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A karyomorphological comparison of seven species of *Achillea* L. from Kurdistan of Iran

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Abstract. We conducted the present study on seven important medicinal species of *Achillea* (in a total of 28 populations) in their natural habitats. The results indicated that the populations had a base number (x= 9) and the diploid, tetraploid, and hexaploidy levels were observed. In addition to the inter-species diversity, there was the intra-species genetic diversity as *A. millefolium* (4*x*, 6*x*), *A. vermicularis* (2*x*, 4*x*), *A. tenuifolia* (2*x*, 4*x*), *A. Aleppica* (2*x*), *A. talagonica* (2*x*), *A. biebersteinii*, and *A. wilhelmsii* (4*x*). Furthermore, studies also indicated that 11 out of 28 populations had 1A symmetry, 15 populations had 2B symmetry, a population had 2A, and another population had 2B. Cluster analysis of cytogenetic variables could differentiate only the species *of A. aleppica, A. talagonica* and *A. wilhelmsii* but others could not separate differences between species appropriately, probably due to the superiority of intra-species diversity of populations to inter-species diversity. Furthermore, we found %TF and DRL are useful parameters for differentiating intrachromosomal variation of species.

Keywords: *Achillea*, cytogenetic, cytomorphology, cluster analysis, chromosome structure.

INTRODUCTION

Achillea genus of family Asteraceae is of about 130 species that are distributed from southeastern Europe to southeastern Asia and has spread to North America through Eurasia. Different species of this genus have shown significant adaptation to different environmental conditions and have spread from deserts and coastal areas to rocky regions. The plants of this genus are perennial, allogamous and they are pollinated by insects (Mozaffaria, V. 2003). There are 19 herbaceous species of this genus available in Iran. Other species of this genus also grow in Anatolia, Syria, Caucasus, Lebanon, Palestine, Central Russia, Transcaucasia, Turkmenistan, Afghanistan, Southwest

Asia, and Central Asia in addition to Iran (Ghahreman 1984). Yarrow is a popular medicinal herb that is widely used in traditional medicine to treat diseases, particularly burns and scars (Muẓaffarīyān 1996).

Cytogenetics is study of relationship of chromosome structure with cellular function. Karyotype, the highest level of functional and structural organization of nuclear genome, that is essential for studying chromosomal characterization of plant species (Altınordu et al. 2016). Comparative chromosomal taxon has been providing useful knowledge about patterns and evolutionary mechanisms in speciation (Flavell 2021). Chromosomal features, such as chromosome length, centromere index, number of chromosomes are crucial variables for investigating interrelationships and intrarelationships of taxa. However, in addition of chromosomal morphotype, population geographical origin is an important parameter in interpreting taxon's diversity (Ramsey 2011) .

The basic chromosome number $x = 9$ is commonly reported for *Achillea* but variation in chromosome numbers and different ploidy levels are frequently occurring in this genus. Unfortunately, only few cytological studies have been published concerning karyological aspects on this genus in Iran. Ploidy and number of chromosomes in *A. aleppica* DC. species has been varied from 2*x* to 8*x* and with high symmetrical characteristic karyotype (2A), however in some population asymmetrical chromosomes (2B) have been reported (Rad and Javaheri 2014). *A. vermicularis*, *A. wilhelmsii* and *A. millefolium* species have shown different levels of ploidies 2*x*, 4*x*, 6*x* and 8*x* with 2A symmetrical Stebbins's index (Afshari et al. 2013). Meanwhile, study of nine populations of *A. biebersteinii* species demonstrated a diploid (2*x*); however, chromosomal interspecies variation has been observed and there has been a symmetrical Stebbins's index 1A and 2A (Chehregani Rad et al. 2017). The results of an another study, that was conducted on 14 populations of 8 *Achillea* species showed *A. talagonica* and A. *berbersteinii* species have been 2*x* ploidy, meanwhile *vermicularis* and *wilhelmsii* showed 4*x*, 6*x* ploidy levels respectively (Sheidai et al., 2009). Unfortunately, we have few studies on different species of *Achillea* particularly in Iran. Therefore, to fill this gap, we try to conducted this study on 28 population of seven species of *Achillea* in their natural habitats in Sanandaj find out the cytogenetical characteristics features.

MATERIALS AND METHODS

Plant materials

All of the 28 samples in this study, including seven species of *Achillea* (*A. millefolium, A. vermicularis, A. tenuifolia, A. aelppica, A. biebersteinii, A. wilhelmsii*, *and A. talagonica)*, with four replicates in each species, were collected in west of Iran, Kurdistan, Sanandaj. This region is located at a longitude of 46° 59' 45" E and latitude of 35° 19' 00" N. To identify every species, a sample was collected from each point. Figs 1, 2 represent the exact position of each location and morphological population features. Furthermore, we recorded the geographical position of each location using the GPS. Table (1) presents the latitude and longitude of each site.

Cytogenetical study of species

The seeds obtained from every point were disinfected employing the solution of the Sodium hypochlorite 2%, under sterile conditions, inside a Petri dish, and on the filter paper. Afterwards, the seeds germinated at room temperature. Following two to five days,

Table 1. Details on population sites including geographical coordinates, altitude and origin of samples.

Population	Longitude	Latitude	Altitude/m	Origin
W13	46.71	35.29	2,208	Klatei
W12	46.88	35.36	1,974	Arandan
W11	47.00	35.51	1,999	Sofla Mamox
W14	46.79	35.51	2,026	Gav Dareh
TA1	46.94	35.47	1,628	Chrandoo
TA2	46.98	35.58	1,980	Biaenchob
TA4	46.99	35.51	2,145	Chrandoo
TA3	46.98	35.49	1,919	Sofla Mamox
TE4	46.96	35.46	1,577	Sarab Ghamish
TE2	46.96	35.51	1,841	Chrandoo
TE1	46.99	35.49	1,866	Mamox
TE3	47.02	35.57	1,934	Bazi Rabab
AL3	46.94	35.28	1,829	Hassan Abad
AL4	46.58	35.32	1,607	Goyran
AL1	46.98	35.30	1,653	Pakr kodak
AL2	47.13	35.29	2,095	Salvat Abad
BI3	46.58	35.30	1,436	Danikesh
BI2	46.60	35.32	1,436	Pichon
BI1	46.89	35.13	1,334	Savarian
BI4	46.93	35.46	1,593	Chelgazii
VE4	46.97	35.30	1,838	Dole Rahman
VE1	46.92	35.55	2,334	Ghalvazei
VE3	46.92	35.58	2,152	Sangi Sefied
VE2	46.99	35.51	2,145	Sofla Mamox
MI2	47.07	35.36	1,498	Babareiz
MI1	47.15	35.25	1,985	Salvat Abad
MI4	47.12	35.54	1,642	Jebreillian
MI3	47.11	35.49	1,993	Dolbandi

their roots reached the proper size for sampling (roots with a length of 0.5-1 cm are appropriate for sampling). After applying the pre-treatment, the root samples were exposed to a 0.5% α-bromonaphthalene solution for 4 h, and running water for 30 min to remove the remains of the solution. Subsequently, we performed the fixation. Thus, we used Levitsky solution as a suitable fixator for karyotypic studies, and the samples were in the for 16 h (Levitsky 1931, Levitus et al. 2010). Following the fixation, the samples were rinsed with running water for 3 h to eliminate the residuals of the fix. Then we used squash at an optimal level to separate the cells and put them at the same level, and make staining better. To this end, we removed the roots from 70% ethyl alcohol, rinsed them with running water for 30 minutes, put them in a hydrolyzer (1 M NaOH), and placed them in the oven at 60 °C for 8 min. After hydrolysis, the samples were dried with filter paper and placed in hematoxylin for 3-4 h, to stain the chromosomes (Abbaszade et al. 2017). Chromosomal images were transferred to the monitor and saved with a digital color CCD camera mounted on a light microscope. The chromosomes of each cell were cut in Photoshop and arranged in a separate file. Using Micro Measure software and specifying the beginning to end of chromosomes and their centromere locations, certain characteristics such as short and long arm length, the total chromosome length, and relative chromosome length were calculated. The results were stored in Excel. In the present study, five cells (replications) were selected and evaluated from each slide to measure chromosomal parameters. The parameters calculated for the karyotypes were as follows: Short arm relative length percentage (SA%), Long arm relative length percentage (LA%), total length (TL), Relative length percentage (RL%), Arm ratio (AR), Total form percentage (TF%), Centromere index (CI), Difference of the range of relative length (DRL), Value of relative chromatin (VRC), Intrachromosomal asymmetry Index (A1) and Interchromosomal asymmetry Index (A2) (Altınordu et al. 2016).

According to the number of the replications, we calculated the standard deviation for the traits and the confidence interval for some of them. The chromosome form was determined using a method by Levan (Levan 1964). After measuring the chromosomes, we drew the ideogram associated with the karyotype of the populations based on the lengths of short and long arms, in which the order of chromosomes was considered based on the length of the short arm (from large to small). We utilized the Stebbins method for comparing the karyotypic symmetry in the species (Stebbins 1971).

Statistical data analysis

We performed all the statistical analyses by employing R software. The cluster analysis was performed for cytogenetic data series using the statistical packages, factoextra, FactoMineR, and devtools (Kassambara 2017). To map extract geological we used raster and MapTool packages by using R software. (Bivand and Lewin-Koh 2013) (Fig. 1).

RESULTS

Comparison of cytogenetic parameters between species

Mitotic metaphase chromosomes, ideograms, and morphological diversity of chromosomes were showed in Fig. 3, 4. The comparison of the results of karyotypic characteristics in the populations indicated that the base chromosome number was x=9 in all the populations and there were hexa-, tetra- and diploid levels for the populations. Regarding the ploidy level, there was diversity not only among the species, but also among the populations of the three species, *A. millefolium* (tetra- and hexa-ploidy), *A. vermicularis* (tetra and diploidy), and *A. tenuifolia* (tetra and diploidy). *A. alepine* and *A. talagonica* species were diploid and *A. biebersteinii* and *A. willhelmsii* species were tetraploid (Table 2). Karyotype formulas of inter-species and intra-species populations were different and all the chromosomes were metacentric only in populations AL2, TA1, VE1, TE1, and TE4; the karyotype consisted of a large number of metacentric chromosomes and a small number of chromosomes were submetacentric in other populations. According to the Stebbins' s index, most of the populations were in 1A and 1B, only population BI4 was in 2B, and MI1 in the 2A (Table 2). Therefore, a symmetrical karyotype was observed for the species of this genus. The highest relative amount of chromatin belonged to population AL3 with an average of 4.15 µm whereas the lowest relative amount of chromatin belonged to population TA2 with an average of 2.55. Except for population AL3, the relative chromatin levels of the populations were less than 4 and more than 2 µm. Since the relative difference in the lengths of chromosomes had an inverse relationship with intra-species ploidy levels, the most asymmetric chromosomes among the hexaploidy populations, based on DRL index, belonged to population MI4 with an average of 38.3% (Tables 2, 3). For diploid populations, BI1 population had the highest rate of chromosomal asymmetry with the highest DRL (5.32 %). Among the diploid populations, VE1 population and four populations of *A. talagonica* species had the highest rate of

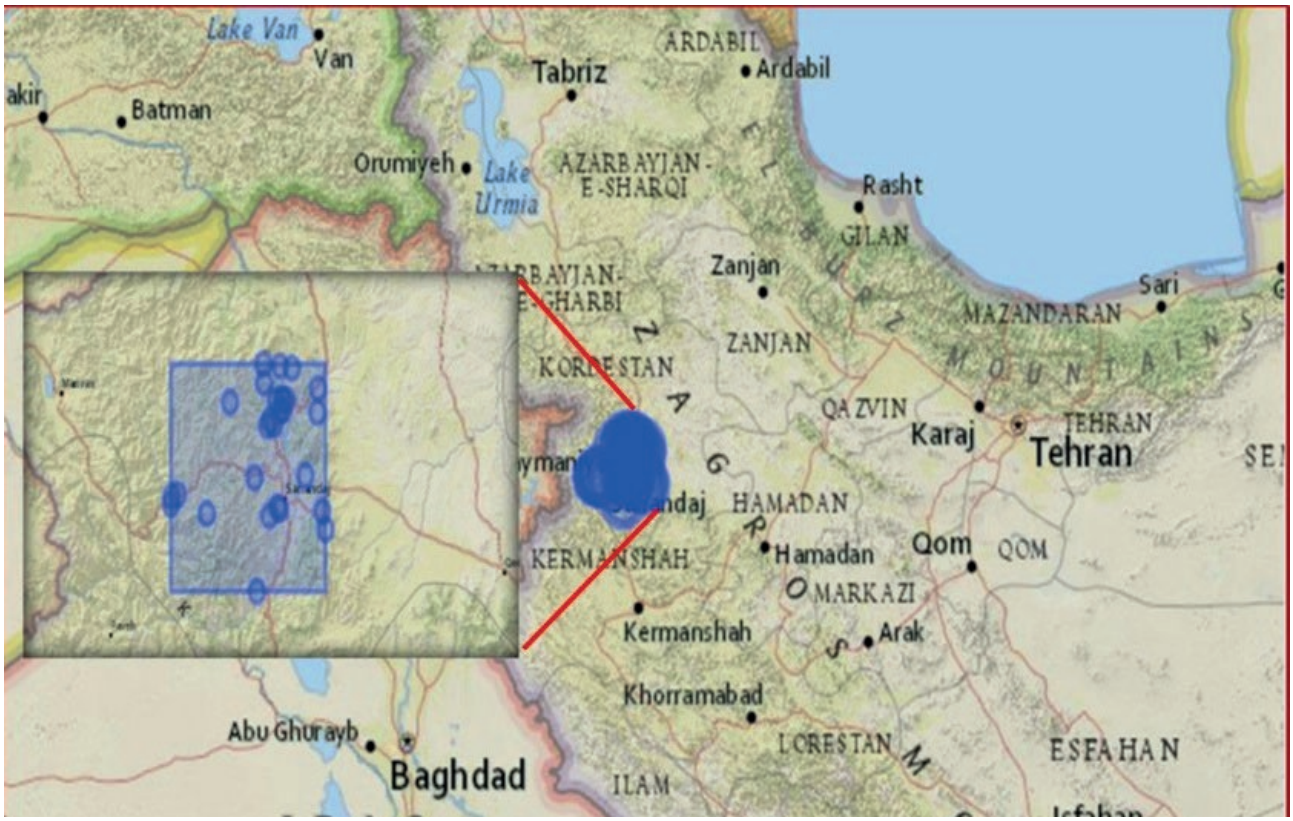

Figure 1. Positions the population samples were collected on the map.

DRL, and the most asymmetric chromosomes. The percentage of overall chromosomes form ranged from 37.6 to 46.11, and the highest percentage of overall chromosome form belonged to populations TE4, TE1, and TA1; thus, they had a more symmetrical karyotype compared to the other populations. On the contrary, AL3, MI2, MI4, and WI4 had the lowest percentage of overall chromosome form; therefore, they had the most asymmetric karyotypes (Tables 2, 3). The lowest A1 belonged to populations TE4, TE1, and TA1; consequently, they had more symmetrical karyotypes than the other populations. Based on index A1, AL3 and Wl4 had the highest chromosomal asymmetry. Hence, it was found that the intra-species diversity was high for A1 and TF%, and the species were indistinguishable based on the parameters. For the A2, the intra-species diversity was somewhat lower, and the species could be divided into three categories; the species of the first class included *A. aleppica* and *A. millefolium*, whose populations had an A2 of less than 0.2 and symmetrical chromosomes based on the index. On the contrary, the populations of two species, *A. talagonica* and *A. vermicularis*, had an index A2 of over 0.2 and asymmetric chromosomes based on the index. However, the populations of other species had higher intra-species diversity compared to the above-mentioned four species populations and also had populations with low inter-chromosomal asymmetry and high A2 (Table 2).

In terms of the CI (Table 3), populations TE4, MI1, TE1, VE3, and TA1 had a centromeric index between 0.42 and 0.46 and they had symmetrical chromosomes based on the index. Meanwhile, populations AL3, BI3, MI2, MI4, WI2, and WI4 with a CI of 0.38 had the most asymmetric chromosomes based on the index. The lowest ratio of long to short arm belonged to TE4, TE1, and TA1 populations with average values of 1.19, 1.37, and 1.4, respectively, and had symmetrical chromosomes based on the index. On the other hand, the highest value for the index with long to short arm ratio between 1.6 and 1.69 belonged to populations AL3, BI3, MI2, MI4, WI4, TE3, and WI2. Thus, they had asymmetric chromosomes. The highest average total chromosome length belonged to population AL3 with an average of 4.15 μm, and other populations had an average total chromosome length between 2.55 and 3.64 μm, among which populations TA2, TE1, TA4, TE4, BI2, VE2, MI1, VE1, BI4,

Figure 2. Shows different morphological attributes of seven species of *Achillea* L. in west of Iran, Kurdistan in their natural habitats. (a) *A. tenifolia* (b) *A. vermicularis* (c) *A. allepica* (d) *A. biebesteinii* (e) *A. wilhielmsii* (f) *A. millefolium* (g) *A. talagonica*.

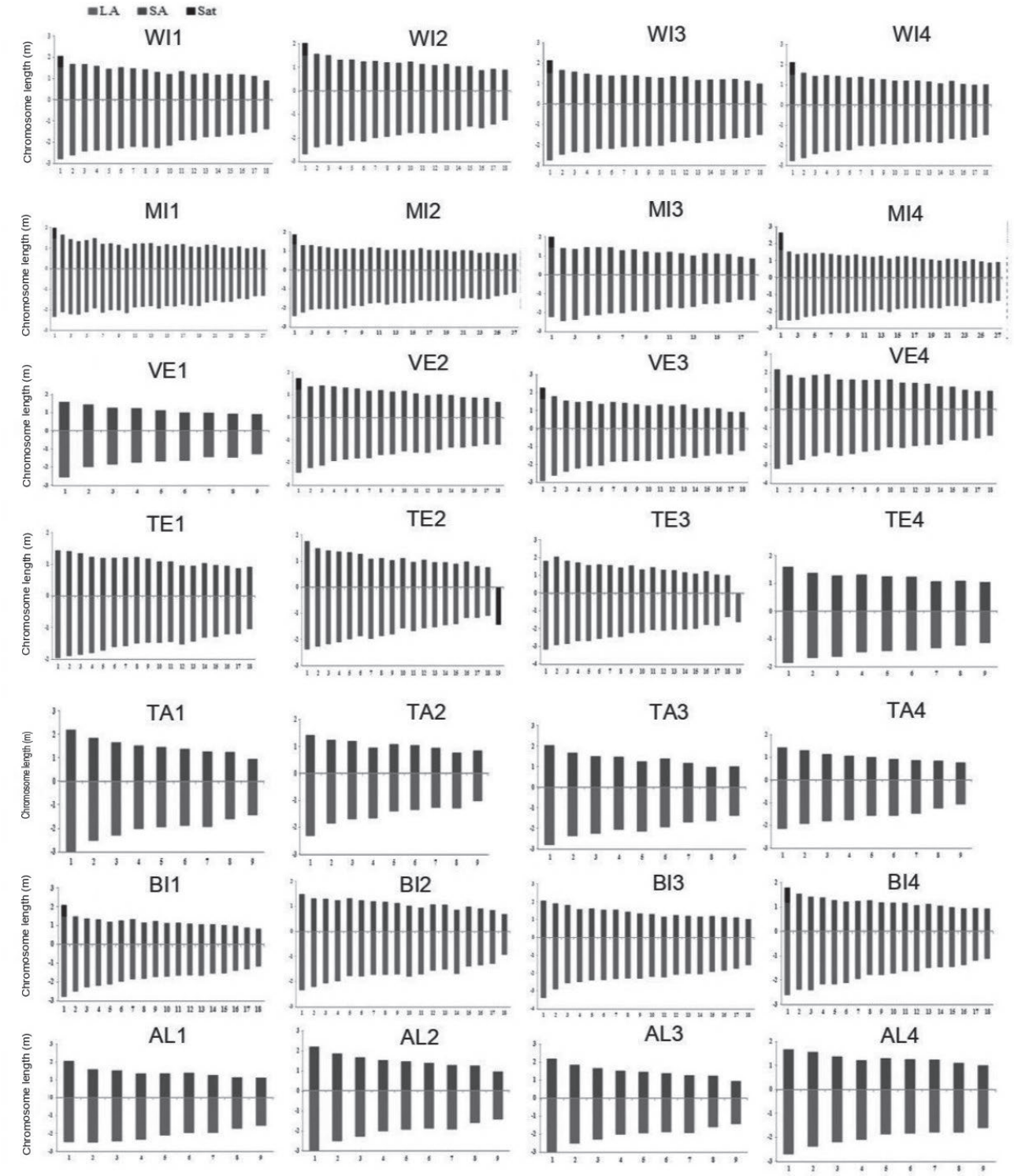

Figure 3. Haploid ideogram of seven species population samples. (Red (SA): Relative length of short arm, Blue (LA): Relative length of long arm, Black (Sat): satellite chromosome, Scale bar = 5 μm, X-axis express No. of chromosome, Y-axis express Relative of long and short arm scale bar = 5 μm).

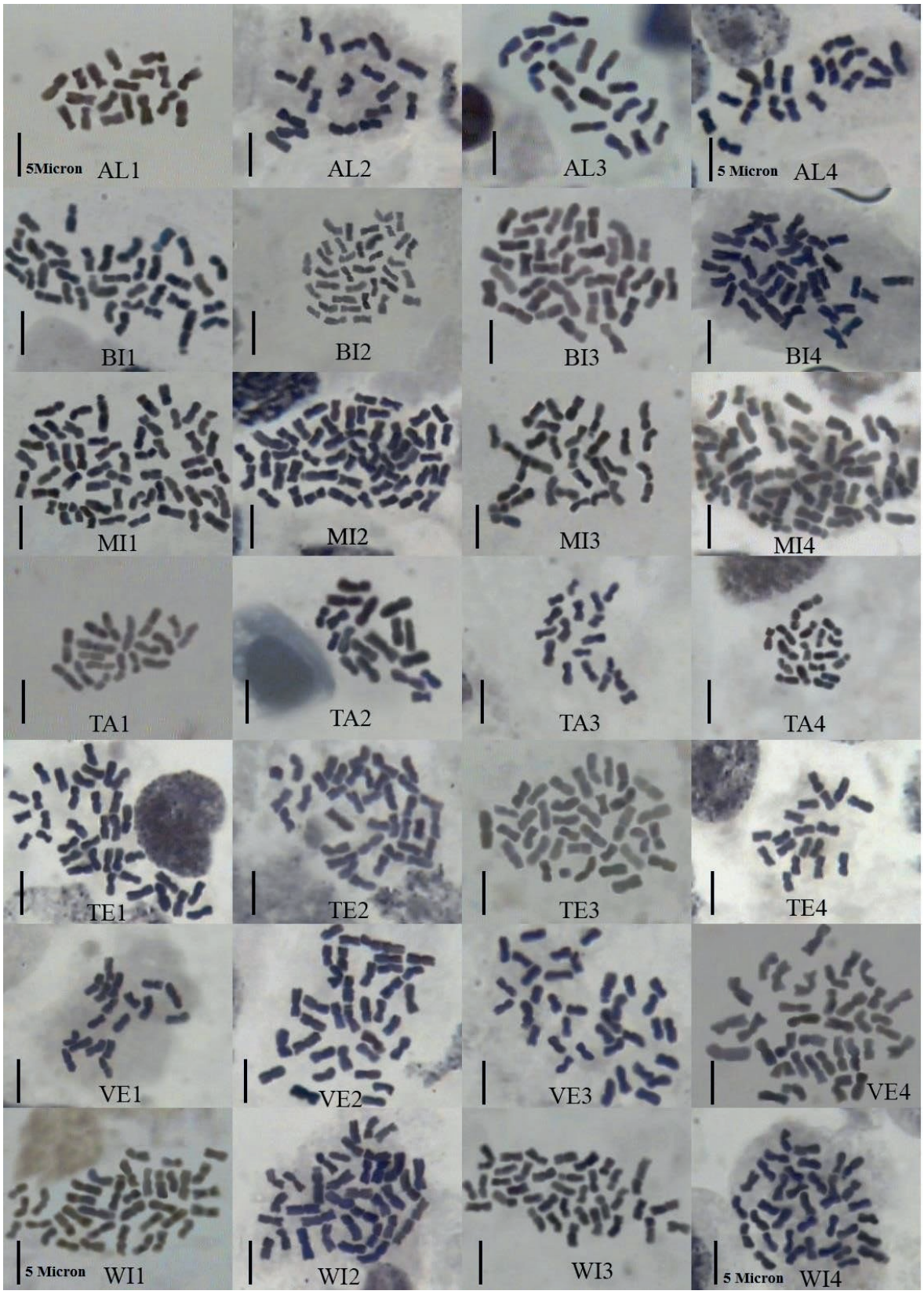

Figure 4. The morphological diversity metaphase chromosomes between and within species (Scale bar = 5μm).

Table 2. Cytogenetic indices data A2, A1, % TF, DRL, VRC, SC, and KF.

Species	KF	2n	SC	VRC	DRL	%TF	A1	A2	Population
A. aleppica	8m+1sm	2*x*=18	1A	3.49	5.90	39.97	0.334	0.170	AL_1
	9m	2*x*=18	1A	3.27	5.67	39.78	0.335	0.162	AL_2
	6m+3sm	2*x*=18	1A	4.15	5.90	37.60	0.395	0.169	AL_3
	8m+1sm	2*x*=18	1A	3.30	5.92	39.27	0.351	0.166	AL_4
A. biebersteinii	14m+4sm	4*x*=36	1B	2.98	5.32	38.42	0.356	0.228	BI_1
	15m+3sm	4*x*=36	1B	2.76	4.43	39.43	0.343	0.190	BI_2
	14m+4sm	4*x*=36	1B	3.62	4.39	38.44	0.376	0.195	BI_3
	14m+4sm	4*x*=36	2B	2.97	4.39	38.62	0.343	0.217	BI_4
A. millefolium	20m+7sm	6*x*=54	2A	3.02	2.54	38.80	0.355	0.153	MI_1
	17m+10sm	6*x*=54	1B	2.79	2.94	37.89	0.378	0.167	MI_2
	15m+3sm	4*x*=36	1A	3.06	3.67	39.29	0.337	0.183	MI_3
	20m+7sm	6*x*=54	1B	3.16	3.38	37.85	0.375	0.194	MI_4
A. talagonica	9m	2*x*=18	1B	3.53	8.80	42.02	0.277	0.236	TA_1
	7m+2sm	2*x*=18	1B	2.55	8.07	40.37	0.311	0.224	TA_2
	7m+2sm	2*x*=18	1B	3.38	8.05	40.68	0.315	0.223	TA_3
	6m+3sm	2*x*=18	1A	2.62	7.34	38.86	0.363	0.209	TA_4
A. vermicularis	9m	2*x*=18	1A	2.85	7.53	39.74	0.339	0.210	VE_1
	15m+3sm	4*x*=36	1B	2.76	4.59	39.84	0.321	0.218	VE_2
	17m+1sm	4*x*=36	1B	3.19	4.94	41.20	0.281	0.224	VE_3
	17m+1sm	4*x*=36	1B	3.64	4.49	40.52	0.317	0.218	VE_4
A. tenuifolia	18m	4*x*=36	1A	2.62	3.04	42.50	0.257	0.159	TE_1
	14m+4sm	4*x*=36	1B	2.82	4.50	39.36	0.347	0.223	TE_2
	11m+7sm	4*x*=36	1B	3.70	3.96	38.56	0.369	0.199	TE_3
	9m	2*x*=18	1A	2.69	5.23	46.11	0.141	0.147	TE_4
A .wilhelmsii	15m+3sm	4*x*=36	1B	3.41	4.17	39.00	0.346	0.190	WI_1
	14m+4sm	4*x*=36	1B	3.08	4.61	38.13	0.371	0.205	WI_2
	16m+2sm	4*x*=36	1A	3.35	3.95	39.13	0.342	0.168	WI_3
	14m+4sm	4*x*=36	1A	3.28	4.04	37.75	0.380	0.183	WI_4

and BI1 had an average total chromosome length of less than 3 μm. The other populations had an average total chromosome length between 3 and 3.64 μm. Therefore, it was found that intra-species diversity was high for AR, CI, and TL indices (Table 3). Based on the parameters, the species were indistinguishable. The range of the total chromosome length varied widely from a minimum range of 1.59 μm in the population BI2 to a maximum of 9.85 μm in the population VE1; hence, the longest chromosome was 6.19 times higher than the shortest chromosome.

Results of analysis of cytogenetic variables

Fig. 5 depicts the results of cluster heatmap (based on Euclidian distance and ward method) analysis for cytogenetic variables of different species. A total of 10 attributes were included in the analysis. The results exhibited that the accessions were assigned two main distinct groups. According to the figure 5, in the first cluster *A. talgonica* (2*x*) species was separated from others. The second group consists of all other species with different ploidy levels, but interestingly in contrary side of first group (maximum distance from first group) a diploid species *A. aleppica* (2*x*) was located. In other words, two diploid species were assigned to different sides of clustering. Obviously, cluster analysis could not detect differences between interspecies, however three species including *A. talagonica*, *A. aleppica* and *A. willhelmsii* were clearly discriminated from others. It can be noted that cluster analysis could not detect ploidy pattens among the *Achillea* populations (annotation group in cluster analysis) (Fig.5). Among the attributes a few variables showed the highest interrelationship variation population accessions which are including %TF, LA and DRL.

Table 3. Karyotypes and chromosomal parameters in this study for each species.

Population	CI	AR	%RL	TL	%SA	%LA	Chromosome range length (µm)
AL1	0.4 ± 0.02	1.52 ± 0.1	11.11 ± 1.23	3.49 ± 0.39	4.44 ± 0.59	6.67 ± 0.71	2.63 – 4.48
AL2	0.4 ± 0.01	1.52 ± 0.05	11.11 ± 1.17	3.27 ± 0.34	4.42 ± 0.41	6.69 ± 0.77	2.53 – 4.19
AL3	0.38 ± 0.01	1.69 ± 0.09	11.11 ± 1.23	4.15 ± 0.46	4.18 ± 0.44	6.93 ± 0.81	3.18 – 5.39
AL4	0.39 ± 0.01	1.56 ± 0.07	11.11 ± 1.2	3.3 ± 0.36	4.36 ± 0.46	6.75 ± 0.76	2.58 – 4.33
BI1	0.39 ± 0.01	1.59 ± 0.07	5.59 ± 0.58	2.98 ± 0.31	2.14 ± 0.16	3.36 ± 0.36	1.95 – 4.81
BI2	0.4 ± 0.01	1.56 ± 0.08	5.56 ± 0.49	2.76 ± 0.24	2.19 ± 0.19	3.37 ± 0.31	1.59 – 3.79
BI3	0.38 ± 0.01	1.63 ± 0.06	5.56 ± 0.5	3.62 ± 0.33	2.14 ± 0.21	3.42 ± 0.3	2.52 – 5.38
BI4	0.39 ± 0.02	1.56 ± 0.1	5.56 ± 0.56	2.97 ± 0.3	2.15 ± 0.15	3.35 ± 0.37	2.01 – 4.35
Ml1	0.44 ± 0.01	1.59 ± 0.07	3.7 ± 0.21	3.02 ± 0.17	1.44 ± 0.08	2.24 ± 0.13	2.22 – 4.29
MI2	0.38 ± 0.01	1.63 ± 0.05	3.7 ± 0.23	2.79 ± 0.18	1.4 ± 0.07	2.28 ± 0.14	2.02 – 4.24
Ml3	0.4 ± 0.01	1.53 ± 0.06	5.56 ± 0.47	3.06 ± 0.26	2.18 ± 0.16	3.32 ± 0.28	2.15 – 4.18
Ml4	0.38 ± 0.01	1.63 ± 0.05	3.7 ± 0.27	3.16 ± 0.23	1.4 ± 0.09	2.25 ± 0.14	2.24 – 5.12
TA1	0.42 ± 0.01	1.4 ± 0.05	11.11 ± 1.71	3.53 ± 0.54	4.67 ± 0.75	6.44 ± 0.97	2.34 – 5.14
TA2	0.41 ± 0.02	1.49 ± 0.14	11.11 ± 1.63	2.55 ± 0.37	4.49 ± 0.58	6.63 ± 1.09	1.83 – 3.68
TA3	0.41 ± 0.01	1.49 ± 0.09	11.11 ± 1.62	3.38 ± 0.49	4.52 ± 0.72	6.59 ± 0.92	2.35 – 4.8
TA4	0.39 ± 0.01	1.59 ± 0.07	11.11 ± 1.52	2.62 ± 0.36	4.32 ± 0.61	6.79 ± 0.92	1.8 – 3.54
VE1	0.4 ± 0.01	1.53 ± 0.06	11.11 ± 1.52	2.85 ± 0.39	4.41 ± 0.59	6.7 ± 0.95	4.92 – 9.85
VE2	0.4 ± 0.01	1.52 ± 0.08	5.56 ± 0.56	2.76 ± 0.28	2.21 ± 0.19	3.29 ± 0.33	2.35 – 3.29
VE3	0.42 ± 0.01	1.42 ± 0.07	5.56 ± 0.58	3.19 ± 0.33	2.29 ± 0.19	3.23 ± 0.36	2.11 – 4.95
VE4	0.41 ± 0.01	1.49 ± 0.06	5.56 ± 0.56	3.64 ± 0.37	2.25 ± 0.23	3.3 ± 0.34	2.36 – 5.30
TE1	0.43 ± 0.01	1.37 ± 0.05	5.56 ± 0.41	2.62 ± 0.19	2.36 ± 0.17	3.19 ± 0.25	1.94 – 3.37
TE2	0.39 ± 0.01	1.56 ± 0.07	5.41 ± 0.61	2.75 ± 0.31	2.19 ± 0.23	3.37 ± 0.34	1.82 – 4.1
TE3	0.39 ± 0.01	1.61 ± 0.06	5.39 ± 0.58	3.59 ± 0.39	2.14 ± 0.2	3.41 ± 0.31	1.6 – 4.93
TE4	0.46 ± 0.01	1.19 ± 0.05	11.11 ± 1.06	2.69 ± 0.26	5.12 ± 0.47	5.99 ± 0.61	2.16 – 3.43
WI1	0.4 ± 0.1	1.56 ± 0.06	5.56 ± 0.49	3.41 ± 0.3	2.17 ± 0.16	3.34 ± 0.3	2.25- 4.85
WI2	0.38 ± 0.01	1.6 ± 0.05	5.56 ± 0.53	3.08 ± 0.29	2.12 ± 0.17	3.39 ± 0.31	2.1 – 4.65
WI3	0.39 ± 0.01	1.54 ± 0.05	5.56 ± 0.43	3.35 ± 0.26	2.17 ± 0.13	3.33 ± 0.25	2.46 – 4.84
WI4	0.38 ± 0.01	1.63 ± 0.05	5.56 ± 0.47	3.28 ± 0.28	2.1 ± 0.14	3.4 ± 0.27	2.45 – 4.84

DISCUSSION

There are high diversity and differences in chromosomal length characteristics of the inter and intra-species of this genus Fig. 3, 4. Given that the existence of diversity and difference in chromosome length indicates an advanced karyotype and has chromosomes in different sizes (Afshari et al. 2013), the species of this genus have advanced karyotypes. The existence of x=9 as the base chromosome number on the yarrow genus has been proven in several reports, yet the number of chromosomes and ploidy levels vary among different species of this genus, which could range from 2n=2x= 18 to 2n=8x=72 even though most species are Diploid(Guo et al. 2005, Baltisberger and Widmer 2016). In addition to inter-species diversity in ploidy levels, there are numerous reports of ploidy level diversity in populations within a species. In other words, different ploidy levels are reported for populations of a species (Hoshi et al. 2010, Ebrahim et al. 2012). Accordingly, a range between diploid to hexaploidy has been reported for *A. aleppica* species (Rad and Javaheri 2014); however, all the accessions of the species were diploid in the present study. The tetraploid level was reported for *A. bieberestini* species (Afshari et al. 2013), which was consistent with the present result. Afshari reported diploid and tetraploid levels for *A. millefolium* species. In another study, hexa and octa-ploidy levels were reported for the species (Ebrahim et al. 2012). The two reports were consistent with the present study in terms of *A. millefolium* species. For four populations of *A. talagonica* species, the diploid level was in accordance with results of studies by Sahin et al. (2006). Finally, the results obtained for ploidy levels of two species, *A. vermicularis* and *A. tenuifolia*, were in agreement with other reports (Afshari et al. 2013, Rad and Javaheri

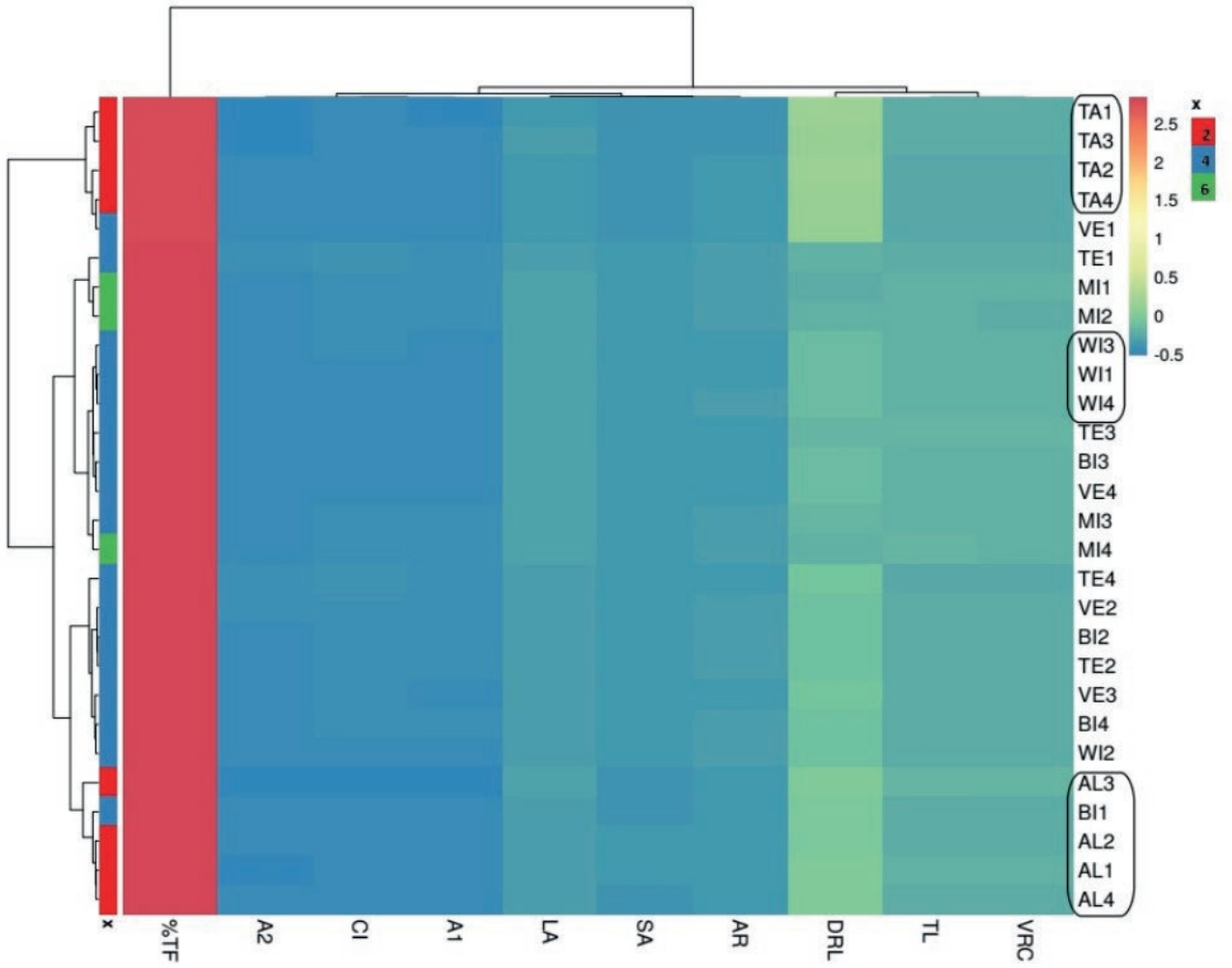

Figure 5. Depicts the results of cluster heatmap (based on Euclidian distance and ward method analysis for cytogenetic parameters).

2014). Therefore, no new reports were found for the ploidy levels of the species.

The karyotypic formulas of all the species consisted of a large number of metacentric chromosomes and a small number of sub-metacentric chromosomes (Table 2). On this basis, the populations of the species of this genus had symmetrical karyotypes, and there were diverse karyotypic formulas for both species and intra-species populations. In several reports on the cytogenetic analysis of species of the yarrow genus, more metacentric chromosomes and less submetacentric chromosomes have been reported (Sahin et al. 2006, Afshari et al. 2013, Rad and Javaheri 2014). Moreover, there were some reports on the subtelocentric chromosomes (Baltisberger and Widmer 2016). However, there were almost symmetrical chromosomes for species of the genus. According to the Stebbins table regarding 28 populations, 11 populations had A1 symmetry, 15 had B1, a population had A2, and a population had B2 symmetry (Table 2); hence, there were more symmetrical chromosomes in the present research than other reports since the karyotype A2 was mostly reported in other reports, and fewer cases had A1 and B1 symmetries (Kiran et al. 2012, Sahin et al. 2006). Accordingly, no obvious differences were reported in karyotype asymmetry between yarrow species; all the species had symmetrical karyotype structures because most chromosomes were metacentric and sub-metacentric (Kiran et al. 2012).

Satellites were observed more in populations with tetra- and hexaploidy levels and on chromosome 1 (Fig. 3). No satellites were observed in diploid populations, and there was only a satellite for each population. Our results were consistent with those of a report by Sahin et al. (2006). On the contrary, no satellites were reported in certain studies (Hoshi et al. 2010, Kiran et al. 2012, Afshari et al. 2013, Rad and Javaheri 2014) whereas one

to three satellites have been reported in some other researches (Afshari et al. 2013). Additionally, more satellites were observed in submetacentric chromosomes and the results were consistent with those of the present study (Hoshi et al. 2010).

There were chromosomes B in two populations of *A. tenuifolium* species (Table 2). A chromosome B was also reported for the species in some populations (Chehregani Rad et al. 2017), and there were some reports on the existence of B chromosome in other species on the genus (Baltisberger and Widmer 2016). Nevertheless, there was no B chromosome in some reports (Kiran et al. 2012).

There was no inter-species diversity for the chromatin content, arm length, and chromosome length. Furthermore, the intra-species populations showed more diversity (Table 3), but there was inter-species diversity for ratios to arms (and large to small); however, the intra-species populations had diversity. Therefore, the evolution and speciation of the genus was through A1 rather than increasing or decreasing the chromatin content and chromosome length. The average length of each chromosome ranged from 2.93 to 3.55 mμ for the species, which was consistent with other reports (Sahin et al. 2006 Afshari et al. 2013) . Meanwhile, the chromosome length range was higher in certain reports than that in the results of the present study, and longer chromosomes were reported for the species (Aksu et al. 2013). Based on the karyotypic characteristics, the *A. aleppica* had more karyotypic evolution in terms of chromatin content, and three species, *A. biebersteinii*, *A. wilhemsii*, and *A. millefolium*, had more complete karyotypes due to the A1 and a higher evolution in terms of chromosome length characteristics and chromatin content. *A. talangonica*, *A. tenifolia*, and *A. vermicularis* had karyotypic evolution due to the chromosomal asymmetry; thus, *A. biebersteinii*, *A. wilhemsii* and *A. millefolium* had more evolved karyotypes than the other species. According to the results, the karyotypic characteristics could not separate the populations of yarrow species due to the intra-species diversity, and the populations of different species were in the same group in several cases. Despite the lack of comprehensive reports on the study of inter and intra-species relationships for the *Achillea* genus based on karyotypic characteristics, the few available reports indicated that the karyotypic characteristics were unable to completely separate the populations of species of the genus (Ebrahim et al. 2012, Kiran et al. 2012).

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Table 1. Details on population sites including geographical coordinates, altitude and origin of samples

Table 2. Cytogenetic indices data A2, A1, % TF, DRL, VRC, SC, and KF.

Table 3. Karyotypes and chromosomal parameters in this study for each species.