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Population structure and genetic diversity of *Ferula gummosa* (Apiaceae) in North Khorasan, Iran: Insights from morphological and ISSR markers

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Keywords: DNA extraction, genetic distance, ISSR, marker, morphology.

Summary. In the watershed of North Khorasan, Iran, *Ferula gummosa* is a perennial plant with deep roots whose presence stabilises the soil texture. The study of its molecular and morphological characteristics is crucial to find effective methods to protect it and prevent its extinction. *Ferula gummosa* is a species native to Iran and an important medicinal plant in Iranian traditional medicine. The morphological and genetic diversity of 6 populations of this species was studied in North Khorasan. Multivariate statistical analyses were performed on 15 quantitative and qualitative traits selected for biometric and morphological studies. Four primers (ISSR13, ISSR826, ISSR810, ISSR7) were used to assess genetic diversity, which resulted in 19 bands. According to the present study, the morphological traits and ISSR molecular data are useful to differentiate the studied population of *F. gummosa*. Both the quantitative and qualitative morphological characteristics of the studied species can be used to differentiate between populations. In the present study, a high degree of genetic diversity between populations was found. Using morphological and molecular data, WARD cluster analysis provided important information on community relationships. Based on the results of this study, there is a high degree of diversity between the existing ecotypes of *F. gummosa*. Due to the fact that the ecotypes of *F. gummosa* originate from different geographical regions, the morphological diversity confirms that the morphological differences between the samples are not only due to environmental influences but also to genetic influences.

Структура популяций и генетическое разнообразие *Ferula gummosa* (Apiaceae) в Северном Хорасане, Иран: выводы с использованием морфологических и ISSR-маркеров

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Ключевые слова: выделение ДНК, генетическая дистанция, маркер, морфология, ISSR.

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Аннотация. В водоразделе Северного Хорасана (Иран) *Ferula gummosa* – многолетнее растение с глубокими корнями, присутствие которых стабилизирует текстуру почвы. Изучение молекулярных и морфологических характеристик вида имеет решающее значение для поиска эффективных методов его защиты и предотвращения его исчезновения. *Ferula gummosa* – вид, произрастающий в Иране, и важное лекарственное растение в иранской традиционной медицине. Морфологическое и генетическое разнообразие 6 популяций этого вида было изучено в Северном Хорасане. Многомерный статистический анализ был проведён по 15 количественным и качественным признакам, выбранным для биометрических и морфологических исследований. Для оценки генетического разнообразия использовали четыре праймера (ISSR13, ISSR826, ISSR810, ISSR7), что привело к получению 19 полос («бэндов»). Согласно настоящему исследованию, морфологические признаки и молекулярные данные ISSR используются для дифференциации изучаемой популяции *F. gummosa*. Как количественные, так и качественные морфологические характеристики изучаемых видов могут быть использованы для дифференциации популяций. В настоящем исследовании была обнаружена высокая степень генетического разнообразия между популяциями. Используя морфологические и молекулярные данные, кластерный анализ WARD предоставил важную информацию о взаимоотношениях в сообществе. Основываясь на результатах настоящего исследования, существует высокая степень разнообразия между существующими экотипами *F. gummosa*. В связи с тем, что экотипы *F. gummosa* происходят из разных географических регионов, морфологическое разнообразие подтверждает, что морфологические различия между образцами обусловлены не только влиянием окружающей среды, но и генетическим влиянием.

Introduction

Ferula L. belongs to the family Apiaceae Lindl. with 172 species in the world and 30 species in Iran, so that 15 species are endemic (Mozaffarian, 1984). The most important species of this genus is *Ferula gummosa*, whose extract is widely used in Iran. Its flowers are yellow and complex in the form of compound clusters with high density. This plant grows at an altitude of 1000–2000 m with a rainfall of about 250–400 mm. This genus is a perennial plant. In the first few years it grows vegetatively and forms ring-shaped leaves. In the last year, it turns into a stem in which flowers and fruits are formed, then the root rots and the plant die. The root of this plant is massive and has a fibrous tissue that penetrates the soil to a depth of 1.5 meters. The skin of the root is brown and 1 to 3 mm thick. The diameter of the root is 6 to 17 cm (Ghahreman, 2003). *Ferula gummosa* is a valuable forage plant that is widespread in mountainous regions, especially in the Zagros and Alborz mountain ranges in the Iranian and Turanian vegetation regions. This plant is native to the eastern region of Iran (Mozaffarian, 1984).

Nowadays, DNA molecular markers are widely used to study the genetic diversity of plant and animal communities. The ISSR method, for example, is widely used and has been shown to be useful for analysing genetic diversity (Williams et al., 1990; Tahmasebi, Nasrollahi, 2021). Considering the importance of genetic diversity for the advancement of breeding programs, it will be important to study this diversity using molecular and morphological methods. Genetic diversity in plant populations can arise through various mechanisms such as mutation, sexual recombination, migration and gene flow, genetic drift and selection. By studying genetic diversity in plants using molecular methods, it is possible to understand the extent of diversity in plant populations.

Mustafina et al. (2021) studied the morphological characteristics of the fruits of *Ferula* species. This research was carried out with the aim of identifying the species groups of the genus *Ferula* from the point of view of economic, complex, molecular and chemical classification.

Some researchers investigated the genetic diversity of *F. gummosa* communities in Iran using RAPD molecular markers. In this study, RAPD was used to determine the genetic diversity of 13 populations. The cluster analysis divided the different populations into three main groups. The results of this study show the effectiveness of this method in determining the genetic diversity of the studied *F. gummosa* populations (Talebi Kohyakhy et al., 2008). A group of researchers used AFLP markers to study *F. gummosa* ecotypes from different regions of Iran. Their results revealed significant genetic diversity between ecotypes. This genetic diversity correlates with differences in essential oil composition, confirming that phytochemical variations are controlled by genetic factors (Khounani et al., 2011). Elibol et al. (2012) conducted a phylogenetic study of *Ferula* species using nuclear sequencing in Turkey and showed that this genus is monophyletic. *Ferula* has long been considered a monophyletic genus because its members are similar in habitat and morphology, but recent molecular studies have shown that there are inconsistencies in the taxonomy of the high and low

levels. For example, Kurzyna-Młynik et al. (2008) found that *Ferula* appears to be multiple in recent molecular systematic research based on nrDNA ITS data.

In light of the paucity of research on *F. gummosa* in Iran and its wide distribution in North Khorasan province, the main objectives of this study are as follows: 1) to identify the population genetic structure and gene flow in six local populations of this species using ISSR molecular markers; 2) to investigate the morphological diversity of these populations in northeastern Iran, and 3) to compare the genetic diversity shown by ISSR and morphological data. This information can be used for conservation, breeding and sustainable management of this indigenous plant species.

Materials and Methods

Plant material

The genetic and morphological data analysed in the present study were based on 18 samples from six populations (three plants from each population) of *F. gummosa* from different parts of North Khorasan province (Table 1, Fig. 1).

DNA extraction and ISSR assay

DNA extraction from the leaves of the samples was performed using a DNA extraction kit (Tiangen, Korea) for plants. A Nanodrop device was used to determine the quantity and quality of DNA. Four ISSR primers were used (Table 2). The PCR amplification reaction was performed in a reaction mix of 25 µl volume containing 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 1 mM dNTP mix (Cinna GenCo, Iran), 0.2 µM primers, 1 U Taq DNA polymerase-500, and 15–40 ng of sample DNA. ISSR-PCR was performed in a thermocycler (Bio-Rad, USA) for 40 cycles consisting of denaturation at 94 °C for 60 second, annealing at 56 °C for 60 seconds, extension at 72 °C for 90 seconds and 72 °C for 3 minutes for the final extension. The PCR products were visualized on a 2 % agarose gel followed by ethidium bromide staining. The size of the fragments was determined using a 100 bp molecular ladder (Fermentas, Germany). The experiment was repeated three times, and the resulting stable ISSR bands were used for subsequent analyses.

Data analyses

Morphological analysis

Morphological studies were carried out on six populations in Iran (Figs. 2, 3, 4). By studying the species description in the flora and conducting the necessary investigations, 15 quantitative and qualitative traits were selected for biometric studies and examined using Dino-Lite model AM413 digital stereomicroscope and Olympus model B×51 light microscope. Qualitative traits were coded in two or more modes to perform multivariate statistical analyses. For quantitative traits, mean values in population units were used. Morphological characteristics were standardized (Mean = 0, variance = 1) and used to estimate Euclidean distance for clustering and ordination analyses (Podani, 2000). Quantitative and qualitative traits were entered into SPSS Ver. 16 software (1988) and multivariate statistical analyses were performed. Cluster analysis and ranking methods based on principal components (PCA) from factor analysis

Table 1. Investigated *Ferula gummosa* populations

Voucher No.	Altitude (m)	Latitude	Longitude	Locality	Pop
803700(GKUH)	1326	37°58'35"	57°28'48"	North Khorasan Province: Raz and Jargalan, Taklah Quz	1
803701 (GKUH)	1378	37°58'44"	57°12'33"	North Khorasan Province: Raz and Jargalan, Tazeh Qaleh	2
803702 (GKUH)	1270	37°45'47"	57°32'33"	North Khorasan Province: Bojnord, Kuh Kamar	3
803703 (GKUH)	1310	37°45'32"	57°32'29"	North Khorasan Province: Bojnord, Baghjiq	4
803704 (GKUH)	1720	37°33'23"	56°55'11"	North Khorasan Province: Maneh and Samalqan Ashkhaneh	5
803705 (GKUH)	1745	37°33'40"	56°55'46"	North Khorasan Province: Maneh and Samalqan, Pishqaleh	6

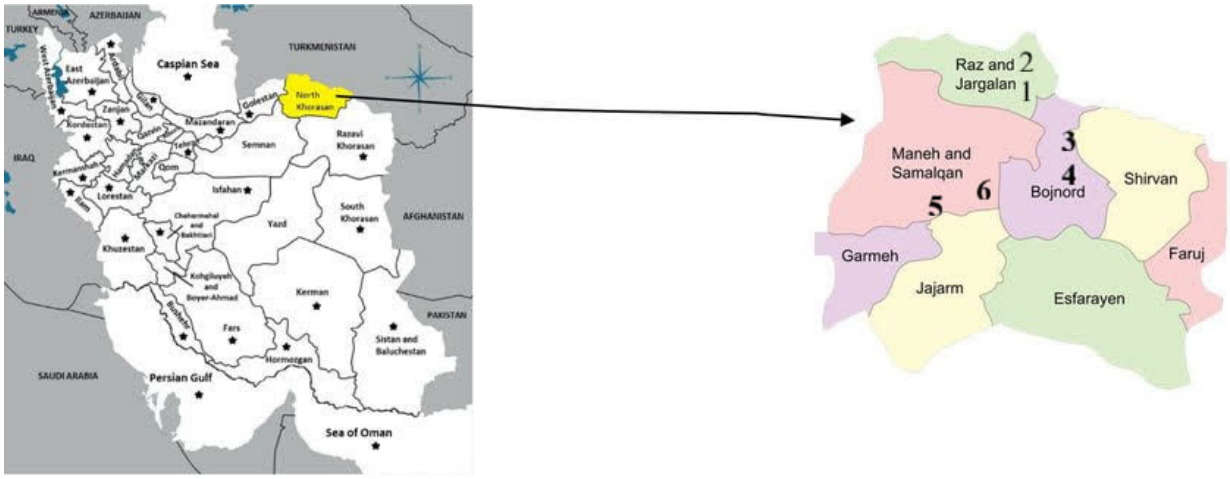

Fig. 1. Distribution map of the studied *Ferula gummosa* populations in North Khorasan province. Populations are marked with numbers from 1–6 according to the Table 1.

Table 2. The used ISSR primers

Sequences	Code
(ACG) 6Ga	ISSR13
CCCGGATCC (CA)8	ISSR826
(GA) 8T	ISSR810
(AC) 8AG	ISSR7

were used to determine the degree of relatedness between species and population units. Factor analysis was performed to determine the most diverse characteristics between population units. For clustering of the plant specimens, UPGMA (unweighted paired group using average) method was used. PAST version 2.17 (Hamer et al., 2012) was used for multivariate statistical analyses of morphological data. Table 3 shows the list of quantitative and qualitative characteristics used.

Molecular analysis

The obtained ISSR bands were treated as binary traits (presence = 1, absence = 0). Genetic diversity parameters were calculated for each population, e.g. Nei genetic diversity (H), Shannon information index (I), number of effective alleles and percentage of polymorphism (Freeland et al., 2011). Nei's genetic distance was used for clustering (Weising et al., 2005). Neighbour Joining (NJ), WARD and UPGMA (Unweighted Paired Group using average) clustering as well as Principal Coordinate Analysis (PCoA) and Multidimensional Scaling (MDS) were used to group populations by 100-fold permutation (Freeland et al., 2011). The Mantel test was used to estimate the correlation between the geographical distance and genetic distance of the populations studied (Podani, 2000). PAST ver. 2.17 (Hammer et al., 2012) was used for these analyses. AMOVA (analysis of molecular variance) (with 1000 permutations), as implemented in GenAlex 6.4 (Peakall, Smouse, 2006), was used to determine the genetic differentiation of population.

Results

Morphometry

In the morphological study, six populations of *F. gummosa* species were examined. For this purpose, quantitative and qualitative traits (Table 3) were used to determine traits with higher differentiation value. A cluster analysis such as WARD (Minimum Variance Spherical Cluster) was used to determine the degree of relatedness of the species and populations. The resulting phenogram shows two main clusters (Fig. 5). The populations 5 and 6 formed the first main cluster. These populations have the same distribution area and are located in the west of North Khorasan province. Populations 1, 2, 3, 4 have morphological similarities and form the second main cluster. These populations are close to each other and are located in the north and center of North Khorasan Province.

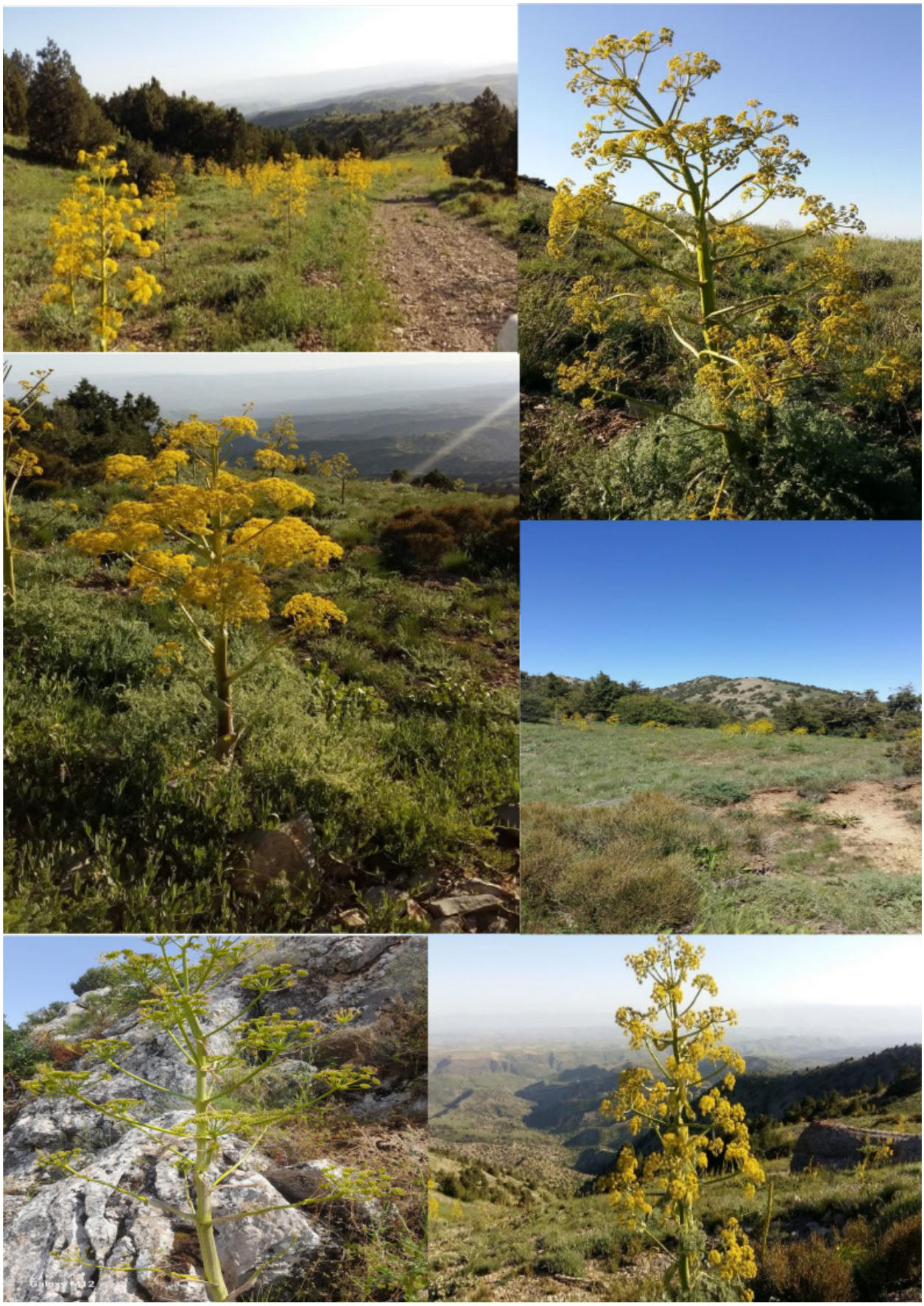

Fig. 2. The natural habitat of *Ferula gummosa*.

Fig. 3. The aerial organs of *Ferula gummosa*.

Fig. 4. A dried sample of *Ferula gummosa* in the full ripening stage.

The factor analysis method was used to determine the most diverse characteristics between species and populations, and the results obtained were used to rank the principal components of PCA (Principal Component Analysis) (Fig. 6). The resulting ranking, based on the first two principal components, shows the degree of convergence and divergence of the populations studied. When analysing the factors and comparing the respective contribution to the resulting diversity (Table 4), it was found that the first two factors cause a total of 44.87 % of the observed diversity. For the first factor (Table 5), which determines about 23.77 % of the total variation, the traits root diameter, seed length and inflorescence length have the highest correlation coefficient (< 0.7). For the second factor,

Table 3. The morphological characters

State of character and coding	Character
Cylindrical (0), Conical (1), Spindle (2)	Root Shape
(1) Dark Brown; (2) Light Brown	Root Color
Oblong (1), Elliptic (2)	Seed Shape
Brown (1), Yellow (2)	Seed Color
(mm)	Under Leaf Length
(mm)	Under Leaf Width
(mm)	Root Diameter
(mm)	Branches Diameter
(mm)	Branches Length
(mm)	Inflorescence Length
(mm)	Inflorescence Width
(mm)	Seed Length
(mm)	Seed Width
Number	Main Umbrellas
Number	Sub Umbrellas

which accounts for 21.10 % of the total variation, the traits number of secondary umbrellas, number of main umbrellas and length of basal leaf provide the highest correlation coefficient (< 0.7).

ISSR assay

The results of the DNA extraction showed an acceptable quality of the molecules obtained for use in the genetic diversity study. The gel electrophoresis results showed that the nucleic acid molecules obtained had no breaks and streaks and could be used in the polymerase chain reaction. The ISSR primers generated 19 bands. Almost all ISSR loci had excellent discriminatory power. Therefore, the ISSR markers are efficient in discriminating the studied population of *F. gummosa*. In total, 19 ISSR loci were produced, with the highest number of loci (16 bands) belonging to population 2 and 3, followed by population 1 (14 bands). A few private bands occurred in some of the population (Table 6).

Genetic diversity parameters determined in *F. gummosa* are reported in Table 7. The percentage of genetic polymorphism taken ranged from 10.51 % in population 6 to 38.77 % in population 3. A good level of genetic polymorphism (36.24 %) arised in population 2. The same populations had higher worth of gene diversity (He).

Nei, genetic distance and genetic identity determined among *Atriplex canescens* populations revealed that genetic similarity among populations ranged from 0.12 to 0.98. Population 2 and 3 show the most genetic similarity (Table 8).

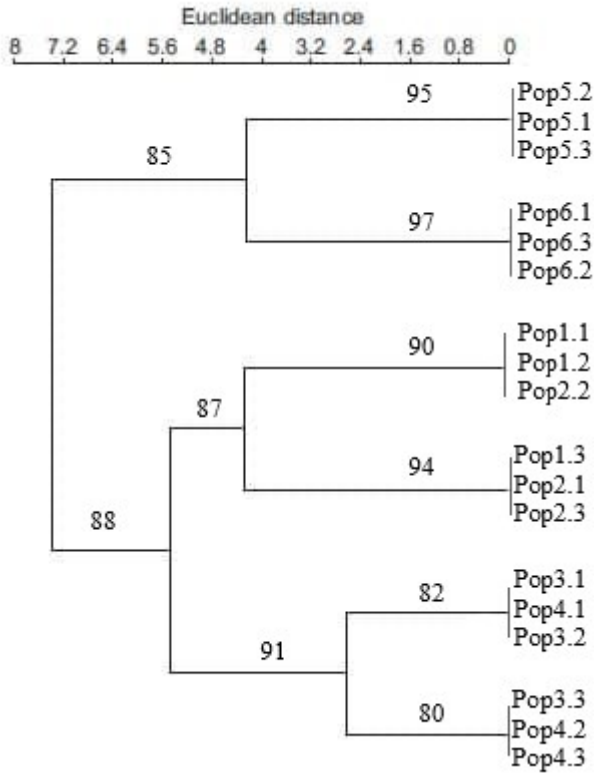

Fig. 5. UPGMA dendrogram of the studied populations based on morphological data.

The AMOVA test revealed a significant genetic difference between the studied populations (P = 0.001), indicating that the studied populations are genetically distinct. The AMOVA showed that

91 % of the total genetic variation was due to genetic differences between populations, while 9 % was due to genetic variation within species. These results indicate a high degree of genetic diversity between populations of *F. gummosa*.

The UPGMA clustering based on the ISSR data shows the existence of two main clusters (Fig. 7). Populations 1, 2, 3 and 4 show genetic similarity and form the first main cluster. On the other hand, populations 5, 6 form the second main cluster.

Table 4. Results of factor analysis based on quantitative and qualitative morphological trai

Cumulative, %	%Variance, %	Parameter
23.77	23.77	1
44.87	21.10	2

Table 5. Morphological data based on factor analysis

Factor 2	Factor 1	Character
–	0.87	Root Diameter
–	0.85	Seed Length
–	0.84	Inflorescence Length
0.74	–	Number of Sub Umbrellas
0.73	–	Number of Main Umbrellas
0.71	–	Under Leaf Length

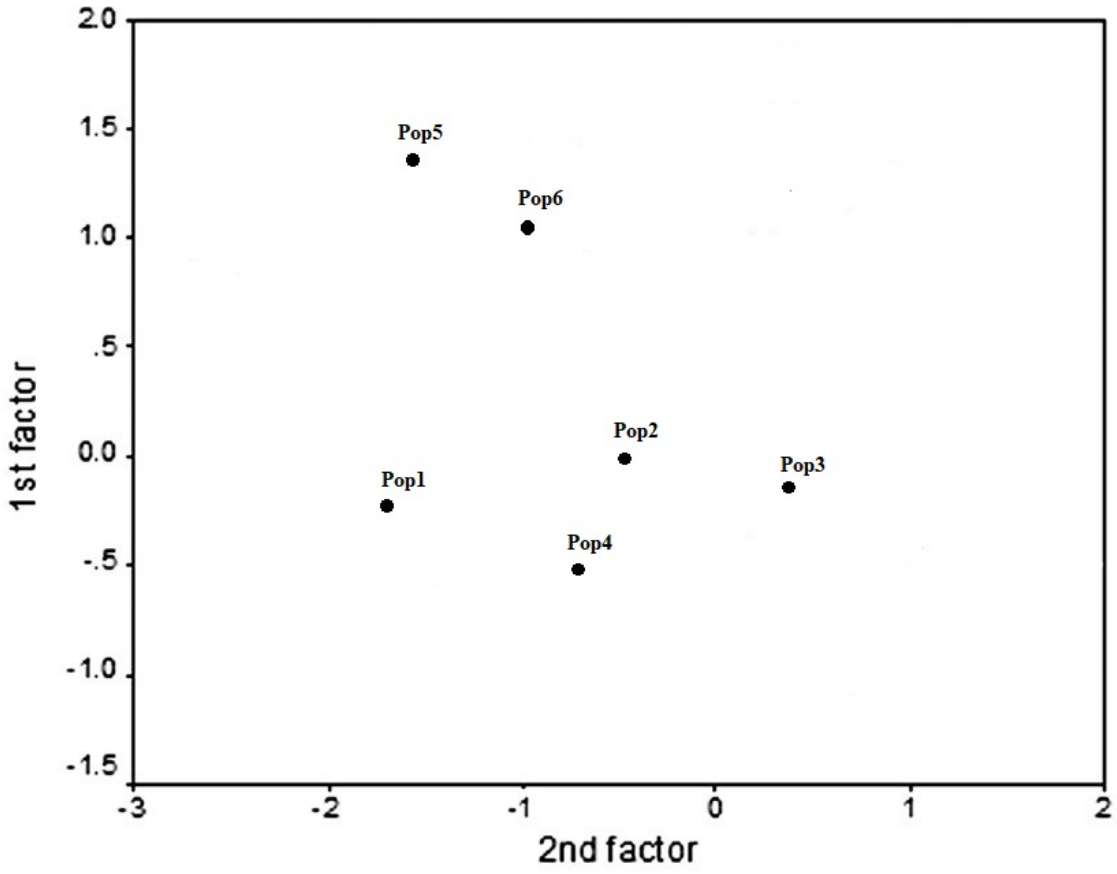

Fig. 6. PCA analysis of morphological data.

Table 6. Details of ISSR bands in *Ferula gummosa* populations (Pop. 1–6 according to Fig. 1)

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
No. Bands	14	16	16	8	9	11
No. Bands Freq. (≥ 5 %)	14	16	16	10	13	9
No. Private Bands	1	2	0	1	0	2
No. Common Bands (≤ 25 %)	0	2	3	0	1	0
No. Common Bands (≤ 50 %)	9	11	11	3	6	3

Table 7. Genetic diversity parameters determined in *Ferula gummosa* populations (Pop1–6 shown in Fig. 1)

Population	N	Na	Ne	I	He	uHe	% P
Pop1	10.000	1.024	1.186	0.175	0.113	0.119	32.03
Pop2	10.000	1.049	1.229	0.221	0.143	0.151	36.24
Pop3	10.000	1.122	1.311	0.270	0.182	0.191	38.77
Pop4	10.000	0.610	1.160	0.124	0.086	0.091	18.51
Pop5	5.000	0.902	1.231	0.199	0.134	0.149	26.52
Pop6	10.000	0.530	1.152	0.144	0.096	0.081	10.51

Notes: N = No. of studied plants; Na = No. of polymorphic alleles; Ne = effective No. of alleles; He = new gene diversity; uHe = unbiassed gene diversity, and % P = percentage of polymorphism.

Table 8. Genetic distance versus genetic identity in *Ferula gummosa* populations (populations numbers are according to Fig. 1)

Pop ID	1	2	3	4	5	6
1	***	0.8525	0.7343	0.7222	0.8262	0.7023
2	0.7646	***	0.8494	0.8515	0.8686	0.8545
3	0.4622	0.7484	***	0.7892	0.7597	0.8329
4	0.3108	0.2219	0.1551	***	0.8434	0.7202
5	0.3210	0.1674	0.1808	0.696	***	0.7070
6	0.2597	0.2354	0.3909	0.3212	0.7152	***

The relationship between the populations represented by the clusters based on morphological characteristics (Fig. 5) and molecular data (Fig. 7) is contradictory in some cases and compatible in most cases. For example, the subclades containing populations 1, 2, 3, and 4 differ in the morphological tree and the molecular tree. This is probably due to the fact that a number of selected morphological traits are mainly influenced by the environment and not by the genotype. This finding is also clearly visible in the consensus tree (Fig. 10). This tree shows that the populations studied have the same relationship in both the morphological tree and the molecular tree. For example, populations 1, 2, 3 and 4 were close to each other. The same applies to populations 5 and 6. The suitability of the ISSR markers for *F. gummosa* was also confirmed by the DCA (Determined Correspondence Analysis) diagram of these markers (Fig. 8). As can be seen from the DCA diagram, the ISSR positions obtained are sparsely distributed and not clustered. Therefore, they represent different regions of the nuclear genome in the analysed *F. gummosa* samples and can be considered as suitable genetic markers for these plants.

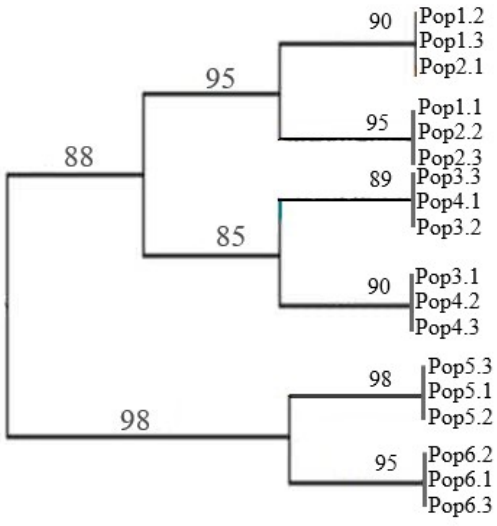

Fig. 7. UPGMA dendrogram of the studied populations based on ISSR data.

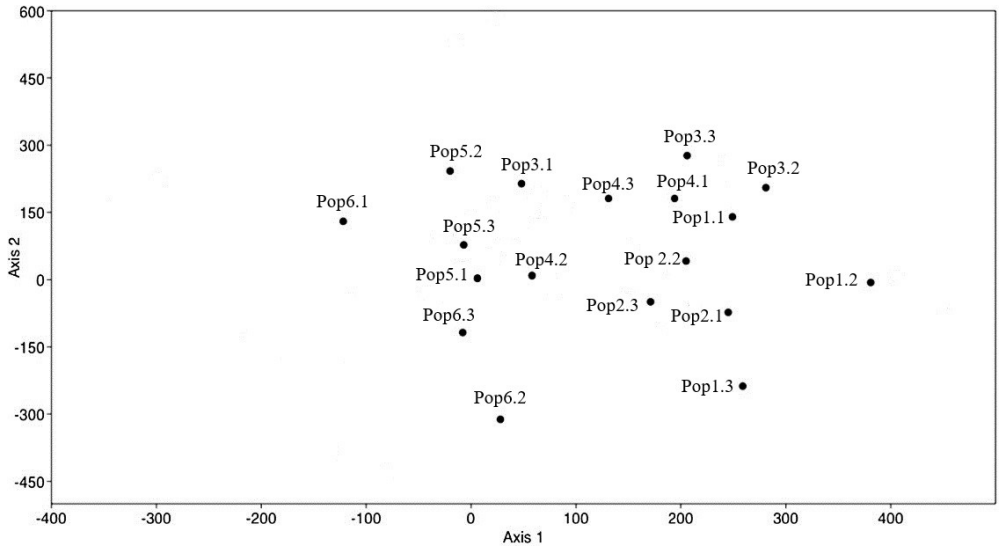

Fig. 8. DCA plot of ISSR loci in *Ferula gummosa*.

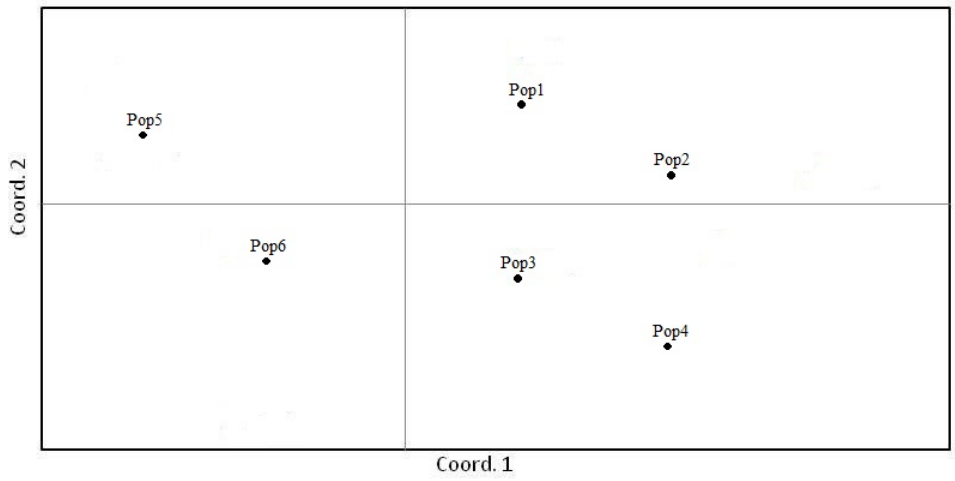

Fig. 9. PCoA plot after 1000 times permutation.

The genetic distance of the studied *F. gummosa* populations was also analysed using the PCoA diagram after 1000-fold permutation (Fig. 9). The PCoA diagram agrees with the UPGMA cluster and shows that the populations studied can be divided into two groups. Populations 1, 2, 3, and 4 form the first group, while populations 5 and 6 form the second group.

The Mantel test was performed 10,000 times and showed a significant correlation between the geographical distance and the genetic distance of the populations (r = 0.798, P = 0.02). These results show that populations which are separated from each other differ in terms of their genetic characteristics.

Discussion

The findings collectively validate the unique value of the morphological characteristics employed. The diversity in morphology plays a crucial role in the survival of plant species, facilitating their adjustment to varying environmental conditions (Nasrollahi et al., 2020). This investigation has demonstrated that both quantitative and qualitative morphological characteristics are effective in distinguishing the various populations of the species under examination.

In accordance with the findings of this investigation, Mustafina et al. (2021) demonstrated that utilizing a combination of fruit characteristics offers a more reliable indication of the genetic similarity among *F. gummosa* species compared to a singular characteristic. Their research revealed a lack of association between the microstructure of fruit surface, the shape of the mericarp, and the chemical components in *Ferula*. Instead, they argued that molecular data, rather than morphological traits and chemical information, is more effective in distinguishing between *Ferula* species at a more specific taxonomic level and elucidating their interrelationships.

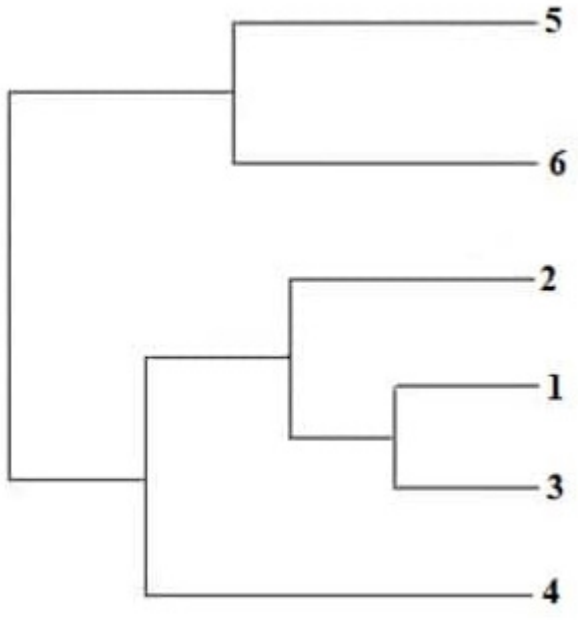

Fig. 10. Consensus tree based on morphological and ISSR dendrograms in studied populations.

Morphological evidence has confirmed the resemblance among populations 1, 2, 3, and 4. These populations are in close geographical proximity to each other, situated in the northern and central regions of North Khorasan province. Similarly, populations 5 and 6 exhibit morphological similarities, occupying a comparable distribution area in the western part of North Khorasan province. It can be deduced that the genetic and morphological characteristics of populations are influenced by environmental factors such as latitude, longitude, and altitude. Consequently, these distinct populations may form separate taxonomic groups below the species level within *F. gummosa*. Previous studies have identified different ecotypes resulting from genetic variations between populations leading to morphological divergence (Sheidai et al., 2012, 2013, 2014; Minaeifar et al., 2015, 2016; Tahmasebi et al., 2023).

Numerous factors influence the estimation of genetic relationships among individuals in the realm of popular genetics. These factors encompass the diversity of markers utilized, the genomic distribution of markers, and the evolutionary mechanisms that underlie the diversity calculated (Powell et al., 1996). The quantity of markers used plays a crucial role, as the information derived from the ISSR technique heavily relies on the number of primers employed. As demonstrated by Ellis et al. (1997), utilizing six primers combinations can justify up to 80 % of the anticipated relationships. In the present study, four primers were employed, aligning with prior research, thus ensuring reliable and consistent results can be attained (Antonio et al., 2004; Ahkami et al., 2007; Talebi Kohyakhy et al., 2008).

In this study, the extent of genetic diversity among populations was found to be 91 %. Khounani et al. (2009) observed a range of genetic similarity between genotypes varying from 56 to 87 %. It is important to note that the samples were collected from various regions of Iran. According to Melchinger (1997), the precision of estimating genetic similarity among individuals relies on the number of markers utilized, the level of polymorphism, and the genomic coverage provided by the markers.

ISSR data has verified the genetic resemblance among populations 1, 2, 3, and 4, indicating distinctiveness from other populations based on morphological characteristics. These populations are geographically proximate to each other, situated in the northern and central regions of North Khorasan province (Fig. 1). Similarly, populations 5 and 6 exhibit genetic similarity according to ISSR statistics, aligning with morphological observations, and are geographically clustered in the western part of North Khorasan province (Fig. 1). The influence of environmental factors such as latitude, longitude, and altitude on the genetic structure of populations is evident. Consequently, these distinct populations may give rise to distinct taxonomic groups within the species *F. gummosa*. Previous studies on wheat plants (*Triticum turgidum* L. and *T. durum* Desf.) have demonstrated a correlation between genetic diversity and geographical distribution, whereby genotypes from different regions and latitudes share genetic similarities (Ahkami et al., 2007; Omid Bakhsh Fard et al., 2009). In the study of Khounani et al. (2009), the analysis of ecotypes revealed that molecular diversity did not align with geographic diversity, highlighting a lack of association between molecular diversity and geographical distribution. This lack of correlation had been also noted by Koyakhi et al. in 2008.

In PCO, the less the principal components justify the percentage of changes. It means that they have a proper distribution at the genome level, for this reason, it is better for examining genetic diversity.

Therefore, according to the following statements, the results from the genetic point of view show the optimal sampling of markers from the whole genome. In this way, each of the markers used are from different parts of the genome, so they have less correlation. Messmer et al. (1992) suggested that Principal Component Analysis as a complementary method for cluster analysis will lead to optimal use and maximum information extraction from molecular data. According to the PCO plot and the cluster diagram, the distribution of genotypes in the two-dimensional axis is consistent with the dendrogram.

The results of this study show high diversity among the population groups of *F. gummosa*. These genetically distinct populations belong to different geographical areas, so it can be concluded that the existence of diversity is not only due to the environmental effect but is also controlled by genetic factors. In this study, it was proved that the ISSR method is fast and reliable, which identifies many gene loci. So that this will be difficult or impossible with other methods at the same time. 'The study of Powell et al. (1996) also showed that this technique is an effective and powerful method due to its high repeatability and the large number of gene locations it evaluates in a short time with one test. Because repeatability is the only simple method to measure the quality of the indicator, as a result of which it is possible to achieve correct clustering for different samples based on the resulting data. The results of the present research can help in the management of *F. gummosa* germplasm. However, given the very small sample size (n = 3 per population), this result must be interpreted as preliminary and highlights the need for future studies with larger sample sizes to confirm the robustness of this pattern.

Conclusion

This study demonstrated that the morphological and statistical properties of ISSR are a useful investigation in the differentiation of *F. gummosa* populations. Every aspect of fitness and quality is important and relevant for distinguishing populations of evolving species. Analysis showed that in the first stage of development, Root Diameter, Seed Length and Length of Inflorescence showed a positive coefficient, while in the second stage, Number of Sub Umbrellas, Number of Main Umbrellas and Under Leaf Length showed a positive coefficient. Current observations indicate a high degree of heterogeneity among populations in *F. gummosa*.

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