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First karyomorphological analysis of French and Russian tarragon (*Artemisia dracunculus* L.)

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Abstract

Artemisia L. is interesting in cytogenetic research due to having a variety of chromosome numbers and ploidy levels. In this research, the karyomorphological characteristics of nine accessions of French and Russian tarragon (*A. dracunculus* L.) collected from different locations in Iran were analyzed for the first time. The basic chromosome number was x = 9, with two ploidy levels of 4x (French accessions) and 10x (Russian accessions). The mean of chromosome length and the total haploid chromosome length of the French accessions ranged from 2.32 to 3.39 μm and 40.26 to 61.13 μm, respectively, while these values were 3.99 to 4.22 μm and 179.72 to 190.13 μm, respectively, for the Russian accessions. Chromosome types of French tarragons were determined as metacentric (dominant) and submetacentric, whereas they were metacentric (dominant), submetacentric, and subtelocentric in Russian tarragons. French accessions were classified as 4A, 3B, and 4B according to the Stebbins classification, while all the Russian accessions have a 3B type karyotype. Russian accessions present the most asymmetrical karyotype based on biplot analysis of asymmetry indices. Cluster analysis according to all karyotypic parameters revealed that the French and Russian accessions were placed in two separate groups. Principal components analysis showed that the first two components possessed 95.8% of the total variation. The PCA score plot generated from the first two principal components not only supported the clustering results but also distinguished the French accessions of Arak, Isfahan, and Tehran, which have more symmetrical karyotype, from the remaining French accessions.

Keywords *Artemisia* · Chromosome number · Evolution · Karyotype asymmetry · Polyploidy

Introduction

The genus *Artemisia* L. is a large and diverse genus of the family *Asteraceae*. Although the center of diversification of *Artemisia* is Central Asia, its species grow mainly in temperate areas of the northern hemisphere including North West America, Irano-Turanian, and Mediterranean regions (Zeb et al. 2018). The taxonomy of *Artemisia* is difficult and complex, and contains about 350–500 species (Shultz 2006). Around 43 species of *Artemisia* have been identified in Iran (Mozaffarian 2005).

Some species of the genus *Artemisia* are economically important (Zeb et al. 2018). *Artemisia dracunculus* L. or tarragon (belongs to subgenus *Dracunculus*) is a perennial herb known as a medicinal and spice species (Zeb et al. 2018; Ekiert et al. 2021). The essential oil of its aerial part and leaves contains secondary metabolites such as monoterpenoids, flavonoids, phenolic acids, coumarins, and alkamides (Ekiert et al. 2021; Kara and Çağlak 2022). This species has two main varieties: French (or German) tarragon and Russian tarragon (designated as *A. dracunculoides* L. in some literature) (Zeb et al. 2018). French tarragon is sterile, propagates vegetatively by rhizome cuttings, and is preferred in cooking use for its stronger aroma, while Russian tarragon is fertile, but rarely is used in culinary (Engels and Brinckmann 2014; Ekiert et al. 2021). They are also characterized by a wide range of morphological, anatomical, phytochemical and cytogenetical variabilities (Obolskiy et al. 2011).

Knowledge of chromosome numbers, ploidy levels and karyotypic characteristics is very important in

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understanding the species' evolution and their relationships (Abdali and Miri 2020; Ebrahimi et al. 2021), as well as solving classification problems of some closely related taxa (Oroji Salmasi et al. 2019; Rajabi Mazaher et al. 2021). The genus *Artemisia* has two basic chromosome numbers, x = 8 (less frequent) and x = 9 (most common) (Matoba et al. 2007). Polyploidy has been identified as a common phenomenon in *Artemisia* species, ranging from 2× to 6× for x = 8 and from 2× to 12× for x = 9 (Matoba et al. 2007; Hayat et al. 2009). The ploidy level and number of chromosomes of French tarragon are reported as 2n = 4x = 36 and Russian tarragon as 2n = 10x = 90 (Rousi 1969). Although many karyological studies have been carried out on *Artemisia* species (Ghasemi et al. 2005; Saedi et al. 2005; Matoba et al. 2007; Pellicer et al. 2007, 2008; Naseri et al. 2009; Zhen et al. 2010; Tabur et al. 2012; Dolatyari et al. 2013; Yazdani et al. 2014; Sancar et al. 2021), however to the best of our knowledge, the karyotypic analysis of *A. dracunculus* remain unknown. Therefore, this study was conducted for the first time to determine the karyotype characteristics of French and Russian tarragon.

Materials and methods

Plant materials

Nine accessions of *A. dracunculus* including six French tarragons, and three Russian tarragons were investigated. Transplants of French tarragon accessions were collected from natural populations, and deposited at the herbarium of the University of Tehran (UTFH). The seeds of Russian tarragons were collected from the Iranian Biological Resource Center (IBRC) (Table 1).

Chromosome counts

Root tips were immersed in α-bromonaphthalene 1% for 5 h, and fixed in Carnoy's fixative (3:1 alcohol: glacial acetic acid, v/v) overnight at 4 °C. The root tips were hydrolyzed in 1 N HCl for 25 min at 60 °C, stained by aceto-orcein for 24 h, and then squashed onto slides in acetic acid 45% for observation. At least five metaphase plates per individual and five plants per accession were examined and their average data were used for karyotype analysis. Photos were taken under a Canon digital camera (Powershot SX50 HS) mounted on a CX52 Olympus microscope.

Karyotype analysis

For the numerical characterization of the karyotypes, short arm length (SA) and long arm length (LA) were measured using MicroMeasure 3.3 software. The following parameters were calculated to identify the chromosomal parameters: mean chromosome length (CL = LA + SA), total chromosome length of the haploid complement (HCL = $\sum$CL), centromeric index (CI = SA/CL), arm ratio (AR = LA/SA), r-value (SA/LA), relative length of chromosome (RL% = (CL/$\sum$CL) × 100) and chromosome type (Levan et al. 1964). Asymmetry indices were calculated using: chromosome form percentage (F% = (SA/$\sum$CL) × 100), total form percentage (TF%; Huziwara 1962), percentage karyotype asymmetry index (AsK%; Arano 1963), intrachromosomal asymmetry index (A_1), interchromosomal asymmetry index (A_2) (Romero-Zarco 1986), percentage of karyotype symmetry (S% = (CL$_{min}$/CL$_{max}$) × 100), degree of karyotype asymmetry (A; Watanabe et al. 1999), mean centromeric index (X$_{CI}$ = $\sum$CL/n), mean centromeric asymmetry (X$_{CA}$; Peruzzi and Eroğlu 2013), coefficient of variation of chromosome length (CV$_{CL}$), coefficient of variation of centromeric index (CV$_{CI}$), asymmetry index (AI) (Paszko 2006),

Table 1 Provenance of the studied *A. dracunculus* accessions

Accession	Province, City	Latitude	Longitude	Altitude (m)	Collector	Date of collection	Symbol
French tarragon	Markazi, Arak	34°05′	49°42′	1737	-	2015	FR-Arak
	Isfahan, Isfahan	32°40′	51°39′	1579	-	2015	FR-Isfahan
	Khorasan Razavi, Mashhad	36°14′	59°39′	972	-	2015	FR-Mashhad
	Mazandaran, Sari	36°34′	53°03′	40	-	2015	FR-Sari
	Fars, Shiraz	29°36′	52°31′	1544	-	2015	FR-Shiraz
	Tehran, Tehran	35°38′	51°06′	1140	-	2015	FR-Tehran
Russian tarragon	Markazi, Arak	34°03′	49°37′	1837	A. Dolatyari & H. Ramezani	2014	RUS-Arak
	Alborz, Karaj	35°49′	50°59′	1341	A. Dolatyari & H. Ramezani	2014	RUS-Karaj
	Tehran, Tehran	35°35′	51°26′	1065	A. Dolatyari & H. Ramezani	2014	RUS-Tehran

Stebbins' class asymmetry index (Stebbins 1971), the difference between minimum and maximum relative length of chromosomes (DRL = RL%$_{max}$–RL%$_{min}$), and centromeric gradient (CG; Lavania and Srivastava 1992).

Idiograms were drawn using Excel based on chromosome length. The cluster analysis was carried out by the nearest neighbor method using Minitab ver. 16 software. Pearson correlation among some karyotype characters between karyotypic parameters with geographical coordinates was computed by SPSS ver. 23 software. Principal components analysis (PCA) was performed to evaluate the contribution of all karyological variables to the diversity of accessions and the first two principal component scores were plotted to identify the grouping pattern among the accessions using Minitab ver. 16 software.

Results

Our results showed that French tarragon accessions were tetraploid with $2n = 4x = 36$, while Russian tarragons were decaploid with $2n = 10x = 90$ (Table 2). Karyotype characters of the French and Russian tarragons were reported for the first time (Table 2). Their mitotic metaphase chromosomes and haploid ideograms are shown in Fig. 1. All investigated tarragon accessions had small chromosomes (≈ 2 μm or less; Stace 2000). The mean size of the chromosomes in Russian tarragon accessions was higher than that of French. The highest LA (2.4 μm), SA (1.8 μm), CL (4.2 μm), and HCL (190.1 μm) were obtained for RUS-Arak, and the lowest of these values were obtained for FR-Shiraz (1.3 μm, 0.9 μm, 2.2 μm an, 40.2 μm, respectively). The length range of chromosomes was 1.10 to 2.89 μm and 2.10 to 6.33 μm in FR-Shiraz and RUS-Arak, respectively. The highest CI and r-value were observed in FR-Isfahan, while the lowest values were found in RUS-Karaj and RUS-Tehran. In addition, FR-Isfahan (1.2) and RUS-Tehran (1.5) had the lowest and highest values of AR, respectively. Pearson's correlation coefficients showed a significant positive correlation between both parameters of ploidy level and chromosome number with CL ($r = 0.84$, $P < 0.01$) and HCL ($r = 0.99$, $P < 0.01$).

The presence of satellites was clearly observed in some accessions such as FR-Mashhad and RUS-Karaj, but in other accessions, due to the low resolution of the images of metaphase plates, they could not be reliably detected, so they were not used for chromosome identification and analysis.

According to the nomenclature of chromosomes by Levan et al. (1964), three chromosome types were found in French tarragons (Table 2): metacentric (M and m) in FR-Arak and FR-Tehran, metacentric (M and m) and submetacentric (sm) in FR-Isfahan and FR-Sari, as well as metacentric (m) and submetacentric (sm) in FR-Mashhad and FR-Shiraz. On the other hand, four chromosome types of metacentric (M and

Table 2 Karyotype features of the nine studied *A. dracunculus* accessions

Accession	2n	CN	LA (μm)	SA (μm)	CL (μm)	HCL (μm)	CI	AR	r-value	RL%	KF
FR-Arak	4x	36	$1.87 \pm 2 \times 10^{-5}$*	$1.52 \pm 1 \times 10^{-5}$	$3.39 \pm 4 \times 10^{-5} (2.42–5.00)$**	$61.13 \pm 7 \times 10^{-4}$	$0.45 \pm 1 \times 10^{-6}$	$1.23 \pm 4 \times 10^{-6}$	$0.81 \pm 2 \times 10^{-6}$	5.55	1M + 17m
FR-Isfahan	4x	36	$1.41 \pm 1 \times 10^{-5}$	$1.20 \pm 1 \times 10^{-5}$	$2.61 \pm 3 \times 10^{-5} (2.00–3.20)$	$47.01 \pm 5 \times 10^{-4}$	$0.46 \pm 4 \times 10^{-7}$	$1.18 \pm 2 \times 10^{-6}$	$0.85 \pm 1 \times 10^{-6}$	5.55	2M + 15m + 1sm
FR-Mashhad	4x	36	$1.94 \pm 1 \times 10^{-5}$	$1.36 \pm 2 \times 10^{-5}$	$3.30 \pm 4 \times 10^{-5} (2.60–4.10)$	$59.50 \pm 7 \times 10^{-4}$	$0.41 \pm 2 \times 10^{-6}$	$1.43 \pm 1 \times 10^{-5}$	$0.70 \pm 7 \times 10^{-6}$	5.55	17m + 1sm
FR-Sari	4x	36	$1.64 \pm 2 \times 10^{-5}$	$1.15 \pm 1 \times 10^{-3}$	$2.79 \pm 1 \times 10^{-3} (1.82–3.70)$	$50.30 \pm 3 \times 10^{-2}$	$0.41 \pm 4 \times 10^{-4}$	$1.45 \pm 2 \times 10^{-3}$	$0.70 \pm 1 \times 10^{-3}$	5.55	1M + 15m + 2sm
FR-Shiraz	4x	36	$1.29 \pm 2 \times 10^{-2}$	$0.94 \pm 1 \times 10^{-5}$	$2.23 \pm 2 \times 10^{-2} (1.10–2.89)$	$40.27 \pm 4 \times 10^{-1}$	$0.42 \pm 8 \times 10^{-3}$	$1.37 \pm 2 \times 10^{-2}$	$0.72 \pm 1 \times 10^{-2}$	5.55	17m + 1sm
FR-Tehran	4x	36	$1.77 \pm 1 \times 10^{-5}$	$1.45 \pm 1 \times 10^{-2}$	$3.22 \pm 1 \times 10^{-2} (2.24–5.00)$	$57.96 \pm 2 \times 10^{-1}$	$0.45 \pm 1 \times 10^{-3}$	$1.23 \pm 8 \times 10^{-3}$	$0.81 \pm 7 \times 10^{-7}$	5.55	2M + 16m
Mean	4x	36	$1.65 \pm 2 \times 10^{-1}$	$1.27 \pm 2 \times 10^{-1}$	2.92 ± 0.45	52.70 ± 8.2	$0.43 \pm 2 \times 10^{-2}$	$1.31 \pm 1 \times 10^{-1}$	$0.76 \pm 6 \times 10^{-2}$	5.55	
RUS-Arak	10x	90	$2.43 \pm 2 \times 10^{-6}$	$1.79 \pm 3 \times 10^{-6}$	$4.22 \pm 5 \times 10^{-6} (2.10–6.33)$	$190.13 \pm 2 \times 10^{-4}$	$0.42 \pm 2 \times 10^{-7}$	$1.46 \pm 1 \times 10^{-5}$	$0.73 \pm 7 \times 10^{-7}$	2.22	2M + 33m + 9sm + 1st
RUS-Karaj	10x	90	$2.32 \pm 2 \times 10^{-4}$	$1.66 \pm 3 \times 10^{-5}$	$3.99 \pm 1 \times 10^{-4} (1.93–5.59)$	$179.72 \pm 7 \times 10^{-3}$	$0.41 \pm 5 \times 10^{-5}$	$1.52 \pm 2 \times 10^{-4}$	$0.71 \pm 7 \times 10^{-5}$	2.22	3M + 30m + 11sm + 1st
RUS-Tehran	10x	90	$2.33 \pm 2 \times 10^{-6}$	$1.67 \pm 3 \times 10^{-6}$	$4.00 \pm 5 \times 10^{-6} (1.91–6.14)$	$180.25 \pm 2 \times 10^{-4}$	$0.41 \pm 3 \times 10^{-7}$	$1.53 \pm 2 \times 10^{-6}$	$0.71 \pm 8 \times 10^{-7}$	2.22	1M + 31m + 12m + 1st
Mean	10x	90	$2.36 \pm 6 \times 10^{-2}$	$1.71 \pm 7 \times 10^{-2}$	4.07 ± 0.13	183.37 ± 5.86	$0.41 \pm 5 \times 10^{-2}$	$1.50 \pm 4 \times 10^{-2}$	$0.71 \pm 1 \times 10^{-2}$	2.22	

2n ploidy level, *CN* somatic chromosome number, *LA* mean long arm, *SA* mean short arm, *AR* arm ratio, *RL%* relative length of chromosome, *KF* karyotype formula, *CI* centromeric index, *HCL* total chromosome length, *CL* mean chromosome length, *HCL* total chromosome length of the haploid complement, *CI* centromeric index of the haploid complement, *CI* centro-

*: Mean value ± standard deviation (SD); **: Shortest chromosome-longest chromosome

Fig. 1 Somatic metaphase chromosomes and haploid idiograms of nine *A. dracunculus* accessions. **A**: FR-Arak, **B**: FR-Isfahan, **C**: FR-Mashhad, **D**: FR-Sari, **E**: FR-Shiraz, **F**: FR-Tehran, **G**: RUS-Arak, **H**: RUS-Karaj, **I**: RUS-Tehran. Scale bar = 5 μm
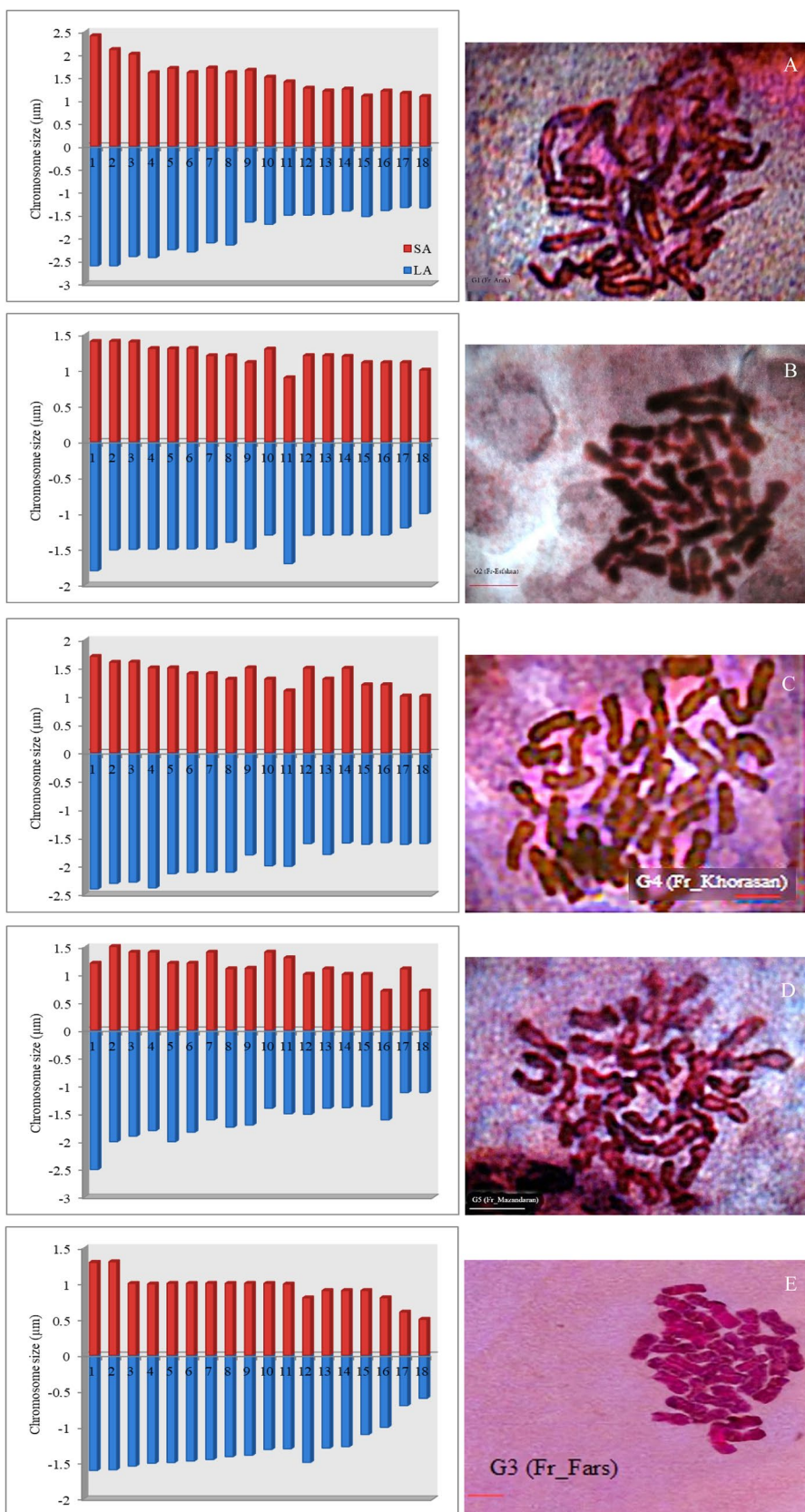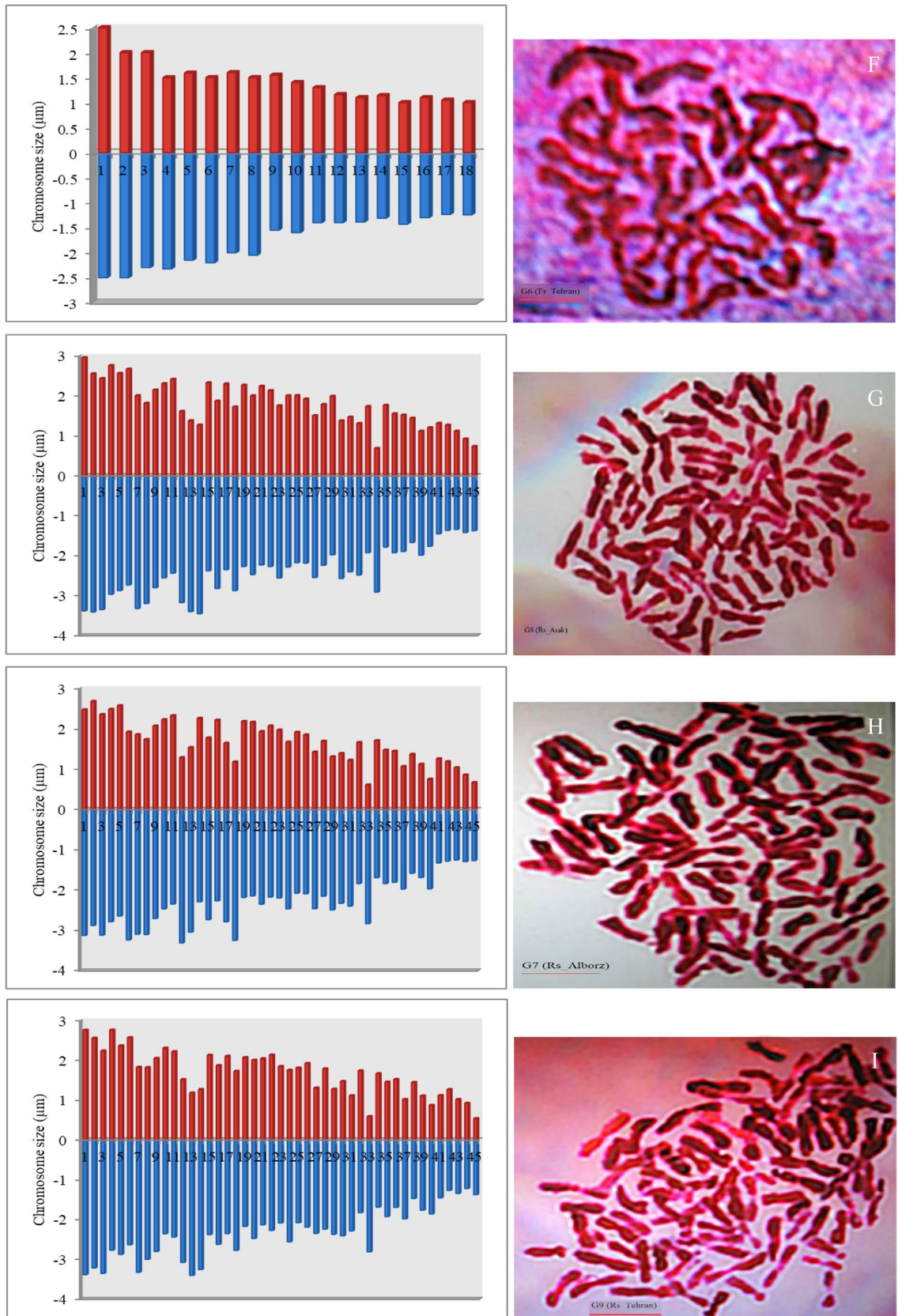
Fig. 1 (continued)

Table 3 Symmetry/asymmetry indices of the nine studied *A. dracunculus* accessions

Population	F%	TF%	AsK%	S%	X_{CI}	X_{CA}	CV_{CL}	CV_{CI}	AI	SC	A_1	A_2	DRL	CG
FR-Arak	2.49	22.45	55.08	48.48	3.39	9.99	23.98	5.91	1.41	4B	0.82	0.24	2.10	47.38
FR-Isfahan	2.56	23.03	53.94	62.59	2.61	7.73	10.21	7.60	0.77	4A	0.86	0.10	1.27	45.94
FR-Mashhad	2.29	20.62	58.75	63.40	3.30	17.42	13.74	8.29	1.14	4A	0.71	0.13	1.26	42.25
FR-Sari	2.30	20.67	58.63	49.22	2.79	17.11	17.41	12.44	2.16	3B	0.72	0.17	1.86	41.07
FR-Shiraz	2.33	21.07	58.07	37.98	2.25	15.71	20.64	6.65	1.37	4B	0.73	0.20	2.22	42.12
FR-Tehran	2.49	22.50	55.09	44.88	3.12	10.18	26.22	6.66	1.74	4B	0.82	0.26	2.34	47.54
Mean	2.41	21.72	56.59	51.09	2.91	13.02	18.71	7.92	1.43	-	0.77	0.18	1.84	44.38
RUS-Arak	0.94	21.21	57.57	33.30	4.22	15.52	23.35	16.68	3.89	3B	0.75	0.23	1.11	41.16
RUS-Karaj	0.92	20.87	58.24	34.52	3.99	17.03	23.65	18.32	4.33	3B	0.73	0.23	1.02	40.38
RUS-Tehran	0.92	20.83	58.32	31.12	4.00	17.37	25.04	18.53	4.64	3B	0.73	0.25	1.17	40.05
Mean	0.92	20.97	58.04	32.98	4.07	16.64	24.01	17.84	4.28	-	0.73	0.24	1.10	40.53

F% chromosome form percentage, *TF%* total form percentage, *AsK%* percentage karyotype asymmetry index, *S%* percentage of karyotype symmetry, X_{CI} mean centromeric index, X_{CA} mean centromeric asymmetry, CV_{CL} coefficient of variation of chromosome length, CV_{CI} coefficient of variation of centromeric index, *AI* asymmetry index, *SC* Stebbins' classification, A_1 intrachromosomal index, A_2 interchromosomal index, *DRL* the difference between minimum and maximum relative length of chromosomes, *CG* centromeric gradient

m), submetacentric (sm), and subtelocentric (st) were found in all Russian tarragons. Overall, metacentric (m) chromosome pairs were dominant in all tarragon accessions.

The karyotype asymmetry was evaluated according to Stebbins' classification (Stebbins 1971) and 13 quantitative indices (Table 3). Based on the Stebbins' class asymmetry index, French tarragon accessions of Isfahan and Mashhad were classified as category 4A, Sari as category 3B, and the other three French accessions as category 4B. All Russian tarragon accessions were located in class 3B. They also presented more asymmetrical karyotypes, as shown in the biplot diagram (Fig. 2), which is indicated by lower values of F%, TF%, S%, A_1, DRL, and CG, and higher values of AsK%, X_{CI}, X_{CA}, CV_{CL}, CV_{CI}, AI and A_2 (Table 3). FR-Isfahan was the most symmetrical karyotype based on values

of F%, TF%, AsK%, X_{CA}, X_{CA}, AI, A_1 and A_2, while RUS-Tehran was the most asymmetrical karyotype according to F%, TF%, AsK%, S%, X_{CA}, CV_{CL}, CV_{CI}, AI, A_1, A_2 and CG parameters.

In French accessions, a positive correlation was observed between latitude with chromosome size (LA, SA, CL and HCL) and X_{CI} (Table 4). In addition, a negative correlation was found between longitude with CI and TF%, whereas X_{CA} showed a positive relationship. A negative correlation was achieved between altitude with AR, CV_{CI} and AI. In Russian accessions, latitude and longitude were negatively correlated with S%, while altitude had a positive correlation. In the case of DRL, the opposite of this correlation was found. Longitude and altitude showed a significant positive and negative correlation with A_1, respectively.

Fig. 2 Biplot analysis of karyotype asymmetrical indices of the nine *A. dracunculus* accessions. Symbols as in Tables 1 and 3
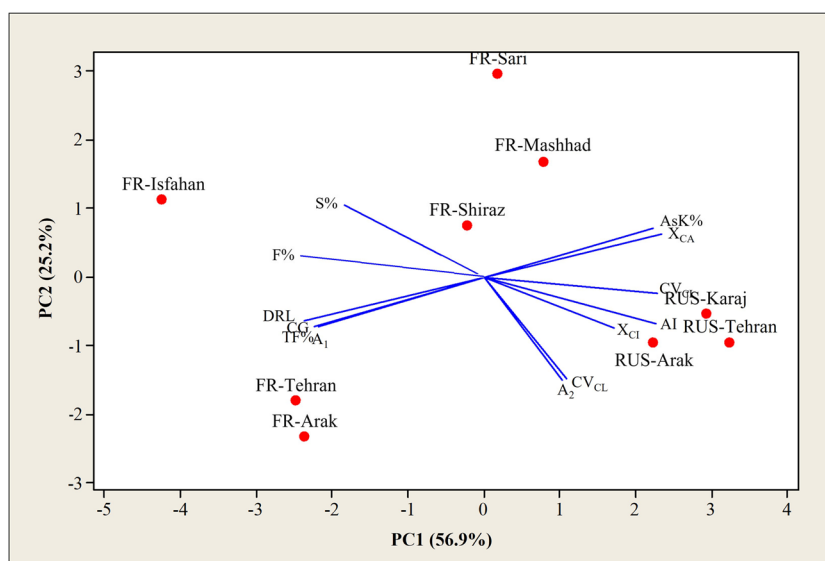
Table 4 Pearson's correlation coefficients between geographical coordinates and karyological variables

Accession	Geographical coordinates	Karyological variables																		
		LA	SA	CL	HCL	CI	AR	r-value	F%	TF%	AsK%	S%	X_{CI}	X_{CA}	CV_{CL}	CV_{CI}	AI	A_1	DRL	CG
French	Latitude	0.80**	0.69*	0.78*	0.78*	-0.04	0.10	-0.01	-0.01	-0.03	0.00	0.50	0.77*	0.03	-0.03	0.44	0.33	-0.02	-0.27	0.13
	Longitude	0.32	-0.09	0.14	0.14	-0.70*	0.68	-0.67	-0.68	-0.70*	0.68	0.52	0.17	0.70*	-0.50	0.34	-0.15	-0.53	-0.60	-0.62
	Altitude	-0.19	0.15	-0.04	-0.04	0.65	-0.70*	0.62	0.61	0.63	-0.61	-0.06	-0.03	-0.63	0.13	-0.93**	-0.72*	0.14	0.09	0.61
Russian	Latitude	0.66	0.65	0.63	0.60	0.64	-0.49	0.60	0.60	0.52	-0.52	-0.69*	0.63	-0.45	0.23	-0.51	-0.22	0.39	0.86**	0.34
	Longitude	0.29	0.27	0.24	0.21	0.25	-0.07	0.21	0.21	0.11	-0.11	-0.93**	0.24	-0.03	0.62	-0.10	0.21	0.74*	0.99**	-0.08
	Altitude	-0.24	-0.23	-0.20	-0.16	-0.20	0.03	-0.16	-0.16	-0.06	0.06	0.95**	-0.20	-0.01	-0.65	0.06	-0.26	-0.77*	-0.99**	0.13

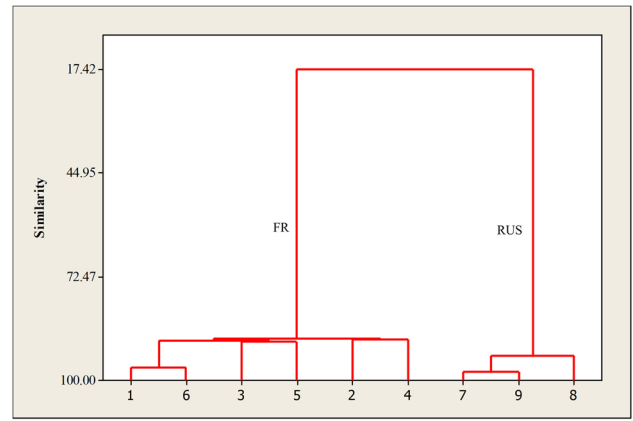*: $P < 0.05$, **: $P < 0.01$, Symbols as in Tables 2 and 3

Fig. 3 Dendrogram of cluster analysis of the nine *A. dracunculus* accessions based on analyzing all karyological parameters by the nearest neighbor method

The results of cluster analysis showed a clear distinction between the studied tarragons, and French and Russian accessions were classified into two major groups (Fig. 3). PCA of all karyological parameters revealed that the first three principal components (with eigenvalues ≥ 1) accounted for 95.8% of the total variance. The first component (64.5%) emphasized the chromosomal parameters, while the second component (22.0%) and especially the third component (9.3%) accentuated the asymmetry indices (Table 5). Based on the results of score plot analysis, the French and Russian accessions were also separated like the cluster analysis, however, accessions FR-Mashhad, FR-Sari and FR-Shiraz (having almost shorter chromosome length and a more symmetrical karyotype) were distinguished from the remaining French accessions (Fig. 4).

Discussion

Chromosome number

The obtained results indicated that all of the studied tarragon accessions have x = 9, which was confirmed by Rousi (1969), Kreitschitz and Vallès (2003), Eisenman and Struwe (2011) and Pellicer et al. (2013) in *Artemisia dracunculus* and other species such as *A. annua* L. (Kreitschitz and Vallès 2003), *A. campestris* L. (Kreitschitz and Vallès 2003; Tabur et al. 2011), *A. abrotanum* L., *A. absinthium* L. (Kreitschitz and Vallès 2003; Tabur et al. 2012), *A. armeniaca* Lam., *A. chamaemelifolia* Vill., *A. tournefortiana* Rchb. and *A. arborescens* L. (Tabur et al. 2012). In addition, Pellicer et al. (2007) found that the basic chromosome number of *A. dracunculus* and 18 other studied species is x = 9. However, x = 8 has been reported in some species such as *A. scoparia* Waldst.et Kit (Tabur et al. 2011) and *A. vulgaris* L., *A.*

Table 5 Principal component analysis of karyological variables in nine *A. dracunculus* accessions

Variable	PC1	PC2	PC3
2n	0.238	-0.188	-0.072
CN	0.238	-0.188	-0.072
LA	0.223	-0.143	-0.062
SA	0.181	-0.265	-0.062
CL	0.209	-0.195	-0.063
HCL	0.238	-0.137	-0.074
CI	-0.187	-0.292	-0.042
AR	0.227	0.195	0.007
r-value	-0.174	-0.308	-0.091
RL%	-0.238	0.118	0.072
F%	-0.247	0.069	0.056
TF%	-0.172	-0.314	-0.065
AsK%	0.169	0.318	0.081
S%	-0.186	0.085	-0.359
X_{CI}	0.213	-0.185	-0.075
X_{CA}	0.185	0.291	0.068
CV_{CL}	0.124	-0.187	0.506
CV_{CI}	0.239	-0.015	-0.111
AI	0.243	-0.082	0.051
A_1	-0.163	-0.322	-0.099
A_2	0.120	-0.199	0.502
DRL	-0.164	0.012	0.509
CG	-0.201	-0.243	0.082
Eigenvalue	15.477	5.284	2.228
Variance (%)	64.5	22.0	9.3
Cumulative variance (%)	64.5	86.5	95.8

Symbols as in Tables 2 and 3

austriaca Jacq., *A. incana* (L.) Druce, *A. splendens* Willd., *A. caucasica* Willd. and *A. haussknechtii* Boiss. (Tabur et al. 2012). The most common and primitive basic chromosome number of *Artemisia* is x = 9, while x = 8 is advanced (Zhen et al. 2010).

Genome size is generally correlated with chromosome length and ploidy level (Torrell and Vallès 2001). Our results showed that the mean and total chromosome length of decaploid Russian tarragons were higher than tetraploid French accessions, so with a 2.5-fold increase in ploidy level, the mean chromosome length increased 1.4-fold. Garcia et al. (2007) and Naseri et al. (2009) noted that the total karyotype length in some North American and Iranian *Artemisia* L. significantly correlated with genome size and DNA content. Mas de Xaxars et al. (2016) also observed that genome size in Alpine *Artemisia* L. was positively and significantly correlated with ploidy level. Pellicer et al. (2010) found that the increase in genome size in *Artemisia* polyploids followed a non-linear relationship with saturation behavior. However, some *Artemisia* species do not follow this pattern (Vallès et al. 2012). These contradictions may be due to the fact that variations in genome size are affected by several factors such as systematic and evolutionary implications, or ecological selection pressures (Torrell and Vallès 2001; Fallahi et al. 2020).

Polyploidy

Polyploidy has played an important role in the evolution, speciation, and biodiversity of higher plants (Afshar 2015; Miri 2020; Shamsolshoara et al. 2020), and is thought to contribute in ecological adaptation and consequently geographical expansion (Vallès et al. 2012; Roughani et al. 2021). It is very prevalent in some *Artemisia* species,

Fig. 4 Score plot of the nine *A. dracunculus* accessions for the first two principal components. Symbols as in Table 1
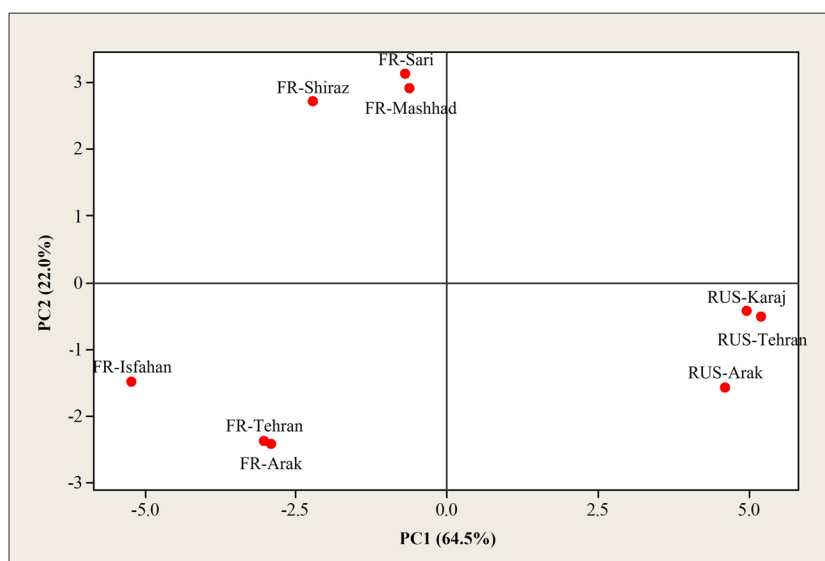
such as *A. dracunculus* (Vallès et al. 2012). *Artemisia dracunculus* is a karyotaxonomically interesting species, as Eisenman and Struwe (2011) and Pellicer et al. (2013) have reported the presence of different ploidy levels, from di- to deca-ploidy depending on the origin of the populations. According to our results, Rousi 1969) detected French and Russian tarragon as tetra- and deca-ploid, respectively. Kreitschitz and Vallès (2003) found di- and tetra-ploidy in *A. abrotanum* and *A. absinthium* populations and suggested that the increase in polyploidy level is an adaptation mechanism to drought conditions. Similarly, Dolatyari et al. (2013) identified two ploidy levels, 2× and 4x, in *A. oliveriana* J.Gay ex Besser accessions, and stated that the doubling of the ploidy level could indicate a speciation process. Russian tarragon is higher vigorous and tolerant (Bown 2001; Tucker and DeBaggio 2009), which could be explained as a result of the polyploidization phenomenon.

Karyotypic variation

Karyotypic variation occurs widely in the genus *Artemisia*, so the karyotype may vary in different populations or among different individuals (Zhen et al. 2010). Other than the reports on the *A. dracunculus* karyotypic formula by Wang (2000) and its karyological data by Pellicer et al. (2013), no data have been reported on the karyological characteristics of *A. dracunculus* so far, which may be due to the small size of chromosomes and high ploidy level. The present study is the first to report the karyomorphology of *A. dracunculus*. Although Tabur et al. (2012) stated that it is difficult to determine the systematic relationships using karyotypes due to the inter- and intra-specific similarity of *Artemisia* L. chromosome morphology, however, the results of the karyotypic formula allowed us to compare and differentiate the French and Russian tarragons. Among the accessions studied, the karyotype morphology of French accessions was more homogeneous, as we detected the presence of 16 to 18 metacentric (88.9–100%) and none to two submetacentric (0–11.1%) chromosome pairs. This is while there were 32 to 35 metacentric (71.1–77.7%), 9 to 12 submetacentric (20.0–26.6%) and 1 subtelocentric (2.2%) chromosome pairs in Russian accessions. Wang (2000) reported the karyotypic formula of *A. dracunculus* as 14m + 4sm. The existence of these karyological differences may be due to the different habitat and climatic conditions of the accessions (Tabur et al. 2012). The genus *Artemisia* has an almost symmetrical karyotype and most of the chromosomes are metacentric and submetacentric (Vallès et al. 2012). However, Dolatyari et al. (2013) reported that the chromosomes of 28 *Artemisia* species are mainly meta- or submetacentric except for three species, which have one or two pair(s) of subtelocentric

chromosomes, which is similar to the results of Russian tarragon karyotype.

According to Stebbins (1971), higher karyotypic asymmetry can be considered as an evolutionarily derived state. Therefore, it seems that the Russian tarragon originated from the French tarragon. French tarragon accessions were placed into three classes based on Stebbins classification, which these changes in the centromere position and chromosome size may be attributed as an evolutionary trend (Ghorbani Sini and Arzani 2015). Oliva and Vallès (1994) found that the tetraploid karyotype of *A. umbelliformis* Lam. is more asymmetric than the diploid *A. eriantha* Ten, and concluded that the latter is at least one of the likely ancestors of the former. These statements agree with the hypothesis of Torrell et al. (2001) that *A. campestris* (2n = 2x) is the origin of *A. campestris* (2n = 4x), *A. crithmifolia* L. (2n = 6x) and *A. monosperma* Delile (2n = 4x) populations. This shows the importance of karyotype analysis to determine the evolutionary status of different accessions.

Although genome size has often been associated with environmental or ecological variables (Mas de Xaxars et al. 2016), the genus *Artemisia* has been less evaluated. Several chromosomal parameters and asymmetry indices in the studied tarragons showed significant correlations with geographic coordinates and altitude (as a set of environmental variables), e.g., French accessions collected from higher latitudes had a longer chromosome size or tarragons prepared from higher altitudes had a relatively more symmetric karyotype (based on 2 to 3 asymmetry indices). This relationship was less in Russian accessions, which may be due to the small number of accessions and the proximity of the sample collection site. Hamidi et al. (2018) did not identify any significant correlation between genome size and environmental conditions in 18 populations of *A. khorassanica* Podlech, and concluded that their 2C DNA amounts were independent of environmental conditions. However, the observed relationships indicate that adaptation to habitat could influence karyotypic characteristics, possibly because different populations encounter different environmental variables that may promote genetic diversity (Fallahi et al. 2020). Oyundelger et al. (2021) in a study on environmental effects on genetic diversity and structure of *A. frigida* Willd. using SSR markers reported that there are significant correlations between genetic structure and environmental conditions.

Grouping analysis

Cluster analysis and score plot based on karyotypic characteristics were able to separate clearly French and Russian tarragon accessions. The genus *Artemisia* is one of the most complex genera from a taxonomic classification viewpoint (Dolatyari

et al. 2013). The taxonomic delimitation between French and Russian tarragons is ambiguous and they are classified as varieties, cultivars, subspecies, or even species (Obolskiy et al. 2011). These can be distinguished from some morphological characteristics such as height at maturity, branching habit, color and consistency of leaves, hairiness of mature leaves, stems and pedicels, length of the pedicel, the tip of the involucral bracts, the diameter of opening flower head and amount of pollen (Rousi 1969), however, there is a great similarity in the morphology of secretary structures of Russian and French tarragons (Obolskiy et al. 2011). Therefore, in addition to morphological, anatomical and phytochemical characteristics (Werker et al. 1994; Fraternale et al. 2015), karyotype analysis is also clearly able to distinguish between French and Russian tarragons.

Conclusion

In conclusion, we presented the first karyomorphology of French and Russian tarragons, in terms of chromosome formula and asymmetry indices, which could be useful in providing insights into the evolution and systematics of *A. dracunculus*. The findings of ploidy levels and karyotypic asymmetry indices indicated that Russian tarragons are more evolved than French tarragons. However, we believe that further research on molecular cytogenetics and phylogenetics analyses are necessary to identify the taxonomical relationships and evolution of *A. dracunculus*.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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