Research Article

Investigations on chromosome variation in *Achillea tenuifolia* Lam and *A. bieberestinii* Afan (Asteraceae) from Hamedan and Kermanshah in the West of Iran

Abdolkarim Chehregani-Rad*, Fariba Mohsenzadeh, Hajar Salehi

Laboratory of Plant Cell Biology, Department of Biology, Bu-Ali Sina University, Hamedan, Iran

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Abstract

Chromosome counting was performed in nine populations of *Achillea tenuifolia* Lam and eight populations of *A. bieberestinii* Afan (Asteraceae) collected from Hamedan and Kermanshah provinces in the west of Iran. Chromosome numbers in both species varied from 2n=2x=18 to 2n=4x=36. Some populations of both species showed (2n=4x=36) chromosome number that is the first report as polyploidy levels. Aneuploidy is also the first report for both species. Diploid and tetraploid individuals were observed in some populations at the same locality. B-chromosomes were observed in some populations of both species. The results indicated that polyploidy is a common future in this species similar to other Asteraceae plants.

Keywords: *Achillea bieberestinii*, *A. tenuifolia*, Chromosome number, Polyploidy

Introduction

The Compositae (Asteraceae) is the largest and most successful flowering plant family in the world with about 1700 genfera and 25,000 species. They grow everywhere but they prefer open areas and are common garden plants (i.e., sunflowers, daisies, artichokes, thistles, lettuce) (Funk et al., 2009). Yarrow (Achillea L.) is one of the youngest evolutionary genera of the Asteraceae family, which is present throughout the world (Goli et al., 2008; Rahimmalek et al., 2009).

The genus Achillea L. has approximately 130 perennial herb species (Saukel et al., 2004; Guo et al., 2004, 2005). Most of the its species are distributed in Eurasia, some in North Africa, and a few can be found in North America and in the Southern Hemisphere (Post 1933; Bremer and Humphries 1993; Zheng-Yi and Raven 1994). Bumadaran is a popular name for several species of Achillea in Persian language. The majority of Achillea species are of medicinal values having therapeutic applications. They have been used as anti-inflammatory (Benedek and Kopp 2007), anti-spasmodic (Karamenderes and Apaydin 2003), diaphoretic, diuretic, emmenagogic agents and for treatment of hemorrhage, pneumonia, rheumatic pain and wounds since antiquity (Zargari 1996).

The most common basic chromosome number in the Anthemideae is x=9, although x=8 and x=10 have also been reported by some researchers (Carr et al., 1999; Vallès et al., 2005; Chehregani and Hajisadeghian 2009). The basic chromosome number in Achillea is x=9, with polyploidy (4x, 6x, 8x) occurring frequently that most of the species being diploid. The genus exhibits wide ecological ranging from deserts to aqueous habitats and from level of sea to the high mountains (Lawrence 1947; Contandriopoulos and Martin 1967; Liove 1972, 1973; Oswiecimska 1974; Halliday and Beadle 1980; Tutin et al., 1980; Androschchuk and Kostinenko 1981; Danihelka and Rotreklová 2001, 2002; Constantinidis and Kalpoutzakis 2005; Magulaev 1982; Dąbrowska 1989, 1992; Maffei et al. 1993; Ehrendorefer and Guo 2006; Chehregani et al., 2013). The genus is also widespread in different regions of the Iran, with 19 species (Podelech 1986), out of which 7 species are endemic to Iran (Mozaffarian 2005, 2007).

Polyploidy is currently considered as a prominent force in plant evolution and represents the most common mode of sympatric speciation in plants (Wendel and Doyle 2005 and references cited there in; Chehregani et al., 2010).

*Corresponding author E-mail: chehregani@basu.ac.ir

Table 1- The populations of *Achillea tenuifolia* and *A. bieberestinii* that were subjected for karyological studies

Sr.#	Name of species	Locations	Geographical characters	Altitude (m)	Collector	Chromosome number	Date of collection
1	*Achillea tenuifolia*	Hamedan, Razan, Boghati mountain	N: 35^0 32.8' 58." E:48^0 43.1' 63"	2385	Salehi	*2n=18*	89.4.19
2	*A. tenuifoila*	Hamedan, Malayer to Arak road, Protected área of Lashkardar, Border Road	N: 34^0 08' 28.5" E: 48^0 13' 03.2"	1852	Salehi	*2n=36*	89.4.23
3	*A. tenuifoia*	Hamedan to Kermanshah, Near to Asadabad city	N: 34^0 49' 23.3" E: 48^0 11' 04.7"	2315	Salehi	*2n=18,36*	89.4.20
4	*A. tenuifolia*	Hamedan, 40 km from Asadabad to Kermanshah, Viilage of Tajabad, after Almagholagh mountain	N: 34^0 53' 15.8" E: 48^0 11' 66.6"	2012	Salehi	*2n=18,36*	89.420
5	*A. tenuifoila*	Hamedan, Kabudarahang, Soubashi mountans	N: 35^0 11' 61.5" E: 48^0 16' 24.7"	2295	Salehi	*2n=18,36*	89.4.16
6	*A. tenuifoila*	Hamedan, Road of Malayer to Arak, village of Marvil , Malvak mountain	N:34^0 02,2' 77.9" E: 48^0 50' 53.1"	2249	Salehi	*2n=16,36*	89.4.18
7	*A. tenuifolia*	Hamedan, Kabudarahang, Gholi abad mountain	N: 35^0 15' 15.1" E: 48^0 50' 30.1"	2304	Salehi	*2n=18,36*	89.4.22
8	*A. tenuifoila*	Hamedan, road of Malayer to Arak, village of Marvel, slopes of Malvak mountain	N: 34^0 02' 97.6" E: 48^0 50' 18	2148	Salehi	*2n=18,36*	89.43.18
9	*A. tenuifoila*	Hamedan, road of Malayer to Arak, Ahangaran Lead and Zink mine area	N: 34^0 47 ' 29.2" E: 48^0 29' 19"	2003	Salehi	*2n=18,36*	89.4.18
10	*Achillea bieberestinii*	Hamedan to Kermanshah road, 40 km after Asadabad, village of Tajabad , after Almagholagh mountain, beside of raceway	N: 340 53' 15" E: 480 11' 65"	2012	Salehi	2n=18	89.4.20
11	*A. bieberestinii*	Hamedan city, Ganjnameh area	N:340 43' 90.3" E: 480 25' 88.7"	2356	Salehi	2n=18	89.4. 22
12	*A. biebrestinii*	Hamedan, Ganjnameh area, right sides of waterfall	N: 340 45' 82.0" E: 480 26' 23.9. "	2248	Salehi	2n=18,36	89.4.21
13	*A. bieberestinii*	Hamedan, Ganjnameh, 300m higher than waterfall, beside of Kiwarestan campus	N: 340 45' 93.9" E: 480 25' 91.1"	2367	Salehi	2n=18,36	89.4.21
14	*A. bieberestinii*	Hamedan, road of Ganjnameh ,the border of garden	N: 340 45' 52.6." E:480 26' 34.3"	2100	Salehi	2n=18,36	89.4.21
15	*A. bieberestinii*	Hamedan, village of Heiydarh, beside of road	N: 340 11' 15.1" E: 480 20' 15"	1890	Salehi	2n=18,36	89.4.22
16	*A. bieberestinii*	Hamedan, village of Moradbayg	N: 340 44' 89.4" E: 480 30' 17"	2059	Salehi	2n=18,36	89.4.21
17	*A. bieberestinii*	Hamedan, Nahavand city, Gian forest, right side of valley	N: 340 08' 03.5" E: 480 13' 05.2"	1730	Salehi	2n=18,36	89.4.18

Polyploidy and hybridization have been essential for massive secondary evolutionary radiation in Achillea as in many other angiosperm clades (Leitch and Bennett 1997; Wendel 2000; Soltis et al., 2003). Therefore polyploidy has an important role in the evolution and speciation in higher plants, which are estimated from 35 to 80% of all species (Stebbins 1971; Soltis and Soltis 2000). The native flora of Iran comprises about 8000 angiosperm species. Chromosome counts on Iranian material have so far been carried out for about 1500 species, but in many cases only a single chromosome count has been studied (Ghaffari and Kelich 2006).

An aneuploid is an unbalanced polyploidy in cells or individuals having chromosome numbers different than basic number (Rieger et al., 1968). In aneuploids changing of chromosome numbers are occurred and it involves the addition or subtraction of chromosomes or the gain or loss of genetic material by means of a centromere without changing the total number of chromosome (Moore 1976). According to the original definition, aneuploidy refers to organisms having one or two or a few chromosomes more or less than the basic chromosome number in the species (Dyer et al., 1970).

Nevertheless, Achillea species are poorly known in terms of karyology and ploidy level; and there are few chromosome counts that were reported different chromosome number for a species. Our aims in this paper was to contribute to the general knowledge of chromosome number in Achillea tenuifolia and A. bieberestinii, to provide more information about their chromosome number variation. Therefore, the main objective of the study was to determine chromosome number in the different populations of Achillea tenuifolia and A. bieberestinii growing in West provinces of Iran.

Materials and methods

Plant materials- Plant samples of Achillea tenuifolia and A. bieberestinii plants were collected from the populations growing in natural areas in the West of Iran, Hamedan and Kermanshah provinces. Specimens were prepared and dried in a local herbarium at Bu-Ali Sina University and were determined using taxonomical keys and related references (Mozafarian 2005, 2007; Podlech 1986). Determination of the collected plant materials was also confirmed by the scientists at Rangeland Research Center of Hamedan.

Voucher specimens of the all studied materials were deposited in the Herbarium of Department of Biology, Faculty of Science, Bu-Ali Sina University, Iran (BHU). The locations, collectors and dates are shown in Table 1. Seeds were collected from at least ten plants in each population.

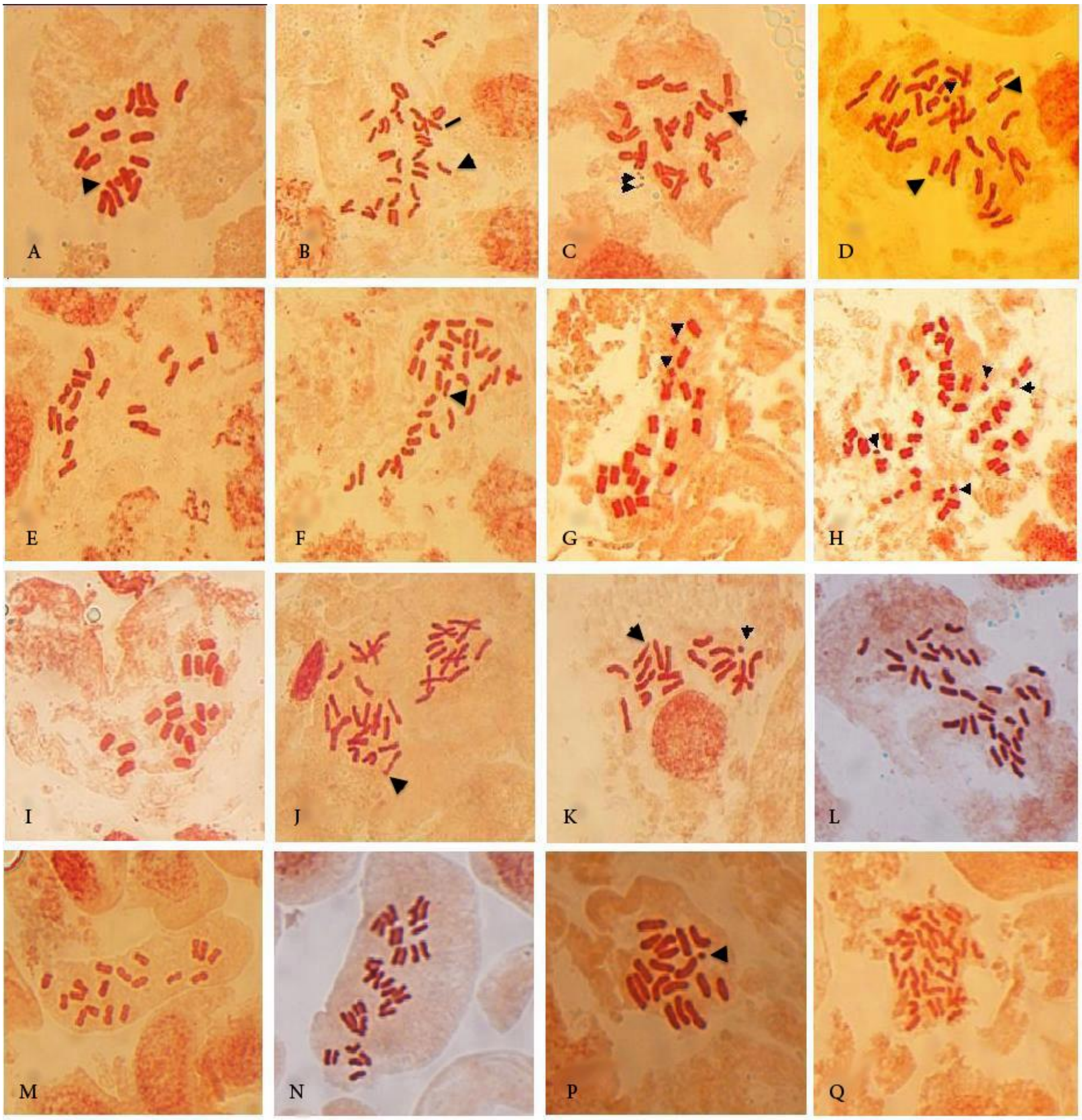

Fig. 1- Light micrographs of mitotic metaphase chromosomes prepared from different populations of Achillea tenuifolia. A, Population No. 1 with chromosome number 2n=2x=18; B, Population No. 2 with chromosome number 2n=4x=36. C-P, The populations 3-9 with two different chromosome numbers 2n=2x=18 and 2n=4x=36. Arrow indicated B-chromosomes or satelites chromosomes in the figures. Bar in all figures= 5µm.

Karyological studies: Chromosome counts were made on somatic metaphases using standard squash techniques. Seeds collected from wild samples were used in the present study. Root-tip meristems were obtained by germinating seeds on the wet filter paper in Petri dishes at approximately 20°C. Samples were pretreated with 0.05% colchicine for 2.5 h at room temperature. The materials were fixed in 3:1 v/v absolute ethanol: glacial acetic acid for a minimum of 24 h at 4°C.

Meristems were hydrolysed in 1 M hydrogen chloride (HCl) for 30 min at room temperature. They were then stained in 2% acetic orcein for a minimum of 3 h at 4°C (Chehregani et al. 2012). Squashes were made in 45% acetic acid. Photographs were taken through a Zeiss Axiostra microscope (Germany) with a Canon G11 (Japan) digital camera.

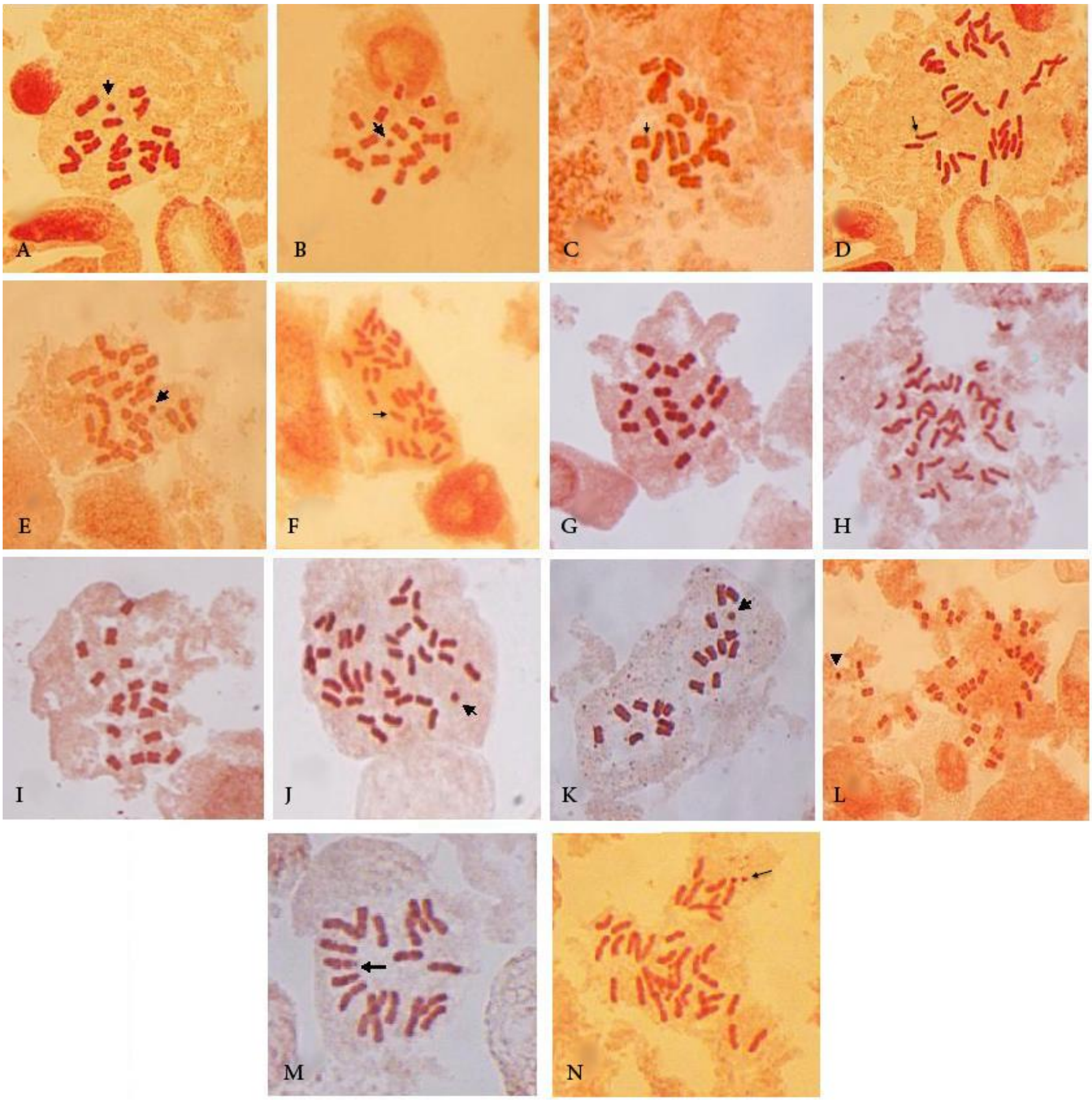

Fig. 2- Light micrographs of mitotic metaphase chromosomes prepared from different populations of Achillea biebersteinii. A, Population No. 1 with chromosome number 2n=2x=18; B, Population No. 2 with chromosome number 2n=2x=18. C-N, The populations 3-8 with two different chromosome numbers 2n=2x=18 and 2n=4x=36. Arrow indicated B-chromosomes or satelites chromosomes in the figures. Bar in all figures= 5µm.

Table 2-Somatic chromosome number in the 9 populations of *Achillea tenuifolia* and as detailed form.

Pop No.	Chromosome number / Name of species	18	36	17	16	15	19	Number of B chromosome	Number of Satellite
1	***A. tenuifolia***	18(8/10) 80%	-	17(1/10) 10%	-	15(1/10) 10%	-	-	-
2	***A. tenuifoila***	-	36(10/10) 100%	-	-	-	-	-	-
3	***Achillea tenuifoia***	18(37/50) 74%	36(13/50) 26%	-	-	-	-	(3/50)=6% 0-1B	(5/50)=10% 0-1
4	***A. tenuifolia***	18(78/93) 83.87%	36(5/93) 5.37%	17(6/93) 6.45%	16(3/93) 3.22%	-	-	-	(12/78)=12.9% 0-1
5	***A. tenuifoila***	18(24/34) 70.58%	36(2/34) 5.88%	17(3/34) 8.82%	16(4/34) 11.76%	-	19(1/34) 2.94%	(18/34)=52.94% 0-4B	(1/34)=2.94% 0-2
6	***A. tenuifoila***	18(18/30) 60%	36(4/30) 13.33%	17(2/30) 6.66%	16(3/30) 10%	-	19(3/30) 10%	-	-
7	***A. tenuifolia***	18(9/13) 69.23%	36(4/13) 30.76%	-	16(1/13) 7.69%	-	-	(1/13)=7.69% 0-1B	-
8	***A. tenuifoila***	18(26/40) 65%	36(7/40) 17.50%	17(2/40) 5%	16(3/40) 7.5%	15(2/40) 5%	-	(3/40)=7.5% 0-1B	(2/40)=5% 0-1
9	***A. tenuifoila***	18(47/52) 90.38	36(4/52) 7.69%	17 (1/52) 1.92%	-	-	-	(2/52)=3.84% 0-1B	-

To access the existence of published chromosome counts in the studied species, we used the most recent reports of chromosome numbers for this species (Chehregani and Mehanfar 2008; Chehregani and Hajisadeghian 2009), as well as the chromosome number databases, Index to Plant Chromosome Numbers (Missouri Botanical Garden, http://mobot.mobot.org/ W3T/Search/ipcn.html) and Index to Chromosome Numbers in the Asteraceae (Watanabe 2002, http:// www- asteraceae.cla.kobe-u.ac.jp/index.html).

Results

Nine populations of Achillea tenuifolia Lam and eight populations of A. bieberestinii Afan in Iran were studied in this research. Based on our results the basic chromosome number in the all studied populations was x=9, however, they were different regarding polyploid level from each other and two different ploidy level was determined in the studied populations. The results were represented as following.

Achillea tenuifolia

Nine populations of *Achillea tenuifolia* were studied for chromosome counting. In a population that was collected from Boghati mountain at the north of Hamedan province, chromosome number was 2n=18 (Fig.1A). A population with tetraploid chromosome number (2n=36) was also found in a protective area namely Lashkardar between the Malayer and Arak cities at the South of Hamedan provinve (Fig. 1B). In the all other studied populations that were collected from different regions of Hamedan and Kermanshah, two different chromosome numbers (2n=2x=18 and 2n=4x=36) were observed (Fig. 1 & Table 1). A population with just chromosome number 2n=2x=18 (Fig. 1a) and a population with chromosome number 2n=4x=36 (Fig. 1b) were determined, but the most common chromosome number among the seven remained populations is both 2n=18 and 2n=36 (Fig. 1 and Table 1). The chromosome number obtained for populations of A. tenuifolia (2n=2x=18) not supports the earlier report 2n=3x=27 by Khaniki (1995), but is accordance with chromosome number 2n=2x=18 reported by some prior researchers (Sheidai et al., 2009). While the chromosome number 2n=4x=36 is the first report for the species Achillea tenuifolia.

In addition to above-mentioned results, the study showed a regular aneuploid series 2n=15, 16, 17 and 19 within the some populations of this species (Table 2), that indicated occurrence conditions of aneuploidy in this species. In aneuploidy, reducing of chromosome number has been considered to be more common than increasing chromosome number since a descending change is simpler to produce than an ascending chromosome number.

B-chromosomes (Bs) were also observed in the some populations of this species (Fig. 1 and Table 2).

Table 3- Somatic chromosomes in the 8 population of *Achillea biberestinii* as detailed form.

Pop No.	Different Ch. number / Name of species	18	36	32	34	17	16	15	20	19	Number of B chromosome	Number of Satellite
1	***Achillea bieberestinii***	18 (9/9) 100%	-	-	-	-	-	-	-	-	(2/9)=22.22% 0-1B	(1/9)=11.11% 0-1
2	***A. bieberestinii***	18 (19/24) 79.16%	-	-	-	-	16(3/24) 12.5%	15 (1/24) 4.16%	-	19(1/24) 4.16%	(2/24)=8.33% 0-1B	(8/24)=33.33% 0-1
3	***A. bieberestinii***	18(17/25) 68%	36(2/25) 8%	-	-	-	16(3/25) 12%	-	20(3/25) 12%	-	(4/25)=16% 0-1B	(4/25)=16% 0-1
4	***A. bieberestinii***	18 (15/25) 60%	36(4/25) 16%	-	34(1/25) 4%	17(2/25) 8%	16(2/25) 8%	15(1/25) 4%	-	-	(1/25)=4% 0-2B	(3/25)=12% 0-2
5	***A. bieberestinii***	18 (42/51) 82.35%	36(3/51) 5.88%	32(1/51) 1.96%	-	-	16(5/51) 9.80%	-	-	-	(1/51)=1.96% 0-1B	(13/51)= 25.4% 0-1
6	***A. bieberestinii***	18 (27/36) 75%	36(3/36) 8.33%	-	34(4/36) 11.11%	-	16(2/36) 5.55%	-	-	-	(1/36)=2.77% 0-1B	(2/36)=5.55% 0-1
7	***A. bieberestinii***	18 (22/30) 53.33%	36(4/30) 13.33%	-	-	17(2/30) 6.66%	16(1/30) 3.33%	15(1/30) 3.33%	-	-	(4/30)=13.33% 0-1B	-
8	***A. bieberestinii***	18 (24/28) 85.71%	36(2/28) 7.14%	-	-	-	16(2/28) 7.14%	-	-	-	(1/28)=3.57% 0-1B	(12/28)=42.85% 0-2

The B-chromosomes were observed as much smaller than the A-chromosome , have a shape of round and did not pair with the A-chromosomes. B-chromosomes are accessory chromosomes existing in more than 1300 species of plants and 500 species of animals (Cammacho et al., 2000).

B-chromosomes (0-1) were reported in A. tenuifolia in a prior researche (Sheidai et al., 2009), while we were able to detect 0-4 B-chromosomes in the one population of this species. Variation in number of Bs among cells of the same root meristem was observed in some of the plants examined, which may be explained by nondisjunction during mitotic division of the meristem cells. Besides the variation in number from cell to cell and among populations, the B-chromosomes of A. tenuifolia diverged in size (from micro-size to about 0.7 μm) and morphology. The maximum number of cells containing Bs (52.94%) and the highest number Bs (0-4) were observed in the population collected from Soubashi in Hamedan province (see Fig. 1F).

Four populations of this species showed 0-1 satellaits chromosomes and one population have also 0-2 satellaits chromosomes. The population that has the highest number of cell contained satellaits chromosome (12.9%) collected from Assadbaad at the west of Hamedan province (Table 3).

Achillea biebersteinii

Chromosome counts were performed for the 8 populations of this species. Members of two populations, belonging to this species, showed chromosome number 2n=2x=18 (Fig. 2 A, B and Table 1).

One population was collected from Assadabad in the west of Hamedan province and other one collected from Ganjnameh area near the Hamedan city. In the other six populations two chromosome numbers (2n=2x=18 and 2n=4x=36) were observed at the same time (Fig. 2 and Table 1). Finding the chromosome number 2n=2x=18 confirmed the earlier reports (Morton 1981; Sheidai et al., 2009), and the chromosome number 2n=4x=36 for this species confirms the unique earlier report (Efimov 1998), but is opposite with the other report of chromosome number 2n=28 (Efimov 2005). Studies showed that polyploidy is common in this species, therefore existences of two ploidy levels for this species is accordance with prior reports. Otherwise we reported occurrence of B-chromosomes in this species for the first time that was 0-2 B-chromosomes in the most populations of this species. The maximum number of cells (22.22%) that contained Bs were observed in a population collected from Asadabad (Almagholagh montain) at the west of Hamedan province.

Only one population showed 0-2 Bs in their cells while other populations showed 0-1 Bs (Table 3).

In the otherwise the chromosome numbers of 2n=15, 16, 17, 19, 20, 32 and 34 were observed in the some populations (Table 3) that indicated the occurrence of aneuploidy in this species. The most occurrence of aneuploidy that were observed in this species were with chromosome numbers 2n=16 (12%) and 2n=20 (12%) in a population that collected from Ganjnameh at near the city of Hamedan.

In the most of populations of this species, 0-1 satellaits chromosomes were observed expect two populations that have 0-2 satellaits chromosomes. The population that has the highest number of cell contained satellaits chromosome (42.85%) collected from Nahavand (Gian forest) at the southwest of Hamedan province (see table 3).

Discussion

All of the examined populations have the same basic chro¬mosome number x=9 that is the most common basic number in tribe Anthemideae and the Asteraceae family (Inceer, et al. 2007). Polyploidy is another relevant evolutionary mechanism in plants (Wood, et al., 2009). Based on our results, few populations of the both species have chromosome number 2n=2x=18, and the most of populations of the species showed two different chromosome numbers 2n=2x=18 and 2n=4x=36 that suggests existence of different ploidy level among these species. In the same way, the polyploidy is also a response to the eco¬logical tolerances; It is suggested to study of ecological properties of the plant habitats. Chromosome number x=9 is the primitive basic chromosome number from which the others have been derived by aneuploidy (2n=15, 16, 17, 19, 20, 32 and 34) and autoploidy 2n=4x=36. Among the considered populations, most of populations of both species lose or received single or multiple chromosomes that is represented existence of aneuploidy in the genus. Aneuploidy arises from meiotic or mitotic deviations, or radiation and chemical treatment response (Stebbins 1971) and probably played an important role in the speciation mechanism and seeming to be an important component of chromosomal evolution in this genus. The presence of B-chromosomes, which is common in the species of A. tenuifolia and A. bieberestinii, is one of the most particular futures of the both species. Meristem cells showed variation in B-chromosome numbers, size and morphology, both in individual cells of the same plant and in different plants.

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