Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Narges Firoozi, Ghasem Karimzadeh, Mohammad Sadegh Sabet, Vahid Sayadi (2022). Intraspecific karyomorphological and genome size variations of *in vitro* embryo derived Iranian endemic Asafoetida (*Ferula assa-foetida* L., Apiaceae). *Caryologia* 75(4): 111-121. doi: 10.36253/caryologia-1721

Received: June 29, 2022

Accepted: December 06, 2022

Published: April 28, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Intraspecific karyomorphological and genome size variations of *in vitro* embryo derived Iranian endemic Asafoetida (*Ferula assa-foetida* L., Apiaceae)

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Abstract. Asafoetida (*Ferula assa-foetida* L.) is one of the endemic medicinal plants in Iran. Analysis of karyomorphology and 2Cx DNA measurements (monoploid genome size) of 18 Iranian endemic *Ferula assa-foetida* populations were performed. The *in vitro* embryo-derived root tips were examined for karyological studies, via technique of squash and stain with 2% (w/v) aceto-orcein. Seeds of the *Ferula* samples and leaves of *Solanum lycopersicum* as standard reference (2C DNA = 1.96 pg) were stained with propidium iodide (PI), using flow cytometric (FCM) technique. All the studied populations were diploids ($2n = 2x = 22$) with mean chromosome length (CL) of 3.95 µm, varied from 3.05 µm (P7) to 4.94 µm (P18). The mean total chromosome volume (TCV) was 0.98 µm^3, ranged from 0.47 µm^3 (P7) to 1.57 µm^3 (P3). Two-typed chromosomes ("m", "sm") formed three classes of karyotype formula. Karyotypes were mostly symmetrical and fell in 1A and 2A Stebbins category. The monoploid genome size of Iranian endemic *Ferula assa-foetida* populations is being stated for the first time; its mean value was 4.51 pg, ranged from 4.09 (P4) to 4.69 pg (P16). Intraspecific karyomorphological and genome size variations were clearly confirmed in studied *Ferula assa-foetida*.

Keywords: Chromosome, *Ferula assa-foetida*, Flow cytometry, Karyotype, 2Cx DNA.

1. INTRODUCTION

Ferula assa-foetida belongs to Apiaceae family that grows in Iran, Kashmir in Pakistan, and Afghanistan. Asafoetida production from *Ferula assa-foetida* as a source is confined to southern Iran (Farhadi *et al.* 2019; Barzegar *et al.* 2020). Iranian flora consists of 30 species of *Ferula*, most of which are endemic (Khajeh *et al.* 2005; Farhadi *et al.* 2019). It is herbal and permanent and enlarges to 2 cm high (Khajeh *et al.* 2005). Apiaceae family had very low germination owing to seed dormancy (Nadjafi *et al.* 2006). Hence, the germination of *Ferula*'s seeds was complicated. To accelerate the breakage of

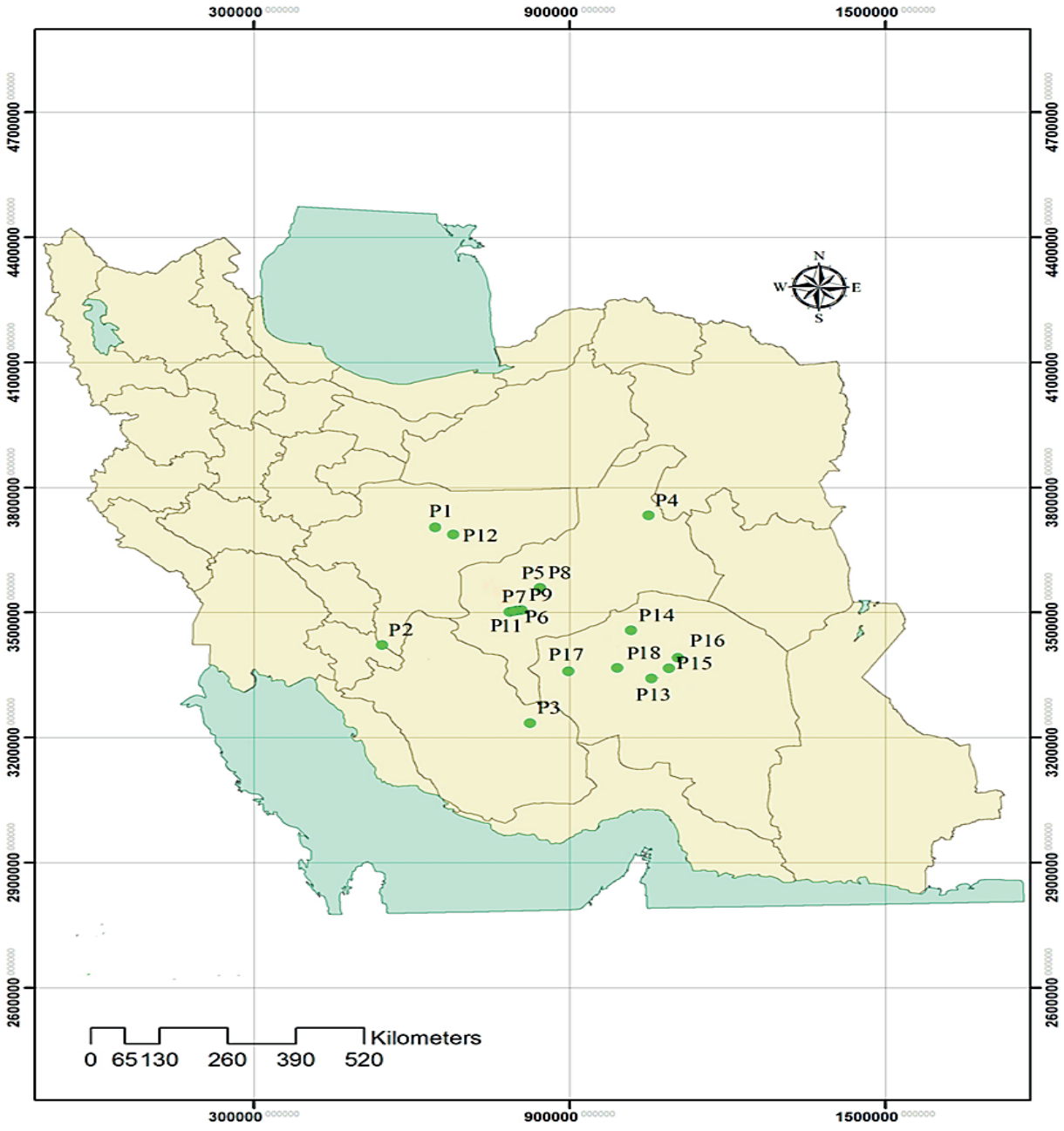

Figure 1. Geographic distribution of sampled *Ferula assa-feotida* L. on the map of Iran, using ArcGIS

its seed dormancy, various methods were applied such as soaking with running water and treating with either chilling temperature or GA_3 (Keshtkar *et al.* 2008; Hassani *et al.* 2009; Zare *et al.* 2011). The oleo-gum-resin has got from taking away of the stems or cut off the roots that have a sulfurous smell and bitter taste. *Ferula* species, due to its chemical compounds, play a useful role in the treatment of various diseases. The oleo-gum-resin is antiseptic, antifungal, antibiotic, laxative, indigestion, antiviral, antidiabetic whooping cough, cramp, infertilitypain, and cancer chemopreventive (Aruna and Sivaramakrishnan 1992; Dehpour *et al.* 2009; Lee *et al.* 2009). The attribute properties of these plants have sesquiterpene coumarins and e few monoterpenes (El-Razek *et al.* 2001). Terpene coumarins have anti-HIV activity (Zhou *et al.* 2000). In plant sciences, for a large number of plants in order to DNA content's screening is used of flow cytometry (FCM) as a powerful and reli-

Table 1. Local information of the collected Iranian endemic *Ferula assa-foetida* L.

Mean rainfall (mm)	Mean Temp (°C)	Altitude (m)	Longitude (E)	Latitude (N)	Local collection locations	Population code
13.66	20.20	1817	52° 33' 00.0"	33° 27' 54.0"	Esfahan, Iran	P1
60.52	15.25	2750	51° 26' 55.3"	30° 55' 49.9"	Kohkiloyeh Boyerahmad, Iran	P2
15.87	20.82	1795	54° 20' 00.0"	29° 12' 00.0"	Fars, Iran	P3
5.54	22.91	669	56° 55' 50.6"	33° 35' 39.1"	Khorasan, Iran	P4
4.32	20.42	2158	54° 38' 23.8"	32° 06' 43.4"	Yazd, Iran	P5
5.08	20.54	1720	54° 09' 53.6"	31° 38' 13.4"	Yazd, Iran	P6
5.08	20.54	831	55° 38' 20.4"	33° 06' 43.2"	Yazd, Iran	P7
4.32	20.42	2158	54° 38' 23.6"	32° 06' 43.4"	Yazd, Iran	P8
5.08	20.54	1950	54° 14' 42.5"	31° 38' 41.7"	Yazd, Iran	P9
5.08	20.54	2160	54° 09' 53.6"	31° 38' 13.5"	Yazd, Iran	P10
5.08	20.54	3279	54° 05' 27.0"	31° 37' 41.2"	Yazd, Iran	P11
9.04	20.00	2164	57° 54' 50.4"	29° 18' 10.8"	Kerman, Iran	P12
12.05	17.07	2000	56° 45' 00.0"	30° 48' 00.0"	Kerman, Iran	P13
9.04	20.00	2200	56° 25' 00.0"	31° 08' 00.0"	Kerman, Iran	P14
12.05	17.07	1900	57° 07' 00.0"	30° 17' 00.0"	Kerman, Iran	P15
4.05	21.05	2600	57° 18' 00.0"	30° 30' 00.0"	Kerman, Iran	P16
11.49	16.67	1850	55° 07' 57.0"	30° 17' 40.0"	Kerman, Iran	P17
7.04	19.23	2335	56° 60' 00.0"	30° 20' 20.4"	Kerman, Iran	P18

able technique (e.g., Loureiro *et al*. 2005; Mahdavi and Karimzadeh 2010; Karimzadeh *et al*. 2010, 2011; Abedi *et al*. 2015; Tarkesh Esfahani *et al*. 2020; Zarabizadeh *et al*., 2022). It mainly focused on cell cycle analysis, measurement of nuclear DNA content, and determination of ploidy level. It can be used to determine monoploid and holoploid genome size (Doležel *et al*. 2003, 2007; Greilhuber *et al*. 2005(. Furthermore, in plant systematics and plant breeding, karyotypes can make available evidence and data for species identification and the study of populations resulting from a cross between individuals (Anjali and Srivastava 2012). In a study on *Ferula assa-foetida*, it was found that this plant is diploid with a chromosome number 22, grouping in 2A class according to Stebbins classification (Zhao *et al*. 2006). Likewise, the same chromosome number of 22 was reported by El-Alaoui-Faris *et al*. (2006) in five different species of *Ferula*; *F. gouliminensis, F. cossoniana, F. tingitana, F. sauvagei,* and *F. atlantica*. Furthermore, in a study conducted in China on two species of *F. liacentiana* and *F. bungeana*, the 22-chromosome number was also reported (Qixin and Menglan, 1993). It is significant to note that some studies and reports on *F. assa-foetida* in outside of its native range have mistakenly identified the species. In other words, some species that produce asafoetida are often misidentified as *F. assa-foetida* (Chamberlain, 1977). Awareness of genetic diversity and the management of genetic resources are considered as the main parts of plant breeding programs. The first step in plant breeding is to understand the genome structure and the germplasm collection (Lee *et al*. 2021). Taken together, these situations indicate the need for basic investigations especially cytogenetic studies. The key aim of the current survey was to study intraspecific genome size and karyo-morphological variations among 18 *F. assa-foetida* populations of Iran.

2. MATERIALS AND METHODS

Seeds of 18 Iranian endemic populations of *Ferula assa-foetida* L. were used for this study. The germplasm collection of the Iranian Biological and Resource Center (IBRC), Tehran, Iran from where the seeds were obtained. Geographical description, climatic information, the population codes used in this study and the gene bank codes are present in Table 1. Since previously reported methods, including chilling temperature and treating $GA_{3,}$ was not satisfactory to achieve good germination, hence, *in vitro* embryo culture was the best, and the most suitable technique (Zare *et al*. 2011; Suran *et al*. 2016).

2.1. In vitro embryo culture

At first, seeds were soaked in running water for 24 h sterilized as follows:

- Wash the seeds with five drops of washing liquid for 2 min.
- Seeds placement under running water for 5 min.
- Seeds placement in ethanol 70% for 2 min.
- Wash seeds with distilled water for 5 min.
- Seeds placement in sodium hypochlorite 5.25 (v/v) for 20 min.
- Wash seeds with dsH_2O three times and each time for 2 min in laminar airflow.

After this step, the embryos were excised by push the bottom of seeds and were transferred to Murashige and Skoog medium (Murashige and Skoog 1962). The embryos in Petri dishes were placed in an incubator at photoperiod with a 16 h light/8 h dark and 25 °C. It was germinated after three to four days.

2.2. Karyomorphological analysis

For the preparations of karyology, actively around one cm-long *in vitro* growing roots were removed and pre-treated in colchicine (0.05% (w/v)) for 4 h in the dark at 4 °C to impel metaphase arrest. After that, the roots were subsequently fixed in freshly Carnoy's fixative (3 absolute ethanol: 1 glacial acetic acid (v/v) ratio) at 4 °C for 24 h (Karimzadeh *et al.* 2010, 2011). Using distilled water, the fixed roots were washed then in a water bath its hydrolyzed in 1M Hydrochloric acid (HCl) for 10 min at 60 °C, after these steps, staining was performed for 3 h with aceto-orcein (2% (w/v)) at 25 °C (Karimzadeh *et al.* 2011). The five well-expand monolayer metaphase plates from various individuals were examined per *Ferula assa-foetida* populations. Photographs were taken in high resolution via a light microscope (BX50 model, Olympus Optical Co., Ltd., Tokyo, Japan) armed with an digital camera (DP12, Olympus Optical Co., Tokyo, Japan).

Eight chromosomal parameters were either investigated as short arm (S) and long arm (L) lengths, or measured as chromosome length (CL = L + S), r-value (S/L), arm ratio (AR; L/S), total chromosome volume (TCV = πr^2 CL, where r = average chromosome radius), form percentage (F% = S/SCL), and centromeric index (CI = S/CL). Chromosome types were determined, via Levan *et al.* (1964) formula and Idiograms were drawn from the mean values. The following parameters were also assessed for analysis karyotype asymmetry: the difference of range relative length ($RL\%_{Max}$ - $RL\%_{Min}$), karyotype total form percentage (TF% = $\Sigma S/\Sigma CL \times 100$), dispersion index (DI = (Mean CI × CV_{CL}) /100), mean centromeric asymmetry (M_{CA}), coefficient of variation of chromosome length (CV_{CL}) (Paszko 2006; Peruzzi *et al.* 2009), Romero-Zarco (1986) intrachromosomal (A1) and interchromosomal (A2) asymmetry indices; and Stebbins asymmetry categories for the investigation of karyotype asymmetry were assessed (Stebbins 1971).

2.3. Flow cytometric analysis

For each Asafetida population, the monoploid 2Cx-value was calculated through Flow cytometric analysis. Hence, to prepare nuclear suspensions, four seeds (Jedrzejczyk and Sliwinska, 2010) of each Asafetida sample along with the healthy fresh young leaves of *Solanum lycopersicum* cv. Stupicke (2C DNA = 1.96 pg; Doležel et al., 2007) as the internal reference standard plant were chopped with a sharp razor blade in ice-cold woody plant buffer (WPB, Loureiro et al., 2007). Flow cytometric analysis was carried out via PI (Propidium Iodide) staining method. The nuclei suspension was examined via the flow cytometer (BD FACSCanto II, BD Biosciences, Bedford, MA, USA), through BD FACSDiva™ Software. The gained data were transferred to Flowing Software (ver. 2.5.0, Cell Imaging Core, Turku Centre for Biotechnology) to be editable in Partec FloMax software (ver. 2.4e, Partec, Münster, Germany). The range of gating zone was calculated on obtained histograms from FCM, via the FloMax. Healthy fresh young leaves of the Asafetida sample's seeds and the standard reference were chopped, using a sharp razor blade. this action performed in ice-cold nuclear extraction buffer WPB (Woody Plant Buffer, Loureiro *et al.* 2007). The chopped seeds and leaves were filtered via a 30 µm green nylon mesh (Partec, Münster, Germany). One ml of staining buffer, 50 µg ml^{-1} of PI (Fluka) solution and 50 µg ml^{-1} of RNase (Sigma-Aldrich Corporation, MO, USA) stock solution were added to each sample. For the stained nuclei, the relative fluorescence intensity was calculated via FCM on a linear scale. For each sample, flow cytometrically minimum 5000 nuclei were evaluated. The values of the means of G1 peak were used to estimate the absolute DNA amount of the sample (Doležel *et al.* 2003, 2007; Greilhuber *et al.* 2005; Doležel and Bartoš 2005; Mahdavi and Karimzadeh 2010; Karimzadeh *et al.* 2011) as follows:

Sample $2C_X$ DNA (pg) = (Sample G1 peak mean/Standard G1 peak mean) × Standard 2C DNA (pg).

Monoploid genome size was estimated based on above converting formula in the form of base pair (Doležel et al. 2003). It should be noted that one pg of DNA equivalent to 978 mega base pairs (Mbp) were considered (Doležel et al. 2003).

2.4. Statistical analysis

In First normality test was carried out for the obtained data from karyotypic and flow cytometric studies and then were investigated for karyological data in five replications and for nuclear DNA content three replications based on a completely randomized design (CRD). Differences between means were measured through the least significant difference (LSD). Minitab 17 software package was used for multivariate statistical analysis. principal components analysis (PCA) was then carried out based on data matrix to evaluate the contribution of each karyotypic parameter to the ordination of populations. A cluster analysis on chromosomal parameters was carried out, through the Ward's method and the Euclidean distance to assess similarities and variations amid the populations.

3. RESULTS

All 18 Iranian endemic populations of *Ferula assa-foetida* were diploid ($2n = 2x = 22$). Obtained karyotypes from somatic complement and the haploid complement's idiograms of studies *F. assa-foetida* populations are demonstrated in figures 2 and 3, respectively. Based on the *ANOVA* results, among populations for either all chromosomal parameters, or monoploid genome size were significant differences ($P < 0.01$; 2Cx DNA; Table 2). The mean chromosome length (CL) was determined as 3.95 µm, varied from 3.05 µm (P7) to 4.94 µm (P18). The mean CI of the complement, varied from 41.15% (P18) to 45.57% (P1). The mean TCV was 0.98 μm^3, ranged from 0.47 μm^3 (P7) to 1.57 μm^3 (P3). According to numerous karyotypic symmetrical indices tested, *F. assa-foetida* populations indicated various symmetrical clusters. The maximum value of TF% was recognized in P1 (45.3%) and the lowest value was identified in P14 (41.3%). The uppermost and the lowermost values of the various range of relative length (DRL) were detected in P8 (4.79%; the most asymmetric) and P1 (2.25%; the most symmetric), respectively. The highest and lowest values of CV_{CL} %were identified in P8 (15.53%; the maximum asymmetric) and P1 (7.17%; the maximum symmetric), respectively. Similar to the results of DRL and

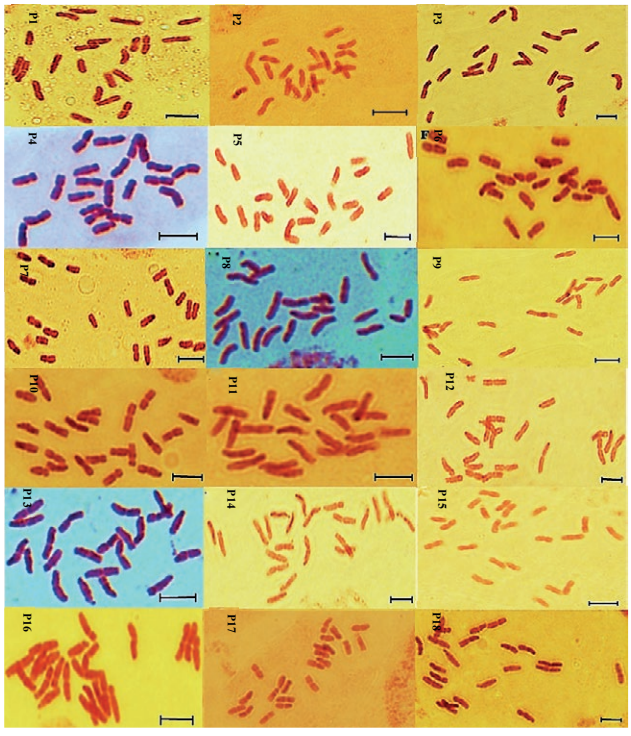

Figure 2. Karyotypes of somatic chromosome complement of 18 Iranian endemic *Ferula assa-foetida* L. ($n = 2x = 22$) populations. Scale bars = 5 µm.

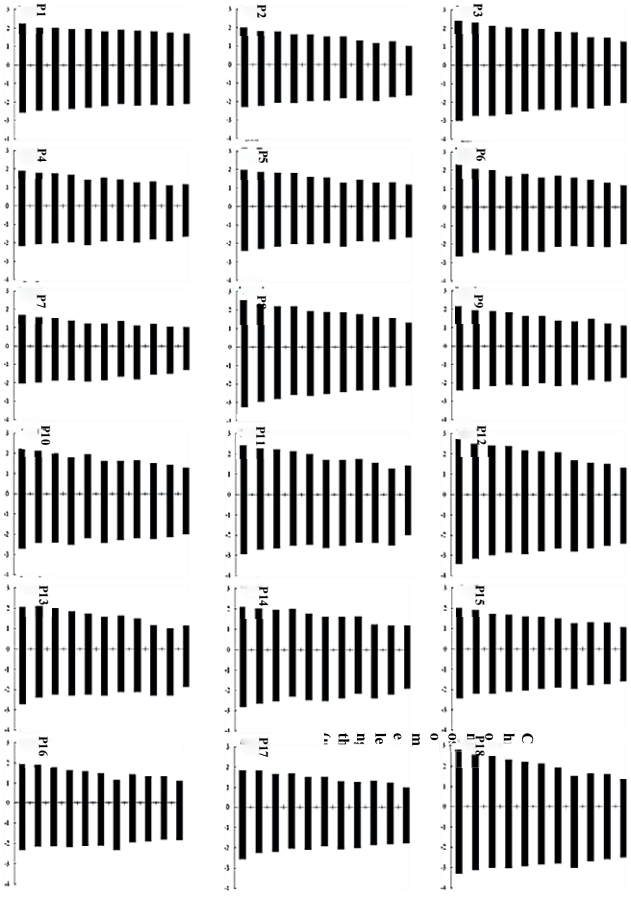

Figure 3. Idiograms of haploid chromosome complement of 18 Iranian endemic *Ferula assa-foetida* L. ($2n = 2x = 22$) populations.

Table 2. *ANOVA* of chromosomal parameters (a) and monoploid genome size (2Cx DNA; b) of *Ferula assa-foetida* L.

S.O.V.	Df	MS							
		S	L	CL	AR	r-value	F%	TCV	CI
a) Chromosomal parameters									
Population	17	12.58**	0.19**	0.18**	2.35**	2.42**	1.85**	20.12**	2.32**
Error	972	0.79	0.005	0.005	0.97	0.97	0.98	0.66	0.97
b) Monoploid genome size (2Cx DNA)									
S.O.V.	Df	2Cx DNA							
Population	17	0.066**							
Error	36	0.004							

** Significant difference ($P < 0.01$).

Table 3. Karyotypic parameters of *Ferula assa-foetida* L. ($2n = 2x = 22$).

Populations	Stebbins' category	Karyotype formula	CV_{CL}%	DI	DRL%	TF%	M_{CA}	Asymmetry Indices (Romero-Zarco, 1986)	
								A_2	A_1
P1	1A	22m	7.17	0.03	2.25	45.30	9.00	0.07	0.16
P2	1A	20m+2sm	13.78	0.06	4.21	43.54	14.11	0.14	0.23
P3	1A	22m	14.45	0.06	4.40	43.34	14.26	0.14	0.24
P4	1A	20m+2sm	10.88	0.05	3.27	43.26	14.24	0.11	0.24
P5	1A	22m	13.16	0.06	3.89	43.21	13.48	0.13	0.23
P6	1A	22m	12.79	0.05	4.03	42.61	15.58	0.13	0.26
P7	1A	22m	14.01	0.06	4.29	42.97	14.92	0.14	0.24
P8	1A	22m	15.53	0.07	4.79	42.95	14.34	0.16	0.24
P9	1A	22m	13.71	0.06	4.22	43.05	14.05	0.14	0.23
P10	1A	22m	11.65	0.05	3.65	42.67	14.89	0.12	0.25
P11	1A	20m+2sm	12.86	0.05	3.99	43.06	15.31	0.13	0.25
P12	1A	20m+2sm	14.94	0.06	4.48	41.78	17.14	0.15	0.27
P13	2A	18m+4sm	13.78	0.06	4.24	41.50	17.59	0.14	0.28
P14	1A	18m+4sm	13.73	0.06	4.07	41.31	18.75	0.14	0.30
P15	1A	22m	14.94	0.06	4.62	42.67	13.03	0.15	0.22
P16	2A	20m+2sm	11.67	0.05	3.43	42.58	16.06	0.12	0.26
P17	1A	20m+2sm	13.37	0.06	4.12	41.39	17.25	0.13	0.28
P18	1A	18m+4sm	13.92	0.06	4.10	41.67	17.69	0.14	0.29

CV_{CL}%, the highest value of dispersion index (DI) was detected in P8 (0.07; the most asymmetric), while P1 displayed the lowest (0.03; the most symmetric). In conclusion, three (DRL, CV_{CL}% and DI) among five karyotypic symmetrical indices examined, confirmed that all 18 *F. assa-foetida* populations examined P8 and P1 performed to have the most asymmetrical and symmetrical karyotypes, respectively. The highest value of M_{CA} was identified in P14 (18.75 %) while P1 demonstrated the lowest value (9.1 %). Two-typed chromosomes were recognized in all populations by using Levan *et al.* (1964) chromosome nomenclature: "m" (centromere at medium region) and "sm" (centromere at sub medium region); formed 3 different karyotypic formulas as follows: 22m (nine populations), 20m+2sm (six populations) and 18m+4sm (three populations; Table 3). Karyotypes of all populations were ordered in the 1A and 2A classes of Stebbins classification (Stebbins 1971). For further detailed studies of asymmetry, A_1 and A_2 indices were also calculated (Romero-Zarco, 1986). The highest $A_1 = 0.30$ was found in P14, showing the highest inter chromosomal difference, resulted in asymmetric karyotype and the lowest $A_1 = 0.16$ is related to the P1 which demonstrations the highest symmetry. The highest $A_2 = 0.16$ was related to

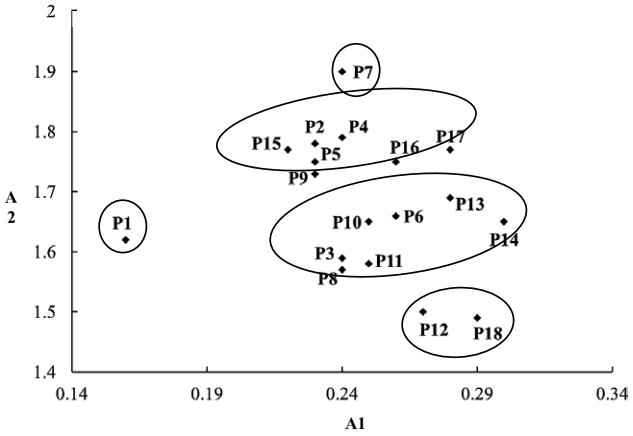

Figure 4. Scatter plot of intrachromosomal (A1) and interchromosomal (A2) asymmetries of 18 Iranian *Ferula assa-foetida* L. populations

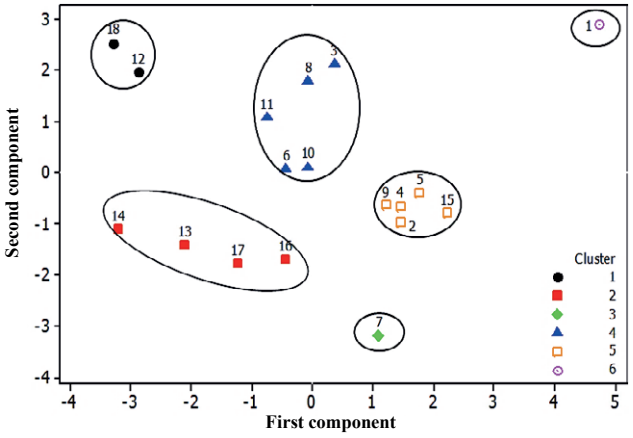

Figure 5. Diagram resulting from principal components analysis (PCA) of *Ferula assa-foetida* L. populations.

P8; they have the highest chromosomal asymmetry and the lowest $A_2 = 0.07$ was related to P1, having the highest intra chromosomal symmetry (Table 3).

The scatter diagram of these indices (Figure 4) shows five groups of populations. The principal component analysis of the karyotypic parameters was carried out to estimate total variation and its parameters quota in populations. The PCA representing that the first three principal components account for 99% of the cumulative variation, and they were plotted in a 2-dimensional graphic (Figure 5). The Ward (Khodadadi *et al.* 2014) phenogram constructed based on karyotype similarities (Figure 6) shows six major clusters. The principal component analysis resulting populations arrangement from this exam entirely fits with that obtained with the Ward grouping analysis. Thus, the obtained results suggested

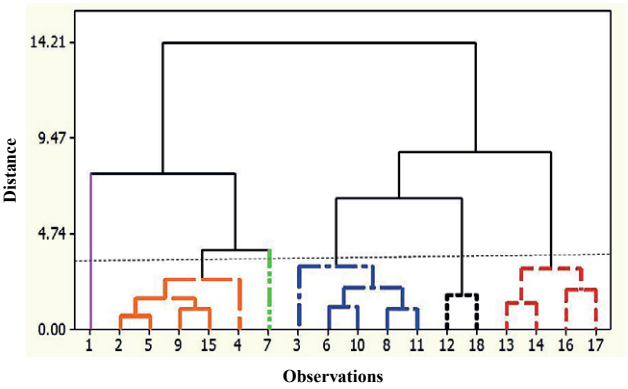

Figure 6. Dendrogram showing the phenetic relationships among the studied populations of *Ferula assa-foetida* L. populations.

Table 4. Mean (± Se) comparisons of monoploid genome size (2Cx DNA) of Iranian endemic *Ferula assa-foetida* L. ($2n = 2x = 22$).

1Cx DNA (Mbp)	1Cx DNA (pg)	Mean 2Cx DNA (pg) ± Se	Population
2166.27	2.215	4.43 ± 0.075^{efg}	P1
2146.71	2.195	4.39 ± 0.047^{fg}	P2
2122.26	2.170	4.34 ± 0.006^{g}	P3
2000.01	2.045	4.09 ± 0.042^{h}	P4
2210.28	2.260	4.52 ± 0.036^{bcde}	P5
2185.83	2.235	4.47 ± 0.010^{def}	P6
2259.18	2.310	4.62 ± 0.035^{ab}	P7
2176.05	2.225	4.45 ± 0.049^{defg}	P8
2244.51	2.295	4.59 ± 0.012^{abc}	P9
2273.85	2.325	4.65 ± 0.015^{a}	P10
2190.72	2.240	4.48 ± 0.060^{cdef}	P11
2176.05	2.225	4.45 ± 0.025^{defg}	P12
2234.73	2.285	4.57 ± 0.019^{abcd}	P13
2229.40	2.280	4.60 ± 0.038^{abc}	P14
2244.51	2.295	4.59 ± 0.026^{abc}	P15
2293.41	2.345	4.69 ± 0.030^{a}	P16
2283.63	2.335	4.67 ± 0.019^{a}	P17
2268.96	2.320	4.64 ± 0.056^{ab}	P18
2000.01-2293.41	2.045-2.345	4.09-4.69	Range

Means with the same symbol letter in a "Mean 2Cx DNA (pg)" column are not significantly different ($P > 0.01$), using LSD test.

that populations within one cluster, having the high homology in chromosomal differences.

The result of FCM data was confirmed for normality and analyzed based on completely randomized design (CRD) with three replicate cells. *ANOVA* showed among- populations high significant difference ($P < 0.01$) for monoploid genome size (2Cx DNA content; Table 2). The mean value was 4.51 pg (Table 4), varied from 4.09 pg in P4 to 4.69 pg in P16. The histograms obtained for

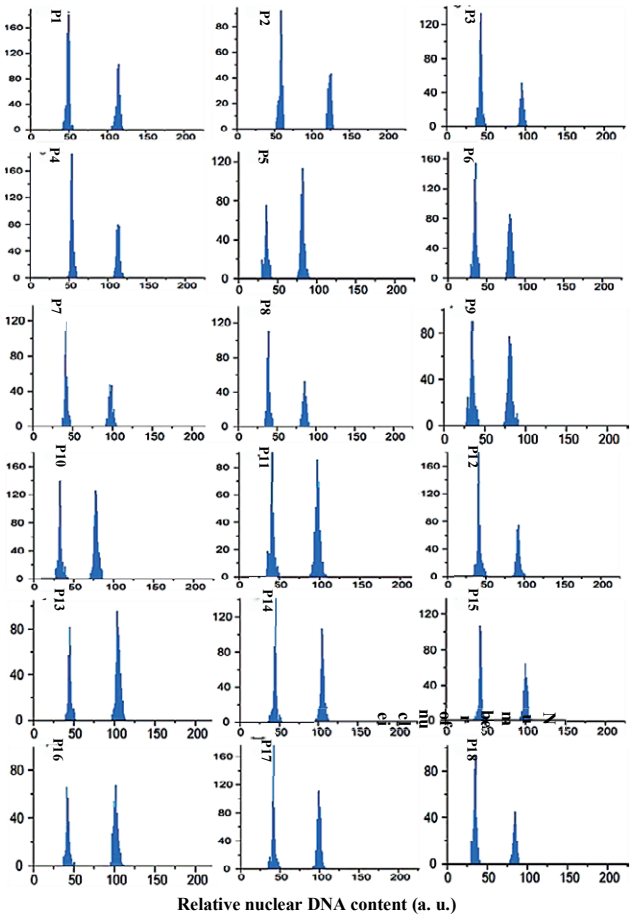

Figure 7. Flow cytometric histograms of monoploid genome size (2Cx DNA (of 18 Iranian endemic *Ferula assa-foetida* L. populations. The left peaks refer to G1 of the *Solanum lycopersicum* cv. Stupicke (2C DNA = 1.96 pg) reference standard and the right peaks refer to G1 of *Ferula assa-foetida* L. samples.

analyzing nuclear DNA amount included two peaks (Figure 7): the left peaks refer to the G1 of *Solanum lycopersicum* cv. Stupicke as reference standard plant (Doležel *et al.* 2007) and the right peaks to the G1 of *F. assa-foetida* samples. In other words, using 1Cx DNA monoploid genome size in Mbp, the mean value of all populations was 2205.39 Mbp (Table 4).

4. DISCUSSION

Ferula assa-foetida belongs to Apiaceae family includes about 170 identified taxa, of which 30 species have been detected in different phytogeographical regions in Iran (Zomorodian *et al.*, 2018). It is an important perennial herb with medicinal benefits which is native to central Asia and Iran *(*Farhadi *et al.* 2019*).* Many pharmaceutical properties and medical benefits had been reported for Ferula (Dehpour *et al.* 2009; Lee *et al.* 2009). For increasing the potential applicability of this genus, this perennial plant still needs more investigation on the genetic variability, expanding the range of research on its genetic characteristics as well as develop breeding methods.

In the current study, we studied the Iranian endemic *Ferula assa-foetida* populations. Eighteen Iranian endemic populations of Asafoetida (*F. assa-foetida* L.) were cytogenetically assessed on the basis of karyomorphology and genome size in the current study; they were all diploids ($2n = 2x = 22$). Mostly, in *Ferula* genera the diploids are more frequent and obtained results in the present study are in agreement with those reported on *Ferula assa-foetida* (Zhao *et al.* 2006) and on other species of *Ferula* (Wanscher 1931; Qixin and Menglan 1993; Ghaffari *et al.* 2005; El-Alaoui-Faris *et al.* 2006; Sağiroğlu and Duman 2006; Bernard *et al.* 2007). The obtained results of *ANOVA* show a significant difference in terms of all chromosomal traits. (Table 2), confirming intraspecific karyomorphological diversity in the studied Iranian endemic Asafoetida populations, which would be beneficial for the success of the breeding programs. The mean chromosome length (CL) was 3.95 µm, varied from 3.05 µm (P7) to 4.94 µm (P18). In other words, a remarkable 1.89 µm variation in chromosome size was detectable among such a number of examined Iranian endemic Asafoetida. In the present investigation two-typed chromosomes ("m", "sm") formed three classes of karyotype formula, comprised: 22m for nine populations (P1, P3, P5- P10, P15), 20m+2sm for six populations (P2, P4, P11, P12, P16, P17(, and 18m+4sm for three populations (P13, P14, P18). Zhao *et al.* (2006), studying on *Ferula fukanensis*, similarly reported the two-typed chromosomes of "m" and "sm". The studied karyotypes were grouped in the 1A and 2A classes based on classification of Stebbins. In a study on *Ferula fukanensis*, karyotypes were classified as 2A (Zhao *et al.* 2006), which was consistent with the results of the current study in the P13 and P16 populations. It has been alleged that symmetric karyotypes have a lower grade of evolution in comparison with asymmetric karyotypes (Stebbins 1971). Understanding taxon evolution, and interrelations are facilitated through the information obtained from karyotype, and chromosome morphology (Furo *et al.* 2020; Sayadi *et al.* 2021). In the current investigation, the scatter diagram shown the populations in five groups that exactly fits with the principal component analysis resulting species arrangement. Furthermore, the results noted that populations inside a cluster have the maximum homology of chromosomes. According to

karyotypes studies, the most fertile offspring can be produced by crossing the populations having the maximum chromosomal homology. The results of principal component analysis, and cluster analysis for the chromosomal traits, it is possible to introduce populations in groups 2 and 3 due to the shortest distance, to intersect and produce maximum fertile offspring. Accordingly, crossing between populations in a cluster is recommended, for instance between P13 with P14, P16, or P17, and also P12 with P18. The karyotypes in the primitive species are usually highly symmetrical, but that is not necessarily the case. In other words, a distinct and more evidence is ever required to evaluate the direction in changes of karyotype (Peruzzi and Eroğlu 2013). It has been noted that the evolutionary relationships via asymmetry indices usage for the establishment may not be straightforward. It can be stated that the genus diversity may have resulted from the structural changes. Some differences in asymmetry indices and karyotype formula between species may have contributed to this diversity (Seijo and Fernandez 2003).

As stated in the Stebbins (1971) classification, the karyotypes were mostly symmetric (Table 3). In the present study, to achieve greater measurement accuracy, additional parameters were also assessed in addition to Stebbins asymmetry categories, including TF%, DI, CV_{CL}, M_{CA}, A_1, and A_2 asymmetry indices for karyotype asymmetry analysis (Table 3). Some have argued and suggested that Stebbins' (1971) classification as a qualitative method is not so strong and lower flexible regarding the types of conclusions it can provide (Paszko, 2006). The average of DI% was 5.6% (from 3% in P1 to 7% in P7). All of the populations were symmetric based on TF% parameter, that the mean value was 42.71%, ranging from 41.3% (P14) to 45.3% (P1). Some chromosomal disorders are probably a factor of gradual changes in the amounts of TF%. The appearance changes in the chromosomes morphology were happens due to the various causes such as translocated or duplicated chromosomes (Das *et al*. 1998). When the populations are similar in Stebbins classification, estimates A1 and A2 parameters of Romero-Zarco (1986) to determine the further asymmetric karyotype are necessary. As A1 index gets lower in P1 represents karyotype symmetry and a higher value in P14 assessed greater asymmetry. The A_2 parameter was 13.22%, ranging from 7% (P1) to 16% (P8). Preventing interspecific cross accomplishment and offspring infertility may have ensued from the difference in resulting karyotype symmetry.

Obvious intraspecific diversity was also detected in terms of monoploid genome size (2Cx DNA) among examined Iranian endemic Asafoetida populations. Hence, on the other hand, the 2Cx DNA amounts of 18 Iranian endemic *F. assa-foetida* L. populations are being reported for the first time, having the mean value of 4.51 pg, varied from 4.09 (P4) to 4.69 pg (P16). In other words, in our knowledge, few reports were found in the literature for the genome size estimation even in other *Ferula* species. For example, 2.90 pg was reported for 2C DNA amount of *F. communis* by Olmedilla *et al*.)1985) and 4.92 pg in *F. heuffelii* by Siljak-Yakovlev *et al*. (2010). Changes in the genome size (increases or decreases) may have participated in the genus diversity and evolution (Seijo and Fernandez 2003). Cytogenetical investigations as a valuable method have been Significantly performed in phylogenetic relationships amongst plants; and its obtained information has been of appreciable value in understanding taxon evolution and interrelations. These results may provide relevant information for *F. assa-foetida* L. breeding studies.

ACKNOWLEDGEMENT

Authors gratefully thank Tarbiat Modares University, Tehran, Iran, for their support to the study.

AUTHOR CONTRIBUTION

N. Firoozi carried out the experiments under the supervision of Prof. G. Karimzadeh and the advisory of Assist. Prof. M. S. Sabet. The manuscript prepared and revised by V. Sayadi. All authors approved the final manuscript.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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