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Original Article

Cytogenetic Diversity of *Ferula assa-foetida* L. populations from Fars Province

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ABSTRACT

Ferula assa-foetida is one of the most important medicinal, rangeland and industrial plants that, in addition to its role in soil protection, produces oleo-gum with high medicinal and industrial value. The present study aimed to evaluate karyotypic variation and cytogenetic diversity among natural populations of *F. assa-foetida*. For karyological analysis, eight populations of *F. assa-foetida* were collected from Fars Province, Iran. The results showed that all populations were diploid (2n = 2x = 22), with a basic chromosomes number of x = 11. Despite the uniform chromosome number, significant differences were observed among populations in terms of karyotypic and chromosomal characteristics.

Regarding chromosome length (CL), long arm length (L) and short arm length (S) populations F5 exhibited the largest chromosomes with the highest mean values, whereas population F2 showed the smallest chromosomes with the lowest mean values. Based on intrachromosomal asymmetry parameters, F5 displayed the most asymmetric karyotype with the highest mean A_1 and the lowest TF% values, while F7 showed the most symmetric karyotype with the lowest mean A_1 and the highest TF% values. In terms of interchromosomal asymmetry indices, the highest mean values of A_2 and DRL were observed in F7, whereas the lowest values were recorded in F2. Most karyotypes were relatively symmetrical and classified within the 1A and 2A categories of Stebbins' classification.

Principal component analysis revealed that the first two components explained more than 96.3% of the total variation among populations. The first component was mainly associated with intrachromosomal symmetry parameters, whereas the second component was related to chromosome size and interchromosomal symmetry. Cluster analysis using the UPGMA method grouped the studied populations into two distinct clusters, indicating the presence of cytogenetic differentiation among populations. These results highlight that structural chromosomal variation, rather than changes in chromosome number, plays an important role in population differentiation of *F. assa-foetida*.

Keywords: Chromosome, *Ferula assa-foetida,* Karyotype, Medicinal plants

INTRODUCTION

Iranian flora consists of approximately 30 species of *Ferula*, most of which are endemic. *Ferula assa-foetida* is one of the most important medicinal, rangeland, and industrial plants of the Apiaceae family, and is native to Iran and Central Asia [1, 2]. The importance of this plant is mainly due to oleo-gum that is extracted from the roots of this plant. This oleo-gum contains sulfur compounds and monoterpenes.

The pharmacological effects of *F. assa-foetida* include antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelminitic, aphrodisiac and antiseptic properties [3–7]. It is also used against insects [5, 6].

In plant systematics and plant breeding, karyotypes provide valuable evidence and data for species identification and the study of populations resulting from crosses between individuals [8]. *F. assa-foetida* is diploid with a chromosome number 22, grouping in 1A and 2A class according to Stebbins classification [9, 10]. Similarly, the same chromosome number of 22 was observed by El-Alaoui-Faris *et al.* [11] and Qi-xin *et al*. [12] in different species of *Ferula*.

In plant taxonomy, genetic studies and breeding information about chromosome karyotypes can be useful in species identification and analysis of hybrid populations. The first step in plant breeding is understanding the structure of the germplasm collection [13, 14]. Considering this issue, basic research such as cytogenetics is necessary. Accordingly, this paper presents karyotypic and cytogenetic studies conducted on eight populations collected from Fars province.

MATERIALS AND METHODS

Seeds of eight different population of *Ferula assa-foetida* L. were collected from Fars Province (Table 1). Chromosomal karyotypes were analyzed via the squash technique applied to cells in metaphase. The initial step involved germinating the seeds in petri dishes at

4°C, following a 24-hour leaching period. Subsequently, root tips were subjected to a 4-hour pretreatment with α-bromonaphthalene (1% v/v) at room temperature. Fixation was then carried out using Carnoy's solution (a 1:3 mixture of glacial acetic acid and absolute ethanol), after which the samples were stored in 70% ethanol. The fixed materials were hydrolyzed in 1 N HCl at 60°C for 6 minutes and subsequently stained with Hematoxylin for 4 hours. Finally, the stained root tips were squashed in 45% acetic acid, and optimal metaphase plates were photographed under an Olympus CH30 microscope.

The karyotypes were organized by arranging chromosomes in descending order of length, with homologous pairs classified following Levan's classification [15]. Karyotypic asymmetry was evaluated based on Stebbins' categories [16]. Idiograms were prepared for all populations, and the karyotype parameters including short arm (S) and long arm (L) lengths, were measured, and chromosome length was calculated as CL=L + S. Additional parameters included r-value (S/L), arm ratio (AR=L/S), relative length of short arm (S%=S/ΣCL×100), relative length of long arm (L%=L/ΣCL×100), and centromeric index (CI = S/CL).

The following parameters were also assessed for karyotype asymmetry analysis: the total form percentage (TF%=ΣS/ΣCL×100) [17], the difference of range relative length ($DRL\%=RL\%_{Max}-RL\%_{Min}$), intrachromosomal ($A_1$) and interchromosomal ($A_2$) asymmetry indices [18], dispersion index ($DI=A_2\times(\text{Mean S}/\text{Mean CL}\times100)$) [19], symmetry index ($SI=CL_{min}/CL_{max}\times100$).

Table 1 Geographical characteristics of the areas where *F. assa-foetida* seeds are collected

Code	Location	Longitude (E)	Latitude (N)	Altitude (m)
F1	Larestan	54º 26' 41.69"	27º 45' 43.11"	2187
F2	Neyriz, Deraz Boshneh	54º 44' 44.61"	29º 31' 19.57"	1815
F3	Neyriz, Moshkan, Taft	54º 33' 41.51"	29º 08' 47.30"	2098
F4	Moshkan, Neyriz	54º 28' 32.52"	29º 26' 06.70"	2268
F5	Qotrouyeh, Hasanabad	54º 24' 35.17"	29º 28' 09.24"	1605
F6	Kohmareh Sorkhi	52º 09' 17.05"	29º 20' 55.13"	1500
F7	Neyriz	54º 18' 57.44"	29º 10' 32.08"	2187
F8	Dasht Qotrouyeh	54º 38' 11.15"	29º 10' 25.10"	2058

First, the normality of the karyotypic data was verified. Subsequently, the karyological traits were evaluated in a completely randomized design (CRD) with three replicates. Mean comparisons were performed using Duncan's multiple range test. Multivariate statistical analyses, including principal component analysis (PCA) and cluster analysis, were conducted using SAS 9 and R software. PCA was applied to the data matrix to determine the contribution of each karyotypic parameter in distinguishing the populations. For cluster analysis, chromosomal parameters were analyzed via the UPGMA method with Euclidean distance, thereby elucidating the patterns of similarity and variation among the studied populations.

RESULT

A karyological analysis of eight populations revealed a diploid chromosome number of 2n=2x=22 (Fig 1). Based on the ANOVA results, significant differences were observed among populations for all chromosomal parameters ($P<0.01$ and $P<0.05$; Table 2). This indicates the existence of considerable diversity in chromosomal characteristics among the studied populations. Environmental and climatic conditions prevailing in the regions where these populations grow may have played an important role in generating this diversity.

Karyotypes of all populations were classified into the 1A and 2A classes of Stebbins' classification. Based on the results of mean comparisons (Table 3), in terms of CL, L, and S traits among Fars Province populations, population F5 exhibited the largest chromosomes with the highest mean values, whereas population F2 showed the smallest chromosomes with the lowest mean values.

The highest AR value was observed in population F5 (1.646), whereas the lowest value was recorded in population F7 (1.356), and populations F6 and F7 belonged to the same statistical group with respect to the lowest AR values. Populations F7 and F6 were also placed in the same statistical group in terms of the lowest L% values and the highest S% and r-value parameters, whereas population F5 exhibited the highest L% value and the lowest S% and r-value parameters. In terms of CI, population F7 showed the highest value (0.43), while population F5 showed the lowest value (0.39).

Regarding the two parameters DRL and A_2, as interchromosomal symmetry parameters, among the Fars Province populations, population F7 showed the lowest interchromosomal symmetry, with the highest mean values of the above traits (5.033 and 0.164), whereas population F2 exhibited the highest interchromosomal symmetry, with the lowest mean values of the aforementioned traits (3.293 and 0.103).

Among the Fars Province populations, TF% and A_1, as intrachromosomal symmetry parameters, population F5 exhibited the most asymmetrical and simultaneously the most evolved karyotype, with the lowest mean TF% (39.227) and the highest mean A_1 (0.348). Similarly, population F7 showed the most symmetrical and at the same time the most primitive karyotype, with the highest mean TF% (43.142) and the lowest mean A_1 (0.235).

Based on the results, SI and DI, as two other parameters of interchromosomal symmetry, population F7, with the highest mean DI (7.174) and the lowest mean SI (59.95), showed the lowest degree of symmetry and a more specialized karyotype, whereas population F2, with the highest mean SI and the lowest mean DI, exhibited the highest symmetry and, consequently, a more primitive karyotype.

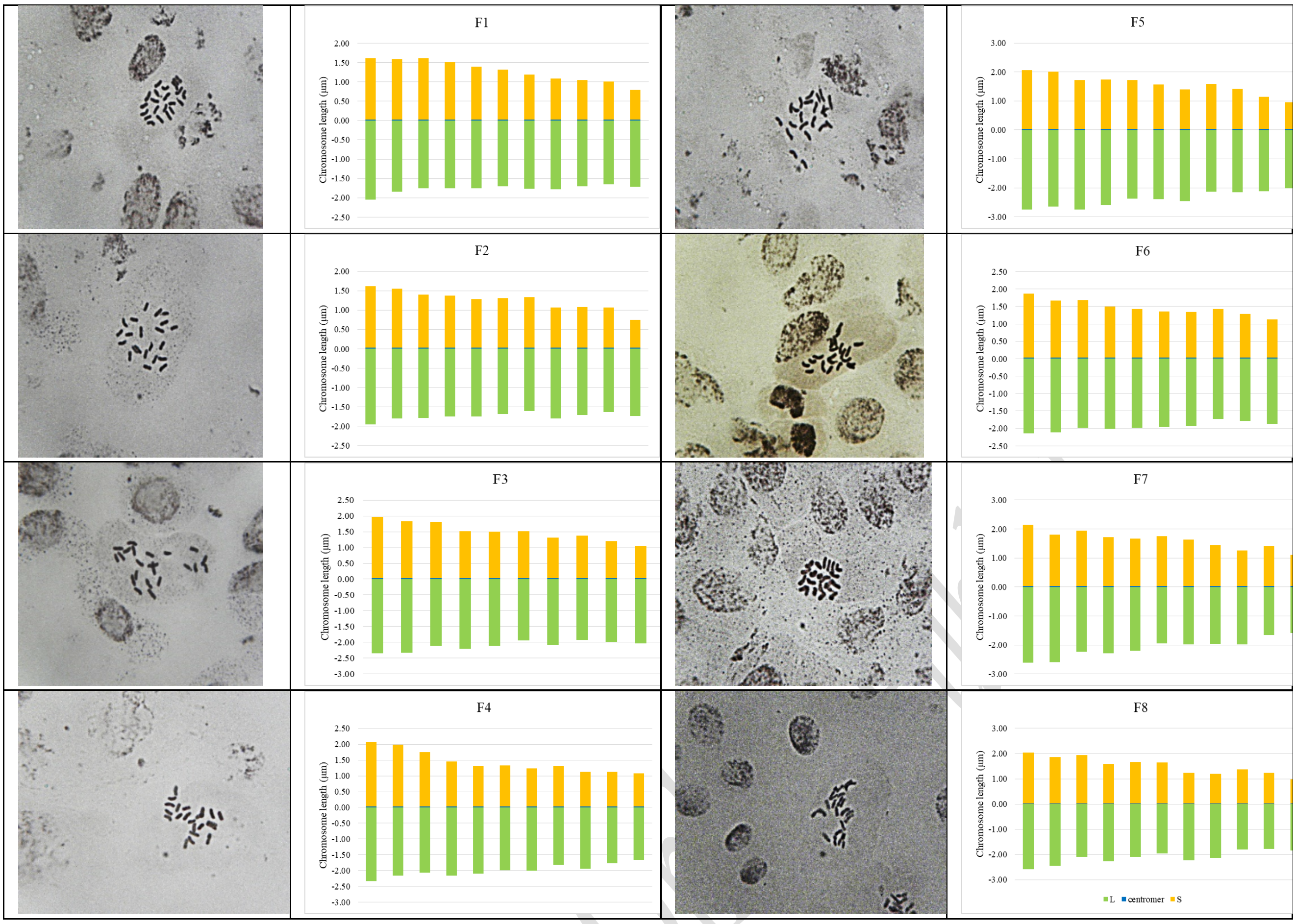

Fig. 1 Karyotypes and ideograms of haploid chromosome of 8 populations in *Ferula assa-foetida* L. (2n=2x=22).

Table 2 ANOVA of chromosomal parameters of *Ferula assa-foetida* L.

SOV	df	Mean of squares													
		L	S	CL	AR	r-Value	S%	L%	CI	TF%	DRL%	A_1	A_2	SI	DI
Population	7	0.132 **	0.05 **	0.323 **	0.0231 *	0.0036 *	0.0348 *	0.0344 *	0.0005 *	4.2836 *	1.7858 *	0.0036 *	0.002 *	63.82 *	2.866 *
error	16	0.008	0.012	0.031	0.0087	0.0013	0.013	0.0122	0.00020	1.5845	0.6703	0.0013	0.0007	25.06	1.013
C.V.%		4.43	7.81	5.14	6.59	6	3.49	2.46	3.63	3.49	21.29	14.65	19.21	9.48	20.32

* and **: significant at 5 and 1% probability levels.

Table 3 Mean comparisons of chromosomal parameters of *Ferula assa-foetida* L.

Population	L	S	CL	AR	r-Value	S%	L%	CI	TF%	DRL%	A_1	A_2	SI	DI	SC
F1	1.769d	1.256b	3.025de	1.492ab	0.711ab	3.774ab	5.317ab	0.41ab	41.509ab	3.392ab	0.289ab	0.115ab	68.799ab	4.951b	2A
F2	1.744d	1.227b	2.971e	1.503ab	0.708ab	3.753ab	5.338ab	0.409ab	41.284ab	3.293b	0.292ab	0.103b	69.577a	4.347b	2A
F3	2.079bc	1.437ab	3.516bc	1.532ab	0.695ab	3.716ab	5.375ab	0.405ab	40.872ab	3.76ab	0.305ab	0.13ab	66.136ab	5.499ab	2A
F4	1.997bc	1.404ab	3.401bc	1.503ab	0.705ab	3.751ab	5.34ab	0.408ab	41.265ab	4.404ab	0.295ab	0.152ab	62.402ab	6.34ab	1A
F5	2.397a	1.545a	3.941a	1.646a	0.652b	3.566b	5.525a	0.389b	39.227b	4.297ab	0.348a	0.148ab	61.193ab	5.742ab	2A
F6	1.924c	1.401ab	3.325cd	1.419b	0.731ab	3.83a	5.261b	0.419a	42.13a	3.578ab	0.269ab	0.115ab	67.175ab	4.838b	1A
F7	2.093b	1.597a	3.69ab	1.356b	0.766a	3.922a	5.169b	0.43a	43.142a	5.033a	0.235b	0.164a	56.95b	7.174a	1A
F8	2.112b	1.493a	3.605bc	1.495ab	0.709ab	3.762ab	5.329ab	0.41ab	41.377ab	4.598ab	0.291ab	0.157ab	61.268ab	6.211ab	1A

In each row, means with common letters are in the same statistical group at 5% probability level (Duncan's test).

Principal component analysis (PCA) showed that the first and second principal components accounted for 53 and 43.4% of the total variance between populations, respectively (Table 4). The results also showed that, in the formation of the first component, AR, r-Value, L%, S%, CI, TF%, and A_1 had the greatest contribution to the formation of this component. This component can be referred to as the intrachromosomal symmetry component because it groups populations based on their evolutionary status and intrachromosomal symmetry.

In the second component, the traits L, CL, S, A_2, SI, DRL, and DI had the highest contribution to the variance between populations, which can also be considered as a component of chromosome length and interchromosomal symmetry because it distinguishes different populations based on their interchromosomal symmetry and chromosome size.

The scatter plot of the distribution of populations based on the values of the first and second components is presented in Fig. 2. The grouping of populations was consistent with the results of cluster analysis, and the populations were placed in two groups.

Table 4 Principal components analysis (PCA) of *Ferula assa-foetida* L. populations

Parameters	Component 1	Component 2
L	-0.164	0.350
S	0.031	0.387
CL	-0.093	0.377
AR	-0.364	0.028
r-Value	0.366	0.003
S%	0.366	-0.021
L%	-0.366	0.021
CI	0.362	-0.007
TF%	0.366	-0.025
DRL%	0.095	0.384
A_1	-0.366	-0.003
A_2	0.013	0.384
SI	-0.076	-0.392
DI	0.121	0.367
Eigenvalue	7.412	6.072
Variance%	53.0	43.4
Cumulative of Variance%	53.0	96.3

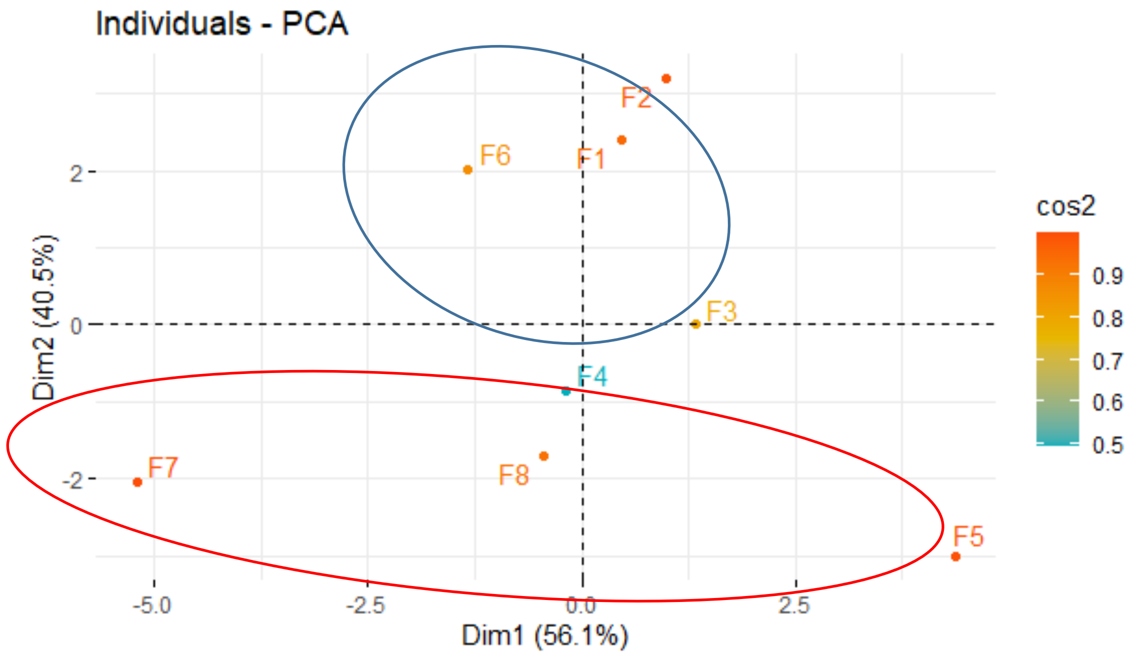

Fig. 2 Diagram resulting from principal components analysis (PCA) of *Ferula assa-foetida* L. populations

In cluster analysis using the UPGMA method, different populations were placed into two distinct groups (Fig. 3). Based on this, the populations F1, F2, F3, and F6 were placed in the first class. The first principal component played an important role in this separation. It appears that the factors that differentiated these populations were the traits AR, L%, S%, and A_1, with the lowest mean value, and the traits r-Value, CI, and TF% with the highest mean value.

Populations F4, F5, F7, and F8 were placed in the second group, and the second principal component played an important role in distinguishing these populations. In other words, the factors that caused the separation were the traits of L, CL, S, DRL, A_2, DI with the highest mean value, and SI with the lowest mean value.

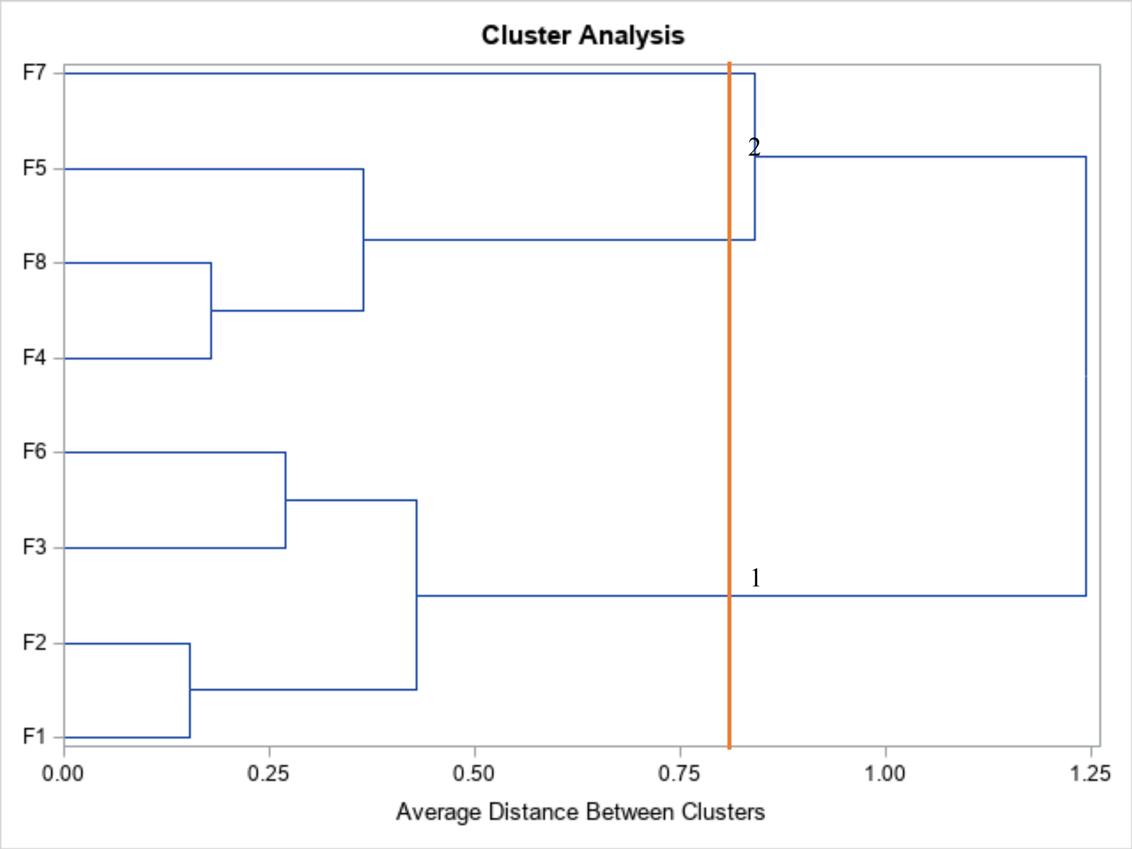

Fig. 3 Dendrogram resulting from cluster analysis (UPGMA) on different *Ferula assa-foetida* L. populations

DISCUSSION

In this study, eight populations of *Ferula assa-foetida*, one of the most important and valuable native medicinal plants of Iran, were investigated using karyotypic and cytogenetic approaches. The results clearly showed that all studied populations were diploid with a chromosome number of 2n = 22. This uniform chromosome number across geographically distinct populations indicates a conserved basic karyotype structure within the species, which is consistent with previous reports on *F. assa-foetida* [10] and other species of the genus *Ferula* [11, 12, 20–23]. The analysis of variance for all karyotypic parameters revealed significant differences among populations, indicating the presence of considerable cytogenetic diversity. Such variation among natural populations, despite a conserved chromosome number, suggests that structural chromosomal changes rather than numerical alterations have contributed to intra-specific differentiation. This level of diversity is particularly valuable for breeding and germplasm management programs, where genetically diverse populations can provide a broader adaptive potential. Classification of karyotypes into the 1A and 2A categories based on Stebbins' system further supports the relative symmetry of chromosomes in *F. assa-foetida*. Similar karyotype classes have been reported for Angus ecotypes and other Ferula species [10, 11, 20], indicating that karyotype symmetry may represent a conserved evolutionary feature within this genus. Although most populations exhibited relatively symmetrical karyotypes based on TF%, notable variation was observed among them. Population F7 showed the highest TF% value and therefore the greatest karyotype symmetry, whereas F5 exhibited the lowest TF% and the most asymmetric karyotype. Gradual shifts in TF% values among populations likely reflect minor structural chromosomal rearrangements, such as duplications or translocations, rather than major evolutionary transitions, as previously suggested for plant karyotype evolution [24]. The dispersion index (DI) proved to be an effective parameter for distinguishing closely related karyotypes that fall within similar asymmetry classes. This highlights the importance of using multiple complementary indices when assessing karyotype variation, particularly in taxonomic and cytogenetic studies of closely related populations or species [19]. Cluster analysis based on all karyotypic parameters divided the studied populations into two main groups, a pattern that was consistent with the results of principal component analysis. The concordance between multivariate statistical approaches strengthens the reliability of the observed population grouping and suggests that karyotypic traits play a meaningful role in differentiating populations of *F. assa-foetida*. From a breeding and conservation perspective, genetic diversity assessments should ideally integrate cytogenetic and morphological data. Populations that are cytogenetically similar but morphologically divergent may offer valuable combinations for breeding programs, as they allow chromosome pairing during meiosis while maintaining phenotypic diversity. However, successful hybridization can be constrained by differences in chromosome structure, centromere position, and overall karyotype organization, which may disrupt meiotic pairing and fertility. Therefore, cytogenetic compatibility should be carefully considered when selecting parental populations for breeding or conservation strategies. Despite the valuable insights provided by this study, some limitations should be acknowledged. The analysis was restricted to populations from a single province, and molecular cytogenetic or genomic data were not included. Future studies integrating molecular markers and broader geographic sampling would provide a more comprehensive understanding of genetic diversity and evolutionary relationships within *F. assa-foetida*.

CONCLUSION

In conclusion, the present study demonstrated that *Ferula assa-foetida* populations from Fars Province share a conserved diploid chromosome number while exhibiting notable variation in karyotypic structure and symmetry. This intra-specific cytogenetic diversity reflects structural chromosomal differentiation rather than numerical changes and underscores the importance of karyotype analysis in understanding population differentiation. The combined use of karyotypic parameters, principal component analysis, and cluster analysis proved effective in revealing relationships among populations. These findings provide a useful cytogenetic basis for future studies on breeding, conservation, and evolutionary analysis of this valuable medicinal species.

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