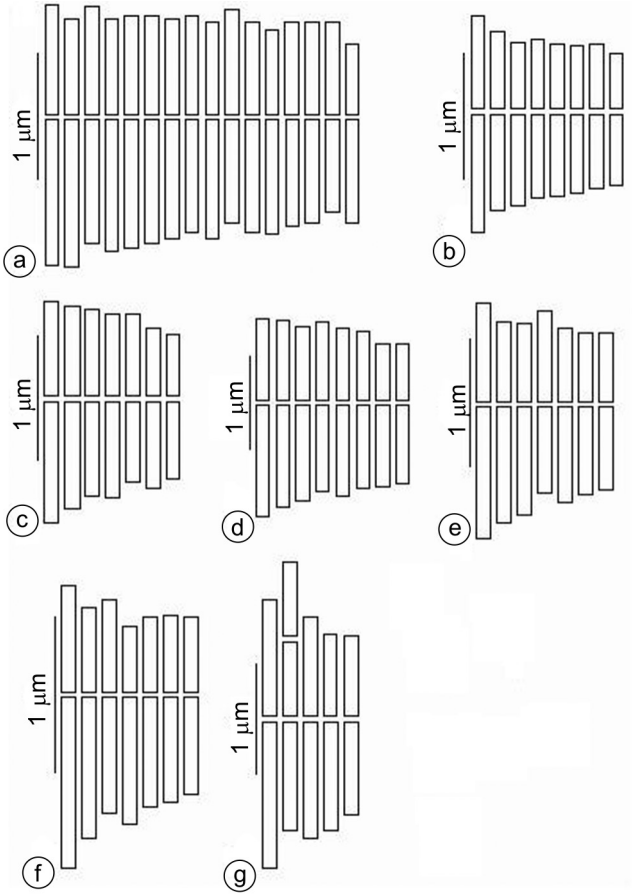


Fig. 8. Haploid idiograms of *Trifolium* taxa. **(a)** *T. repens* var. *repens* (2n=32), **(b)** *T. speciosum* (2n=16), **(c)** *T. campestre* (2n=14), **(d)** *T. physodes* var. *physodes* (2n=16), **(e)** *T. pratense* var. *americanum* (2n=14), **(f)** *T. sylvaticum* (2n=14), **(g)** *T. bocconei* (2n=10). Bar=10 μm.

Trifolium bocconei Savi (Tabs. 1, 2; Figs. 7, 8h)
B7, Elaziğ, Kayabeyli village, 1450 m, M. Kursat, 2006. We determined the chromosome number of this species as 2n=10 (x=5). All chromosomes are metacentric (m) and chromosome II has a satellite. Previous reports give two other chromosome numbers for *T. bocconei*: 2n=12 (Petrova and Kozuharov, 1982; Muñoz-Rodriquez, 1993b) and 2n=14 (Issolah and Abdelguerfi, 1999).

CONCLUSION

The chromosome numbers we found are generally in accord with previous results, except for *T. repens* var. *repens*, *T. pratense* var. *americanum* and *T. bocconei*. Some previous studies reported chromosome counts differing from our findings (Armstrong and Cleveland, 1970; Williams and Verry, 1981; Pandey et al., 1987; Lavrenko et al., 1991; Salimpour et al., 2008). We determined three different basic chromosome numbers (x=5, 7 and 8). *T. speciosum* and *T. physodes* have 2n=16 chromosomes. *T. campestre, T. pratense* var. *americanum* and *T. sylvaticum* have 2n=14. *T. bocconei* has 2n=10, and *T. repens* var. *repens* (tetraploid) has 2n=32. Previous studies suggest that two diploid species, *T. occidantale* (2n=16) and *T. nigrescens* (2n=16) and the tetraploid species *T. uniflorum* (2n=4x=32) are closely related to *T. repens* (Ansari et al., 1999; Jones et al., 2003). All studied chromosomes have the centromeres at the median point (M) and in the median region (m), except for the submetacentric (sm) chromosome of *T. sylvatica*. Only *T. bocconei* was found to have a satellite.

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