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Research Article

Karyomorphological Studies in Genus *Anthemis* spp. (Anthemideae)

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Abstract

Chamomile (*Anthemis* L.), an important medicinal plant belonging to the Asteraceae family, is widely distributed in Iran and other parts of the world, with 175 species. We can study chromosome characteristics such as chromosome numbers and karyotype characteristics to realize genetic variation within and between species and their populations. In the present study, we examined the details of the chromosome number and karyological characteristics analysis of 25 populations representing four *Anthemis* species (*A. altissima, A. haussknechtii, A. trimfettii, and A. pseudocotula*) from geographically isolated regions of Iran. Specific staining method and microscopic observation showed somatic chromosome number 2n=18 for all populations, which were confirmed by the previous data. Analysis of the karyotype formula showed a dominance of metacentric chromosomes in almost all of them. The largest chromosome and genome length belonged to two populations of *A. pseudocotula* species. Populations of *A. pseudocotula* have chromosome size, and populations of *A. haussknechtii* have morphology chromosome variation. A cluster analysis of the tested accessions, at 11.19 genetic distance, created three main groups that showed the similarity of members of each group. Additionally, the level of symmetric karyotypes estimated by karyotype characters and the role of each trait in the variation of species and their populations are argued. Genetic variations were confirmed between diploid populations, for different karyotype characters. Observed variation mainly caused by the morphology of chromosomes, and that, its contribution was important in discriminating the populations. We hope these data will be used in future investigations as basic information for breeding and hybridization between species and their populations.

***Keywords*:** *Anthemis*, Chamomile, Chromosome, Cytogenetics, Karyotype, Symmetric

Introduction

The family Asteraceae, or sunflower family, also known as Compositae is one of the largest and most widespread plant families with almost 10% of all flowering plants worldwide (1700 genera and 25000 species) (Funk et al., 2009, 2005; Mandel et al., 2019). In the flora of Iran, Asteraceae is represented by about 1123 species (Rechinger, 1986). The majority of plant members representing this family are herbaceous plants, but some species, such as woody shrubs and trees, as well as creepers and climbers, are also reported (Garcia et al., 2010). *Anthemis* L. is one of the largest genus in the Compositae family and Anthemideae tribe, including about 175 species in the world (Jeffrey, 2007). *Anthemis* species are found throughout Europe, Southwest Asia, North America, Australia, New Zealand, North, East and South Africa. The center distribution of *Anthemis* is in Southwest Asia (Oberprieler et al., 2007; Presti et al., 2010). *Anthemis* L., "*Baboneh*" in Persian, consists of 39 annual and perennial herbaceous species (15 species endemic), distributed all over Iran (Mozaffarian, 2006).

The study of the structure, characteristics, and behavior of chromosomes during cell division (meiosis and mitosis) is called cytogenetics. Also, chromosomal changes are studied (Cruz-Sánchez et al., 2020). The results obtained from karyological studies (counting and characterization of chromosomes) are used to present the hypotheses of the evolution of the genus (Goula et al., 2022). The results and information obtained from Karyological studies (the somatic chromosome number 2n, the haploid number n, the basic number x, morphology, and the length of the genome), and classical cytogenetic approaches are used in plant taxonomy, cytogeography, and cytoecology to explain the course of evolution within species, genera, and families (Peruzzi and Eroglu, 2013).

Based on information obtained from the literature and 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 family

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Asteraceae, the count of chromosomes has been reported as 2n: 10, 14, 18, 22, 24, 26, 30, 34, 36, 38, 54 (Javadi, 2017; Chehregani-Rad et al., 2014; Sanchez-Jimenez et al., 2009; Tantray et al., 2021; Watanabe, 2002; Watanabe et al., 2007). In the genus *Anthemis*, many karyological investigations have been carried out on the chromosome numbers and karyological data, chromosome numbers of many thousands of *Anthemis* species are estimated as *2x* to *8x* with two basic chromosome numbers (*x=8* and *9*) (Chehregani-Rad and Mehanfar, 2008; Inceer and Hayirlioglu-Ayaz, 2007; Javadi et al., 2013; Peruzzi et al., 2016; Tantray et al., 2021). Extremely high chromosome numbers were reported in the *Anthemis* species is 2n=72. It seems that changes in the basic chromosome number of species take place in the final stages of their evolution (Javadi et al., 2013; Inceer and Hayirlioglu-Ayaz, 2007; Vallès et al., 2005; Vallès and McArthur, 2001; Vallès and Šiljak-Yakovlev, 1997). Nevertheless, it seems, that only, less than 40% of Anthemideae species, have studied chromosome numbers, and for the rest of the species, chromosome counting has not been done or is unclear (Vallès et al., 2005).

Based on numerous cytogenetic studies, few studies have dealt with the genetic diversity of chamomile in Iran, and new studies in cytogenetics are still strongly needed. Therefore, the present work aimed at increasing the knowledge about chromosome numbers and comparative karyological analysis between *Anthemis* species (*A. altissima*, *A. haussknechtii*, *A. trimfettii*, *A. pseudocotula*), and their populations, to find out possible variability among these populations. Additionally, the symmetric karyotypes will be estimated by karyotype characters. We hope such findings will help researchers promote an understanding of the relationships between the chromosomal criteria and taxonomic delimitations.

Materials and Methods

Plant Material

Karyotype study was performed on 25 populations of *Anthemis altissima* L. (Three populations), *A. haussknechtii* Boiss. & Reut. (Four populations), *A. trimfettii* (L.) All. (Eight populations) and *A. pseudocotula* Boiss. (10 populations). The seeds of plants were obtained from the Natural Resources Gene Bank of Iran. The collected locations of seeds are given in Table 1.

Method of Study

A technique for studying mitosis in plant species involved the squashing of root tips. This method permits the separation of cells and facilitates the spreading of their chromosomes. To stimulate cell divisions, root tips were pre-treatment with 0.5% saturated Apha-bromonaphthalene solution (1/100 ml in 10 ml of water) for 4 hr. at 4°C. After pre-treatment, root tips were transferred to a 1:1 fixative (Chromic acid 1%, Formaldehyde 10%), 24 hr. at 4°C. In this stage, the chromosomes, are washed under tap water for 3 hr. The tips of fixed roots were hydrolyzed in 1N NaOH at 60 °C for 5 min, and stained in Aceto-Iron-Hematoxylin for 24 hr. at room temperature. The stained roots were squashed in 45% acetic acid under a stereo microscope and per population, ten well-spread metaphase plates were selected and used to prepare the karyotype by Adobe Photoshop v.7.0 software. Micro Measure 3.3 software was used for measuring karyotype characters (Javadi et al., 2013; Reeves, 2001).

Karyotype Characterization

The following factors were calculated.

A diploid number of chromosomes (2n): The number of somatic chromosomes.

Length of arms: Length of long arm (L), short arm (S), and total length of chromosome (T=L+S).

The ratio of long arm to short arm (AR=L/S), and index of centromere [CI=S/ (L+S)].

The difference in range relative length of longer chromosome to shorter chromosome (DRL=Max RL%-Min RL%), that Max RL% and Min RL% are the relative lengths of longer and shorter chromosomes respectively.

Karyotype formula (KF): Differences in the types of chromosomes were determined by using the method of Levan et al. (1964).

Percentage of the length of the long and short arm of the total length of the karyotype (L%)=[(∑L/∑(L+S))*100] and (S%)=[(∑S/∑(L+S))*100].

Table 1. Populations of *Anthemis* spp. karyologically studied

No.	Population	Gene bank code (RIFR)	Location City, Province	Altitude (m)	Longitude	Latitude	2n	x	∑TL	SC	KF
1	*Anthemis altissima*	9885	Sanandaj, Kordestan	1331	46˚ 58′51″ E	34˚58′95″ N	18	9	55.93	1A	6m+3sm
2	*A. altissima*	12790	Asadabad, Hamadan	1600	48˚11′56″ E	34˚78′00″ N	18	9	59.28	1A	6m+3sm
3	*A. altissima*	29610	Shahreh Kord, Chaharmahale Bakhtiyari	2095	50˚55′56″ E	32˚18′00″ N	18	9	56.07	2A	6m+3sm
4	*A. haussknechtii*	10058	Baneh, Kordestan	1540	45˚53′00″ E	35˚59′00″ N	18	9	63.45	2A	9m
5	*A. haussknechtii*	26044	Divandareh, Kordestan	1850	47˚01′00″ E	35˚54′00″ N	18	9	61.03	3A	3m+6sm
6	*A.haussknechtii*	13818	Gorgan, Golestan	160	54˚46′00″ E	36˚50′00″ N	18	9	55.10	2A	6m+3sm
7	*A. haussknechtii*	10791	Ivan, Ilam	1140	46˚17′00″ E	33˚49′00″ N	18	9	61.69	2A	6m+3sm
8	*A. triumfettii*	30017	Gorgan, Golestan	176	54˚25′00″ E	36˚50′00″ N	18	9	54.78	2A	6m+3sm
9	*A. triumfettii*	23955	Gorgan, Golestan	165	54˚46′18″ E	36˚50′41″ N	18	9	57.11	2A	6m+3sm
10	*A. triumfettii*	14170	Urumiyeh, West	1332	45˚02′00″ E	37˚32′00″ N	18	9	55.42	2A	3m+5sm+1st
11	*A. triumfettii*	11900	Sarein, Ardabil	1800	48˚04′00″ E	38˚09′00″ N	18	9	64.25	2A	3m+6sm
12	*A. triumfettii*	16684	Ramiyan, Golestan	1368	55˚03′25″ E	36˚51′55″ N	18	9	59.76	2A	4m+3sm+2st
13	*A. triumfettii*	21605	Semnan, Semnan	2015	53˚34′02″ E	36˚01′25″ N	18	9	57.51	2B	4m+4sm+1st
14	*A. triumfettii*	27544	Shahroud, Semnan	1815	55˚03′25″ E	36˚42′57″ N	18	9	66.49	3A	5m+2sm+2st
15	*A. triumfettii*	29705	Loshan, Gilan	490	49˚53′25″ E	36˚65′57″ N	18	9	55.78	2A	5m+3sm+1st
16	*A. pseudocotula*	19269	Zanjan, Zanjan	1640	47˚31′00″ E	36˚40′00″ N	18	9	79.23	2A	5m+3sm+1st
17	*A. pseudocotula*	19320	Zanjan, Zanjan	1560	48˚15′00″ E	37˚15′00″ N	18	9	64.23	2A	5m+4sm
18	*A. pseudocotula*	19907	Galikesh, Golestan	160	54˚46′00″ E	36˚50′00″ N	18	9	64.85	2A	6m+3sm
19	*A. pseudocotula*	20136	Minodasht, Golestan	157	55˚37′00″ E	44˚36′00″ N	18	9	56.94	2A	6m+3sm
20	*A. pseudocotula*	20172	Minodasht, Golestan	160	54˚46′18″ E	36˚50′52″ N	18	9	60.37	2A	6m+3sm
21	*A. pseudocotula*	16744	Marave tapeh, Golestan	587	56˚05′52″ E	37˚49′37″ N	18	9	62.25	2A	5m+4sm
22	*A. pseudocotula*	18831	Tehran, Tehran	165	51˚05′52″ E	35˚07′37″ N	18	9	58.22	2B	3m+6sm
23	*A. pseudocotula*	20137	Aliabad, Golestan	140	54˚57′52″ E	36˚94′37″ N	18	9	51.59	2A	3m+6sm
24	*A. pseudocotula*	21071	Shahdyeh, Yazd	1210	54˚15′45″ E	37˚55′56″ N	18	9	73.15	2A	5m+3sm+1st
25	*A. pseudocotula*	29717	Roudbar, Gilan	1074	49˚44′53″ E	36˚46′52″ N	18	9	52.79	2B	3m+6sm

Asymmetric indexes: Percentage of the total form (TF %)=[(∑S/∑T)*100] (Huziwara, 1962), intra-chromosome asymmetry index (A_1)=1-[∑(S/L)/n], where S and L are the mean lengths of short and long arms of each pair of homologous, respectively and n is the number of homologous, inter-chromosome asymmetry index (A_2)=s/x, where s and x are the average of standard deviation and mean of chromosome length respectively (Romero Zarco, 1986). Another index that was used for determining karyotype asymmetry was Stebbins classes (SC) (Stebbins, 1971).

Analysis of Karyotype Characteristics

To calculate the variation between populations, and compare of means of parameters, an unbalanced completely randomized design (CRD), and Duncan's test were used. Principal Components Analysis (PCA) and clustering analysis were used to determine the contribution of each karyotype parameter to the ordination of populations and examine karyotype similarity among populations, respectively. Numerical analyses were performed using SAS, JMP, and Excel software.

Results

25 populations of four *Anthemis* species (*A. altissima, A. haussknechtii. A. triumfettii, A. pseudocotula*) were analyzed for features of chromosome (chromosome counts and morphology), and characters of karyotype (length of chromosome and genome, symmetry of karyotype).

Analysis of Chromosome Features

Table 1 offers information about the Gene bank code and details of location collecting seeds, number of somatic chromosomes (2n), haploid chromosomes numbers (x), genome length (∑TL), Stebbins class (SC), karyotype formula of tested populations, and figures 1 and 2, show the metaphase plates and karyotypes of them.

The total length of haploid chromosomes (length of the genome) varied from 51.59 to 79.23 µm in populations 20137 (Aliabad, Golestan) and 19269 (Zanjan, Zanjan) of *A. pseudocotula*, respectively. That showed the longest genome is 1.5x the shortest one. Four classes of Stebbins (1A, 2A, 3A, and 3B) were distinguished in populations. In the karyotype formula of populations, three types of chromosomes (m: metacentric, sm: sub-metacentric, and st: sub-telocentric) were determined. More information is given in Table 1.

Analysis of Karyotype Traits

The results of analysis variation based on an unbalanced completely randomized design, revealed the existence of significant differences for all the traits (TL, LA, SA, AR, CI, TF%, DRL, A_1, A_2, LA%, and SA%), between the populations. The difference between species was also significant, except for TL, SA, and A_2 traits (Table 2). The significant difference between populations for all traits showed the importance of studied traits in evolution and the affinity between different species and their populations.

The mean values of chromosome lengths (arms and total length of chromosomes), the ratio of arms (AR), index of centromere (CI), percent of total form (TF%), difference between relative length of chromosomes (DRL), intra and inter asymmetric chromosome index (A_1 and A_2), percent length of long and short arms (LA % and SA %), between the species are given in Table 3. The total length of chromosomes (TL) in species, varied from 6.34 to 6.83 µm, and *A. pseudocotula* showed the longest total length (6.83 µm) and long arm (4.19 µm). Most values of AR, A_1, LA% were obtained in *A. pseudocotula* and *A. triumfettii* species, but in CI, TF%, DRL, and SA% traits, *A. haussknechtii* and *A. altissima* was best.

Table 4 shows the mean of traits between populations of species. The length of the total chromosome (TL), varied from 5.73 in 20137 to 8.80 µm in 19269 populations of *A. pseudocotula*. So, the longest chromosomes with 8.80 and 8.13 µm were shown in populations 19269 and 21071 of *A. psedocotula*, respectively. In addition, these two populations of *A. pseudocotula* had the longest length of the long arm (5.46 and 5.41 µm) and short arm (3.34 and 2.91 µm). The populations 20137, and 29717 of *A. pseudocotula* had the shortest chromosome (5.73, and 5.86 µm). The long genome and chromosome complement were found in populations 19269, and 21071 of *A. pseudocotula* from Zanjan and Yazd provinces (∑TL=79.23, TL=8.80 and ∑TL=73.15, TL=8.30 µm), respectively, while the shortest one was observed in population 20137 of *A. pseudocotula* from Golestan province (∑TL=51.59, TL=5.73 µm).

To show the extent of karyotype asymmetry, we used Arm ratio (AR), CI (Centromeric Index), TF% (Total form percent), A_1, and A_2 (intra and inter-chromosomal index). The range of variation in mean AR was from 1.35 to 2.01. The highest value of

mean AR “2.01 and 1.87” belonging to populations 26044 of *A. hausskenchtii* and 21071 of *A. pseudocotula* species. These populations (26044 and 21071), also had the most value of A_1 (0.46). AR and A_1 show an asymmetric karyotype for chromosome morphology.

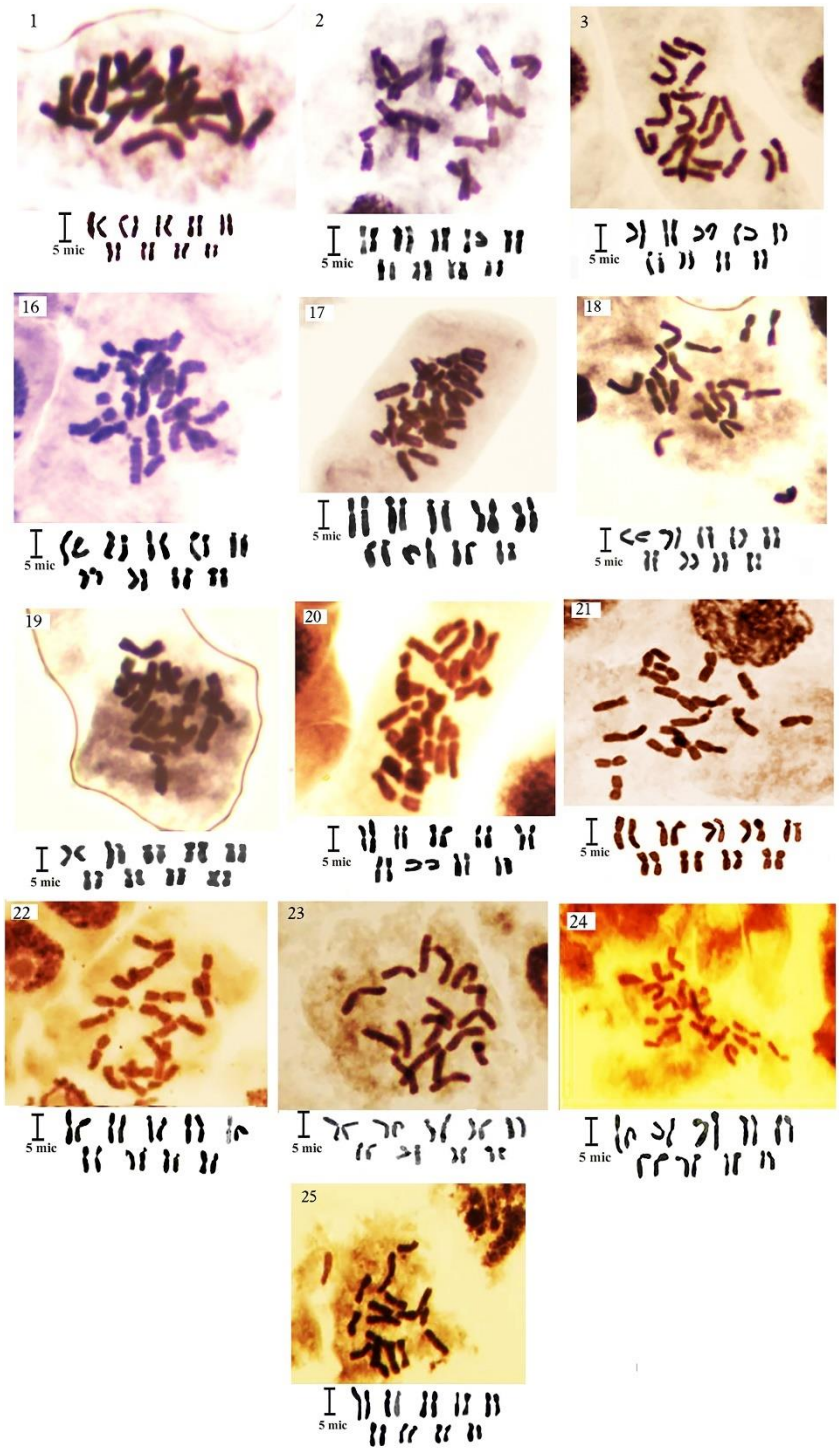

Figure 1. Somatic metaphases and karyotypes: **1.** *A. altissima* (9885), **2.** *A. altissima* (12790), **3.** *A. altissima* (29610), **16.** *A. pseudocotula* (19269), **17.** *A. pseudocotula* (19320), **18.** *A. pseudocotula* (19907), **19.** *A. pseudocotula* (20136), **20.** *A. pseudocotula* (20172), **21.** *A. pseudocotula* (16744), **22.** *A. pseudocotula* (18831), **23.** *A. pseudocotula* (20137), **24.** *A. pseudocotula* (21071), **25.** *A. pseudocotula* (29717).

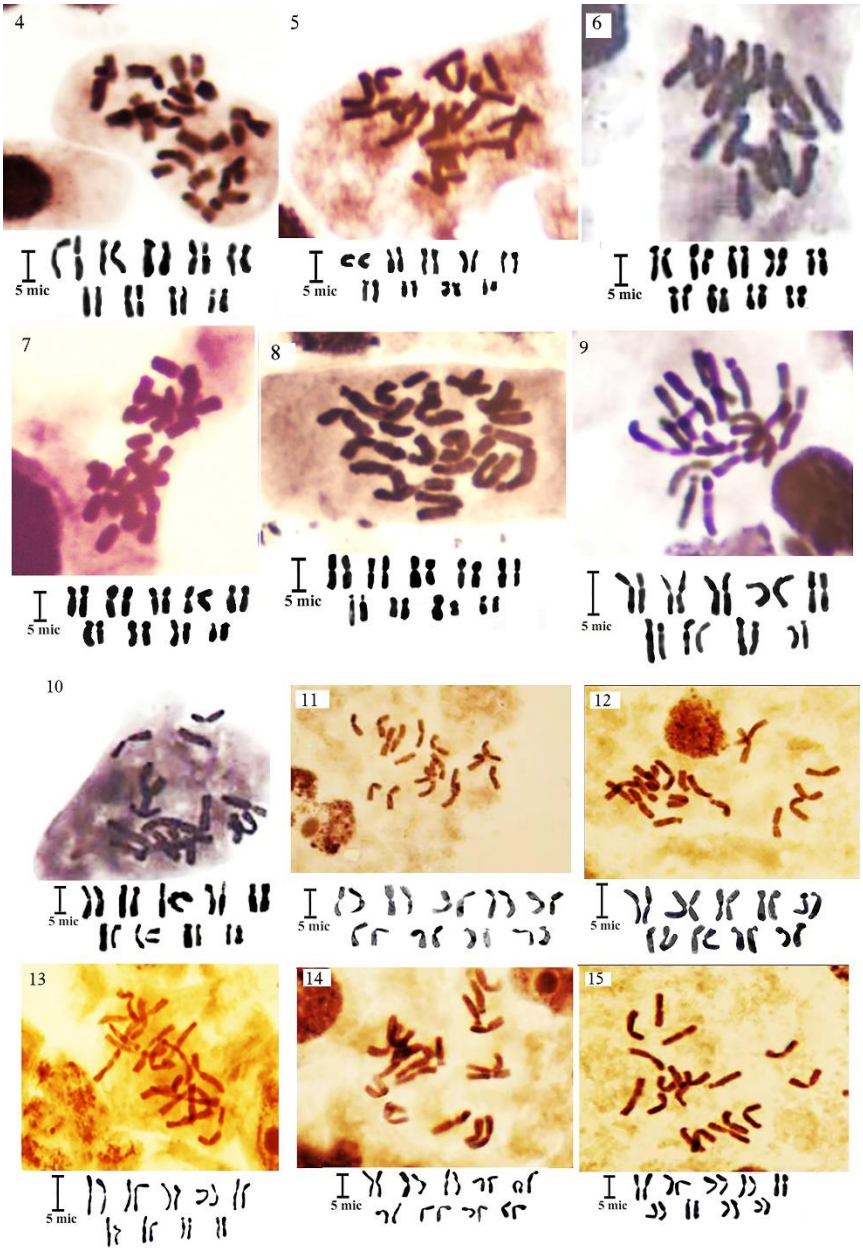

Figure 2. Somatic metaphases and karyotypes: **4.** *A. haussknechtii* (10058), **5.** *A. haussknechtii* (26044), **6.** *A. haussknechtii* (13818), **7.** *A. haussknechtii* (10791), **8.** *A. triumfettii* (30017), **9.** *A. triumfettii* (23955), **10.** *A. triumfettii* (14170), **11.** *A. triumfettii* (11900), **12.** *A. triumfettii* (16684), **13.** *A. triumfettii* (21605), **14.** *A. triumfettii* (27544), **15.** *A. triumfettii* (29705).

CI and TF% traits show a symmetric karyotype in terms of chromosome morphology, and they have an indirect relationship with A_1 and AR traits. The range values of mean CI and TF% varied between 0.34 to 0.43 and 33.90 to 42.65, respectively and the highest values of CI (0.43, 0.42) and TF% (42.65, 42.33), were shown in populations 10058 of *A. haussknechtii* and 19907 of *A. pseudocotula species*. The value of A_2 indicates the multi-form of karyotype chromosomes in the length of view that overlaps with the value of DRL (Difference value of relative length). DRL indicates the difference between chromosomes of a karyotype in terms of length. The value of DRL differed from 2.10 in population 20137 to 8.45 and 8.57 µm in 19269 and 19320 of *A. pseudocotula species*, respectively. In the A_2 trait that shows asymmetry between chromosomes, we had values between 0.12 and 0.23. Population 19269 of *A. pseudocotula* (Zanjan, Zanjan), with high values of DRL and A_2

(DRL=8.45, A_2=0.23), consists of a karyotype with different chromosomes as long and short chromosomes. This issue is confirmed by the karyotype in Figures 1-16.

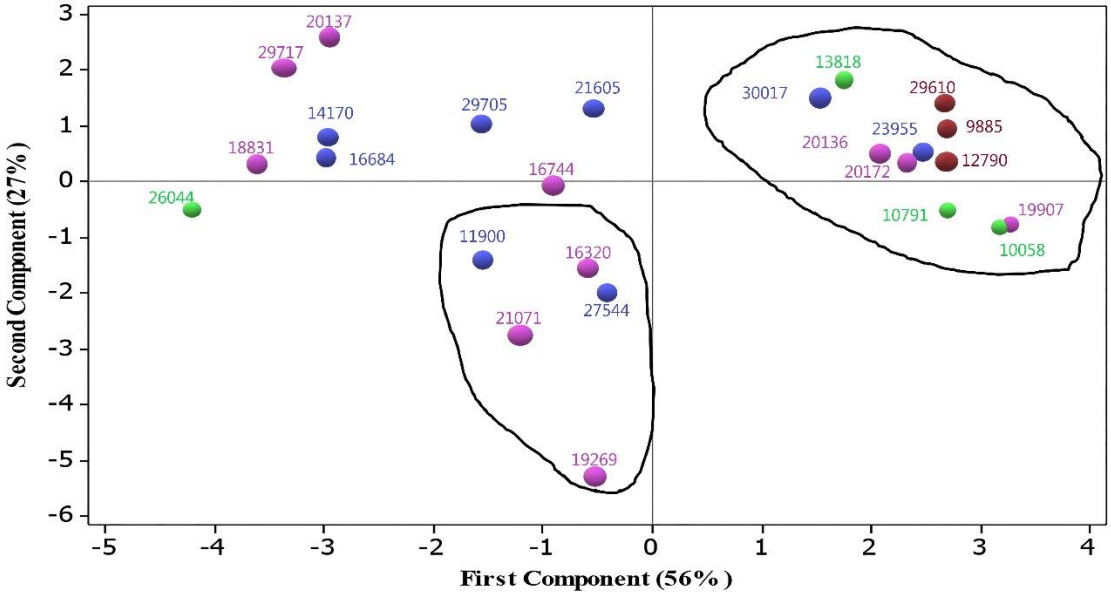

Figure 3. Scatter plot of 25 *Anthemis* populations for the first two principal components. *A.altissima* (9885, 12790, 29610), *A.hussknechtii* (10058, 26044, 13818, 10791), *A.triumfettii* (30017, 23955, 14170, 11900, 16684, 21605, 27544, 29705), *A.pseudocotula* (19269, 19320, 19907, 20136, 20172, 16744, 18831, 20137, 21071, 29717)

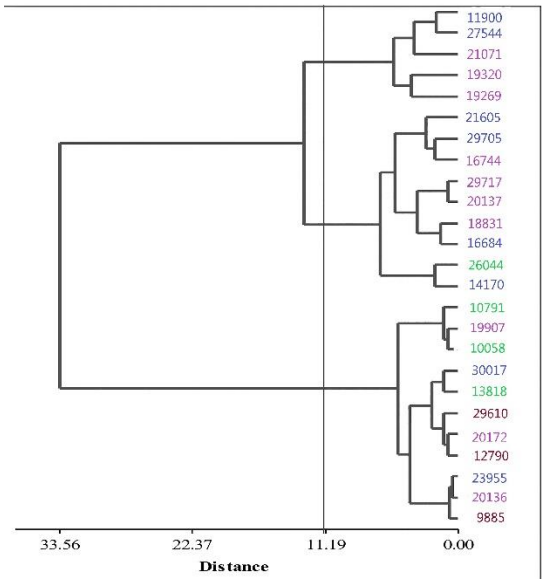

Figure 4. Dendrogram of 25 *Anthemis* populations by analysing eleven karyotype parameters using Ward cluster analysis method. *A.altissima* (9885, 12790, 29610), *A.hussknechtii* (10058, 26044, 13818, 10791), *A.triumfettii* (30017, 23955, 14170, 11900, 16684, 21605, 27544, 29705), *A.pseudocotula* (19269, 19320, 19907, 20136, 20172, 16744, 18831, 20137, 21071, 29717)

The principal component analysis (PCA), of the karyotype traits, showed that the first three components account for more than 90% of the total variance. The first component emphasizes the asymmetric traits (AR, CI, TF%, A_1, LA%, SA%), the second component length traits (TL, LA, SA), and the third component, accentuates DRL and A_2 traits (asymmetric in terms of chromosome length) (Table 5). The two first components had the most significant role in separated classes and this grouping, so the dispersion of populations based on these two components is given in Figure 3.

We used cluster Ward analysis, for grouping populations based on karyotype traits with cophenetic correlation coefficient (r=0.8) in metric distance 11.19, the populations were classified into four groups (Table 4 and Figure.4), which completely fit with results in Figure 3.

Discussion

Chromosome numbers and karyological characters of studied 25 populations of the genus *Anthemis* species were determined. The information obtained from chromosome number, ploidy level, genome size, and karyotype evolution, provides a new perspective on genome function and

its power. The results obtained from this research: 2n=18 (*x=9*) are similar to the data reported by Alizadeh et al., 2018; Iranshahr, 1986; Javadi et al., 2013; Peruzzi et al., 2016 and Tabur et al., 2012. The most species-rich family, Asteraceae, has been intensively studied karyologically over the years, in connection with their taxonomic treatment (Kuzmanov, 1978, 1991; Markova, 1989). Polymorphism in some of their representatives provided a basis for numerous studies. The species in the family Asteraceae are predominantly diploid (2n=18), and tetraploid (2n=36), with *x=9* (Javadi, 2017; Javadi and Salehi Shanjani, 2022; Petrova and Vladimirov, 2020). Lots of research has been done on the genus *Anthemis* from the cytogenetics point of view, and it has been determined that there are two basic chromosome numbers in *Anthemis*: *x=9* and *8* with high, and low frequency, respectively (Chehregani Rad and Mehanfar, 2008; Inceer and Hayirlioglu-Ayaz, 2007; Peruzzi et al., 2016; Tantray et al., 2021; Yousofzadeh et al., 2010).

Morphologically, based on findings of metaphase's plates (Figures 1 and 2), and the length of chromosome arms, (method of Levan), karyotype parameters indicate that all studied species have metacentric (m) and sub-metacentric (sm) chromosomes and a few sub-terminal chromosomes only in *A. triumfettii* and *A. pseudocotula* populations. More metacentric chromosomes in a karyotype formula indicate a more symmetric karyotype, in terms of morphology. Therefore, population 10058 of *A.haussknechtii* (Baneh, Kordestan) with KF (9m) has homogeneous chromosomes. Other research also showed a dominance of metacentric chromosomes in the karyotype of *Anthemis* species (Alizadeh et al., 2018; Javadi et al., 2013; Tabur et al., 2012; Yousofzadeh et al., 2010).

The existence of the longest and shortest genomes and chromosome lengths in the populations of *A. pseudocotula* indicates the existence of karyotype asymmetry in terms of chromosome length among populations of this species. In other species, there was no significant difference in the genome and chromosome length between the populations, which indicates the symmetric karyotype between the populations of these species. The difference in the length of the genome among organisms indicates the difference in the anatomical structure of the cells or their metabolism and it seems unlikely that they are related to the primary forces of genomic evolution (Lynch and Conery, 2003).

Populations 26044 of *A. haussknechtii* from Kurdistan and 21071 of *A. pseudocotula* species from Yazd provinces, with higher values of AR (2.01; 1.87), A_1 (0.46) and less value of CI (0.34; 0.37) and TF% (33.90; 37.20), had more asymmetric karyotypes than other populations. More sub-metacentric and telocentric chromosomes in their karyotypes provide this issue. While karyotypes in population 10058 of *A. haussknechtii* species (Baneh, Kurdistan) and 19907 of *A. pseudocotula* (Galikash, Golestan), with fewer values of AR, A_1 (AR=1.34 and 1.35; A_1= 0.26 and 0.25), and more values of CI (0.43 and 0.42), TF% (42.65 and 42.33) are introduced as populations with more symmetric karyotypes. So, the existence of significant differences in the length of chromosomes and the factors of symmetric karyotype between populations of *A. pseudocotula*, introduced an asymmetric karyotype in *A. pseudocotula species*. The occurrence of asymmetric karyotypes is probably a result of chromosomal structural changes, especially centric fusion, or could be the result of chromosome rearrangements. All these events increase the inter-chromosomal asymmetry by increasing the morphological heterogeneity between chromosomes in a karyotype (Assis et al., 2013; Moraes et al., 2012, 2013, 2016).

In PCA, due to the large contribution of the first component (55%), in the grouping of *Anthemis* populations, it seems that the most important factor in the grouping of populations is chromosome morphology. In the second component, the chromosome length trait was important, which consisted of more than 26% of the variation. The difference in the length of chromosomes is not important in the separation of populations as we can see in the third component (7.8%).

As we can see in Figure 3, the desperation of populations based on two first components (first and second components), shows that the populations located on the right of the x-axis (populations: 98885, 12790, 29610, 10058, 13818, 10791, 30017, 23955 of *A. haussknechtii*) have a positive and direct relationship with traits in the first component and populations: 14170, 16684, 21605, 29705 of *A. triumfettii*, populations: 16744, 18831, 20137, 29717 of *A. pseudocotula* and 26044 of *A. haussknechttii* species are located in the left of x-axis have a direct and negative relationship with the first component traits.

Table 2. The results of analysis of variance for karyotype data in species and populations of *Anthemis* spp.

Source of variation	Degrees of freedom	Mean of squares										
		TL	LA	SA	AR	CI	TF%	DRL	A_1	A_2	LA%	SA%
Popilation	24	1.64^{**}	0.69^{**}	0.39^{**}	0.38^{**}	0.003^{**}	30.85^{**}	18.93^{**}	0.01^{**}	0.003^{*}	30.07^{**}	30.85^{**}
Species	3	1.40^{ns}	1.05^{**}	0.15^{ns}	0.60^{**}	0.005^{**}	52.07^{**}	14.74^{**}	0.03^{**}	0.002^{ns}	46.48^{**}	52.07^{**}
Error	68	0.61	0.23	0.13	0.06	0.00	5.47	1.72	0.002	0.002	5.42	5.47
CV%		11.80	12.05	13.88	14.96	6.46	5.98	28.25	15.28	27.92	3.82	5.98

** and *- Significant at 1% and 5% level of probability, ns- Not significant.

Table 3. Mean of karyotype traits of *Anthemis* spp.

	TL	LA	SA	AR	CI	TF%	DRL	A_1	A_2	LA%	SA%
A. altissima	6.34a	3.68b	2.65a	1.43b	0.41a	41.87a	41.87a	0.27c	0.15a	58.12c	41.87a
A. haussknechtii	6.75a	4.05a	2.70a	1.67a	0.39b	40.00b	40.00b	0.31b	0.16a	59.99b	40.00b
A. pseudocotula	6.83a	4.19a	2.61a	1.83a	0.37c	38.19c	38.19c	0.33b	0.17a	61.57a	38.19c
A. triumfettii	6.54a	4.02a	2.51a	1.82a	0.38c	38.55bc	38.55bc	0.37a	0.17a	61.44ab	38.55bc

Table 4. Mean of karyotype traits of *Anthemis* populations

G.B.C (RIFR)	Population	$\overline{TL}$	$\overline{LA}$	$\overline{SA}$	$\overline{AR}$	$\overline{CI}$	$\overline{TF\%}$	$\overline{DRL}$	$\overline{A1}$	$\overline{A2}$	$\overline{LA\%}$	$\overline{SA\%}$
9885	*A. altissima*	6.21c-e	3.60c	2.61b-f	1.38f	0.41a-c	42.06a-c	5.93b-e	0.27fg	0.18a-c	57.94f-h	42.06a-c
12790	*A. altissima*	6.58c-e	3.83c	2.76a-d	1.38f	0.40a-c	41.84a-d	5.48c-e	0.28e-g	0.15a-c	58.16e-h	41.84a-d
29610	*A. altissima*	6.23c-e	3.63c	2.60b-f	1.40f	0.41a-e	41.71a-d	4.73c-g	0.28e-g	0.13bc	58.29e-h	41.71a-d
10058	*A. haussknechtii*	7.05b-e	4.04c	3.01ab	1.34f	0.43a	42.65a	5.71b-e	0.26g	0.17a-c	57.35h	42.65a
26044	*A. haussknechtii*	6.78b-e	4.48bc	2.31c-f	2.01a	0.34g	33.90i	6.94a-c	0.46a	0.17a-c	66.10a	33.90i
13818	*A. haussknechtii*	6.12c-e	3.65c	2.47b-f	1.48ef	0.41a-e	40.77a-f	4.36e-i	0.29d-g	0.12c	59.23d-h	40.77a-f
10791	*A. haussknechtii*	6.85b-e	3.98c	2.88a-c	1.38f	0.41a-c	41.93a-d	6.25b-e	0.27fg	0.18a-c	58.07e-h	41.93a-d
30017	*A. triumfettii*	6.10c-e	3.61c	2.48b-f	1.46ef	0.40a-e	40.64a-f	4.82c-f	0.31d-g	0.15a-c	59.36d-h	40.64a-f
23955	*A. triumfettii*	6.35c-e	3.71c	2.64b-f	1.40ef	0.41a-c	41.57a-e	6.53a-e	0.28d-g	0.18a-c	58.43d-h	41.57a-e
14170	*A. triumfettii*	6.16c-d	3.98c	2.18d-f	1.80cd	0.34g	35.51g-i	7.69ab	0.45ab	0.16a-c	64.49a-c	35.51g-i
11900	*A. triumfettii*	7.14b-d	4.42bc	2.72a-d	1.60c-e	0.37c-g	38.38b-g	2.49h-j	0.42a-c	0.22a	61.62b-g	38.38b-g
16684	*A. triumfettii*	6.64c-e	4.28bc	2.35c-f	1.82cd	0.37fg	35.49g-i	2.20ij	0.37b-d	0.17a-c	64.51a-c	35.49g-i
21605	*A. triumfettii*	6.39c-e	3.97c	2.42b-f	1.64c-e	0.38b-g	37.97c-i	2.89f-j	0.36c-f	0.12c	62.03b-f	37.97c-i
27544	*A. triumfettii*	7.39b-c	4.48bc	2.91a-c	1.54f	0.39a-f	39.37a-f	2.59g-j	0.45ab	0.20ab	60.63c-h	39.37a-g
29705	*A. triumfettii*	6.20c-e	3.88c	2.32c-f	1.67c-e	0.36e-g	37.49e-i	2.74f-j	0.34c-g	0.20a-c	62.51a-d	37.49e-i
19269	*A. pseudocotula*	8.80a	5.46a	3.34a	1.64c-e	0.38a-g	37.90d-i	8.45a	0.36c-f	0.23a	62.10b-e	37.90d-i
19320	*A. pseudocotula*	7.14b-d	4.45bc	2.69b-e	1.64c-e	0.38a-g	37.87d-i	8.57a	0.36c-e	0.19a-c	62.13b-e	37.87d-i
19907	*A. pseudocotula*	7.21b-d	4.14bc	3.06ab	1.35f	0.42a	42.33ab	5.14c-e	0.25g	0.15a-c	57.67gh	42.33ab
20136	*A. pseudocotula*	6.33c-e	3.71c	2.62b-f	1.40f	0.41a-e	41.40a-e	6.81a-d	0.29d-g	0.18a-c	58.60d-h	41.40a-e
20172	*A. pseudocotula*	6.70c-e	3.93c	2.78a-d	1.40f	0.41a-d	41.42a-e	4.68d-h	0.28d-g	0.14bc	58.58d-h	41.42a-e
16744	*A. pseudocotula*	6.92b-e	4.22bc	2.60b-f	1.61de	0.37d-g	37.31f-i	2.36ij	0.34c-g	0.17a-c	61.23b-h	37.31f-i
18831	*A. pseudocotula*	6.47c-e	4.19bc	2.28c-f	1.84cd	0.35fg	35.11hi	2.61f-j	0.41a-c	0.21ab	64.89ab	35.11hi
20137	*A. pseudocotula*	5.73e	3.70c	2.00f	1.85cd	0.35fg	35.04hi	2.10j	0.29d-g	0.17a-c	64.96ab	35.04hi
21071	*A. pseudocotula*	8.30ab	5.41ab	2.91ab	1.87cd	0.37d-g	37.20f-i	2.77f-j	0.46a	0.15a-c	61.28b-h	37.20f-i
29717	*A. pseudocotula*	5.86de	3.81c	2.05ef	1.84cb	0.34g	34.99hi	2.65f-j	0.32d-g	0.19a-c	65.01a	34.99hi

G.B.C: Gene Bank Code, **$\overline{TL}$**-Mean of total length, **$\overline{LA}$**-Mean of long arm, **$\overline{SA}$**-Mean of short arm, **$\overline{AR}$**-Mean of arm ratio, **$\overline{CI}$**-Mean of ctromeric index, **$\overline{TF\%}$**-Mean of total form percentage, **$\overline{DRL}$**-Mean of difference of relative length, **$\overline{A_1}$**-Mean of intra chromosome asymmetry index, **$\overline{A_2}$**-Mean of inter chromosome asymmetry index, **$\overline{LA\%}$**-Mean of relative length of long arm, **$\overline{SA\%}$**-Mean of relative length of short arm

Table 5. Eigenvectors from the first three principal components analysis for eleven karyotype parameters to classify 25 populations of *Anthemis* spp.

	TL	**LA**	**SA**	**AR**	**CI**	**TF%**	**DRL**	**A_1**	**A_2**	**LA%**	**SA%**	**E.V**	**P.V**	**C.P.V**
F.C	0.013	-0.149	0.235	**-0.397**	**0.401**	**0.399**	0.139	**-0.300**	-0.135	**-0.397**	**0.399**	6.148	0.559	**0.559**
S.C	**-0.572**	**-0.531**	**-0.466**	0.034	-0.023	-0.01	-0.192	-0.233	-0.282	0.032	-0.01	2.918	0.265	**0.824**
T.C	-0.187	-0.133	-0.158	0.042	-0.031	-0.01	**0.839**	-0.121	**0.439**	0.093	-0.01	0.863	0.078	**0.903**

F.C- First Component, **S.C**- Second Component, **T.C**- Third Component, **TL**- Total Length, **LA**- Long Arm, **SA**- Short Arm, **AR**- Arm Ratio, **CI**- Centromeric Index, **TF%**- Total Form percent, **DRL**- Difference of Relative Length, **A_1**- Intra-chromosome asymmetry Index, **A_2**- Inter-chromosome asymmetry Index, **LA%**- Relative Length of Long Arm, **SA%**- Relative Length of Short Arm, **E.V**- Eigen Value, **P.V**- Percent of Variance, **C.P.V**- Cum Percent of Variance

In cluster analysis, all populations were grouped based on karyotype traits. Cluster one consists of populations with long chromosomes, two populations: 11900, and 27544 of *A. triumfettii*, and three populations: 19269, 19320, and 21071 of *A. pseudocotula species*. Members of group two, have more asymmetric karyotypes, including populations: 10058, 10791, and 13818 of *A. haussknechttii*, 9885, 12790, 29610 of *A. altissima*, 19907, 20172, 20136 of *A. pseudocotula* and 23955, 30017 of *A. triumfettii species.* The rest of the populations were located in group three, with the same characteristics.

Conclusions

The basic chromosome number *x*=9, with two ploidy levels (2x and 4x) was shown in all populations of *Anthemis* studied. The longest genome and chromosome were shown in population 19269 of *A. pseudocotula* species from Zanjan. Also, this population (19269), had an asymmetric karyotype, in terms of chromosome length (long and short chromosomes). The asymmetric and symmetric karyotypes, in terms of chromosome morphology, were shown in populations: 26044 and 10058 of *A. haussknechtii* species. Therefore, populations of *A. pseudocotula* and *A. haussknechtii* have variations in the size and morphology of the chromosomes, respectively. It seems that the most important factor in the grouping of populations of *Anthemis* studied was chromosome morphology.

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Conflict of Interests
The authors of this study declare that they have no conflict of interest.

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