Karyotype Analysis in Ten Taxa of *Tanacetum* L. (Asteraceae) from Turkey

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Summary The chromosome number and karyotype analysis of ten taxa of genus *Tanacetum* found native to Turkey were examined in detail. Somatic chromosome numbers were determined as follows: $2n=18$ in *T. balsamitoides* Sch.Bip., *T. parthenifolium* Sch.Bip., *T. zahlbruckneri* (Náb.) Grierson, *T. densum* (Labill.) Sch.Bip. ssp. *laxum* Grierson, *T. densum* (Labill.) Sch.Bip. ssp. *amani* Heywood, *T. argenteum* (Lam.) Willd. ssp. *argenteum*, *T. tomentellum* (Boiss.) Grierson, $2n=36$ in *T. nitens* (Boiss. & Noë) Grierson, *T. polycephalum* Sch.Bip. ssp. *argyrophyllum* (K.Koch) Podlech, *T. abrotanifolium* (L.) Druce. It was observed that chromosomes of *Tanacetum* taxa, of which karyotype analyses were examined, have generally median (m), submedian (sm), and rarely median point (M), subterminal (st) centromeres. In *T. parthenifolium*, *T. nitens*, *T. abrotanifolium* and *T. argenteum* ssp. *argenteum*; one pair of satellite chromosomes was observed.

Keywords Asteraceae, *Tanacetum*, Karyotype analysis, Chromosome number, Turkey.

Asteraceae is the largest plant family with over 1600 genera and 23000–30000 species (Funk *et al.* 2009, Altınordu *et al.* 2015). *Tanacetum* species previously known as *Pyrethrum* Zinn. Has a weak classification in Asteraceae (Brown *et al.* 1999). According to the opinions of the researchers, the *Tanacetum* involving 250–500 taxa is one of the largest genera of the family. (Mcarthur and Plummer 1978, Mabberley 1990, Ling 1991a, b, 1995a, b, Bremer and Humphries 1993, Kadereit and Jeffrey 2007, Funk *et al.* 2009). While it distributes throughout the northern hemisphere, it is rarely represented in the southern hemisphere. Most species of the genus are significantly found as abundant in various habitats and most of them are used as a source in medicine, food, feed and other useful products (Abdolkarim *et al.* 2011).

As well as the most common chromosome number of Anthemideae is $x=8$; $x=9$ and $x=10$ have been also reported by some researchers (Vallès *et al.* 2005, Chehregani and Hajisadeghian 2009). The basic chromosome number in the genus *Tanacetum* is $x=9$ and its species is mostly $2n=2x=18$ (Chehregani *et al.* 2012). Cytogenetic studies on *Tanacetum* generally focused on determination of chromosome numbers (Brown *et al.* 1999, Baltisberger 2006, Li *et al.* 2008) and there was a less detailed study of karyotype analysis. Therefore wide works on karyotype evolution of the genus are insufficient.

The purpose of this study is to determine the chromosome numbers and karyotypes in ten *Tanacetum* taxa,

including five endemic taxa in Turkey. To obtain karyological information contributing to their relationships.

Materials and methods

Plant materials

Plant specimens of ten *Tanacetum* taxa were collected from natural habitats during fruiting season between 2012 and 2013 in Turkey. The voucher specimens were deposited at the Bitlis Eren University Herbarium (BEUH). The Locality and collection date, altitude, and voucher number of the taxa are presented in Table 1.

Chromosome analysis

The karyological studies are conducted on meristematic cells of root tips. Thirty roots of each taxon were used from seeds germinated on a moist filter paper in Petri dishes at 25°C. The actively growing root tips were pretreated with 0.05% colchicine for 2 h at room temperature. Afterward the root tips were fixed with Farmer fixative (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolyzed in 1 M HCl at 60°C for 10 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen reagent for 1 h and mounted in 45% acetic acid (Elçi 1982, Kıran 2018). An Olympus BX51 microscope attached to an Olympus Camedia C-4000 digital camera was used to take microphotographs. Index to chromosomes numbers in Asteraceae (http://www.lib.kobe-u.ac.jp/infolib/meta_pub/result) was checked.

The number of somatic chromosomes, total lengths of the chromosomes [TL: long arm length (LA)+short arm length (SA)], relative length (RL), arm ratio (AR),

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With Supplementary files of Tables S1–S10

Table 1. Localities and voucher numbers of the studied *Tanacetum* taxa.

Taxon	Locality and collection date	Collector (voucher number)
<i>T. balsamitoides</i>	Bitlis; Kambos Mountain, Steppe, 1,400m, 02.07.2013.	M. Kursat 6013
<i>T. parthenifolium</i>	Bitlis; Between Bitlis and Tatvan, Slope, 1,250m, 15.09.2012.	M. Kursat 6011
<i>T. zahlbruckneri</i>	Bitlis; Kambos Mountain, Steppe, 1,400m, 12.06.2013.	M. Kursat 6012
<i>T. nitens</i>	Elazığ; Baskil, Hasan Mountain, Slope, 1,950m, 25.07.2012.	M. Kursat 6007
<i>T. densum</i> ssp. <i>laxum</i>	Elazığ; Baskil-Bolucuk Village, Northern slopes, Rocky, 1,600m, 17.07.2012.	M. Kursat 6003
<i>T. densum</i> ssp. <i>amani</i>	Elazığ; Baskil, Hasan Mountain, Slopes, Rocky, 1,800m, 25.07.2012.	M. Kursat 6008
<i>T. polycephalum</i> ssp. <i>argyrophyllum</i>	Bitlis; Nemrut Mountain, Rocky, 2,200m, 12.08.2012.	M. Kursat 6009
<i>T. abrotanifolium</i>	Bitlis; Tatvan-Garzit Village, Van Lake, Southern slopes, 1,650m, 16.08.2012.	M. Kursat 6010
<i>T. argenteum</i> ssp. <i>argenteum</i>	Elazığ; Baskil-Bolucuk Village, Northern slopes, Rocky, 1,550m, 17.07.2012.	M. Kursat 6004
<i>T. tomentellum</i>	Bitlis; Kambos Mountain, Rocky, 1,450m, 09.09.2013.	M. Kursat 6014

Table 2. Karyological features of the studied *Tanacetum* taxa.

Taxon	$2n$	Ploidy level	Chromosome length (μm)		Total chromosome length (μm)	AR		RL		Haploid Karyotype formulae
			Min	Max		Min	Max	Min	Max	
<i>T. balsamitoides</i>	18	2x	4.30	7.90	111.25	1.04	2.09	3.86	7.10	7m+2sm
<i>T. parthenifolium</i>	18	2x	2.52	4.47	61.41	1.10	1.89	4.10	7.27	8m+1sm
<i>T. zahlbruckneri</i>	18	2x	5.38	9.36	128.88	1.02	2.27	4.17	7.26	8m+1sm
<i>T. nitens</i>	36	4x	4.42	7.15	215.90	1.00	2.21	2.04	3.31	2M+15m+1sm
<i>T. densum</i> ssp. <i>laxum</i>	18	2x	4.57	6.38	98.10	1.00	2.98	4.65	6.50	1M+6m+2sm
<i>T. densum</i> ssp. <i>amani</i>	18	2x	4.40	7.00	107.80	1.00	4.56	4.08	6.49	1M+6m+1sm+1st
<i>T. polycephalum</i> ssp. <i>argyrophyllum</i>	36	4x	4.74	7.02	204.31	1.02	2.26	2.32	3.43	14m+4sm
<i>T. abrotanifolium</i>	36	4x	4.32	6.90	208.88	1.04	2.49	2.06	3.30	16m+2sm
<i>T. argenteum</i> ssp. <i>argenteum</i>	18	2x	5.11	8.49	127.01	1.07	2.58	4.02	6.68	8m+1sm
<i>T. tomentellum</i>	18	2x	5.15	7.73	116.59	1.03	2.10	4.41	6.63	8m+1sm

and centromeric index (CI) were obtained. Karyotype formula was determined based on centromere position (Levan *et al.* 1964). Haploid ideograms were arranged in decreasing lengths according to the metaphase chromosome size (Kıran 2018). Also, karyotype symmetry nomenclature was determined based on Stebbins' classification (Stebbins 1971).

Results

Tanacetum balsamitoides Sch. Bip. is rhizomatous perennial species that grows in moist ground and sometimes is cultivated between altitudes of 1,000 and 3,000 m (Davis 1975). The somatic chromosome number was determined as $2n=2x=18$ and supporting a previous report (Khandjian 1975). Chromosomes of the species had 14m and 4sm. AR was measured as 1.04–2.09, CI varied between 32.42 and 48.95, and RL varied from 3.86 to 7.10%. TL ranged from 4.30 to 7.90 μm and the ratio of the longest to shortest chromosome is 1.8:1 (Tables 2 and S1, Figs. 1A and 2A).

T. parthenifolium (Willd.) Sch. Bip. grows in dry stony or sandy slopes at altitudes of 1,100–2,225 m (Davis 1975). The somatic chromosome number was $2n=2x=18$ and supporting previous reports of the chromosomes (Khandjian 1975, Morton 1977, Gregor and Hand 2007). It consist 16m and 2sm chromosomes.

Chromosome AR was measured as 1.10–1.89, CI varied between 34.74 and 47.54, and RL varied from 4.10 to 7.27%. TL ranged from 2.52 to 4.47 μm and the ratio of the longest to shortest chromosome is 1.7:1. One pair of satellite was observed in the chromosomes of the species (Tables 2 and S2, Figs. 1B and 2B).

T. zahlbruckneri (Nab) Grierson is an endemic species and Iran Turan Element that grows in *stony slopes* at altitudes of 1,300 and 3,000 m (Davis 1975). As a result of the literature review; no data about the chromosome number and morphology of this species was found and the chromosome number and morphology have been determined by the author for the first time. The chromosome number of the species was found as $2n=2x=18$. The chromosomes had 16m and 2sm. Chromosome AR was measured as 1.02–2.27. CI varied between 30.56 and 49.38, and RL varied from 4.17 to 7.26%. TL ranged from 5.38 to 9.36 μm and the ratio of the longest to shortest chromosome is 1.7:1 (Tables 2 and S3, Figs. 1C and 2C).

T. nitens (Boiss. & Noe) Grierson is an endemic species that grows in *Quercus* or coniferous woodland and on limestone ridges at sea level at altitudes of 1,640–2,900 m (Davis 1975). The chromosome number and morphology have been determined by the author for the first time. The chromosome number of the species was found as $2n=4x=36$. The chromosomes had 4M,

Fig. 1. Somatic metaphase chromosomes in ten *Tanacetum* taxa. A: *T. balsamitoides*, B: *T. parthenifolium*, C: *T. zahlbruckneri*, D: *T. nitens*, E: *T. densum* ssp. *laxum*, F: *T. densum* ssp. *amani*, G: *T. polycephalum* ssp. *argyrophyllum*, H: *T. abrotanifolium*, I: *T. argenteum* ssp. *argenteum*, J: *T. tomentellum*. Scale bars=10 μ m.

30m, and 2sm. AR was measured as 1.00–2.21. CI varied between 31.22 and 50.00, and RL varied from 2.04 to 3.31%. TL ranged from 4.42 to 7.15 μ m and the ratio of the longest to shortest chromosome is 1.6:1. One pair of satellite chromosomes was observed at the terminal region of the arm of one pair chromosomes (Tables 2 and S4, Figs. 1D and 2D).

T. densum (Labill.) Sch. Bip. ssp. *laxum* Grierson is an endemic subspecies that grows in limestone rocks and screes at 1,500–2,500m altitudes (Davis 1975). This is the first karyological report for this taxon. The chromosome number of the species was found as $2n=2x=18$. The chromosomes had 2M, 12m, and 4sm. AR was measured as 1.00–2.98. CI varied between 25.05 and 50.00, and RL varied from 4.65 to 6.50%. TL ranged from 4.57 to 6.38 μ m and the ratio of the longest to shortest chromosome is 1.4:1 (Tables 2 and S5, Figs. 1E and 2E).

T. densum (Labill.) Sch. Bip. ssp. *amani* Heywood is

an endemic subspecies that grows in limestone rocks and screes at 1,500–2,500m altitudes (Davis 1975). The chromosome number and morphology have been determined by us for the first time. Chromosome number of the species was $2n=2x=18$. The chromosomes had 12m, 2sm, 2M, and 2st. AR was measured as 1.00–4.56. CI varied between 18.00 and 50.00, and RL varied from 4.08 to 6.49%. TL ranged from 4.40 to 7.00 μ m and the ratio of the longest to shortest chromosome is 1.3:1 (Tables 2 and S6, Figs. 1F and 2F).

T. polycephalum Sch. Bip. ssp. *argyrophyllum* (K. Koch) Podlech. is a perennial subspecies that grows on rocky slopes, stream-sides, and scree at altitudes of 760–2,900m (Davis 1975). In the previous studies conducted on this species; somatic chromosome number was found as $2n=18$ (Inceer *et al.* 2012). In our study, somatic chromosome number was $2n=4x=36$. The chromosomes had 28m and 8sm. AR was measured as 1.02–2.26. CI

Fig. 2. Haploid ideograms of ten *Tanacetum* taxa. A: *T. balsamitoides*, B: *T. parthenifolium*, C: *T. zahlbruckneri*, D: *T. nitens*, E: *T. densum* ssp. *laxum*, F: *T. densum* ssp. *amani*, G: *T. polycephalum* ssp. *argyrophyllum*, H: *T. abrotanifolium*, I: *T. argenteum* ssp. *argenteum*, J: *T. tomentellum*. Scale bars=1 µm.

varied between 30.27 and 49.41, and RL varied from 2.32 µm to 3.43%. TL ranged from 4.74 to 7.02 µm and the ratio of the longest to shortest chromosome is 1.5:1 (Tables 2 and S7, Figs. 1G and 2G).

T. abrotanifolium (L.) Druce is perennial species and grows in banks and rocky igneous slopes and sometimes *Quercus* woods at altitudes of 1,630–2,300 m (Davis 1975). The somatic chromosome number was determined as $2n=4x=36$ and confirmed previous report (Khandjian 1975). The chromosomes had 32m and 4sm. AR was measured as 1.04–2.49. CI varied between 28.59 and 48.99, and RL varied from 2.06 to 3.30%. TL ranged from 4.32 to 6.90 µm and the ratio of the longest to shortest chromosome is 1.6:1. The satellite was observed at the terminal of the short arm of one pair chromosomes (Tables 2 and S8, Figs. 1H and 2H).

T. argenteum (Lam.) Willd. ssp. *argenteum* (L.) All. Is an endemic subspecies and Iran Turan Element that grows in rocks and limestone cliffs at 990 and 2,500 m altitudes (Davis 1975). The chromosome number of the species was found as $2n=2x=18$ and supporting the previous report of Tekin *et al.* (2016). The chromosomes had 16m and 2sm. AR was measured as 1.07–2.58. CI varied between 27.91 and 48.14, and RL varied from 4.02 to 6.68%. TL ranged from 5.11 to 8.49 µm and the ratio of the longest to shortest chromosome is 1.7:1. One pair of satellites at terminal region of short arm observed

(Tables 2 and S9, Figs. 1I and 2I).

T. tomentellum (Boiss.) Grierson is a perennial species that grows metamorphic rock ledges and crevices at altitudes of 1,900–2,300 m (Davis 1975). This is the first karyological report for this taxon. Chromosome number of the species was $2n=2x=18$. The chromosomes had 16m and 2sm. AR was measured as 1.03–2.10. CI varied between 32.23 and 49.32, and RL varied from 4.41 to 6.63%. TL ranged from 5.15 to 7.73 µm and the ratio of the longest to shortest chromosome is 1.5:1 (Tables 2 and S10, Figs. 1J and 2J).

Discussion

The present study confirmed that studied *Tanacetum* taxa have $x=9$ basic number and they have chromosome number of the species was $2n=2x=18$ chromosome number except for *T. nitens*, *T. polycephalum* ssp. *argyrophyllum* and *T. abrotanifolium*, which have polyploid chromosome number of $2n=4x=36$. Studied *Tanacetum* taxa have M, m, sm, and st chromosomes. The present study showed that *T. balsamitoides*, *T. parthenifolium* and *T. nitens* have 1A whilst the others have 2A based on Stebbins' classification. Inceer *et al.* (2012) found that *Tanacetum* taxa were in the 2A to 2B categories. They reported that karyotypes of *Tanacetum* were relatively symmetrical and is primitive. The present study also supported the findings of Inceer *et al.* (2012). However, it also has species showing polyploid forms and *T. polycephalum* varies between $2n=2x=18$ and $2n=6x=54$ (Abdolkarim *et al.* 2011). *T. parthenifolium*, *T. nitens*, *T. abrotanifolium* and *T. argenteum* ssp. *argenteum* have one pair of sat-chromosome (Figs. 1, 2). TL of the species ranged from 2.52 to 9.36 µm and *T. parthenifolium* had the shortest chromosome length with 2.52–4.47 µm and *T. zahlbruckneri* had the longest chromosome with 5.38–9.36 µm size. CI varies between 25.05 and 50.00, and RL varies from 2.04 to 7.27% (Tables 2). These karyological informations obtained in this study will contribute to taxonomical and phylogenetical understanding of *Tanacetum*.

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