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Chromosome, ploidy analysis, and flow cytometric genome size of caper (*Capparis spinosa*) medicinal plant

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Abstract. Caper (*Capparis spinosa*) is a shrubby, deciduous perennial medicinal plant belonging to the Capparaceae family. Its use is in folk medicine, pharmacy, food, and spices. Chromosome, ploidy analysis, and flow cytometric genome size of 10 populations collected from different parts of Iran were analyzed. The results showed that all populations were diploid, with nine populations having $2n = 2x = 30$ (P1-P8, P10) and one population having $2n = 2x = 34$ (P9) chromosomes. The average chromosome length (CL) for these two chromosome groups was 1.05 and 0.97 μm, respectively. The mean monoploid genome sizes for the populations with 30 and 34 chromosomes were 0.646 and 0.633 pg, respectively. As a whole, the mean genome size of all populations was 0.643 pg. The chromosome number as well as the genome size are being reported for the first time. Cluster analysis and principal component analysis revealed a categorization of the caper population into four distinct groups. The first group comprised three populations (P1, P3, and P4), while the second group included only P2 population, the third group was represented by two populations (P5 and P7), and the fourth group encompassed four populations (P6, P8, P9, and P10). Future research on the genetic traits and breeding methodologies of this species can build upon the foundational findings of this study.

Keywords: caper, *Capparis spinosa*, chromosome, karyology, 2Cx DNA, genome size, flow cytometry.

INTRODUCTION

People of every culture have always experimented with endemic plants over thousands of years and have recognized that almost all of nature is used for food, clothing, shelter, and they have adapted based on the available resources. Plants that have beneficial pharmacological effects on the human body are called medicinal plants and are useful almost exclusively due to their natural ability to synthesize secondary metabolites (Sundarrajan and Bhagtaney, 2023). The presence of various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic compounds in medicinal plants has anti-inflammatory, antimicrobial, and antioxidant

effects, confirming that the use of medicinal plants is a suitable alternative to current conventional methods in the treatment of many problems such as wounds (Cedillo-Cortezano *et al*., 2024). Caper (*Capparis spinosa* L.) is a common member of the *Capparis* genus of the Capparaceae (Capparidaceae) family, which is a thorny perennial shrub and an aromatic plant common in many parts of the world, especially the Mediterranean regions (Shahrajabian *et al*., 2021). This shrubby plant has woody stems and herbaceous branches with thick, shiny, bright green, oval-shaped, and alternate simple leaves. It has single, fragrant flowers with white to pinkish-white petals, and numerous long purple stamens. The fruit shape is oval-shaped and it has a dark green color (Condurso *et al*., 2015; Chedraoui *et al*., 2017) (Figure 1).

Although this plant is native to the Mediterranean, it grows well in Italy, North Africa, Greece, Central Asia, and Iran (Zarei *et al*., 2021). Caper prefers a rainy spring and a hot, dry summer with intense sunlight, with temperatures exceeding 40 °C and an average annual rainfall of 350 mm (Barbera and Di Lorenzo, 1984). This plant grows both wild and cultivated, and prefers rocky soils in semi-arid regions, limestone slopes, and crevices in old walls. Additionally, caper is tolerant to both salt and drought stresses. Due to its ability to grow in harsh environments, this plant is recommended to prevent land degradation, control soil erosion, and maintain and promote agriculture in areas exposed to severe climate change (Sakcali *et al*., 2008). *C. spinosa* is one of the most common aromatic plants in Mediterranean cuisine. The flower buds of this plant are edible. The flowers, which are harvested in the spring before they open, are usually processed in brine, pickled in vinegar, or preserved in grain salt and used as a seasoning in salads, pasta, meat, sauces, and condiments to add a spicy and salty flavor and aroma to food (Cincotta *et al*., 2022). The fruit, leaves, and the younger branches of the caper plant are edible and consumed salted or pickled in vinegar, or as fresh or cooked vegetables (Moghaddasi, 2011). Different parts of this plant, including the roots, bark, leaves, buds, and fruits, have traditionally been employed to alleviate conditions such as joint diseases, hemorrhoids, rheumatism, rheumatoid arthritis, gout, fever, cough, asthma, and inflammation (Chedraoui *et*

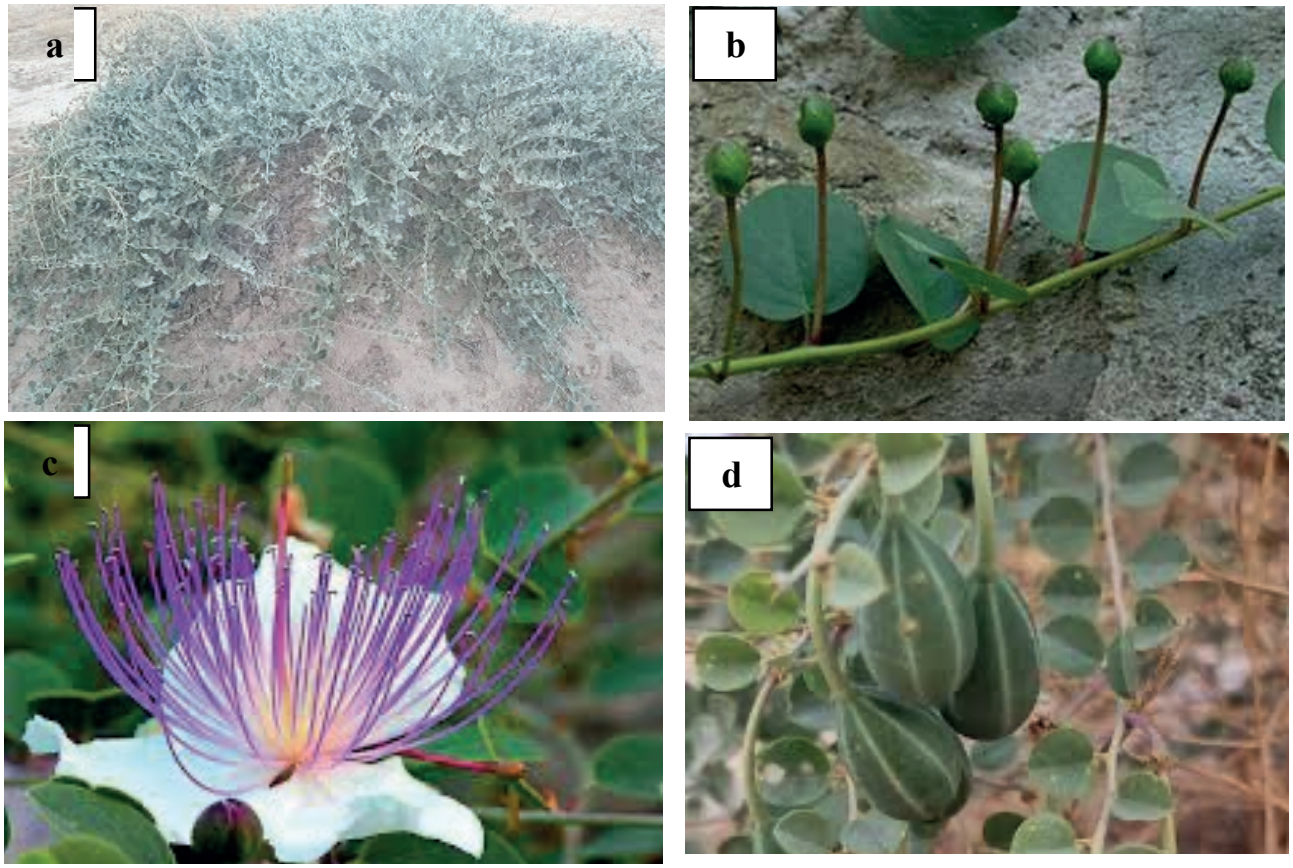

Figure 1. Whole plant (a), bud and leaf (b), flower (c), and fruit (d) of caper (*Capparis spinosa*) medicinal plant.

al., 2017). Furthermore, caper extract exhibits significant properties including antihypertensive, hepatoprotective, antidiabetic, anti-obesity, bronchodilator, antiallergic and antihistamine, antibacterial, antioxidant, and anticancer effects (Nabavi *et al*., 2016; Chedraoui *et al*., 2017; Merlino *et al*., 2024). Caper seeds are rich in antioxidant molecules and a source of omega-6, utilized in both the food and pharmaceutical industries (Ara *et al*., 2013; Tlili *et al*., 2015). On the other hand, *C. spinosa* contains bioactive lipids, glucosinolates (glucocaprin), and flavonoids (rutin); its seed oil is also rich in unsaturated lipids (Argentieri *et al*., 2012; Annaz *et al*., 2022). The most important phytochemical compounds identified, includes quercetin derivatives, kaempferol, isorhamnetin myristicin, eriodictyol, circimaritin, and gallocatechin (Bakr and El Bishbishy 2016). Moreover, it has the alkaloids capariсin A, capariсin B, and capariсin C, which are used in medical treatments (Marir, 2024).

Karyotype, as an important genetic feature, represents the phenotypic appearance of somatic chromosomes, including chromosome number and length (Ning *et al*., 2018; Vimala *et al*., 2021; Abbasi-Karin *et al*., 2022; Rasekh *et al*., 2023; Yari *et al*., 2024; Morovati *et al*., 2024), is used in systematic and evolutionary studies of plants (Peruzzi *et al*., 2017; Wang *et al*., 2020; Yari *et al*., 2024). The simplest technical feature related to the genome of a species is the chromosome number, which is the most fundamental feature. For this reason, since 1882 (Garbari *et al*., 2012), chromosome number data have been collected for many plant organisms worldwide, representing about one-third of the plant currently known in this respect (Stace, 2000). The Capparaceae family consists of about 40-45 genera and 700-900 species, whose members show significant diversity in terms of appearance, fruit, and floral features (Kamel *et al*., 2015). The most important genera of this family include *Capparis*, *Cadaba*, *Boscia*, and *Maerua* (Ali and Amar, 2020). The genus *Capparis* has about 250 species, the chromosome numbers for the few known species of this genus are (2*n* = 18, 30, 38, 40, and 84) (Rock, 2016). *Cadaba indica* and *Cadaba triphylla*, which are both species in the genus *Cadaba*, have chromosome numbers that are both 14 and 34, respectively reported by Subramanian and Pondmudi (1987). The species *Crataeva nurvala* belongs to the genus *Crataeva* has 26 chromosomes (Gupta and Gill, 1981). Another species of this genus, *Crataeva religiosa*, also has 26 chromosomes (Subramanian and Pondmudi, 1987). The species *Maerua arenaria* sensu Baillon (Subramanian and Pondmudi, 1987), (Khatoon and Ali, 1993) *M. arenaria* (DC.) Hook. f. & Thoms. and *M. crassifolia* Forssk. (Khatoon and Ali, 1993), belonging to the genus *Maerua* each have 40, 20, and 20 chromosome numbers, respectively. The species *Niebuhria linearis* DC. of the genus *Niebuhria* has 102 chromosomes (Sharma, 1968). *Capparis*, the largest genus in the Capparaceae family, has 250 species with various chromosome numbers, including *C. brevispina* DC. (2*n* = 36) (Subramanian and Pondmudi, 1987), *C. decidua* Pax (2*n* = 40) (Khatoon and Ali, 1993), (Subramanian and Pondmudi, 1987) *C. divaricata* (2*n* = 160), *C. diversifolia* Wight & Arn. (2*n* = 98) (Subramanian and Pondmudi, 1987), (Subramanian and Pondmudi, 1987) *C. grandis* L. f. (2*n* = 42), *C. leucophylla* DC. (2*n* = 10, 20) (Sandhu, 1989), *C. rotundifolia* (2*n* = 42) (Subramanian and Pondmudi, 1987), *C. sandwichiana* var. Zoharyi, O. Deg. & I. Deg. (2*n* = 40) (Carr, 1978), *C. sepiaria* (2*n* = 40) (Sharma, 1968; Subramanian and Pondmudi, 1987), *C. zeylanica* (2*n* = 40, 44) (Singhal and Gill, 1984; Subramanian and Pondmudi, 1987), *C. spinosa* (2*n* = 24, 38) (Magulaev, 1979; Al-Turki *et al*., 2000), *C. spinosa* subsp. Rupestris (2*n* = 38) (Runemark 1996), and *C. spinosa* var. herbacea (Willd.) (2*n* = 42) (Wang *et al*., 2022). Given the valuable medicinal and nutritional value of caper, this study aimed to investigate intraspecific diversity among Iranian populations in terms of karyotypic characteristics and genome size.

Genome size refers to the amount of genomic DNA present in the gametes of a species, which is generally constant in an organism and is represented as a C-value (Swift, 1950; Greilhuber *et al*., 2005; Pellicer *et al*., 2018; Kocjan *et al*., 2022). The C-value estimation is essential for sequencing and genomic analysis, as well as for plant species identification and classification (Gregory, 2005; Bourge *et al*., 2018; Sliwinska, 2018). Genome sizes vary considerably among the flowering plants overall, as well as within smaller taxonomic groups such as families or even genera. Monoploid genome size is refers to the amount of DNA of one chromosome set, 1 Cx-value, with chromosome base number *x*) and holoploid genome size to the amount of DNA of the whole chromosome complement, 1 C-value, with chromosome number *n*, regardless of the degree of polyploidy, aneuploidies, etc.) as described by Greilhuber *et al*. (2005). Flow cytometry (FCM) has been used to estimate the plant nuclear DNA content since the 1980s. It is commonly used in plant breeding (especially in polyploid and hybrid breeding) (e.g. Doležel and Bartoš, 2005; Doležel *et al*., 2007; Tavan *et al*., 2015; Bourge *et al*., 2018; Javadian *et al*., 2018; Hamidi *et al*., 2018; Tarkesh Esfahani *et al*., 2020) and seed production (Sliwinska, 2018). Recently, studies of karyomorphology and genome size, using flow cytometry technique have been conducted in a diverse array of plant communities, such as: *Thymus* species (Mahdavi and Karimzadeh, 2010; Tavan *et al*., 2015), *Satureja*

(Shariat *et al*., 2013; Zare Teymoori *et al*., 2021), *Tulipa* (Abedi *et al*., 2015, *Papaver bracteatum* (Tarkesh Esfahani *et al*., 2016), *Artemisia khorassanica* (Hamidi *et al*., 2018), *Medicago monantha* (Zarabizadeh *et al*., 2022), *Epilobium* spp. (Abbasi-Karin *et al*., 2022), *Ferula assa-foetida* (Firoozi *et al*., 2022), berry (Mohammadpour *et al*., 2022), *Allium* spp. (Sayadi *et al*., 2022), *Papaver somniferum* (Rasekh and Karimzadeh, 2023), *Cymbopogon olivieri* (Yari *et al*., 2024), *Coriandrum sativum* (Khakshour *et al*., 2024), *Sapindus mukorossi* (Gao *et al*., 2024), *Cyphomandra clade* (Mesquita *et al*., 2024), *Nigella* and *Garidella* species (Aydın *et al*., 2024), and *Datura* spp. (Morovati *et al*., 2024) have been used to identify intra- and inter-specific diversity.

MATERIALS AND METHODS

Plant materials

Seeds from 10 endemic Iranian caper (*Capparis spinosa* L.) populations were collected from various regions of Iran during the growing season within their natural habitats. Population codes, geographical coordinates (latitude, longitude), altitude (m), mean annual temperature (°C), and mean annual rainfall (mm) are presented in Table 1 and illustrated in Figure 2.

Chromosome analysis

Seeds mucilage was first removed by washing with water. The seed coats were then mechanically scarified to break dormancy (Olmez *et al*., 2006; Agah *et al*., 2020; Radmanesh *et al*., 2023). For this purpose, seeds were disinfected by immersing in 5% (v/v) sodium hypochlorite for 5 min, followed by 70% (v/v) ethanol for 1 min (Aguilar-Rito *et al*., 2023; Qi *et al*., 2023). Subsequently, healthy seeds were placed on a layer of Whatman filter paper within 9 cm diameter glass petri dishes (Honarmand *et al*., 2016). To induce germination, seeds were moistened with distilled water at room temperature (RT). Petri dishes were then placed in a growth room under controlled conditions: 16/8 h light/dark at 25 °C (Honarmand *et al*., 2016). Approximately, 0.5-mm length roots were incubated in a 0.002 M 8-hydroxyquinoline solution for 3 h in the dark at RT (Mehravi *et al*., 2022a, b; Anjum *et al*., 2023). Roots were fixed in Carnoy's fixative (glacial acetic acid:ethanol, 1:3 v/v) for a minimum of 24 h at 4 °C (Karimzadeh *et al*., 2011; Firoozi *et al*., 2022; Khakshour *et al*., 2024; Yari *et al*., 2024). Following fixation, Carnoy's fixative was removed by washing with distilled water for 5 min. Subsequently, root meristems were hydrolyzed in 1 M HCl for 10 min at 60 °C. Root samples were stained with 4% (w/v) hematoxylin solution for 3 h at RT in the dark (Mohammadpour *et al*., 2022). Slides were prepared, using the squash method in 45% (v/v) acetic acid. Photomicrographs were taken with a DP12 digital camera (Olympus Optical Co., Tokyo, Japan) mounted on a BX50 Olympus microscope (Olympus Optical Co., Tokyo, Japan). In cytological studies of plants with small chromosomes (almost one micrometer), accurately measuring the lengths of the long and short arms are often challenging due to the difficulty in identifying the centromere (Morales Valverde, 1986; Mahdavi and Karimzadeh, 2010; Abbasi-Karin *et al*., 2022; Rasekh and Karimzadeh, 2023, Yari *et al*., 2024; Morovati *et al*., 2024). Hence, in the current study, the chromosome length (CL) was measured, using MicroMeasure software version 3.3.

Table 1. Geographic distribution and climatic data of endemic Iranian caper (*Capparis spinosa*) populations.

Populations codes	Local collection locations	Latitude (N)	Longitude (E)	Altitude (m)	Mean Temp. (˚C)	Mean rainfall (mm)
P1	Dargaz, Khorasan-e Razavi	37°26'33.25"	59° 6'26.06"	408	12.75	266.70
P2	Balanej, Azarbayjan-e Gharbi	37°24'6.72"	45° 9'54.19"	1303	14.20	341.00
P3	Tehran, Tehran	35°44'38.00"	51° 9'54.71"	1286	18.68	250.98
P4	Torbat-e-Jam, Khorasan-e Razavi	35°15'8.38"	60°35'42.42"	909	26.00	254.00
P5	Ahvaz, Khozestan	31°28'21.02"	48°43'21.48"	17	26.65	191.20
P6	Kharg Island, Bushehr▪	29°14'16.05"	50°18'58.48"	1	26.18	265.60
P7	Borazjan, Bushehr	29°13'12.64"	51°14'34.86"	104	27.63	283.10
P8	Firuzabad, Fars	28°50'23.45"	52°35'30.92"	1333	20.87	379.70
P9	Fathabad-e Deh-e Arab, Fars	28°40'42.01"	52°41'18.26"	1148	21.20	377.00
P10	Dasht-e Lar, Fars	28°22'11.55"	52°46'38.95"	924	25.70	302.20

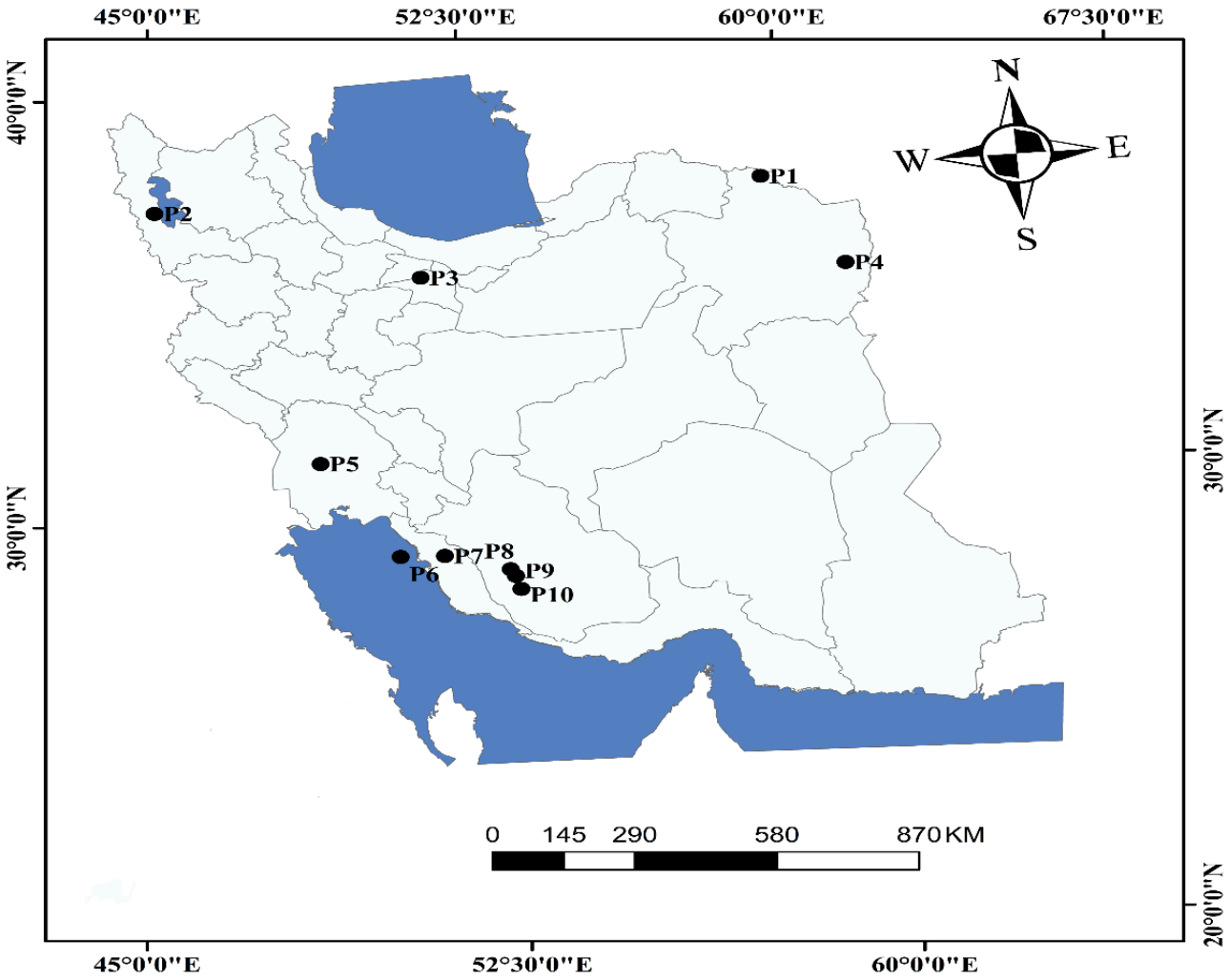

Figure 2. Map of Iran showing the collection locations of endemic Iranian medicinal plant caper (*Capparis spinosa*) populations, using ArcGIS.

Flow cytometric genome size estimation

To estimate genome size, seeds were first germinated in petri dishes. After two weeks, the seedlings were transferred to pots containing a mixture of soil, sand, perlite, and cocopeat in the research greenhouse of the College of Agriculture at Tarbiat Modares University, where suitable growth conditions were maintained. During growth, NPK fertilizer was applied after five months when the grown plants having developed leaves suitable for flow cytometric analysis. Then, 1 cm^2 of young developed leaves from *C. spinosa* and radish (*Raphanus sativus* cv. Saxa; 2C DNA = 1.11 pg) as an internal reference standard (Doležel *et al*., 1992) were chopped simultaneously with a sharp blade in a glass petri dish containing 1 ml of Woody Plant Buffer (WPB; Loureiro *et al*., 2007). No peaks were identified in the analysis. Hence, instead General Plant Buffer (GPB; 0.5 mM Spermine·4HCl, 30 mM Sodium citrate·$3H_2O$, 20 mM MOPS, 20 mM NaCl, 80 mM KCl, 1% PVP-10, and 0.5% v/v Triton X-100, pH 7.0) (Loureiro *et al*., 2007) was used. The resulting nuclear suspension was passed through a 30 µm green nylon filter (Partec, Munster, Germany) to remove large tissue fragments and debris. This was followed by the addition of 50 µg ml^{-1} of RNase (for RNA removal) (Sigma-Aldrich Corporation, MO, USA) and 50 µg ml^{-1} of propidium iodide (PI, Fluka) fluorescent dye (for nuclear DNA staining) to the samples (Loureiro *et al*., 2007). The nuclear suspension was then analyzed using a BD FACSCanto™ flow cytometer (Biosciences, Bedford, MA, USA) with BD FACSDiva™ software. The output data were transferred to FloMax ver. 2.4.1 software for gat-

ing the output histograms. Relative fluorescence intensity measurements of stained nuclei were performed on a linear scale, with at least 5,000 nuclei analyzed for each sample. The absolute DNA content of a sample was calculated based on the average G1 peak values. The following formula was used to determine the genomic DNA content (in pg) of an unreplicated gamete (2Cx DNA) based on the mean G1 peak values in caper (Doležel *et al*., 2007; Loureiro *et al*., 2007; Firoozi *et al*., 2022; Sayadi *et al*., 2022; Mehravi *et al*., 2022a; Mohammadpour *et al*., 2022).

Sample 2Cx DNA (pg) = (Sample G1 peak mean/Standard G1 peak mean) × Standard 2C DNA (pg)

Moreover, the size of the monoploid genome (2Cx DNA) in base pair terms is based on the converting formula proposed by Doležel *et al*. (2003), where 1 pg of DNA is equivalent to 978 Mbp.

Statistical analyses

The normality test was first applied to the residuals data of chromosome length (CL) and genome size data, the data were then analyzed according to a completely randomized design (CRD) with five and three replications, respectively, using Minitab 17 software (Cardoso *et al*., 2023). Chromosome length data were not normalized; instead, they were transformed in the reverse way (Osborne, 2010), resulting in normalized data. Analysis of variance (ANOVA) and subsequent comparison of means, using the least significant difference (LSD) method (Hinkelmann, 2012) were performed with the general linear model (GLM) procedure in SAS 9.1 software (SAS Institute Inc., 2009). Furthermore, multivariate statistical analysis (MANOVA) of mean CL, genome size, and geographical parameters (Karimzadeh *et al*., 2011) was conducted in Minitab 17 software (Yeshitila *et al*., 2023).

RESULTS

Karyotype analysis

The karyotypic study results indicated that all 10 populations of the caper (*Capparis spinosa*) medicinal plant were diploid (2*x*) in terms of ploidy level. The results of ANOVA indicate a significant difference ($P < 0.01$) in chromosome length (CL) among the studied populations, reflecting intraspecific diversity (Table 2). Interestingly, within such a ploidy level, two chromosome numbers were identified. Hence, nine populations had $2n = 2x = 30$ chromosomes, while one population (P9) had $2n = 2x = 34$ chromosomes (Fig. 3, Table 3). This study is being reported for the first time in Iran. The mean chromosome length (CL) in the nine populations (P1-P8, P10) with 30 chromosomes was 1.05 µm, ranging from 0.97 µm (P9) to 1.12 µm (P2). The P9 population, which had 34 chromosomes, also measured 0.97 µm, while the mean CL for all 10 populations was 1.05 µm.

Table 2. Analysis of variance for chromosome length and monoploid genome size in populations of caper (*Capparis spinosa*).

SOV	df	MS CL	df	MS 2Cx DNA
Population	9	0.2819**	9	0.0015^{ns}
Error	750	0.562	20	0.0009
CV%	–	23.4	–	4.71

ns, ** Non-significant at $P < 0.05$, significant difference at $P < 0.01$.

Means with the same symbol letters are not significantly different at either ($P < 0.01$) for CL column or ($P < 0.05$) for 2Cx DNA column, using LSD

Nuclear genome size estimation

The analysis of variance of monoploid genome size (2Cx DNA) revealed no significant differences ($P < 0.05$) among the studied populations (Table 2). The flow cytometric nuclear monoploid DNA amount (2Cx DNA) of the studied populations is shown in Figure 4. The mean genome size in the nine populations (P1-P8, P10) with 30 chromosomes was 0.646 pg, ranging from 0.608 pg (P10) to 0.677 pg (P2; Table 3). It was 0.633 pg in the P9 population with 34 chromosomes. Overall, the mean 2Cx DNA for all 10 populations was 0.643 pg or 628.85 Mbp (Table 3). However, to explore potential differences, mean comparisons were performed, using the LSD method at $P < 0.05$, indicating a significant difference between populations P2, P6, and P10 (Table 3). Additionally, histogram analysis complemented the karyotypic examination, confirming the diploid nature of the studied populations.

Multivariate statistical analysis

The correlation between mean monoploid genome size (2Cx DNA) and either chromosome length (CL), or geographical parameters (latitude, longitude, altitude, mean temperature, and mean rainfall) in the populations of the medicinal plant caper is presented in Table 4. The correlation coefficient between 2Cx

Table 3. Means comparison (± SE) chromosome length (CL) and monoploid genome size (2Cx DNA; pg) of populations of the Iranian endemic caper (*Capparis spinosa*).

Pop.	Local collection locations	2*n*	CL (μm ± Se)	Monoploid 2Cx DNA (pg)	Monoploid 2Cx DNA) (Mbp)
P1	Dargaz	30	1.106 ± 0.033^{ab}	0.659 ± 0.005^{abc}	644.50
P2	Balanej	30	1.122 ± 0.029^{a}	0.677 ± 0.002^{a}	662.10
P3	Tehran	30	1.073 ± 0.027^{abc}	0.639 ± 0.028^{abc}	624.94
P4	Torbat-e-Jam	30	1.117 ± 0.032^{a}	0.652 ± 0.018^{abc}	637.66
P5	Ahvaz	30	0.988 ± 0.030^{c}	0.660 ± 0.008^{abc}	645.48
P6	Kharg	30	1.009 ± 0.030^{bc}	0.620 ± 0.022^{bc}	606.36
P7	Borazjan	30	1.067 ± 0.028^{abc}	0.668 ± 0.015^{ab}	653.30
P8	Firuzabad	30	1.037 ± 0.029^{abc}	0.627 ± 0.009^{abc}	613.20
P9	Fathabad-e Deh-e Arab	34	0.974 ± 0.025^{c}	0.633 ± 0.029^{abc}	619.08
P10	Dasht-e Lar	30	0.975 ± 0.030^{c}	0.608 ± 0.015^{c}	594.62
	Total		1.047	0.643	628.86
Means	P1-P8, P10	–	1.055	0.646	631.78
	P9		0.974	0.633	619.08
LSD	–	–	$LSD_{1\%} = 0.108$	$LSD_{5\%} = 0.052$	–

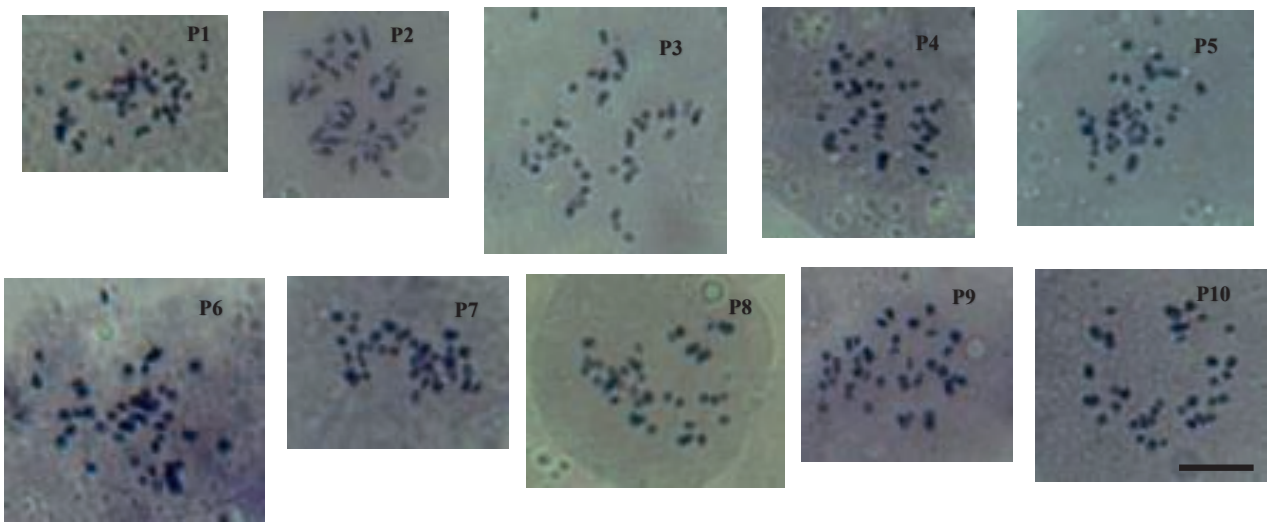

Figure 3. Somatic chromosomes of 10 populations of caper (*Capparis spinosa*). Scale bar = 5 μm.

DNA and CL was positive and significant ($P < 0.05$; $r = 0.66^{*}$). However, no significant differences were found for the geographical parameters. Given the significant correlation between monoploid genome size and CL, linear regression analysis (b = 0.25*) was conducted (Figure 5). Additionally, cluster analysis was performed to identify distinct groups of individuals based on their genetic similarity. This analysis was based on mean original data for 2Cx DNA, chromosome length (CL), and the geographical parameters. To determine the distance between populations, Euclidean distance was calculated, and cluster merging was performed, using the unweighted pair group method with arithmetic mean (UPGMA) method. Moreover, to evaluate the efficiency of the classification method, the cophenetic coefficient was calculated, using NTSYS 2.02e software. Various classification methods were tested with this software. Ultimately, the method that determined the Euclidean distance and merged the average cluster, which had a higher cophenetic coefficient value (r = 0.92), was selected to present the results (Table 5). It should be noted that the higher the cophenetic coefficient for a method, the better it is for cluster analysis (Batagelj, 1988; Gong *et al*., 1995). Subsequently, cluster

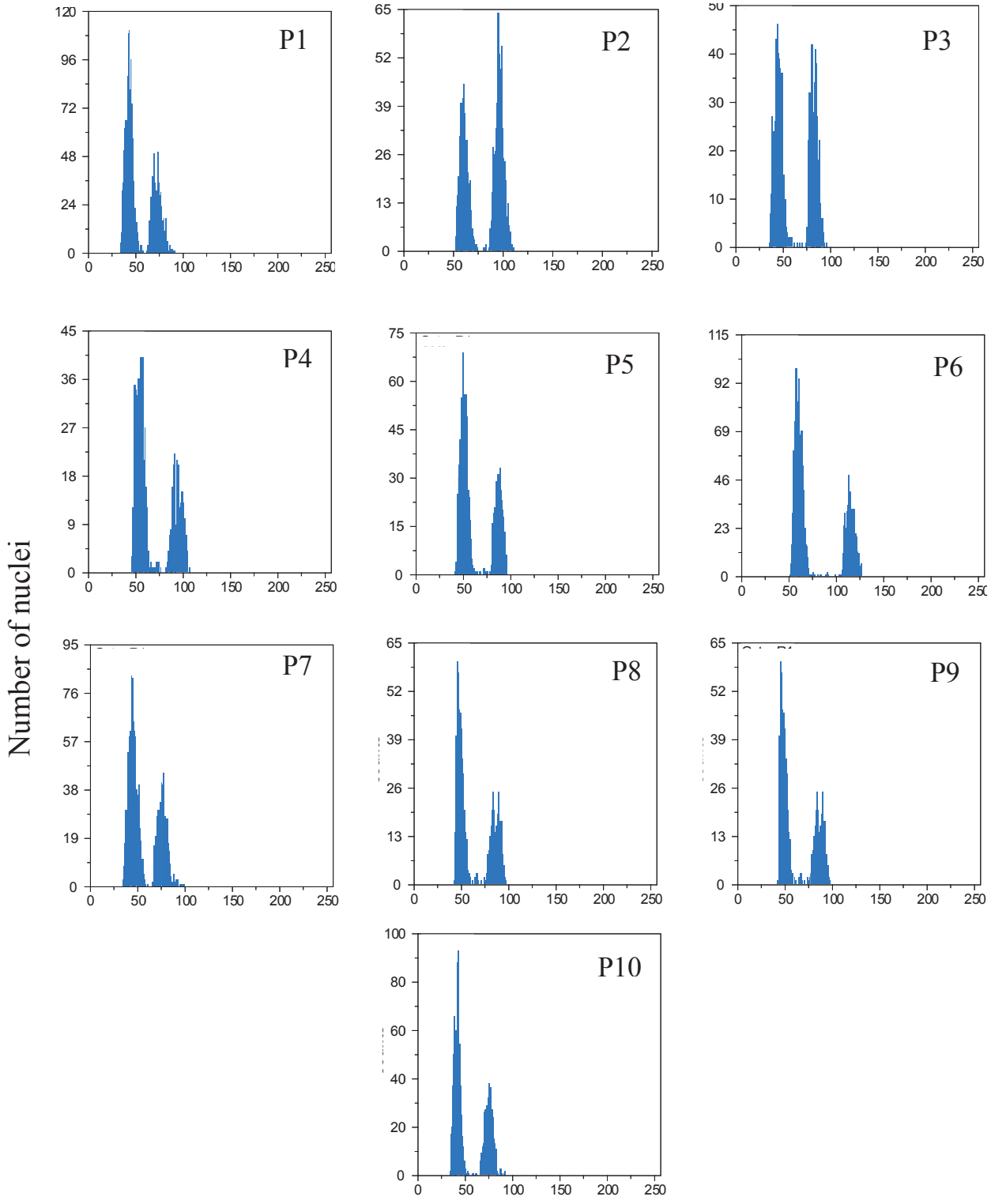

Figure 4. Flow cytometric histograms illustrating the genome size of the medicinal plant caper (*Capparis spinosa*). The left peaks represent the G1 phase of the caper plant, while the right peaks correspond to the G1 phase of the internal standard, radish (*Raphanus sativus* cv. Saxa; 2C DNA = 1.11 pg).

Table 4. Correlation coefficients between monoploid genome size (2Cx DNA; pg) wither either chromosome length (CL; µm) or geographical parameters in populations of caper (*Capparis spinosa*) medicinal plant.

Trait	CL (µm)	Latitude (N)	Longitude (E)	Altitude (m)	Mean Temp. (˚C)	Mean rainfall (mm)
2Cx DNA (pg)	0.66*	0.62 ns	-0.13 ns	-0.16ns	-0.32 ns	-0.23 ns

ns, * Non-significant and significant at $P < 0.05$.

Table 5. Cophenetic coefficient of different clustering methods for populations of the caper (*Capparis spinosa*) related to chromosome length parameter, genome size, and geographical parameters).

Linkage Method	Euclidean Distance			Squared Euclidean Distance		
	UPGMA	Single	Complete	UPGMA	Single	Complete
Cophenetic Coefficient	0.92	0.90	0.91	0.83	0.83	0.82

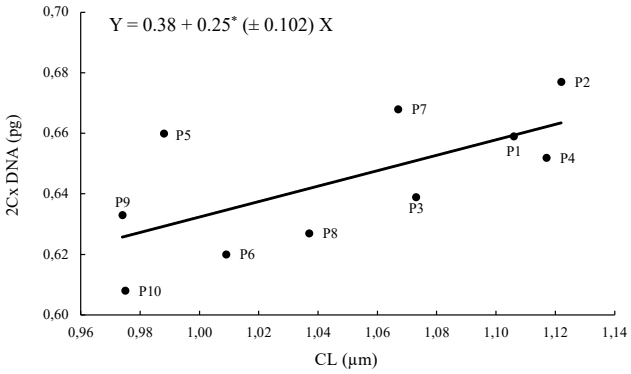

Figure 5. Linear relationship between monoploid genome size (2Cx DNA) and chromosome length (CL) in caper (*Capparis spinosa*) medicinal plant.

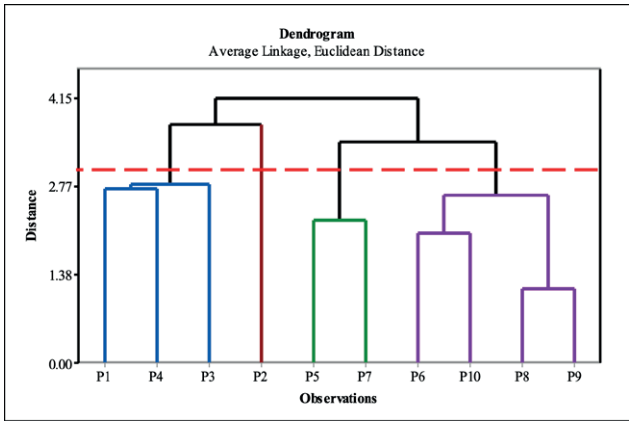

Figure 6. Dendrogram related to chromosome length parameter, genome size, and geographical conditions of the medicinal plant caper (*Capparis spinosa*), using Euclidean distance and unweighted pair group method with arithmetic mean (UPGMA; r = 0.92).

analysis was performed, using Minitab 17.0 software by standardizing the parameters. The results of the cluster analysis are presented in the form of a dendrogram (Figure 6). According to which, the populations of caper medicinal plant were divided into four groups. The first group included three populations of P1, P3, and P4, the second group included P2 population, the third group comprised two populations of P5 and P7, and the fourth group included four populations of P6, P8, P9, and P10 (Figure 7). Furthermore, to determine the total variation in populations and the contribution of parameters to this variation, principal component analysis (PCA) was performed on the 2Cx DNA, CL, and geographic parameters. The analysis revealed that the first four principal components accounted for 93% of the cumulative variation. The first two coordinates were displayed in a 2-dimensional graphic based on the desired parameters in four categories (Figure 6). The results showed that CL (0.53), 2Cx DNA (0.41), latitude (0.55), and mean annual temperature (-0.45) had a stronger correlation with the first coordinate, which accounted for 42% of the variation in the calculated data. In the second component, altitude (0.61), average annual precipitation (0.65), and average annual temperature (-0.31) played a vital role in explaining 20% of the total variation. In the third component, 2Cx DNA (0.38) and longitude (-0.91) had the highest contributions. In the fourth component, 2Cx DNA (-0.51), average annual rainfall (0.53), and average annual temperature (-0.40) played the most significant roles. Together, these two components accounted for 23% of the total variance (Table 6).

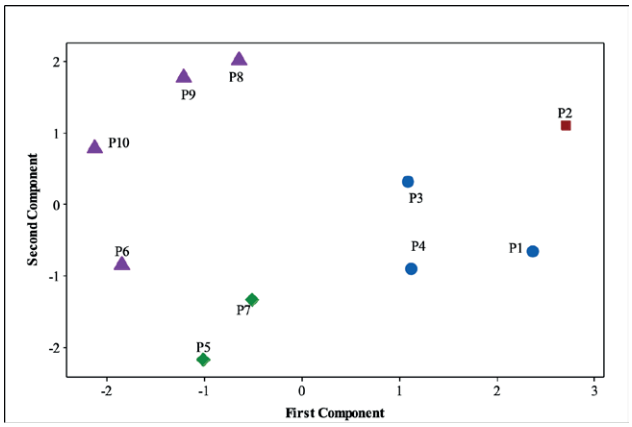

Figure 7. Population classification based on the first and second components in principal component analysis on the chromosome length parameter, genome size, and geographical conditions of populations of the medicinal plant caper (*Capparis spinosa*).

Table 6. Eigenvalues, relative and cumulative variances, and eigenvectors for the four principal components resulting from principal component analysis on the chromosome length, genome size, and geographical conditions of populations of caper (*Capparis spinosa*) medicinal plant.

Characteristics	Components			
	First	Second	Third	Fourth
Eigenvalue	2.93	1.94	1.10	0.49
Relative variance	0.42	0.28	0.16	0.07
Cumulative variance	0.42	0.70	0.86	0.93
CL (µm)	0.53	-0.08	-0.11	-0.33
2Cx DNA (pg)	0.41	-0.28	0.38	-0.51
Latitude (N)	0.55	-0.11	-0.05	0.36
Longitude (E)	0.09	-0.10	-0.91	-0.21
Altitude (m)	0.18	0.61	-0.09	0.11
Mean temp. (°C)	-0.45	-0.31	-0.06	-0.40
Mean rainfall (mm)	-0.03	0.65	0.06	-0.53

DISCUSSION

The results of this study provide, for the first time, accurate snapshots of the chromosome number of endemic Iranian populations of the caper (*Capparis spinosa*) medicinal plant. Little information is available about the chromosome number of this plant. In the studied populations, information on chromosome and monoploid genome size (2Cx DNA) was completely inadequate. As a result, this study provides basic cytogenetic, genetic, and genomic information for these populations, which is useful for constructing genetic and physical maps and for whole genome sequencing in the future. The findings of the present study showed a diploid ($2x$) ploidy level, as well as two different chromosome numbers of 30 and 34. In previous studies, the chromosome numbers reported as 24 (Magulaev, 1979), 38 (Al-Turki *et al*., 2000), and 42 (Wang *et al*., 2022). Variation in somatic chromosome number has been reported in the root tips of many flowering plant species (angiosperms) (e.g. Kula, 1999; Winterfeld *et al*., 2015, 2020; Mehravi *et al*., 2022a). In the present report, the average chromosome length was determined to be 1.055 µm in populations (P1-P8, P10) with 30 chromosomes and 0.974 µm in a P9 population with 34 chromosomes.

Flow cytometry has been successfully employed to estimate nuclear genomic DNA content (Doležel and Bartoš, 2005; Doležel *et al*., 2007; Bourge *et al*., 2018) and to accurately determine ploidy levels in a diverse array of plant species (Mahdavi and Karimzadeh, 2010; Tavan *et al*., 2015; Abedi *et al*., 2015; Tarkesh Esfahani *et al*., 2016, 2020; Javadian *et al*., 2017; Hamidi *et al*., 2018; Mehravi *et al*., 2022a, b; Firoozi *et al*., 2022; Mohammadpour *et al*., 2022; Zarabizadeh *et al*., 2022; Rasekh and Karimzadeh, 2023; Khakshour *et al*., 2024; Morovati *et al*., 2024; Yari *et al*., 2024). In the current report, the genome size of caper (*Capparis spinosa*) populations with 30 chromosomes (P1-P8, P10) was determined to be 0.646 pg (631.78 Mbp) and that of P9 population with 34 chromosomes was 0.633 pg (619.08 Mbp). Following a previous study on *Thymus* species (Lamiaceae) reported by Mahdavi and Karimzadeh (2010), in the present study, we calculated the average genome size per chromosome (pg/chr) by dividing the genome size by the chromosome number. Therefore, the average genome size per chromosome (pg/chr) for populations P1-P8 and P10 (30 chromosomes) and P9 (34 chromosomes) was 0.021 pg/chr and 0.019 pg/chr, respectively, or 20.54 and 18.58 Mbp/chr, respectively. Hence, such a slight smaller genome size of a 34-chr P9 population can be verified by its slight smaller chromosomes (0.974 µm; Table 3). In other words, such a reduction in DNA content in the 34-chr P9 population is fully consistent with the reduction in chromosome length compared to the 30-chr populations (P1-P8, P10). Our results can be compared to a study on caper (*Capparis spinosa* var. herbacea) in China, which reported a chromosome number of 42 ($2n = 2x$), a genome size of 549.06 Mbp, and an average of 13.07 Mbp/chr (Wang *et al*., 2022). Thus, the genome size of the caper population studied in China, with 42 chromosomes, was approximately 57% and 42% smaller than that of 30- and 34-chr caper populations studied in the current report, respectively. Consequently, it can be concluded that the chromosomes of the Iranian endemic caper populations exhibit approximately twice

the genome size compared to the caper plant reported in China. This suggests that the average chromosome length of the endemic Iranian caper plant is likely to be longer than that of the Chinese plants.

Cytogenetic studies can be used to better understand the relationships between different species and populations of a species, and to guide the evolutionary trends of plants (Stebbins, 1971). The first step towards understanding the genetic characteristics of a plant is to determine the status of its chromosomes. Chromosomal information allows for the comparison of species and their populations (Singh 2016). Populations of each species exhibit their own genomic adaptations to the environment in which they grow. As adaptive differences increase, new varieties and even new species may emerge in plant habitats (Weigel and Nordborg, 2015). Therefore, chromosomes are suitable factors on which to determine the evolutionary process of plants (Levin, 2002). Chromosomal differences are different from morphological, physiological, and ecological differences (Caceres *et al*., 1998). Because these differences reflect differences in the products of gene action that change due to environmental factors, while chromosomal differences are more or less due to the genetic content of individuals (Beckmann *et al*., 2007). Differences in chromosome size can indicate differences in the gene products or proteins that an individual produces, or they can indicate duplication of genes that can affect the rate of synthesis of various proteins (Kondrashov *et al*., 2002). Differences in karyotype morphology indicate differences in gene arrangement, which can significantly affect how genes segregate and recombine during Mendelian inheritance. Finally, differences in chromosome number can indicate differences in gene arrangement, gene duplication, or both (Stebbins 1950, 1971; Goldblatt *et al*., 1979; Levin, 2002; Patwardhan *et al*., 2022). The present study revealed significant variation in chromosome length (CL), providing evidence for intraspecific chromosomal diversity (Table 2). Chromosomal number variation among populations indicates that chromosomal structural changes may provide a valuable tool for differentiating closely related populations that exhibit minimal morphological divergence (Mayrose *et al*., 2021). Chromosomal differences, such as variations in chromosome number and length, can be utilized in breeding programs to generate hybrid populations. Parental combinations exhibiting differences in chromosome number/length, particularly those affecting chromosome pairing, can facilitate successful hybridization (Hamidi *et al*., 2018; Akbarzadeh *et al*., 2021). On the other hand, in the present report, the P2 population exhibited the highest average genome size (0.677 pg) and the longest average chromosome length (1.122 μm) among the studied Iranian caper populations. It is noteworthy that this population was placed in a separate group in the principal component analysis and cluster analysis compared to the other populations, as illustrated in Figs. 1 and 2. An increase in nuclear DNA content is typically associated with an increase in total chromosome volume and subsequently cell size, which can lead to larger seed size (Karimzadeh *et al*., 2011). Considering that the fruits of this medicinal caper plant are edible and its seeds contain valuable oil for medicinal and industrial uses (Matthäus and Özcan, 2005; Ara *et al*., 2013). Thus, the P2 population can potentially be a valuable resource for polyploidy induction or hybridization programs aimed at producing larger seeds with enhanced oil content. Furthermore, genome size can serve as an effective marker for identifying hybrids (Ellul *et al*., 2002).

In the present report, the positive and significant correlation between chromosome length (CL) and 2Cx DNA content suggests a strong association between changes in nuclear DNA content and structural alterations in chromosomes. This finding is consistent with previous reports of such correlations in *Vicia* (Naranjo *et al*., 1998), *Tulipa* (Abedi *et al*., 2015), *Hypericum* (Mehravi *et al*., 2022a), and *Pimpinella* (Mehravi *et al*., 2022b). Cluster analysis based on cytological data and geographical conditions revealed four distinct clusters of populations. These results suggest that populations within a cluster exhibit the lowest metric distances and the highest degree of homology in terms of chromosome length, genome size, and geographical parameters. This information can be valuable for selecting parental lines in breeding programs aimed at maximizing genetic diversity. To assess the overall variation within the population and the relative contribution of different karyotypic parameters, principal component analysis (PCA) was also performed. The first two principal components explained 70% of the cumulative variation, and were subsequently visualized in a two-dimensional plot (Fig. 6). Furthermore, PCA analysis applied to different caper populations demonstrated the strong discriminatory power of karyological parameters, genome size, and geographical conditions in distinguishing between these populations. As previously reported, PCA is a valuable tool for establishing karyological relationships (Peruzzi and Altinordu, 2014). While karyological data provide valuable insights into evolutionary relationships, they are not sufficient on their own to establish robust phylogenetic relationships between species. It is crucial to integrate karyological data with independent sources of systematic information, such as morphological, molecular, and ecological data (Siljak-Yakovlev and Peruzzi, 2012;

Peruzzi and Eroğlu, 2013; Harpke *et al*., 2015). Consequently, geographical data were incorporated into the cluster analysis and PCA to enhance the phylogenetic inference.

CONCLUSION

This study examined the genetic diversity of the medicinal plant *Capparis spinosa* across ten distinct populations in Iran, utilizing chromosome analysis, ploidy level assessment, and genome size determination via flow cytometry. The findings revealed that all populations were diploid, with two distinct chromosome counts observed: $2n = 30$ and $2n = 34$. This represents the first documented instances of these chromosome numbers in *C. spinosa*. Genome size assessment indicated that while there were no significant differences in genome size among the populations, notable variations in chromosome length were detected. Clustering and principal component analysis demonstrated significant genetic diversity across the populations, categorizing them into four separate groups. These results contribute essential baseline data regarding the karyotype and genome size of *C. spinosa* in Iran. Such information may be instrumental for future endeavors in genetic mapping, genome sequencing, and breeding programs for this valuable medicinal species. The observed genetic diversity among populations underscores the potential for developing new cultivars with desirable traits.

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AUTHOR CONTRIBTIONS

PR, and GK conceived and designed this study. PR conducted the experiments. PR and GK analyzed the data. PR wrote the manuscript. GK revised the manuscript.

DATA AVILABITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s

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