Research Article

Karyological Data of *Tanacetum polycephalum* Schultz-bip. and *T. parthenium* Schultz-bip. (Asteraceae) Populations

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Abstract

Chromosome numbers in 19 populations of *Tanacetum polycephalum* and *Tanacetum parthenium* from natural resources gene bank, that collected from different regions of Iran, were determined for the first time. The samples prepared by using root tips. After pretreatment, fixation, hydrolysis and staining, the microscopic samples prepared by squash method, metaphases were captured using an optical microscope. The best metaphases plates were selected and used for karyotype analyses. In all of populations the basic chromosome number was x=9 and the populations showed two ploidy levels (diploid & tetraploid). The type of the most chromosomes in all of the populations was metacentric (m) and sub-metacentric (sm) and located in 2A and 2B except for *T. parthenium* (Yazd, Taft) with 5m+3sm+1st karyotype formula and 2C Stebbins classes. In addition, *T. polycephalum* (Esfahan, Golpayegan) with the highest value of AR and A_1 had karyotype heterogeneity, also *T. polycephalum* (Esfahan, Golpayegan) and *T. polycephalum* (West-Azerbaijan, Uromeyeh) had the highest value of chromosome length (TL). Detailed karyotype allows us to group the different populations based on Stebbins classes and asymmetry indices.

***Keywords*:** Chromosome numbers, Ploidy levels, Karyotype, *Tanacetum polycephalum, Tanacetum parthenium,* Microscopic samples, Squash method

Introduction

Tanacetum L. is a genus of about 160 species of flowering plants in the Aster family, Asteraceae native to many regions of the Northern Hemisphere (Watson, 1754), Northern Europe, Canada, Alaska, and Northern Russia (Heywood and Humphries, 1977; Tutin et al., 1976; Hulten, 1968), though the center of diversity for *Tanacetum* is South-West Asia and the Caucasus in the Old World (Soreng and Cope, 1991; Heywood and Humphries, 1977).
Tanacetum L. is a medicinal herb which is found in many old Gardens (Mitich, 1992). The genus *Tanacetum* (Asteraceae) is represented by 26 species in the flora of Iran, as herbal, perennial and sometimes shrub plants that dispersed in many regions of Iran, 12 of them are endemic (Mozaffarian, 2006). These species have traditionally been used as a spicy additive for food. It has been used in folk medicine for reducing fever (Nezhadali and Zarrabi Shirvan, 2010).
Karyological data are essential information for any organism and many karyological investigations have been performed, providing important characters for plant systematic and evolutionary analyses (Stace, 2000).
Many species of Anthemideae have been studied in karyological comparisons (e.g., Carr et al., 1999; Watanabe, 2002; Valles et al., 2005; Chehregani and Mehanfar, 2008; Chehregani and Hajisadeghian, 2009; Chehregani et al., 2013). Since chromosome number of less than 40% of the species of the Asteraceae has been documented, , more studies are still necessary to improve the knowledge of the family as well as the tribe Anthemideae (Volkova and Boyko, 1986; Valles et al., 2005).
Basic chromosome number within Anthemideae tribe is x=9, though x=8 with different ploidy levels have been reported by some researchers (diploid, tetraploid and hexaploid) (Chehregani et al., 2011; Chehregani and Hajisadeghian, 2009; Chehregani and Mehanfar, 2008; Javadi et al., 2013).
In systematic, chromosome number is an important character for plant evolutionary studies and may provide some information about polyploidy and other highly significant genome changes (Guerra, 2008; Louzada et al., 2010) or the benefits of plant chromosome number databases are useful tools for systematic comparisons of geographical and taxonomical groups of plants (Peruzzi et al., 2012).

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Also, chromosome counts can increase our understanding of phylogenetic relationships at different taxonomic levels (Yang et al., 2009).
The purpose of the present study is to determine chromosome number, ploidy levels, karyotype analyses and asymmetry indices in different populations of *Tanacetun polycephalum* Schultz-Bip. and *Tanacetun parthenium* Schultz-Bip. Asymmetry indices are the best knowledge of karyological feature of taxa (Altınordu et al., 2014). In literature, there is no sufficient data about chromosome number and karyotype analyses of studied taxa. We expect that our study will be performed in the light of previous cytotaxonomic studies.

Materials and Methods

In this study, we used root tip meristems from seedling obtained by the germination of ripe seeds collected from various locations (19 populations) on wet filter paper in petri dishes and have been left at 25°C temperature. The studied populations are listed in table 1.

Pretreatment and Preparation

Root tip meristems obtained from seedlings were pretreated with 0.05% (w/v) 8-hydroxyquinoline for 4 to 5 h at 16°C. Pretreated root tips were fixed in a 3:1 (v/v) mixture of 95% (v/v) ethanol and propionic acid for 24 h. Root tips were hydrolyzed in 1M HCl for 5 to 7 min at 60°C and stained in Schiff's reagent for 2 h at room temperature.
Feulgen stain was removed and the root tips were rinsed with cold double-distilled water and stained with Carbol fuchsin stain overnight at 4°C in a refrigerator. After staining, the root tips were washed three to four times with cold double distilled water and stored in cold double-distilled water in a refrigerator.
Root tips were squashed in a droplet of 45% (v/v) acetic acid and lactic acid (10:1). The preparations were observed with an optical microscope (BX41 Olympus supplemented with Digital color video camera) at a magnification of about 2000 X. The best plates were selected and captured. For each population, 5 mitotic metaphase plates were prepared.

Karyotype Analyses

The following parameters were measured in each metaphase plate to characterize the karyotypes numerically: haploid chromosome numbers (n), long arm (LA), short arm (SA), total length (TL=LA+SA), genome size (∑TL), arm ratio (AR=LA/SA), centromeric index [CI=SA/(LA+SA)], difference of range relative length (DRL=MaxRL%-MinRL%), (MaxRL%=[MaxTL/(∑TL)*100], (MinRL%=[MinTL/(∑TL)*100], that MaxRL% and MinRL% are relative length of longer and shorter chromosome respectively, karyotype formula (KF) according to Levan's method (Levan et al., 1964), the classification of chromosomes as median (m), submedian (sm), subterminal (st) and terminal point (T).
For analysis of karyotype asymmetry, the following methods were used. To describe karyotype asymmetry and to determine the relationships of karyotypic between species, Huziwara (1962) developed the total form percent (TF%=[(∑SA/∑TL)*100]). Romero Zarco (1986) also, provided a different method to measure karyotype asymmetry which is the intrachromosomal asymmetry index (A_1=1-[∑(SA/LA)/n]), where SA and LA are the mean length of short and long arms of each pair of homologous, respectively and n is the number of homologous.
The other interchromosomal asymmetry index is (A_2=s/x), whereas s and x are the average of standard deviation and mean of chromosome length, respectively.
Also, karyotypic evolution has been determined using the symmetry classes of Stebbins (SC) (Stebbins, 1971). Stebbins (1971) distinguished 12 categories concerning the karyotype asymmetry and from which 10 categories were known to occur in higher plants.
He established these by recognizing three degrees of difference (A–C) between the largest and smallest chromosome complement, and four degrees (1–4) with respect to the proportion of chromosomes which are median pair with an arm ratio of less than 2:1.

Results

The pictures of the mitotic metaphase samples and their karyotypes were presented in Figures 1and 2. The results showed that in all of populations the basic chromosome number was x=9, and showed two ploidy levels (2x and 4x).
The type of all chromosomes usually were metacentric (m), sub-metacentric (sm) and rarely sub-telocentric (st). The somatic chromosome numbers (2n) and karyotypic details for the studied populations were presented in Table 2.

Table 1. Materials used for chromosomal study of *Tanacetum polycephalum* Schultz-Bip. and *Tanacetum parthenium* Schultz-Bip. populations. (G.B.C:Gene Bank Code, H.C:Hetrbarium Code)

No.	G.B.C	H.C	Population	Location	altitude (m)	Latitude	Longitude
1	1459	102810	*Tanacetum polycephalum*	Hamadan, Malayer	2250	48º 30′ 05″ N	34º 34′ 41″ E
2	17650	102820	*Tanacetum polycephalum*	Qom, Qom	2310	50 08′ 52″ N	34º 08′ 10″ E
3	22613	102816	*Tanacetum polycephalum*	Kordestan, Baneh	1956	45º 57′ 00″ N	36º 03′ 00″ E
4	25991	102818	*Tanacetum polycephalum*	Kordestan, Qorveh	1995	47º 43′ 03″ N	35º 12′ 46″ E
5	31204	102814	*Tanacetum polycephalum*	Kohkiloyeh & Boyerahmad	2435	51º 42′ 37″ N	30º 03′ 33″ E
6	33331	102812	*Tanacetum polycephalum*	Zanjan	2250	48º 13′ 56″ N	36º 36′ 50″ E
7	35122	102819	*Tanacetum polycephalum*	Esfahan, Golpayegan	1970	50º 13′ 39″ N	33º 28′ 30″ E
8	35185	102817	*Tanacetum polycephalum*	West-Azerbaijan,	1648	45º 01′ 55″ N	38º 01′ 31″ E
9	35579	102815	*Tanacetum polycephalum*	Mazandaran, Savadkoh	2024	52º 57′ 16″ N	36º 51′ 52″ E
10	35635	102813	*Tanacetum polycephalum*	Mazandaran, Amol	2570	52º 06′ 00″ N	35º 52′ 13″ E
11	10262	102811	*Tanacetum parthenium*	Yazd, Taft	2310	54º 08′ 01″ N	31º 37′ 97″ E
12	20096	102827	*Tanacetum parthenium*	Qazvin, Qazvin	1700	50º 10′ 00″ N	36º 26′ 00″ E
13	27153	102822	*Tanacetum parthenium*	Gilan, Shaft	970	49º 11′ 12″ N	36º 56′ 40″ E
14	27158	102826	*Tanacetum parthenium*	Gilan, Fuman	1120	49º 02′ 42″ N	37º 08′ 52″ E
15	27162	102825	*Tanacetum parthenium*	Gilan, Astara	1460	48º 33′ 12″ N	38º 15′ 50″ E
16	27173	102821	*Tanacetum parthenium*	Gilan, Talesh	945	48º 42′ 53″ N	37º 58′ 12″ E
17	29813	102823	*Tanacetum parthenium*	Kohkiloyeh & Boyerahmad	1800	51º 10′ 16″ N	31º 50′ 59″ E
18	33183	102814	*Tanacetum parthenium*	Hamadan	21413	48º 29′ 45″ N	34º 43′ 57″ E
19	35190	102824	*Tanacetum parthenium*	West-Azerbaijan,	2167	46º 41′ 72″ N	36º 51′ 36″ E

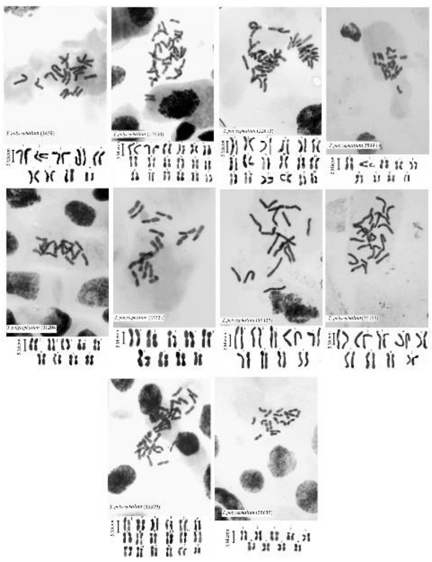

Figure 1. The mitotic metaphase samples and their karyotypes

Table 2. Karyotype characteristics of *Tanacetum polycephalum* Schultz-Bip. and *T. parthenium* Schultz-Bip. populations. 2n- somatic chromosome number, SC- symmetry classes of Stebbins, ∑TL-length of genome(micron), TL-mean of chromosome Length (micron), AR- arm ratio, CI- Centromer Index, TF%- total form percentage, A_1-intrachromosome asymmetry index, A_2- interchromosome asymmetry index, DRL- difference of relative length, KF-karyotype Formula (m: metacentric, sm: submetacentric, st: subtelocentric)

N	Populat	2	S	∑T	TL	A	CI	TF	A_1	A_2	DR	KF
1	*Tanacet*	1	2	73.4	8.1	2.0	0.3	37.	0.3	0.2	7.9	5m(1,2,4,5,6)+3Sm(3,8,9)+1St(7)
2	*Tanacet*	3	2	100.	5.5	1.7	0.3	37.	0.3	0.1	3.9	10m(3,5,7,8,9,10,11,12,13,16)+7Sm(2
3	*Tanacet*	3	2	129.	7.1	1.7	0.3	37.	0.3	0.1	3.3	11m(2,5,6,8,10,11,12,13,15,16,17)+5S
4	*Tanacet*	1	2	46.7	5.1	1.5	0.3	39.	0.3	0.2	7.2	7m(1,2,3,4,5,6,8)+2Sm(7,9)
5	*Tanacet*	1	2	54.9	6.1	1.4	0.4	42.	0.2	0.0	2.3	7m(1,2,3,5,6,8,9)+2Sm(4,7)
6	*Tanacet*	1	2	81.3	9.0	1.4	0.4	42.	0.2	0.1	3.9	7m(1,2,4,5,6,7,8)+2Sm(3,9)
7	*Tanacet*	1	2	94.3	10.	2.0	0.3	35.	0.4	0.1	5.0	4m(1,2,4,5)+4Sm(3,6,7,9)+1St(8)
8	*Tanacet*	1	2	95.4	10.	1.6	0.3	39.	0.3	0.2	8.3	6m(1,2,3,5,7,9)+2Sm(4,8)+1St(6)
9	*Tanacet*	3	2	110.	6.1	1.6	0.3	39.	0.3	0.1	4.2	14m(1,2,5,6,7,8,10,11,12,13,14,16,17,
1	*Tanacet*	1	2	45.6	5.0	1.3	0.4	43.	0.2	0.1	3.2	8m(1,2,3,5,6,7,8,9)+1Sm(4)
1	*Tanacet*	1	2	59.1	6.5	1.7	0.3	38.	0.3	0.3	13.	5m(1,4,7,8,9)+3Sm(2,3,5)+1St(6)
1	*Tanacet*	1	2	50.5	5.6	1.7	0.3	38.	0.3	0.1	4.9	4m(1,2,3,7)+5Sm(4,5,6,8,9)
1	*Tanacet*	1	2	44.0	4.8	1.5	0.4	40.	0.3	0.1	4.5	6m(1,2,3,4,5,6)+3Sm(7,8,9)
1	*Tanacet*	1	2	57.4	6.3	1.6	0.3	38.	0.3	0.1	4.7	5m(3,4,5,6,9)+4Sm(1,2,7,8)
1	*Tanacet*	1	2	44.2	4.9	1.5	0.3	39.	0.3	0.1	6.1	7m(1,3,5,6,7,8,9)+2Sm(2,4)
1	*Tanacet*	1	2	39.0	4.3	1.6	0.3	38.	0.3	0.2	6.3	6m(1,2,4,6,7,9)+3Sm(3,5,8)
1	*Tanacet*	3	2	110.	6.1	1.6	0.3	38.	0.3	0.2	4.8	11m(2,5,6,8,9,10,11,14,16,17,18)+6S
1	*Tanacet*	1	2	43.2	4.8	1.6	0.3	39.	0.3	0.2	8.1	6m(3,4,5,6,7,8)+3Sm(1,2,9)
1	*Tanacet*	3	2	74.2	4.1	1.3	0.4	42.	0.2	0.1	3.9	17m(1,2,3,4,5,6,7,8,10,11,12,13,14,15,

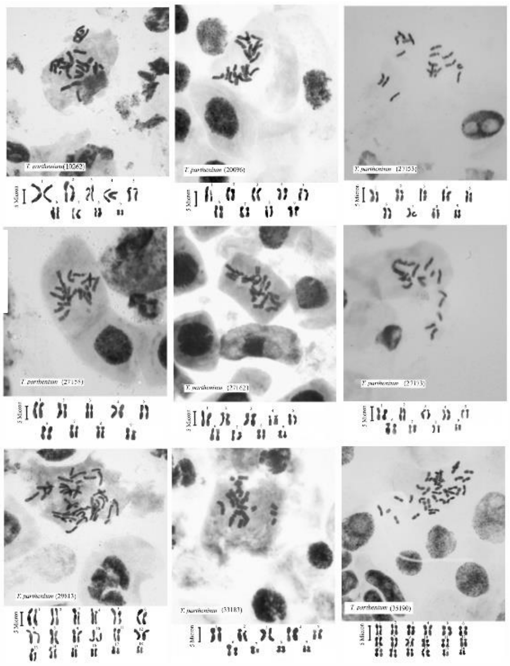

Figure 2. The mitotic metaphase samples and their karyotypes

In the present work, we have provided additional karyomorphological parameters using Symmetry of Stebbin's (SC), Total Form percentage (TF%), asymmetry indices of Romro-Zarco (A_1 and A_2) (Romero, 1986) and Difference Relative Length (DRL), which do not depend on chromosome number or chromosome size. The scatter diagram of populations dispersion based on two components (A_1–A_2) with Stebbins symmetry (SC) are represented graphically in Figure 3.

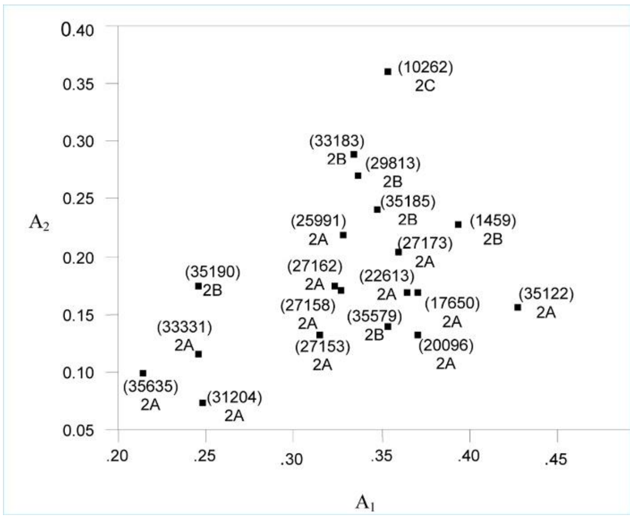

Figure 3. The scatter diagram of populations dispersion based on two components (A_1–A_2) with Stebbins symmetry (SC).

All populations are located in Stebbin's classes (SC) 2A and 2B, except for *T. parthenium* (10262), with the highest DRL value (13.615), and A_2 (0.363), is located in 2C Stebbin's classes. *T. polycephalum* (35122) and *T. polycephalum* (1459) had the highest value of AR and A_1, respectively, while *T. polycephalum* (35635) had the lowest AR value (1.343), and A_1 value (0.214). The differences in AR and A_1 values among the other populations were not significant (AR=1.355-1.799, A_1=0.245-0.371). While the highest TF% value (43.683) and CI value (0.435) belonged to *T. polycephalum* (35635), *T. polycephalum* (35185) and *T. polycephalum* (35122) had the highest of chromosome length (TL) in all populations. So these two populations have the longer chromosome than other populations.

The current available chromosome data showed polyploidy to be the most significant evolutionary trend in chromosome number within the Asteraceae (Carr et al., 1999; Valles et al., 2005; Chehregani et al., 2013).

In this survey, we study variation of chromosome number in *Tanacetum polycephalum* Schultz-Bip. and *Tanacetum parthenium* Schultz-Bip. populations from natural gene bank. We had 10 populations from *T. polycephalum* and nine populations from *T. parthenium*. In all populations the basic chromosome number was x=9 and the populations showed two ploidy levels: Diploid and Tetraploid (Table 2 and Figures 1 and 2). This is the first report of chromosome number in *Tanacetum polycephalum* and *T. parthenium* for populations in natural resources gene bank of Iran, indicating diploid and tetraploid level (2x and 4x) based on x=9. Our results agree with other results, showing the basic chromosome number is 9 with different ploidy levels in Anthemidaea tribe (Goldblat and Johnson, 1979; Torrel et al., 2001; Valles et al., 2005; Yousefzadeh et al., 2010).

The diploid chromosome number (2n=2x=18) has already been reported in *T. albipannosum* Hub.-Mor and Grierson, *T. macrophyllum* Sch. Bip., *T. coccineum* (Willd.) Grierson ssp. chamaemelifolium (Sommier and Levier) Grierson, and *T. sorbifolium* (Boiss.) Grierson from Turkey (Inceer and Hayirlioglu-Ayaz, 2007).

Sonboli and et al., (2011) showed that in *Tanacetum fisherae*, from Kerman province, the constant chromosome number found in all metaphase plates was 2n=44+1B that indicates a pentaploid level (5x) based on x=9. A new ploidy level (pentaploidy) is reported for the first time for the genus.

Chehregani and et al., (2011) showed that in 14 populations of *Tanacetum polycephalum* located in west region, the number of chromosomes was 18 (Diploid), 36 (Tetraploid) and 54 (Hexaploid). Some populations were mixoploidy, such as Diploid and Tetraploid or Tetraploid and Hexaploid.

Finally, in Anthemideae tribe both x=9and x=8 were reported. It seems polyploidy is the cause of polymorphism in this species (Valles et al., 2005; Chehregani and Hajisadegian, 2009).

In Table 2 we showed the Length of Genome ($\sum$TL) existed in tetraploid populations including *T. polycephalum* (Qom, Qom), *T. polycephalum* (Kordestan, Baneh), *T. polycephalum* (Mazandaran, Savadkoh), *T. parthenium* (Kohkiloyeh and Boyerahmad) and *T. parthenium* (West-Azerbaijan, Shahindej) The values of $\sum$TL are high and the highest value of $\sum$TL belonged to *T. polycephalum* (Kordestan, Baneh). In diploid populations, the highest value of $\sum$TL is shown in *T. polycephalum* (West-Azerbaijan, Uromeyeh) and *T. polycephalum* (Esfahan, Golpayegan). So, in tetraploid populations, *T. polycephalum* (Kordestan, Baneh) have the longest chromosome (TL=7.197µm) and in diploid populations, *T. polycephalum* (West Azerbaijan, Uromeyeh) and *T. polycephalum* (Esfahan, Golpayegan), have the longest chromosome with TL=10.602µm and

TL=10.482µm, respectively. The smallest chromosome length (4.124µm) is observed in the population of *T. parthenium* (West-Azerbaijan, Shahindej). Therefore the population of *T. polycephalum* has the longest chromosomes (bigger) than the population of *T. parthenium*.

When we compare the karyotype asymmetry in genus *Tanacetum* according to Stebbins (1971) classification, all of the populations are located in Stebbin's classes (SC) 2A and 2B, except for *T. parthenium* (Yazd, Taft), that is classified to symmetry classes of Stebbins as 2C. Among these classes, 2A class is more symmetrical than 2B class and 2B class is more symmetrical than 2C class, but we cannot determine which 2A class has higher symmetry. So Stebbins classification does not clarify this situation. In order to determine the most symmetrical or asymmetrical karyotype we used other indices. The karyotype asymmetry was evaluated based on six different parameters includes AR (arm ratio), CI (centromeric index), TF% (total form percentage), A_1 and A_2 (intrachromosome and interchromosome asymmetry index), DRL (difference relative length). CI and TF% have a direct relation with symmetry of karyotype based on type of chromosomes. Increase of the CI and TF% values is associated with increased symmetry in karyotype and vice versa. . The AR, A_1–A_2 and DRL values increase with increasing of asymmetry (Zuo and Yuan, 2011).

The AR and A_1 are the asymmetric parameters showing the chromosomes are not the same in type. So according to AR and A_1 indices, *T. polycephalum* (Mazandaran, Amol) has the most symmetrical karyotype and *T. polycephalum* (Esfahan, Golpayegan) has the most asymmetrical karyotype. Also, the karyotype formula confirmed this issue; in population of *T. polycephalum* (Mazandaran, Amol) karyotype formula is 8m+1sm, in which 8 chromosomes are metacentric and one chromosome is sub-metacentric.

T. parthenium (Yazd, Taft) had the highest of A_2 and DRL values. A_2 and DRL also are asymmetric parameter and show that karyotype is asymmetric based on length of chromosomes. So population of *T. parthenium* (Yazd, Taft) have an asymmetric karyotype based on the length of chromosomes and its classification in 2C Stebbins classes.

In addition to various symmetrical states by Stebbins, the variance of different populations according to A_1 and A_2 values are presented in Figure 3. With regard to Figure 3, the pattern of variation of A_1 and A_2 values have been compared with the pattern of Stebbins system. This diagram also shows that the *T. parthenium* (Yazd, Taft) has the most derived karyotype.

In the present research, somatic chromosome number, karyotype analysis and karyotype asymmetries of 19 popilations *T. polycephalum* and *T. parthenium* from the family of Asteraceae were defined for the first time. This study will play a positive role to enlighten this taxonomically revision genus (Taner et al., 2014). Determination of the number of chromosomes in the genus *Tanacetum* also shed light on the opinions of further studies in this regard.

Discussion and Conclusions

In all of the populations the basic chromosome number was x=9, and showed two ploidy levels (2x and 4x).

The populations of *Tanacetum polycephalum* have the longest chromosomes than populations of *T. parthenium*.

The asymmetric and symmetric karyotype is shown in populations of *T. polycephalum* from Esfahan-Golpayegan and *T. polycephalum* from Mazandaran-Amol, respectively.

Populations of *T. parthenium* from Yazd-Taft with the longer and smaller chromosome (highest of A_2 and DRLvalue) also have an asymmetric karyotype.

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