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ARTICLE

Comparative cytogenetic within *Achillea* genus (Asteraceae) in Iran: I. chromosome number and morphology

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ABSTRACT The cytological features including chromosome numbers and detailed chromosome measurements of 46 populations of ten *Achillea* species (*A. aleppica, A. biebersteinii, A. eriophora, A. filipendula, A. millefolium, A. nobilis, A. pachycephala, A. tenuifolia, A. vermicularis*, and *A. wilhelmsii*) were reported from Iran. Sample plants and seeds were collected from different geographical regions of Iran. Total chromosome length, long/short arm ratio, centromere index, total form percentage, inter and intra-chromosomal index, different relative lengths of the mitotic chromosomes were calculated. The populations showed one basic chromosome number *x = 9* with two ploidy levels (*2x* and *4x*). The chromosome numbers of *A. filipendula, A. nobilis* and *A. pachycephala,* for the first time are new to science and new ploidy levels are reported for *A. biebersteinii* (*4x*) and *A. wilhelmsii* (*2x*). The mean of chromosome length ranged from 3.8 µm in *A. millefolium* from Semnan, Chasham, Sirat to 6.37 µm in *A. nobilis* from Golestan, Agh Emam. Karyotype analysis indicated that chromosomes of *Achillea* taxa have predominantly median and sub-median centromeres. Several karyological aspects of these taxa are discussed in the light of the cytogenetic data. **Acta Biol Szeged 66(1):47-56 (2022)**

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Introduction

The genus *Achillea* L. belongs to Asteraceae (Compositae), the largest family of vascular plants. This genus comprises 110-140 flowering and perennial species and has a rather wide geographic distribution spanning Europe, temperate areas of Asia, North Africa, and a few growing in North America (Guo et al. 2004; Ehrendorfer and Guo 2006; Oberprieler et al. 2007). *Achillea* genus is also widespread in different regions of Iran, with 19 species, and seven of them are endemic (Mozaffarian 2006). These plants typically have hairy and aromatic leaves and flat clusters of small flowers on the top of the stem and the taxa vary broadly in morphology (Bremer 1994; Rechinger 1977).

Recent investigations including molecular methods show that *Achillea* has a taxonomic problem, because of being a polyploid complex (*x = 9*). Many races of the genus have high local variability, little consistent morphological differentiation over larger areas, many modifications, and few effective barriers to wide. These features cause some problems in the classification of the *Achillea* genus (Sharma and Sen 2002). Information on chromosome morphology is a powerful tool to characterize genomes. For any organism, karyological data represent essential information and provide important characters for plant systematics and evolutionary analysis (Baltisberger and Widmer 2009; Baltisberger and Hörandl 2016), because polyploidy has been reported as evidence for natural hybridization (Guo et al. 2005), so we must investigate the species and populations of *Achillea* for cytogenetics studies.

Achillea species have been studied extensively in different regions of the world for chromosome number and morphology. The basic chromosome number is often reported in species of *Achillea* is *x = 9* with polyploidy included *2n = 18, 36, 54* and *72* (Dabrowska 1992; Danihelka and Rotreklova 2000, 2001; Baltisberger 2002; Saukel et al. 2004; Guo et al. 2004, 2005; Constantinidis and Kalpoutzakis 2005; Ahmet 2006; Kiran et al. 2008, 2012; Ebrahim et al. 2012; Aksu et al. 2013; Shahin et al. 2019), but Only a few cytological studies have been published on the genus in Iran (Sheidai et al. 2009; Afshari et al. 2013; Chehreganirad and Javaheri 2014; Mazooji et al. 2014, 2016), and no karyological works have been published on *A. filipendula, A. nobilis* and *A. pachycephala* species in Iran. These indicated that the chromosome numbers and chromosome morphology were unknown for many *Achillea* species in Iran.

Therefore, this study aims are to determine the chromosome number and provide karyological data (chromosome numbers and karyotypes) of the genus *Achillea* species and find any relationship between the karyotype

characteristics and asymmetrical index with ploidy levels for the first time.

Materials and Methods

Plant materials

Seeds and clones of plants were collected from various locations of Iran (Table 1). The plants were identified by Flora Iranica (Rechinger 1977). Vouchers are deposited in gene bank RIFR (Research Institute of Forest and Rangelands) from Iran.

Chromosome analyses

Mitotic chromosomes were studied in meristematic cells of root tips (1-2 cm in length) obtained from seeds and rooted cuttings at 20 °C. Root tip meristems were pretreated with 0.5% saturated alpha-bromonaphthalene for 4 h at 4 °C, fixed in chromic acid 1%, formaldehyde 10% (1:1) for 24 h at 25 °C, then the root tips were rinsed in distilled water for 3 h, and were hydrolyzed in 1 M NaOH at 60 °C for 5 min, and then rinsed in distilled water for 2-3 min. Finally, staining was carried out using aceto-iron-hematoxylin for 5 h at room temperature. After staining, the root tips were washed in distilled water for 2 h (Javadi 2006; Javadi et al. 2009, 2019). Then slides were prepared by squashing in a droplet of 45% acetic acid. Metaphases were captured using an optical microscope (BX41 Olympus with a digital color video camera) at a magnification of about 2000x. The best metaphase plates were selected and used to prepare the karyotype by Adobe Photoshop 7.0 software. Finally karyotype characters were measured by Micro Measure 3.3 software (Reeves 2001). For each taxon, five karyograms were drawn based on the length of chromosome size (arranged large to small).

Karyotype characterization

The following parameters were measured in each metaphase plate to characterize the karyotypes numerically: diploid number of chromosomes (2n), long arm (LA), short arm (SA), total length (TL = LA+SA), arm ratio (AR = LA/SA), centromeric index [CI = SA/(LA+SA)], difference of range relative length (DRL = MaxRL%-MinRL%), (MaxRL% = [MaxTL/(ΣTL)×100], (MinRL% = [MinTL/(ΣTL)×100], that MaxRL% and MinRL% are relative length of longer and shorter chromosome respectively, karyotype formula (KF) according to Levan's method. The position of the centromere is the most important feature to characterize morphological differences of chromosomes (Levan et al. 1964), contribution of each arm from each chromosome to the total length of the karyotype (LA%) = [(ΣLA/Σ(LA+SA))×100], (SA%) = [(ΣSA/Σ(LA+SA))×100], total form percentage (TF%) = [(ΣSA/ΣTL)×100] (Huziwara 1962), intra-chromosomal asymmetry index (A_1) = 1-[Σ(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, inter-chromosomal asymmetry index (A_2) = s/x, where s and x are the average of standard deviation and mean of chromosome length respectively (Romero 1986). Numerical analyses were performed using Excel (2007) software.

Results and discussion

Analysis of karyotype of the species and populations

The cytogenetic examination indicated that the populations of the species were diploid, or tetraploid and had *2x* and *4x* chromosomes, respectively. Therefore, we found diversity in species and inside the populations in rare cases in terms of ploidy levels.

Achillea aleppica DC. is represented by five populations:

A. aleppica (9917): This accession was collected from Marivan, Zarivar Lake, and 1348 m. Its chromosome number is *2n = 18*. It consists of five median region (m) chromosomes and four submedian (sm) chromosomes (Table 1, Fig. 1A).

A. aleppica (9940): This accession was collected from Baneh, Siranband Board, and 1640 m. Its chromosome number is *2n = 18*. It consists of seven m and two sm chromosomes (Table 1, Fig. 1B).

A. aleppica (9967): This accession was collected from Kamyaran, 25 km Sanandaj, Kechle fence, and 1543 m. Its chromosome number is *2n = 18*. It consists of eight m and one sm chromosomes (Table 1, Fig. 1C).

A. aleppica (18009): This accession was collected from Naghadeh, Soltan Yaghub, and 1389 m. Its chromosome number is *2n = 18*. It consists of six m and three sm chromosomes (Table 1, Fig. 1D).

A. aleppica (22793): This accession was collected from Mianduab, Dash Alti, and 1449 m. Its chromosome number is *2n = 18*. It consists of seven m and two sm chromosomes (Table 1, Fig. 1E).

A. aleppica with one ploidy level in this study contradicts the study of Chehregani-Rad and Javaheri (2014), which showed *A. aleppica* with different ploidy levels ranging from *2x* to *8x*. The mean length of chromosome differs from 5.76 µ *in A. aleppica* (9940) to 6.62 µ in *A. aleppica* (22793). The mean value of AR, CI, TF%, DRL were 1.53,

Table 1. Karyotype characteristics of 46 populations of *Achillea* species.

Taxa	GBC	x	2n	TCL (µm)	MCL (µm)	AR	CI	TF%	A_1	A_2	DRL	KF	
												m	Sm
A. aleppica DC.	9917	2	18	54.55	6.06	1.57	0.40	41.42	0.28	0.46	6.45	5	4
A. aleppica DC.	9940	2	18	51.82	5.76	1.61	0.39	38.10	0.35	0.48	8.02	7	2
A. aleppica DC.	9967	2	18	54.84	6.09	1.42	0.42	42.67	0.25	0.47	7.11	8	1
A. aleppica DC.	18009	2	18	56.79	6.31	1.51	0.41	41.08	0.26	0.48	8.20	6	3
A. aleppica DC.	22793	2	18	59.61	6.62	1.53	0.41	40.84	0.29	0.41	5.65	7	2
A. biebersteinii Afan	4280	2	18	48.55	5.39	1.62	0.39	38.51	0.35	0.41	5.53	6	3
A. biebersteinii Afan	10140	4	36	82.97	4.61	1.54	0.41	40.29	0.32	0.42	5.59	12	6
A. biebersteinii Afan	14831	2	18	42.56	4.73	1.39	0.42	42.88	0.23	0.43	5.12	8	1
A. biebersteinii Afan	17310	2	18	37.92	4.21	1.61	0.39	39.27	0.34	0.44	5.99	5	4
A. biebersteinii Afan	18478	4	36	85.79	4.76	1.57	0.40	40.42	0.29	0.18	3.50	13	5
A. eriophora DC.	15531	2	18	49.09	5.45	1.95	0.35	37.27	0.37	0.49	9.32	1	8
A. eriophora DC.	15924	2	18	60.19	6.69	1.67	0.39	41.81	0.28	0.46	7.38	4	5
A. eriophora DC.	26471	2	18	41.94	4.66	1.46	0.42	42.43	0.23	0.45	5.22	8	1
A. filipendula Lam.	8372	2	18	48.85	5.43	1.64	0.39	39.32	0.33	0.27	2.84	7	2
A. filipendula Lam.	19490	2	18	46.59	5.18	1.68	0.39	39.43	0.32	0.27	2.60	6	3
A. filipendula Lam.	27498	2	18	45.81	5.09	1.73	0.38	38.28	0.35	0.39	3.04	6	3
A. filipendula Lam.	27505	2	18	42.56	4.73	1.43	0.42	41.70	0.27	0.41	2.42	8	1
A. filipendula Lam.	22622	4	36	68.42	3.90	1.49	0.41	40.87	0.29	0.23	1.91	16	2
A. millefolium L.	11277	2	18	53.12	5.90	1.74	0.38	41.32	0.29	0.43	5.58	4	5
A. millefolium L.	14303	4	36	83.71	4.65	1.62	0.39	41.04	0.29	0.37	3.61	13	5
A. millefolium L.	14921	2	18	53.91	5.99	1.61	0.40	41.63	0.33	0.43	5.21	5	4
A. millefolium L.	15847	2	18	58.47	6.50	1.79	0.37	37.54	0.37	0.43	6.70	4	5
A. millefolium L.	17225	2	18	34.20	3.8	1.60	0.41	42.54	0.32	0.32	3.66	5	4
A. millefolium L.	18453	4	36	79.10	4.39	1.62	0.39	39.30	0.34	0.35	3.98	12	6
A. nobilis L.	16273	2	18	60.59	6.73	1.51	0.42	42.02	0.27	0.29	2.22	7	2
A. nobilis L.	23156	2	18	50.44	5.61	1.65	0.39	39.58	0.32	0.24	2.46	6	3
A. nobilis L.	27019	2	18	36.29	4.03	1.74	0.39	38.64	0.33	0.24	2.48	5	4
A. nobilis L.	27023	2	18	49.45	5.49	1.55	0.40	40.31	0.31	0.28	2.32	7	2
A. nobilis L.	27030	2	18	54.66	6.07	1.79	0.36	37.41	0.36	0.26	2.03	4	5
A. pachycephala Rech.f.	10208	4	36	69.36	4.32	1.52	0.41	42.32	0.25	0.21	1.92	17	1
A. pachycephala Rech.f.	18642	4	36	69.12	4.09	1.32	0.44	43.83	0.21	0.20	1.77	17	1
A. tenuifolia Lam.	9053	2	18	45.52	5.06	1.41	0.42	41.74	0.27	0.26	2.09	7	2
A. tenuifolia Lam.	17006	2	18	53.36	5.92	1.40	0.42	42.47	0.24	0.28	2.23	8	1
A. tenuifolia Lam.	21695	4	36	73.01	4.09	1.38	0.44	42.90	0.23	0.29	2.72	17	1
A. tenuifolia Lam.	22829	2	18	46.59	5.20	1.41	0.42	42.81	0.24	0.30	2.02	6	3
A. tenuifolia Lam.	24760	2	18	53.90	6.00	1.44	0.39	38.80	0.34	0.30	2.72	6	3
A. vermicularis Trin.	9093	4	36	76.07	4.22	1.36	0.43	43.42	0.23	0.17	1.72	15	3
A. vermicularis Trin.	16992	4	36	74.09	4.12	1.38	0.43	43.19	0.23	0.21	1.95	17	1
A. vermicularis Trin.	19488	2	18	57.96	6.44	1.49	0.42	41.68	0.26	0.26	1.69	6	3
A. vermicularis Trin.	22833	4	36	86.24	4.81	1.70	0.39	39.46	0.34	0.31	2.02	11	7
A. vermicularis Trin.	27459	4	36	85.19	4.73	1.56	0.41	40.90	0.29	0.24	1.90	13	5
A. wilhelmsii C. Koch	12725	4	36	85.76	4.76	1.53	0.41	41.44	0.29	0.35	3.39	14	4
A. wilhelmsii C. Koch	15588	2	18	56.11	6.24	1.56	0.38	40.80	0.31	0.41	4.90	5	4
A. wilhelmsii C. Koch	15623	4	36	87.81	4.88	1.61	0.40	41.33	0.33	0.37	3.95	13	5
A. wilhelmsii C. Koch	15672	4	36	103.89	5.77	1.53	0.40	39.09	0.30	0.36	3.65	12	6
A. wilhelmsii C. Koch	15923	2	18	46.31	5.15	1.69	0.38	40.07	0.35	0.45	4.86	5	4

GBC: Gene Bank Code, **x**: Basic Chromosome number, **2n**: Somatic Chromosome number, **TCL**: Mean of Total Chromosome Length, **MCL**: Mean of Chromosome Length, **AR**: Mean of Arm Ratio, **CI**: Mean of Centromere Index, **TF%**: Mean of Total Form percent, **A_1**: Mean of intra-chromosome asymmetry Index, **A_2**: Mean of inter-chromosome asymmetry Index, **DRL**: Mean of Different of Relative Length, **KF**: Karyotype Formula (**m**: metacentric, **Sm**: Sub-metacentric).

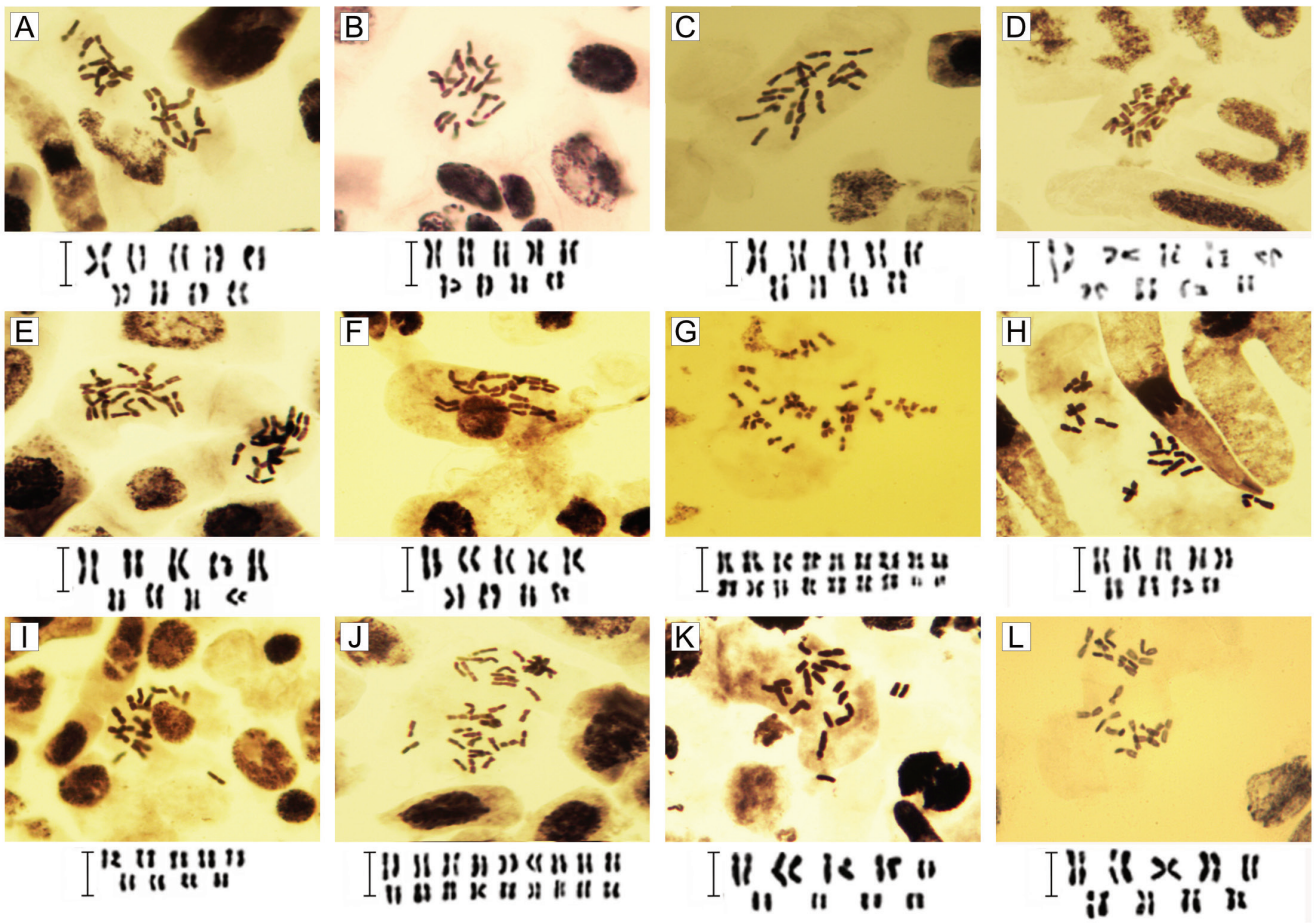

Figure 1. Metaphases of 12 taxa of Iranian *Achillea* L. Scale bar = 5 µm

0.41, 40.82, 7.09, and the asymmetry indices, A_1 and A_2, had values of 0.29 and 0.46, respectively (Table 1).

A. biebersteinii Afan is represented by five populations:

A. biebersteinii (4280): This accession was collected from Jafar Abad, Anjileh, Qahan region, and 2480 m. Its chromosome number is *2n = 18*. It consists of six m and three sm chromosomes (Table 1, Fig. 1F).

A. biebersteinii (10140): This accession was collected from Minodasht, Golestan National Park, Sharlegh, 1120 m. Its chromosome number is *2n = 36*. It consists of 12 m and six sm chromosomes (Table 1, Fig. 1G).

A. biebersteinii (14831): This accession was collected from Markazi, Arak, Maste Sofla region, and 1700 m. Its chromosome number is *2n = 18*. It consists of eight m and one sm chromosomes (Table 1, Fig. 1H).

A. biebersteinii (17310): This accession was collected from Shahroud, Kalpoush, Hosein Abad, and 1550 m. Its chromosome number is *2n = 18*. It consists of five m and four sm chromosomes (Table 1, Fig. 1I).

A. biebersteinii (18478): This accession was collected from Minodasht, Golestan National Park, and 837 m. Its chromosome number is *2n = 36*. It consists of 13 m and five sm chromosomes (Table 1, Fig. 1J).

Sheidai et al. (2009), and Aksu et al. (2013), showed one ploidy level *2x* for *A. biebersteinii*, but we reported two ploidy levels (*2n = 2x* = 18 and *2n = 4x* = 36) for this species that are new to science. The mean length of the chromosome in *A. biebersteinii* differs from 4.21 µm *in A. biebersteinii* (17310) to 5.39 µm in *A. biebersteinii* (4280). The mean value of AR, CI, TF%, DRL were 1.55, 0.40, 40.27, 5.15, and the asymmetry indices, A_1 and A_2, had values of 0.31 and 0.37, respectively (Table 1).

A. eriophora DC. This species is endemic of Iran and is represented by three populations:

A. eriophora (15531): This accession was collected from

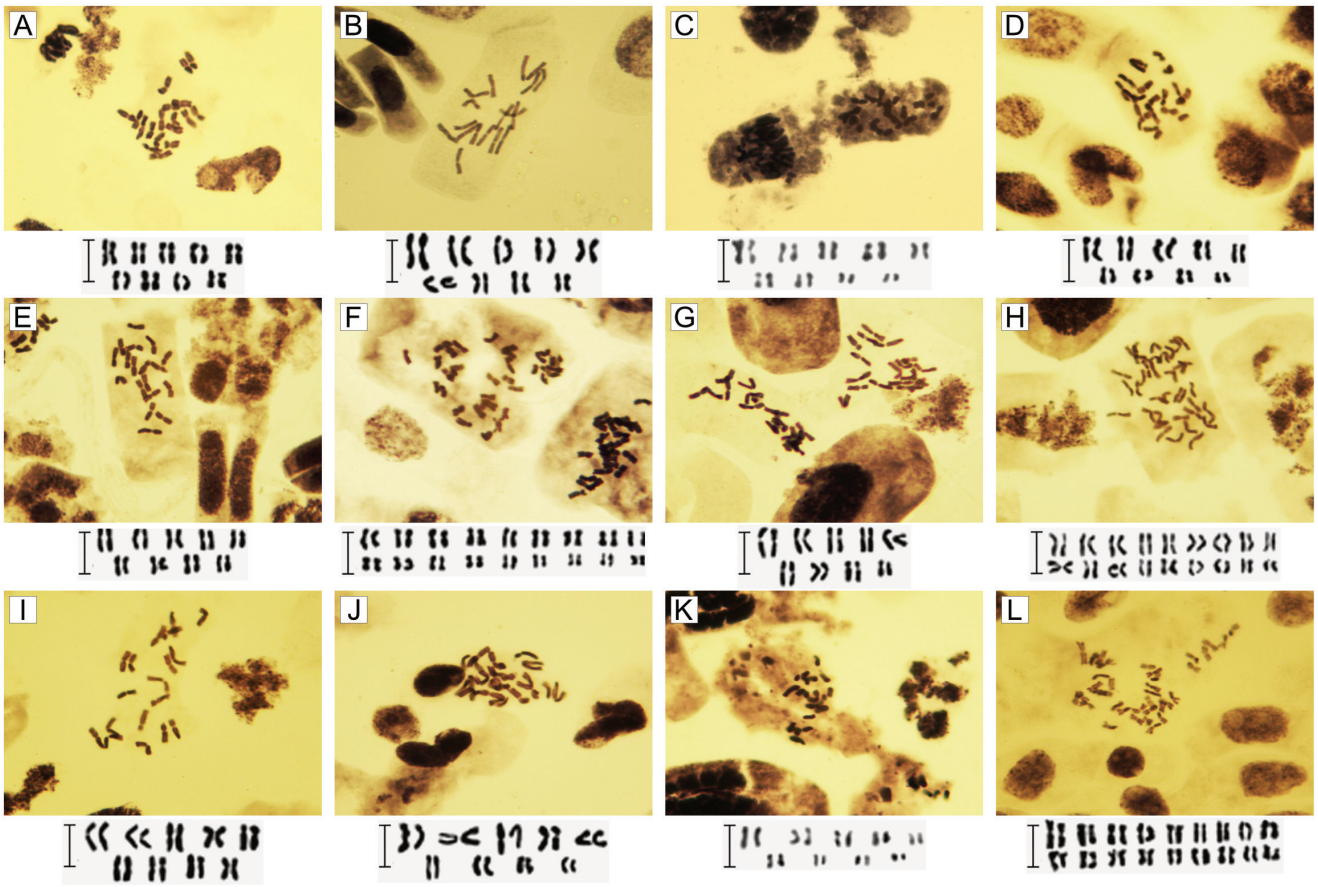

Figure 2. Metaphases of 12 taxa of Iranian *Achillea* L. Scale bar = 5 µm

Khatam, Harat, Road of Khansar, and 1838 m. Its chromosome number is *2n = 18*. It consists of one m and eight sm chromosomes (Table 1, Fig. 1K).

A. eriophora (15924): This accession was collected from Khatam, Harat, Harat to Khansar, 1795 m. Its chromosome number is *2n* = 18. It consists of four m and five sm chromosomes (Table 1, Fig. 1L).

A. eriophora (26471): This accession was collected from Baft, Khabar, Khabar National Park, and 2600 m. Its chromosome number is *2n* = 18. It consists of eight m and one sm chromosomes (Table 1, Fig. 2A).

Our findings were confirmed by Sheidai et al. (2009), that showed *2n = 2x* = 18 chromosome number for *A. eriophora*. The mean length of the chromosome *in A. eriophora* differs from 4.66 µm *in A. eriophora* (26471) to 6.69 µm in *A. eriophora* (15924). The mean value of AR, CI, TF%, DRL were 1.69, 0.39, 40.50, 7.31, and the asymmetry indices A_1 and A_2 had values of 0.29 and 0.47, respectively (Table 1).

A. filipendula Lam. is represented by five populations:

A. filipendula (8372): This accession was collected from Ardabil, Samiran, and 1320 m. Its chromosome number is *2n = 18*. It consists of seven m and two sm chromosomes (Table 1, Fig. 2-B).

A. filipendula (19490): This accession was collected from Baneh, Marivan, before Bayan valley, 1785 m. Its chromosome number is *2n = 18.* It consists of six m and three sm chromosomes (Table 1, Fig. 2C).

A. filipendula (27498): This accession was collected from West Azerbaijan, Sardasht, Mirabad, 1221 m. Its chromosome number is *2n = 18.* It consists of six m and three sm chromosomes (Table 1, Fig. 2D).

A. filipendula (27505): This accession was collected from Piranshahr, 25 km Sardasht, Tarkesh, and 1500 m. Its chromosome number is *2n = 18.* It consists of eight m and one sm chromosomes (Table 1, Fig. 2E).

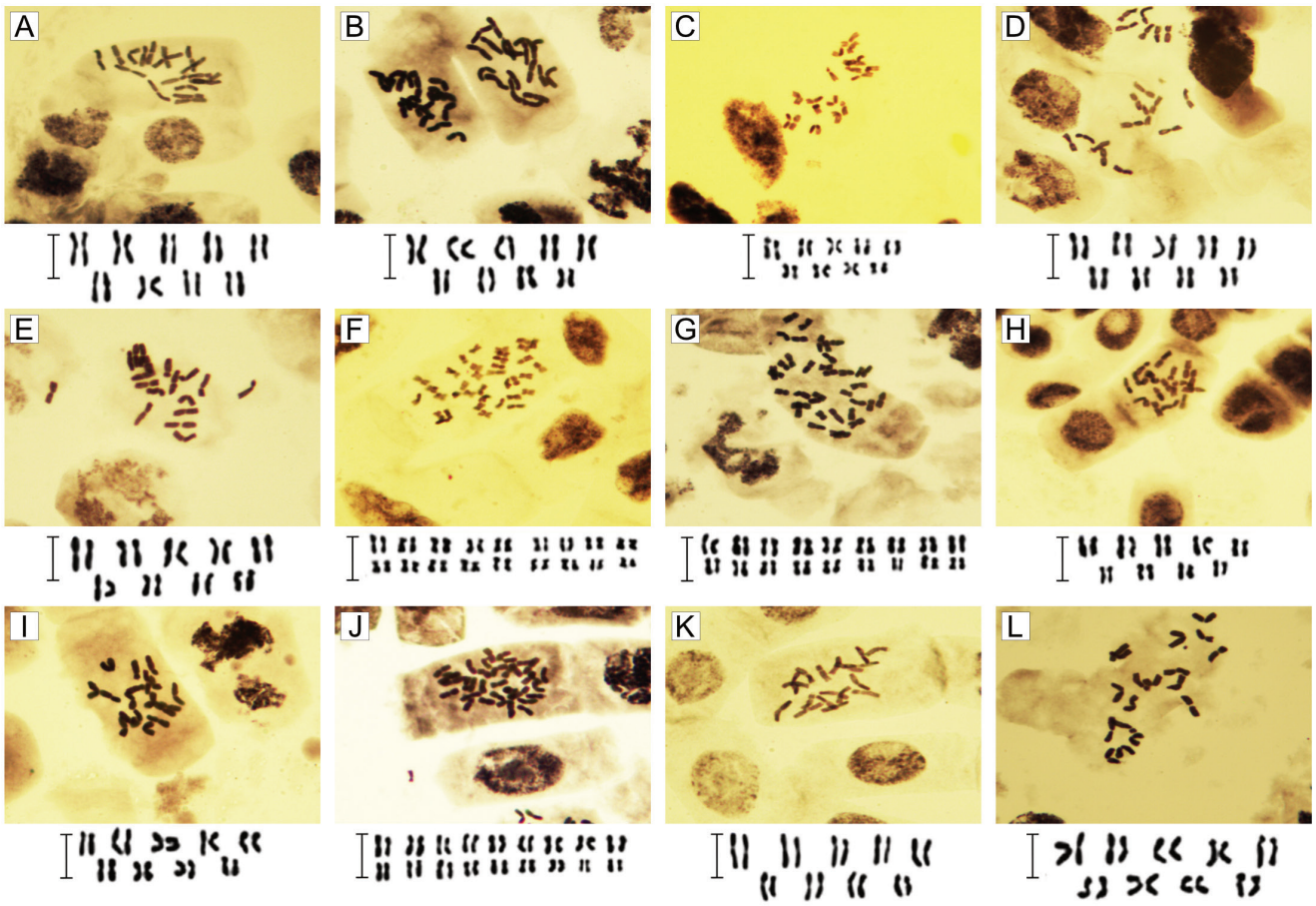

Figure 3. Metaphases of 12 taxa of Iranian *Achillea* L. Scale bar = 5 µm

A. filipendula (22622): This accession was collected from Marivan, Bilchasor, and 1500 m. Its chromosome number is *2n = 36.* It consists of 16 m and two sm chromosomes (Table 1, Fig. 2F).

This is the first report on chromosome number and morphology of *A. filipendula*. The mean length of chromosome differs from 3.90 µm *in A. filipendula* (22622) to 5.43 µm in *A. filipendula* (8372). The mean value of AR, CI, TF%, DRL were 1.59, 0.40, 39.92, 2.56, and the asymmetry indices A_1 and A_2 had values of 0.31 (Table 1).

A. millefolium L. is represented by six populations:

A. millefolium (11277): This accession was collected from Esfahan, Kashan, Qamsar, 1850 m. Its chromosome number is *2n = 18.* It consists of four m and five sm chromosomes (Table 1, Fig. 2G).

A. millefolium (14303): This accession was collected from Sanandaj, Research Station Kharkeh, and 1700 m. Its chromosome number is *2n = 36.* It consists of 13 m and five sm chromosomes (Table 1, Fig. 2H).

A. millefolium (14921): This accession was collected from Hamadan, Malayer, and 1725 m. Its chromosome number is *2n = 18.* It consists of five m and four sm chromosomes (Table 1, Fig. 2I).

A. millefolium (15847): This accession was collected from Khatam, Harat, Siyahobe to Chehelgoeieh, and 1743 m. Its chromosome number is *2n = 18.* It consists of four m and five sm chromosomes (Table 1, Fig. 2J).

A. millefolium (17225): This accession was collected from Semnan, Chasham, Sirat, 3000 m. Its chromosome number is *2n* = 18. It consists of five m and four sm chromosomes (Table 1, Fig. 2K).

A. millefolium (18453): This accession was collected from Gorghan, Chahar Bagh, Yargoli Garden, and 1450 m. Its chromosome number is *2n = 36.* It consists of 12 m and six sm chromosomes (Table 1, Fig. 2L).

Diploid and tetraploid levels (*2x* and *4x*) for *A. millefolium* are confirmed by studies of Afshari et al. (2013), while other ploidy levels (*6x*, *7x* and *8x*) were proved by Panihelka and Rotrekolva (2001), Mazooji et al. (2016), Aksu et al. (2013), Sheidai et al. (2009), Nezhadi et al. (2021), and Baltisberger (2002). The mean length of the chromosome differs from 3.8 μm in *A. millefolium* (17225) to 6.50 μm in *A. millefolium* (15847). The mean value of AR, CI, TF%, DRL were 1.66, 0.39, 40.56, 4.79, and the asymmetry indices A_1 and A_2, had values of 0.32 and 0.39, respectively (Table1).

A. nobilis L. is represented by five populations:

A. nobilis (16273): This accession was collected from Golestan, Agh Emam, Maraveh tappeh, Alteraghchin, 1039 m. Its chromosome number is *2n = 18.* It consists of seven m and two sm chromosomes (Table 1, Fig. 3A).

A. nobilis (13156): This accession was collected from Zanjan, Abdal, and 1850 m. Its chromosome number is *2n = 18*. It consists of six m and three sm chromosomes (Table 1, Fig. 3B).

A. nobilis (27019): This accession was collected from Talesh, Navan Mountains, and 1360 m. Its chromosome number is *2n = 18*. It consists of five m and four sm chromosomes (Table 1, Fig. 3C).

A. nobilis (27023): This accession was collected from Astara, Minoohony Mountains, and 1460 m. Its chromosome number is *2n = 18.* It consists of seven m and two sm chromosomes (Table 1, Fig. 3D).

A. nobilis (27030): This accession was collected from Talesh, Navan, Navan Mountains, and 1380 m. Its chromosome number is *2n = 18*. It consists of four m and five sm chromosomes (Table 1, Fig. 3E).

This is the first report on chromosome number and morphology of *A. nobilis* from Iran. Chromosome number 18 for *A. nobilis* from Turkey was recorded by Kiran et al. (2012) and Aksu et al. (2013). The mean length of the chromosome differs from 4.03 μm *in A. nobilis* (27019) to 6.73 μm in *A. nobilis* (16273). The mean value of AR, CI, TF%, DRL were 1.65, 0.39, 39.59, 2.30, and the asymmetry indices, A_1 and A_2, had values of 0.32 and 0.26, respectively (Table 1).

A. pachycephala Rech. f. This species is endemic of Iran and is represented by two populations:

A. pachycephala (10208): This accession was collected from Minoodashat, Golestan National Park, Sharlegh, and 1120 m. Its chromosome number is *2n = 36.* It consists of 17 m and one sm chromosomes (Table 1, Fig. 3F).

A. pachycephala (18642): This accession was collected from Golestan, 837 m. Its chromosome number is *2n = 36.* It consists of 17 m and one sm chromosomes (Table 1, Fig. 3G).

This is the first report on chromosome number and morphology of *A. pachycephala*. Two populations have the same karyotype formula (17m + 1sm). The mean length of chromosome in *A. pachycephala* (10208) and *A. pachycephala* (18642) were 4.32 μm and 4.09 μm, respectively. In comparing two populations, the most value of AR (1.52), A_1 (0.25), A_2 (0.21), DRL (1.92) and CI (0.44), TF% (43.83) was obtained in *A. pachycephala* (10208) and *A. pachycephala* (18642), respectively (Table 1).

A. tenuifolia Lam. is represented by five populations:

A. tenuifolia (9053): This accession was collected from Sanandaj, Divandarreh, after Vahdat dam, 1590 m. Its chromosome number is *2n* = 18. It consists of seven m and two sm chromosomes (Table 1, Fig. 3H).

A. tenuifolia (17006): The collection place of this accession is unknown. Its chromosome number is *2n = 18*. It consists of eight m and one sm chromosomes (Table 1, Fig. 3I).

A. tenuifolia (21695): This accession was collected from Semnan, Shirin Chah, and 1402 m. Its chromosome number is *2n = 36*. It consists of 17 m and one sm chromosomes (Table 1, Fig. 3J).

A. tenuifolia (22829): This accession was collected from Khoy, Qator, Hendovan, 1949 m. Its chromosome number is *2n = 18.* It consists of six m and three sm chromosomes (Table 1, Fig. 3K).

A. tenuifolia (24760): The collection place of this accession is unknown. Its chromosome number is *2n = 18*. It consists of six m and three sm chromosomes (Table 1, Fig. 3L).

Our findings (*2x* and *4x*) for *A. tenuifolia* are confirmed by studies of Nezhadi et al. (2021), also one ploidy level (*2x*) was proved by Sheidai et al. (2009). The mean length of the chromosome differs from 4.09 μm *in A. tenuifolia* (21695) to 6.00 μm in *A. tenuifolia* (24760). The mean value of AR, CI, TF%, DRL were 1.41, 0.42, 41.74, 2.36, and the asymmetry indices, A_1 and A_2, had values of 0.26 and 0.29, respectively (Table 1).

A. vermicularis Trin is represented by five populations:

A. vermicularis (9093): This accession was collected from Baneh, 15 km to Saqqez, 1860 m. Its chromosome number is *2n = 36*. It consists of 15 m and three sm chromosomes (Table 1, Fig. 4A).

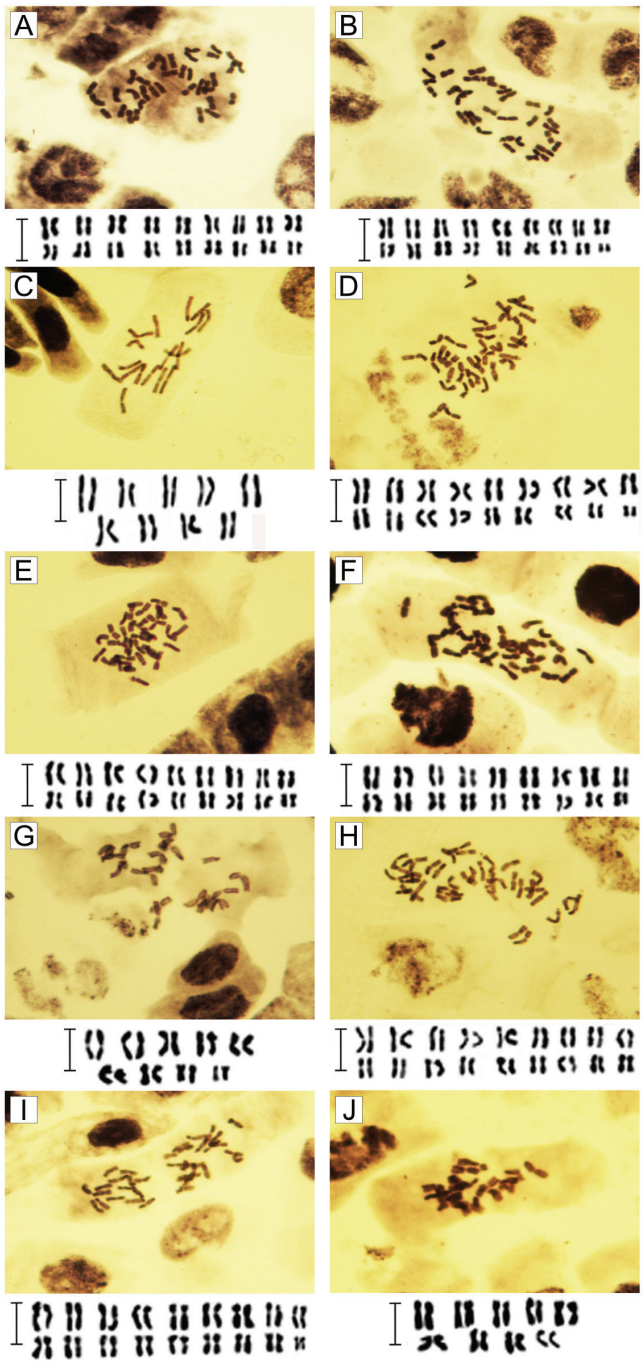

Figure 2. Metaphases of 12 taxa of Iranian *Achillea* L. Scale bar = 5 µm

A. vermicularis (16992): This accession was collected from Kordestan, 2400 m. Its chromosome number is *2n = 36.* It consists of 17 m and one sm chromosomes (Table 1, Fig. 4B).

A. vermicularis (19488): This accession was collected from Piranshahr to Sardasht, Mirabad, and 1245 m. Its chromosome number is *2n = 18*. It consists of six m and three sm chromosomes (Table 1, Fig. 4C).

A. vermicularis (22833): This accession was collected from Uromeyyeh, Qasemlo Valley, Nij Valley, 1631 m. Its chromosome number is *2n = 36*. It consists of 11 m and seven sm chromosomes (Table 1, Fig. 4D).

A. vermicularis (27459): This accession was collected from West Azerbaijan, Sardasht, Inspect Station, 1830 m. Its chromosome number is *2n = 36.* It consists of 13 m and five sm chromosomes (Table 1, Fig. 4E).

We find two ploidy levels (*2x* and *4x*), as findings of Nezhadi et al. (2021), while Sahin et al. (2006) and Sheidai et al. (2009), recorded *2x* and *4x* ploidy levels for *A. vermicularis* from Turkey and Iran, respectively. The mean length of the chromosome differs from 4.12 µm *in A. vermicularis* (16992) to 6.44 µm in *A. vermicularis* (19488). The mean value of AR, CI, TF%, DRL were 1.50, 0.42, 41.73, 1.86, and the asymmetry indices, A_1 and A_2, had values of 0.27 and 0.24, respectively (Table 1).

A. wilhelmsii C. Koch is represented by five populations:

A. wilhelmsii (12725): This accession was collected from Korramabad, Robat, and 1350 m. Its chromosome number is *2n = 36*. It consists of 14 m and four sm chromosomes (Table 1, Fig. 4F).

A. wilhelmsii (15588): This accession was collected from Tafresh, garden in entrance of Tafresh, and 1978 m. Its chromosome number is *2n = 18.* It consists of five m and one four chromosomes (Table 1, Fig. 4G).

A. wilhelmsii (15623): This accession was collected from Arak, Miqan desert, 1676 m. Its chromosome number is *2n = 36*. It consists of 13 m and five sm chromosomes (Table 1, Fig. 4H).

A. wilhelmsii (15672): This accession was collected from Abyek Abad, between Mashhad and Miqan, 1680 m. Its chromosome number is *2n = 36*. It consists of 12 m and six sm chromosomes (Table1, Fig. 4I).

A. wilhelmsii (15923): This accession was collected from Taft, Sanij, after sanij pool, 2250 m. Its chromosome number is *2n = 18*. It consists of five m and four sm chromosomes (Table 1, Fig. 4J).

Our finding, two ploidy levels (*2x* and *4x*) is the first record for *A. wilhelmsii* from Iran. However, Nezhadi et al. (2021) and Sheidai et al. (2009) proved only *4x* for *A.*

wilhelmsii. The mean length of the chromosome differs from 4.76 μm in *A. wilhelmsii* (12725) to 6.24 μm in *A. wilhelmsii* (15588). The mean value of AR, CI, TF%, DRL were 1.58, 0.39, 40.55, 4.15, and the asymmetry indices, A_1 and A_2, had values of 0.32 and 0.39, respectively (Table 1).

Comparison of cytogenetic parameters between species

The comparison of the results of chromosome numbers between populations, indicated two ploidy levels (*2x* and *4x*) with one basic chromosome number (*x = 9*). Regarding the ploidy level, there was diversity not only among the species but also among the populations, except for *A. aleppica*, *A. eriophora*, *A. nobilis* and *A. pachycephala*, with diploid and tetra-ploidy levels, respectively. *A. bibersteinii*, *A. filipendula*, *A. millefolium*, *A. tenuifolia*, *A.vermicularis*, and *A. wilhelmsii* were tetra and diploid levels.

Karyotype formulas of intra-species and populations were different, and all the chromosomes were metacentric and sub-metacentric. Karyotypes consisted of many metacentric chromosomes and a small number of chromosomes were sub-metacentric in all populations.

The highest of chromosome length belonged to populations *A. nobilis* (16273), *A. eriophora* (15924), and *A. aleppica* (22793) with an average of 6.73, 6.69 and 6.62 μm, respectively, whereas the lowest of chromosome length belonged to population *A. millefolium* (17225) with an average of 3.8 μm. *A. eriophora* (15531) (AR = 1.95, A_1 = 0.37), *A. millefolium* (15847) (AR = 1.79, A_1 = 0.37), and *A. nobilis* (27030) (AR = 1.79, A_1 = 0.36) with the highest average for arms ratio (AR) and intra-chromosomal asymmetry index (A_1), had asymmetrical chromosomes based on these indexes. On the contrary, arms ratio (AR) and intra-chromosomal asymmetry indexes (A_1) belonged to *A. pachycephala* (18642) (AR = 1.32, A_1 = 0.21), *A. tenuifolia* (21695) (AR = 1.38, A_1 = 0.23), *A. vermicularis* (9093) (AR = 1.36, A_1 = 0.23), and *A. vermicularis* (16992) (AR = 1.38, A_1 = 0.23) populations were the lowest values of AR and A_1, so had symmetrical chromosomes based on these indexes.

In terms of the centromeric index and total form percentage (TF%), populations *A. pachycephala* (18642), *A. tenuifolia* (21695), *A. vermicularis* (9093) and *A. vermicularis* (16992) with a 0.44, 0.44, 0.43, 0.43 for centromeric index and 43.83, 42.90, 43.42, 43.19 for TF% had symmetrical chromosomes. Meanwhile, populations *A. eriophora* (15531), *A. melifolium* (15847) and *A. nobilis* (27030) with the lowest of a centromeric index (0.35, 0.37, 0.36) and TF% (37.27, 37.54, 37.41), had the most asymmetric chromosomes based on these indexes (Table 1). So, based on asymmetric indexes (AR, CI, TF%, A_1 and A_2), three populations *A. eriophora* (15531), *A. millefolium* (15847), *A. nobilis* (27030) and four populations *A. pachycephala* (18642), *A. tenuifolia* (21695), *A. vermicularis* (9093 and 16992), have asymmetric and symmetric karyotypes, respectively. Asymmetric karyotype in *A. eriophora* (15531), is confirmed by eight sub-meta and one metacentric chromosome in karyotype formula.

Since the relative difference in the lengths of chromosomes (DRL) had an inverse relationship with intra-species ploidy levels, the most asymmetric chromosomes among the tetra-ploidy populations, based on DRL index, belonged to population *A. biebersteinii* (10140) with an average of 5.59 (Table 1). For diploid populations, *A. eriophora* (15531) had the highest rate of chromosomal asymmetry with the highest DRL (9.32), so *A. eriophora* (15531) has a more asymmetrical karyotype than the other populations (size and morphology of chromosomes).

Conclusion

The results of the present study illustrated two ploidy level (*2n = 2x = 18* and *2n = 4x = 36*) in Iranian *Achillea* spp. No previous studies have been performed on the chromosomes of *A. filipendula*, *A. nobilis* and *A. pachycephala*. In all of the examined populations of species, the basic chromosome number was *x* = 9, with *A. aleppica, A. eriophora,* and *A. nobilis* being diploid, *A. pachycephala* tetraploid, and *A. biebersteinii*, *A. filipendula, A. millefolium*, *A. tenuifolia*, *A. vermicularis* and *A. wilhelmsii* mixoploid (*Diploid and Tetraploid*). The longest length of genome in tetraploid and diploid populations were observed in *A. wilhelmsii* (15672) and *A. nobilis* (16273). According to asymmetric indexes, *A. eriophora* (15531), *A. millifolium* (15847), *A. nobilis* (27030) and *A. pachycephala* (18642), *A. tenifolia* (21695), *A. vermicularis* (9093 and 16992) had asymmetric and symmetric karyotype, respectively.

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