

Karyological notes on seven *Trifolium* L. taxa from Turkey

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Abstract

Three basic chromosome counts (x:5, 7 and 8) were determined in this study. It was found that *T. resupinatum* L. var. *resupinatum*, *T. stellatum* L. var. *stellatum* and *T. purpureum* Lois. var. *purpureum* have 2n: 16; *T. arvense* var. *arvense*, *T. haussknechtii* Boiss. var. *haussknechtii* and *T. pauciflorum* d'Urv. have 2n:14; *T. hirtum* has 2n:10 chromosome numbers according to the present results. All chromosomes have median point (M), median region (m) centromeres and satellites were not observed in the studied *Trifolium* taxa. The chromosome number and morphology of *T. haussknechtii* Boiss. var. *haussknechtii* and *T. pauciflorum* d'Urv. are reported for the first time.

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

Türkiyedeki Yedi Türkiyedeki Yedi *Trifolium* L. Üzerine Karyolojik Notlar

Özet

Bu çalışmada üç temel kromozom sayısı belirlendi (x:5,7 ve 8). Mevcut bu çalışmaya göre *T. resupinatum* L. var. *resupinatum*, *T. stellatum* L. var. *stellatum* ve *T. purpureum* Lois. var. *purpureum* 2n: 16; *T. arvense* var. *arvense*, *T. haussknechtii* Boiss. var. *haussknechtii* ve *T. pauciflorum* d'Urv. 2n:14; ve *T. hirtum* 2n:10 kromozom sayısına sahiptir. Çalışılan *Trifolium* türlerinde bütün kromozomlar, median noktalı (M), median (m) olarak bulundu ve satellit gözlenmedi. *T. haussknechtii* Boiss. var. *haussknechtii* ve *T. pauciflorum* d'Urv.'nin kromozom sayıları ve morfolojileri ilk kez rapor edilmiştir.

Anahtar Kelimeler: Karyotip, Kromozom sayısı, *Trifolium* L.

1. Introduction

The genus *Trifolium* L. belongs to the tribe *Trifolieae* (*Fabaceae* family), and contains approximately 250 species with a wide distribution and adaptation to different agro-ecological regions [1-3]. Approximately 250 species and 80 infraspecific taxa are separated into eight sections, namely *Lotoidea*, *Paramesus*, *Mystillus*, *Vesicaria*, *Chronosemium*, *Trifolium*, *Tricocephalum* and *Incolucrarium* [4]. (Lange and Schifino-Wittmann 2000). In the Flora of Turkey, *Trifolium* is represented by 118 taxa, including 96 species, 22 subspecies and varieties [5-6].

Cytogenetically, the majority of *Trifolium* species have a diploid chromosome count of 16 [3,6]. In about 80 % of the *Trifolium* species, x:8 has been determined and it is regard as the primitive basic number [8,9]. The other basic numbers are determined as x:5, 6 and 7 [9-11]. Cytogenetic studies of *Trifolium* chiefly include chromosome numbers, with some reports on meiotic behaviour (especially polyploid species) and karyotypes, owing to the small chromosome size [9, 12-17]. The goal of present study is to determine karyotypes of seven *Trifolium* species grown in Turkey.

2. Materials and Methods

Trifolium specimens and seeds were collected from natural habitats. Collected samples were analysed morphologically and karyological techniques were applied on the seeds. Collected specimens and seeds were stored in the Firat University Herbarium.

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 room temperature and fixed with acetic alcohol (1:3) for 24 h and stored in 70 % alcohol at 2-4 °C. Then root tips are washed and hydrolysed in 1 N HCl for 10-18 min. at 60 °C and rinsed in tap water for a minimum of 3-5 min. Staining was carried out in Feulgen for 1-2 h and root tips were taken in 1 % aceto-orcein for 5-10 min. to better staining. Lasty, squash preparations were made with 1 % aceto-orcein [18-20].

Microphotographs of good quality metaphase plates of each specimen (normally at least three) were taken using an Olympus BX51

microscope and were recorded with an Olympus Camedia C-4000 digital camera. Karyotypes were obtained well-spread metaphase plates. The long arm, short arm and total lengths of of each chromosomes were measured and the relative lengths, arm ratios, and centromeric indices were determined. The chromosome nomenclature followed Levan et al [21]. The intra-chromosomal asymmetry index (A_1) was calculated according to the formula proposed by Romero Zarco[22] and the inter-chromosomal asymmetry index (A_2) was measured as the ratio of chromosome length/mean chromosome length.

3. Results and Discussion

Somatic chromosome number, ploidy level, karyotype formula, ranges of chromosome lengths, 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 this study (Tables 1,2; Figures 1-8).

Table 1. Somatic chromosome number, ploidy level, karyotype formula, ranges of chromosome length, total karyotype length (TKL), assymetry indexes (A_1 , A_2) of Romero Zarco (1986) for *Trifolium* species investigated

Taxa	2n	Ploidy level	Karyotype formula	Range of Chromosome length (µm)	TKL (µm)	A1	A2
<i>T. resupinatum</i> L. var. <i>resupinatum</i>	16	2X	8m	1.08-1.54	10.45	0.21	0.12
<i>T. stellatum</i> L.var. <i>stellatum</i>	16	2X	8m	1.18-1.73	11.81	0.27	0.12
<i>T. hirtum</i> All.	10	2X	5m	1.45-1.94	8.45	0.19	0.10
<i>T. arvense</i> L. var. <i>arvense</i>	14	2X	2M+5m	1.37-1.85	10.90	0.19	0.11
<i>T. purpureum</i> Lois.var. <i>purpureum</i>	16	2X	1M+7m	1.17-1.70	11.14	0.21	0.12
<i>T. haussknechtii</i> Boiss. var. <i>haussknechtii</i>	14	2X	7m	1.37-2.47	12.90	0.24	0.19
<i>T. pauciflorum</i> d'Urv.	14	2X	7m	1.11-1.60	9.49	0.33	0.11

Table 2. Karyomorphological parameters of species *Trifolium* species Abbreviations: RL, relative length; AR, arm ration (L/S); CI, centromerik index (100S/TL); m, median region; M, median point. Chromosome pairs are assigned Roman numerals

Pair No	RL	AR	CI	Type	Pair No	RL	AR	CI	Type
<i>T. resupinatum</i> var. <i>resupinatum</i>					<i>T. stellatum</i> var. <i>stellatum</i>				
I	1.54	1.40	41.55	m	I	1.73	1.40	41.61	m
II	1.44	1.32	43.05	m	II	1.65	1.32	43.03	m
III	1.44	1.25	44.44	m	III	1.60	1.42	41.25	m
IV	1.35	1.28	43.70	m	IV	1.51	1.28	43.70	m
V	1.28	1.24	44.53	m	V	1.43	1.50	39.86	m
VI	1.17	1.29	43.58	m	VI	1.38	1.33	42.75	m
VII	1.15	1.16	46.08	m	VII	1.33	1.29	43.60	m
VIII	1.08	1.16	46.29	m	VIII	1.18	1.26	44.06	m
<i>T. hirtum</i>					<i>T. arvense</i> var. <i>arvense</i>				
I	1.94	1.36	42.26	m	I	1.85	1.40	41.62	m

2n: 16. All chromosomes are median region (m) and no satellite was observed. (Tables 1,2; Figures 1, 8A).

T. stellatum* L. var. *stellatum

B7 Elazığ, Karga Mountain, 730 m, İ. Türkoğlu, 2006.

The present study determined chromosome number as 2n: 16. It was found that all chromosomes are median region (m) and satellites were not observed (Tables 1,2; Figures 2, 8B).

***T. hirtum* All.**

B7 Elazığ, Baskil, 1500m, M. Kurşat, 2006.

The chromosome count was determined as 2n:10. All chromosomes are median region (m) and satellites were not observed in this species (Tables 1,2; Figures 3, 8C).

T. arvense* L. var. *arvense

B7 Elazığ, Baskil-Belhan village, 1900 m M. Kurşat, 2006.

The chromosome number observed for *T. arvense* var. *arvense* was 2n:14. It consists of two median point (M) and five median region (m) chromosomes. No satellite was observed in this taxon (Tables 1,2; Figures 4, 8D).

T. purpureum* Lois. var. *purpureum

B7 Elazığ, Baskil-Bolucuk village, 1750 m, M. Kurşat, 2006.

The chromosome number was found to be 2n: 16, consisting of one median point (M) and seven median region (m) chromosomes. No satellite was observed (Tables 1,2; Figures 5, 8E).

T. haussknechtii* Boiss. var. *haussknechtii

B7 Elazığ, Kamışlık Mountain, 990 m, İ. Türkoğlu, 2006.

The chromosome morphology of this species are reported for the first time in this study. The chromosome count was determined as 2n:14. All chromosomes are median region (m) and satellites were not observed. (Tables 1,2; Figures 6, 8F).

***T. pauciflorum* d'Urv.**

B7 Elazığ, Kuşakçı Mountain, 1300 m., İ. Türkoğlu, 2006.

The chromosome morphology of this species are reported for the first time in this study. The chromosome number of this taxon was determined as 2n: 14. All chromosomes are median region (m) and satellites were not observed (Tables 1,2; Figures 7, 8G).

The present study revealed that the seven investigated *Trifolium* taxa have three different basic chromosome numbers, x:5, x:7 and x:8, respectively. It was found that the chromosome numbers of *T. resupinatum* var. *resupinatum*, *T. stellatum* var. *stellatum* and *T. purpureum* var. *purpureum* were 2n: 16; *T. arvense* var. *arvense*, *T. haussknechtii* var. *haussknechtii* and *T. pauciflorum* were 2n: 14; and *T. hirtum* was 2n:10. All chromosomes have median point (M), median region (m) centromeres and satellites were not observed.

In general, chromosome numbers agreed with previously published findings. For example, chromosome number of *T. resupinatum* var. *resupinatum* were reported as 2n: 16 in previous studies [16,17, 23]. Also, previous studies [17, 24] reported the chromosome counts of *T. stellatum* var. *stellatum* as 2n: 16. However, Ellison et al. [25] reported the chromosome number of *T. stellatum* as 2n:14. Furthermore, Salimpour et al. [17] determined that *T. stellatum* has two satellites. The chromosome numbers observed for *T. hirtum* has 2n:10 chromosome number [17,26,27]. In contrast, Zohary and Heller [9] determined the chromosome count of *T. hirtum* as 2n:16. In addition, in contrast to the present findings, Salimpour et al. [17] found that *T. hirtum* (2n:10) has four median point and one submedian region chromosomes. Similarly, chromosome number of *T. arvense* var. *arvense* was reported to be 2n:14 by [14, 24, 28]. In contrast, Salimpour et al. [17] found that *T. arvense* var. *arvense* has 2n:16 chromosomes (six median point and two submedian region). In contrast to present results (2n:16), the chromosome number of *T. purpureum* var. *purpureum* was found to be 2n:10 by Zohary and Heller [9] and to be 2n:14 by Strid [29]. However, Ellison et al. [25] reported that *L. haussknechtii* has 2n: 16 chromosomes and *L. pauciflorum* has 2n: 16 chromosomes in contrast to the present findings (2n:16).

Average of chromosomal lengths of studied taxa varied from 1.08 µm to 2.47 µm. Among these, while *T. resupinatum* var. *resupinatum* has the smallest chromosome (Tables 1,2; Figures 1, 8A), the biggest chromosome was observed on *T. haussknechtii* var. *haussknechtii* (Tables 1,2; Figures 6, 8F). In addition, AR (the ratio of long arm/short arm chromosomes) results of present

study showed that there was no differences among the investigated *Trifolium* taxa.

The intrachromosomal asymmetry indexes (A_1) varied from 0.19 to 0.33 and the interchromosomal asymmetry index (A_2) varied from 0.10 to 0.19 (Table 2).

The total karyotype length which shortly determines the chromatin content amongst the investigated diploid taxa with $x:7$ was in the range of 9.49 μm in *T. pauciflorum* and to 12.90 μm in *T. haussknechtii*. Also, the total karyotype length of the studied diploid taxa with $x:8$ was in the range of 10.45 μm in *T. resupinatum* var. *resupinatum* and to 11.81 μm in *T. stellatum* var. *stellatum*. Furthermore, it was determined that the total karyotype length of *T. hirtum* ($2n:10$) with $x:5$ was 8.45. μm .

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