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Original Article

Genetic Diversity of Iranian Cumin (*Cuminum cyminum* L.) Accessions, using Inter-Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) Markers

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Abstract

Cuminum cyminum L. (cumin) is an aromatic plant, commonly used in food industries and traditional medicine, especially in tropical Asia. Various accessions of *C. cyminum* with different aromatic properties could be found in Iran, as a main region of cumin production. This study was conducted to evaluate genetic diversity of 22 accessions of *C. cyminum* from different parts of Iran. The seeds were cultivated in a randomized complete block design (RCBD), with 22 accessions and three replicates, and their agro-morphological traits were measured. Genetic variations of the studied accessions were evaluated using inter simple sequence repeat (ISSR) and start codon targeted (SCoT) markers. Estimate of molecular variance showed a significant genetic difference between the studied accessions, whereby 57% of total variance was occurred between the populations. Based on the Mantel test for association of genetic diversities and geographical distances, increase of geographical distance did not influence the genetic differentiation. Significant differences were observed between the studied agro-morphological traits, other than the number of branches. Canonical correspondence analysis of genetic features and environmental factors, including five geographic and climatic factors of the seed's origin, showed significant influences of altitude and latitude on genetic variation of the studied accessions. However, despite the observed genetic variations, the studied cumin accessions, are not totally isolated and hence some amount of gene flow has been occurred between them. Therefore, no isolation by distance exists between the studied accessions. Generally, the results confirmed that both ISSR and SCoT markers were reliable and useful tools for analyzing the genetic diversity of cumin in Iran.

Keywords: Genetic difference, Genetic markers, Medicinal and aromatic plants, Molecular variance

Introduction

Cumin (*Cuminum cyminum* L.) is the second most popular spice in the world and belongs to Apiaceae (Umbelliferae) family, which is considered as a medicinal and economical plant [1,2], especially in tropical Asia [3]. The cumin seed is widely used in food and medicine purposes, because of its phytochemical c
omponents, minerals (such as Fe, Cu, Ca, K, Mg, Se, Zn and Mn), vitamins, and many flavonoid phenolic anti-oxidants [4,5]. Geographical distribution of cumin in Iran is mainly confined to arid and semi-arid regions in eastern, south-eastern, and central provinces [3,6].
Estimation of genetic diversity of various plant cultivars is necessary for utilization and conservation of plant genetic resources [7,8]. On the other hand, morphological and molecular surveys are used to study genetic diversity of genotypes and ecotypes of different crop plants, for upgrading of the cultivars or varieties in conservation and breeding programs [9]. Molecular

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markers are widely used in assessment of genetic diversity, phylogenetics, fingerprinting, variation and differentiation in biology [10-12]. Molecular markers, as reliable tools in detecting the polymorphism at DNA level, could be helpful in estimation of genetic relationships within and between species and ecotypes [13]. DNA fingerprinting of all genetic stocks is imperative to prepare a powerful molecular database for medicinal plants [13]. The most important characteristic of molecular markers is neutrality, which is not influenced by age, environment, economical beneficiary and is more informative than the morphological traits [14,15]. Inter simple sequence repeat **(**ISSR**)** marker is preferred by many researchers due to many advantages such as simplicity, quickly, less costly and high reproducibility [16,17]. Start codon targeted (SCoT) polymorphisms are other types of reproducible markers, which can be used as strong polymorphic markers for detection of differences between individuals [18].

Variation of cumin ecotypes can be investigated using agro-morphological traits [19] and molecular markers, such as RAPD, SCoT, CCMP, ISSR and SSR [19-22]. Each genetic marker has a specific ability for determination of genetic variations. On the other hand, understanding of genetic diversity is necessary to plan conservation and breeding programs for native populations. The present study was conducted to evaluate agro-morphological traits and genetic variations of Iranian cumin accessions, using ISSR and SCoT markers.

Materials and Methods

Plant Materials

A total of 22 cumin accessions were collected from nine Iranian provinces (Fig. 1, Supplementary 1). Accession numbers, origins and ecological conditions of the collected accessions are presented in the Table 1. The seeds were cultivated in an agricultural research field, based on a randomized complete block design (RCBD), with 4 m long plots, 50 cm row spaces and 5 cm distances between the plants. The research field was located in Semnan province, Iran, 53° 23' 20.077" W, 35° 35' 0.752" N, with altitude 1165 m, average temperature 172 °C and annual precipitation 130 – 145 mm.

Agro-morphological traits, including plant height, numbers of branches, umbels, mini-umbels and seeds, 1000-seed weight, seed yield per plant and grain yield, were measured by morphometric methods. The photos of the studied accessions are illustrated in Supplementary data 1.

DNA Extraction and Markers Assessment

Genomic DNA was extracted from young leaves, using the cetyltrimethylammonium bromide method [23], with some modifications as described by Ghasemzadeh-Barkaki *et al*. [24]. The DNA quality was evaluated using 0.8% agarose gel electrophoresis. Genetic diversities of the studied samples were investigated using 10 genetic markers, including five SCoT and five ISSR primers (Table 2). The SCoT primers were selected from those studied by COLLARD and MACKILL (2009). The studied primers were provided by Invitrogen, Thermo Fisher Scientific Co., USA. Polymerase chain reaction was performed in 25 µL reaction volumes, containing 10 mM Tris-HCl, pH 8.3, 2.5 mM Mg Cl_2, 1 mM dNTPs mix, 0.2 µM primer, 1U *Taq* DNA polymerase, 25 ng template DNA and distilled water. Polymerase chain reactions (PCR) were run on a Bio-Rad T100 thermocycler (Bio-Rad Laboratories Inc., USA) using the programs presented in Table 3.

Data Analysis

Analysis of variance (ANOVA) for the agro-morphological data was carried out using three plants per accession, by SPSS 19.0 software. The obtained ISSR and SCoT bands were coded as binary characters (absence = 0, presence = 1). Genetic diversity parameters, including allele diversity [7], Nei's gene diversity, Shannon information index, number of effective alleles, and genetic polymorphism percentage were determined for different populations. Nei's genetic distance was used for clustering the populations [7, 25]. Neighbour Joining clustering were used for grouping [25].

The Mantel test was performed to check correlation between geographical and genetic distances of the studied accessions [26]. PAST ver. 2.17 [27] and GeneALEx 6.4 [28] programs were used for these analyses.

The Pearson coefficient of correlation was determined between geographical features (longitude and latitude) and genetic diversity parameters. Genetic differentiation of the studied accessions was studied by the analysis of molecular variance (AMOVA) test (with 1000 permutations) as performed in GenAlex 6.4 [28].

Canonical correspondence analysis (CCA) was done using the PAST software v. 2.17 [27] to determine the relative importance of geographical factors in the spatial organization of genetic diversity between the studied accessions.

The DARwin program (Version 5) was used to compare the accessions which are genetically and agro-morphologically differentiated from the others [28]. A consensus tree was conducted from the obtained agro-morphological and genetic trees.

Table 1 Geographic information of the studied cumin accessions

Row	Province	Location	Accession No.	Longitude	Latitude	Elevation (m)	AMT (°C)	AP (mm)
1	Razavi-Khorasan	Khaf	100	60° 8' 10.509"	34° 34' 17.431"	972	16.7	200
2	Razavi-Khorasan	Khoshab	101	57° 59' 26.287"	36° 25' 30.785"	1187	13.8	207
3	South-Khorasan	Chah-Dashi	102	59° 43' 20.238"	31° 28' 14.567"	1065	20.05	86
4	South-Khorasan	Ayesk	103	58° 22' 58.742"	33° 53' 14.868"	1368	16.7	135
5	East-Azarbayjan	Tabriz- Ilkhchi	104	45° 58' 35.354"	37° 56' 19.467"	1308	11.6	318
6	East-Azarbayjan	Shabistar-Shendabad	105	45° 37' 44.730"	38° 8' 39.281"	1309	10.9	320
7	Isfahan	Khur and Biabanak	106	55° 5' 6.655"	33° 46' 25.764"	831	20.2	79
8	Isfahan	Mobarakeh-Talkhuncheh	107	51° 33' 36.569"	32° 15' 44.604"	1731	15.1	154
9	Kerman	Rig-Mahan	108	57° 17' 29.504"	30° 3' 40.064"	1901	15.4	156
10	Kerman	Kuhbanan	109	56° 16' 58.527"	31° 24' 37.072"	1990	14.1	146.1
11	Yazd	Yazd	110	54° 21' 20.827"	31° 53' 15.208"	1222	18.9	55
12	Semnan	Dlązyạn	111	53° 24' 23.810"	35° 29' 53.300"	1042	17.2	130
13	Semnan	Damghan	112	54° 20' 27.193"	36° 9' 49.639"	1154	15.4	213
14	Semnan	Sorkheh-Biabanak	113	53° 16' 6.488"	35° 24' 42.841"	1034	17.4	129
15	South-Khorasan	Birjand1	2001	59° 13' 0.940"	32° 52' 24.009"	1454	17	129
16	South-Khorasan	Birjand3	2003	59° 13' 0.940"	32° 52' 24.009"	1454	17	129
17	South-Khorasan	Boshruyah	2004	57° 25' 41.322"	33° 52' 6.185"	879	19.7	96
18	South-Khorasan	Ferdows	2005	58° 10' 0.473"	34° 0' 20.273"	1269	17.2	130
19	South-Khorasan	Nehbandan	2006	60° 3' 1.431"	31° 32' 26.144"	1185	18.4	93
20	South-Khorasan	Faizabad	2007	58° 47' 33.697"	35° 1' 10.119"	942	26	220
21	Tehran	Tehran	2008	51° 22' 50.599"	35° 42' 2.604"	1214	16.4	220
22	North-Khorasan	Bojnord	2014	57° 19' 44.809"	37° 28' 20.124"	1071	13.2	257

AMT (Ambient temperature); AP (Average precipitation).

Table 2 The primers used for polymerase chain reaction.

Primer	Sequence 5′-3′	%GC
ISSR810	GAGAGAGAGAGAGAGAT	47
ISSR834	AGAGAGAGAGAGAGAGYT	44
ISSR(AGC)5GG	AGCAGCAGCAGCAGCGG	70
ISSR(AGC)5GC	AGCAGCAGCAGCAGCGC	70
ISSR(GA)9C	GAGAGAGAGAGAGAGAGAC	52
SCoT1	CAACAATGGCTACCACCA	50
SCoT2	CAACAATGGCTACCACCC	55
SCoT3	CAACAATGGCTACCACCG	55
SCoT7	CAACAATGGTCACCACGG	56
SCoT18	ACCATGGCTACCACCGCC	66

Table 3 PCR programs for the studied primers

Primer	Step	Temperature	Duration	Cycle
ISSR	Initial denaturation	94 ˚C	5 min	1
	Denaturation	94 ˚C	1 min	-
	Annealing	57 ˚C	1 min	40
	Extension	72 ˚C	2 min	-
	Final extension	72 ˚C	10 min	1
SCoT	Initial denaturation	94 ˚C	5 min	1
	Denaturation	94 ˚C	1 min	-
	Annealing	60 ˚C	1 min	36
	Extension	72 ˚C	1.5 min	-
	Final extension	72 ˚C	10 min	1

Results

Phenotypic Data

ANOVA results showed significant differences among the accessions for all the studied traits except number of branches (Table 4), which indicated high genetic diversity among the studied accessions. Plant height in Boshruyah (28.39 cm) was significantly higher than that in other experimental accessions. The Tabriz- Ilkhchi with 16.66 umbels represented the maximum value as compared with other accretions, whereas its lowest amount (3.33 umbels) was observed in Faizabad. Like umbels, mini-umbels in Tabriz- Ilkhchi with 47.66 umbels was higher as compared to other accessions. Among all accretions, Khoshab represented the highest seed number (127 seeds). The maximum 1000-seed weight was observed in Mobarakeh-Talkhuncheh to be 4.30 g. Seed weight per plant in Rig-Mahan (0.16 g) was higher as compared with other accessions. In addition, Rig-Mahan showed the highest grain yield to be 162.23 kg ha^{-1} (Table 5).

In the WARD tree of morphological characters, two major clusters were formed. Whereby, the accessions 1, 2, 3, 4, 6, 8, 10, 11, 18, 19, 20 showed morphological similarity and were placed in the first major cluster, while accessions 5, 7, 9, 12, 13, 14, 15, 16, 17, 21, 22 formed the second major cluster (Fig. 2). Principal components analysis (PCA) biplot supported the grouping result, made by WARD tree and also revealed that these two groups were separated based on agro-morphological traits, including seed weight per plant, grain yield and number of seed per plant (Fig. 3). According to the PCA, 76 % of total variance was explained by the first two components.

Fig. 1 Distribution map of the studied cumin accessions in Iran

Seed weight per plant and grain yield had the highest positive correlation (>0.54) with the first principal component. Number of mini-umbels in umbel and the number of seeds per plant had the highest positive correlations (>0.42) with the second PC.

Molecular Markers Polymorphism in the Studied Accessions

Genetic diversity parameters determined for ISSR, SCoT and combined ISSR-SCoT data markers are presented in Table 6. The highest values for effective number of the alleles (1.19), Shannon Information Index (0.16) and gene diversity (0.11) were observed in the accession 10 (Kuhbanan), while the lowest levels of the effective number of alleles (1.02), Shannon Information Index (0.02) and expected heterozygosity as genetic diversity (0) were occurred in the accession 16 (Birjand 1).

Different results of genetic diversity were obtained by the SCoT and ISSR methods. So that in some accessions the SCoT markers were more efficient than the ISSR marker and in other accession, a different result was obtained. For example, polymorphism percentages in the accessions 1 (Khaf), 4 (Ayesk), 5 (Ilkhchi), 6 (Shabistar), 7 (Khur), 9 (Rig), 11 (Kuhbanan), 12 (Dlazyan) and 13 (Damghan), obtained by the SCoT markers were higher than the ISSR markers, while in other accessions the ISSR markers showed higher polymorphism percentage values. A similar status was exhibited for other genetic diversity parameters (Table 5).

The AMOVA results showed a significant genetic difference between the studied accessions (PhiPT=0.56, *P*=0.01). This analysis indicated that 57 % of total variation was due to diversity within accession and 43% was due to genetic differentiation between the populations (Fig. 4). Significant differences between the studied populations were also observed by pairwise AMOVA.

Intra and inter-accessions variations was also observed in principal coordinates analysis (PCoA) plot for the combined SCoT-ISSR data. The grouping of the accessions obtained by these two procedures produced two major clusters (Fig. 5). The accessions Khaf, Khoshab, Chah-Dashi, Ayesk, Ilkhchi, Shabistar, Khur, Mobarakeh, Rig, Kuhbanan, Dlazyan, Damghan, Sorkheh and Nehbandan comprised the first major cluster. In this cluster, the accessions Dlazyan, Damghan, Sorkheh and Nehbandan showed a higher genetic similarity and were joined to each other. The same holds true for the accessions Khur, Mobarakeh, Rig and Kuhbanan. The Shabistar was the most distinct accession in this cluster. In the other major cluster, the accessions Yazd, Birjand 1, Birjand 3, Boshruyah, Ferdows, Faizabad, Tehran, and Bojnord showed higher genetic similarities and were joined to each other (Fig. 5). The photos of banding pattern in ISSR and SCoT analysis are given in Supplementary data 2.

Environmental Factors and Genetic Diversity

Based on the Mantel test, which was performed between genetic and geographical distances of the origin location of the studied accessions, the results showed no significant correlations (R2=0.005, Fig. 6), which have been indicated an increase in geographical distance of collected seeds of accessions did not influence in genetic differentiation. Therefore, no isolation by distance was observed between the samples.

The main aim of the present study was to compare the effect of different environmental factors on the genetic features of the cumin ecotypes; thus, five geographic and climatic factors of the seed's origin habitat were examined. The CCA biplot of the genetic features and environmental factors showed that the mentioned parameters had no strong effect on the plant genotypes (Axis 1= 32.1% and Axis 2= 28.7% of the total variance), (Fig. 7). However, it seems that the genetic clustering in two major groups is considerably affected by elevation and latitude of the ecotype's origins. The clustering of accessions Ilkhchi and Shabistar (East Azarbayjan province) was related to influence of altitude. These two accessions had higher altitude than the other accessions. Also, longitude influence the grouping of the accessions Mobarakeh, Rig, Kuhbanan and Damghan.

Combined Molecular and Morphological Results

The consensus tree of both molecular and morphological data is presented in Figure 8. The accession specimens Chah-Dashi, Damghan, Ferdows and Nehbandan formed separate clusters. These accessions belong to the South Khorasan province, except ecotype Damghan which belongs to the Semnan province. The results differed from the other studied populations in both genetic and morphological features. This result indicates that genetic background is main cause of the agro-morphological differences in these four accessions.

Table 4 Analysis of variance for the agro-morphological traits in the studied accessions

Source	df	Mean of Square						
		PH	NU	NMU	NS	SW	SWP	GY
Rep	2	1.93^{ns}	10.65^{*}	60.36^{ns}	87.31^{ns}	0.82^{*}	0.002^{ns}	1939.48^{ns}
Accession	21	66.82^{*}	13.68^{*}	196.90^{*}	2843.71^{*}	1.02^{*}	0.035^{*}	35142.63^{*}
Error	42	2.36	2.56	25.36	240.36	0.12	0.004	3880.77
CV (%)		2.3	2.4	2.7	3.3	1.8	3.7	3.9

df: degree of freedom; ^{ns} and *: non-significant and significant at 5%, respectively; PH: plant height; NU: number of umbels; NMU: number of mini-umbels; NS: number of seeds; SW: 1000-seed weight; SWP: seed weight per plant; GY: grain yield; CV: coefficient of variation of the residual effects.

Table 5 Agro-morphological traits of the experimental accessions of Iranian cumin

Accession	PH (cm)	NU	NMU	SN	SW (g)	SWP (g)	GY (kg/ha)
Ayesk	16.6 bc	8.33 de	29.66 b-d	72.33 b-e	3.28 bc	0.14 a-c	137.63 b
Boshruyah	28.39 a	13 a-c	3.66 f	16 h	2.89 b-e	0.12 b-e	114.8 b-d
Sorkheh-Biabanak	14.39 c-e	6.66 d-f	2 4d	55.33 d-g	2.42 ef	0.093 ef	118.33 b-d
Birjand1	11.01 c-e	9 c-e	32 b-d	98.67 ab	3.09 b-d	0.136 a-d	119.1 b-d
Birjand3	13.96 c-e	10.3 b-e	30.33 b-d	94.33 bc	3.06 b-e	0.133 a-d	120.87 b-d
Chah-Dashi	14.50 c-e	9.33 c-e	33.33 b-d	91.67 bc	2.75 c-f	0.103 d-f	112.67 b-d
Damghan	14.38 c-e	11 b-d	42.33 a-c	87.33 b-d	2.81 b-e	0.11 b-f	108.77 cd
Dlązyạn	17.45 b	7.33 d-f	27.33 cd	69C b-e	3.09 b-d	0.116 b-e	114.97 b-d
Faizabad	12.88 d-f	3.33 f	9 ef	27 gh	3.07 b-e	0.123 b-e	118.33 b-d
Ferdows	15.72 b-d	6 ef	20 de	56.67 d-g	2.72 c-f	0.143 ab	121 b-d
Tabriz- Ilkhchi	13.07 d-f	16.66 a	47.66 a	123.67 a	2.56 d-f	0.096 ef	94.97 de
Khaf	12.33 ef	8.33 de	30.66 b-d	73.67 b-e	2.42 ef	0.11 b-f	110.37 b-d
Khur and Biabanak	13.22 d-f	9.66 c-e	28 cd	56.67 d-g	2.74 c-f	0.106 c-f	104 de
Khoshab	15.44 b-d	14.33 ab	44 ab	127 a	2.67 c-f	0.06 g	61.2 f
Kuhbanan	13.70 c-f	6.66 d-f	23.33 d	53.67 e-g	2.61 d-f	0.113 b-f	110.2 b-d
Mobarakeh-Talkhuncheh	15.26 b-f	9 c-e	30.33 b-d	76.33 b-e	4.30 a	0.1 ef	98.5 de
Nehbandan	16.30 bc	8.66 c-e	27 d	56.67 d-g	3.0 b-e	0.126 b-e	118.43 b-d
Rig-Mahan	15.20 b-e	7.66 de	25.66 d	65.33 c-f	3.45 b	0.16 a	162.23 a
Shabistar-Shendabad	14.47 c-e	7 d-f	23 d	35.67 f-h	2.12 f	0.136 a-d	136.53 bc
Yazd	14.73 b-e	9.66 c-e	33 b-d	89.33 bc	2.80 c-e	0.083 fg	82.6 ef
Tehran	14.38 c-e	8.66 c-e	28 cd	53.66 e-g	2.62 d-f	0.114 b-f	136.54 bc
Bojnord	13.95 c-e	9 c-e	30.66 b-d	76.34 b-e	3.01 b-e	0.126 b-e	108.28 cd

PH: plant height; NU: number of umbels; NMU: number of mini-umbels; NS: number of seeds; SW: 1000-seed weight; SWP: seed weight per plant; GY: grain yield. Different letters show significant difference among the accessions ($P \leq 0.05$).

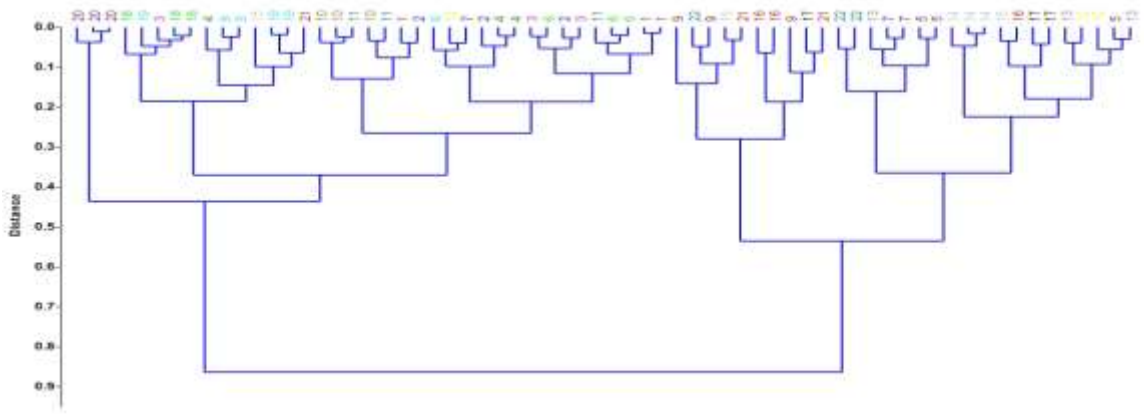

Fig. 2 Representative WARD dendrogram of agro-morphological data showing grouping of the studied accessions. Accession numbers are 1: Khaf, 2: Khoshab, 3: Chah-Dashi, 4: Ayesk, 5: Ilkhchi, 6: Shabistar, 7: Khur, 8: Mobarakeh, 9: Rig, 10: Kuhbanan, 11: Yazd, 12: Dlazyan, 13: Damghan, 14: Sorkheh, 15: Birjand 1, 16: Birjand 3, 17: Boshruyah, 18: Ferdows, 19: Nehbandan, 20: Faizabad, 21: Tehran, 22: Bojnord.

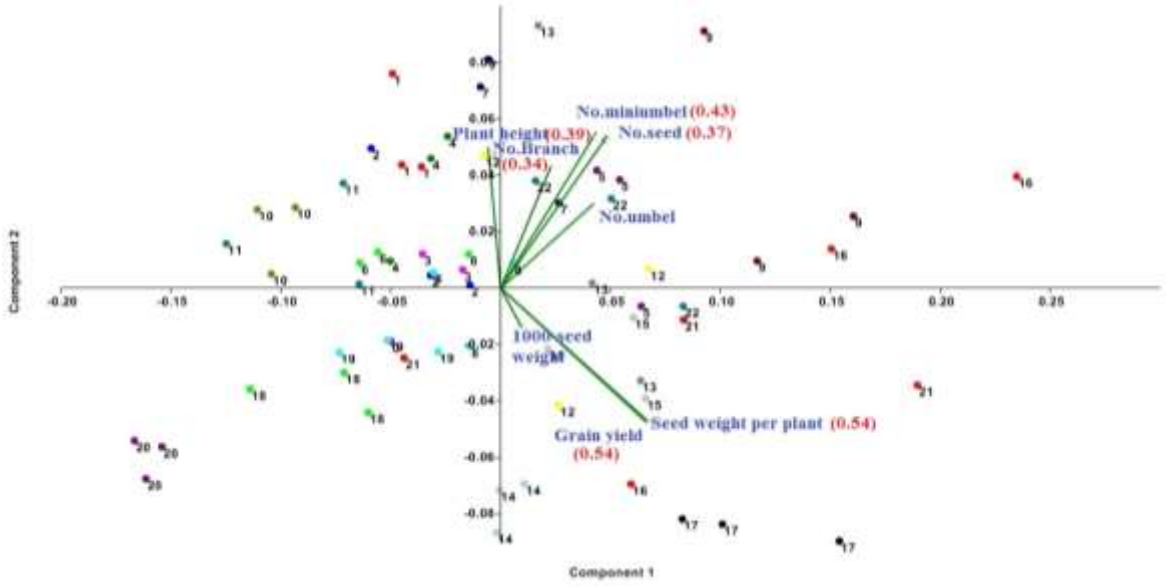

Fig. 3 PCA biplot of accessions based on the agro-morphological characters. Contribution of each trait in variation of the accessions is shown based on the components 1 and 2. Accession numbers are 1: Khaf, 2: Khoshab, 3: Chah-Dashi, 4: Ayesk, 5: Ilkhchi, 6: Shabistar, 7: Khur, 8: Mobarakeh, 9: Rig, 10: Kuhbanan, 11: Yazd, 12: Dlazyan, 13: Damghan, 14: Sorkheh, 15: Birjand 1, 16: Birjand 3, 17: Boshruyah, 18: Ferdows, 19: Nehbandan, 20: Faizabad, 21: Tehran, 22: Bojnord.

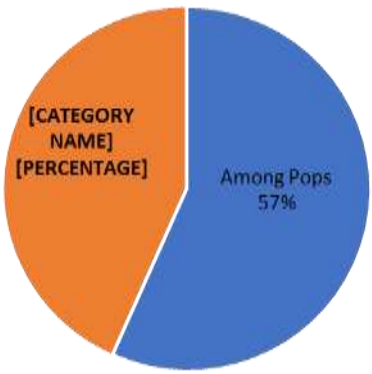

Fig. 4 Percentages of molecular variance among the studied accessions analyzed by AMOVA test (with 1000 permutations).

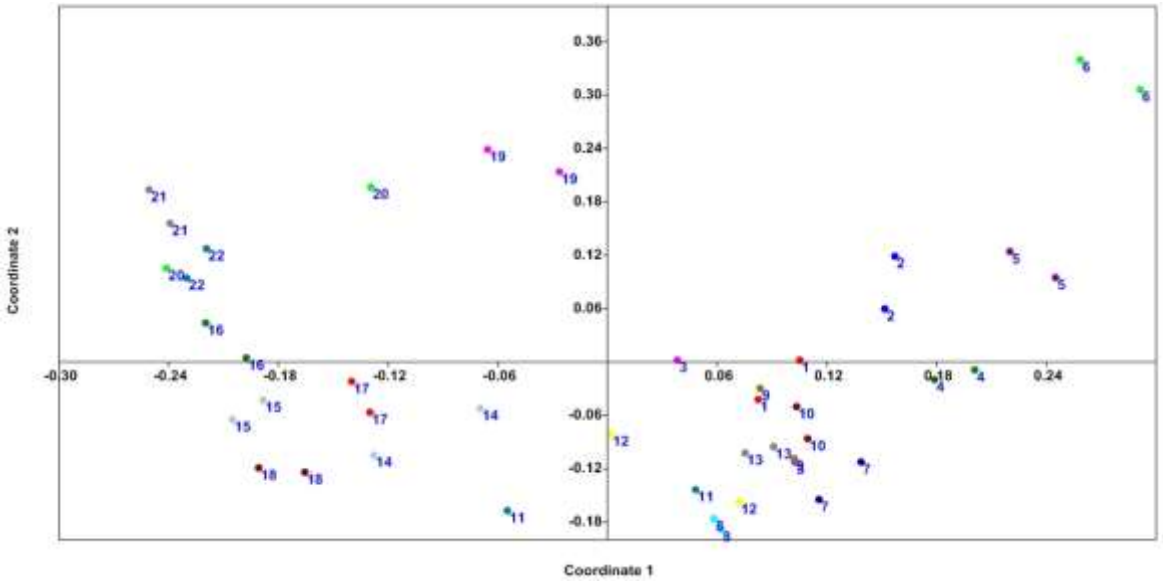

Fig. 5 PCoA plot of the combined ISSR-SCoT data, on the basis of Dice coefficient. The accession numbers are: 1: Khaf, 2: Khoshab, 3: Chah-Dashi, 4: Ayesk, 5: Ilkhchi, 6: Shabistar, 7: Khur, 8: Mobarakeh, 9: Rig, 10: Kuhbanan, 11: Yazd, 12: Dlazyan, 13: Damghan, 14: Sorkheh, 15: Birjand 1, 16: Birjand 3, 17: Boshruyah, 18: Ferdows, 19: Nehbandan, 20: Faizabad, 21: Tehran, 22: Bojnord.

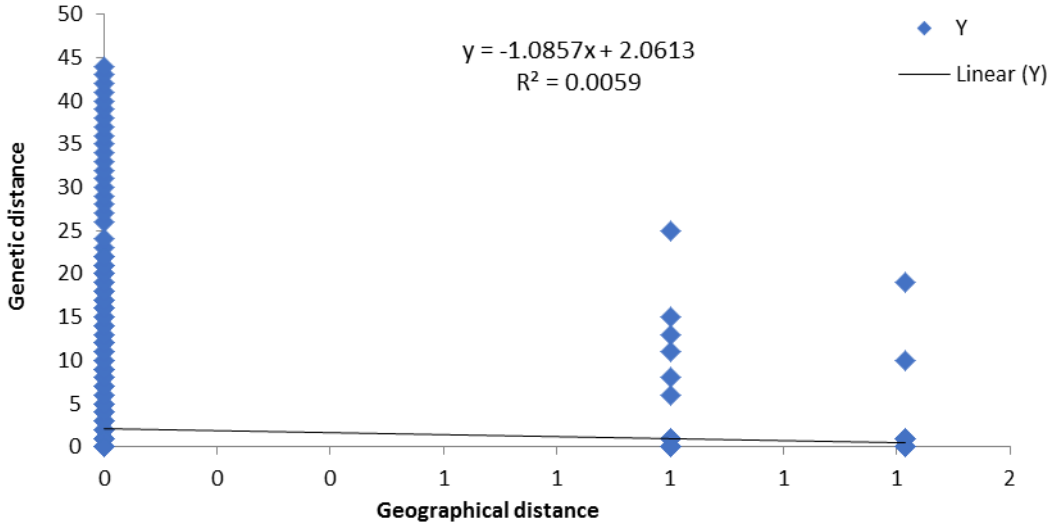

Fig. 6 Mantel test results between genetic and geographical distance of the studied accessions.

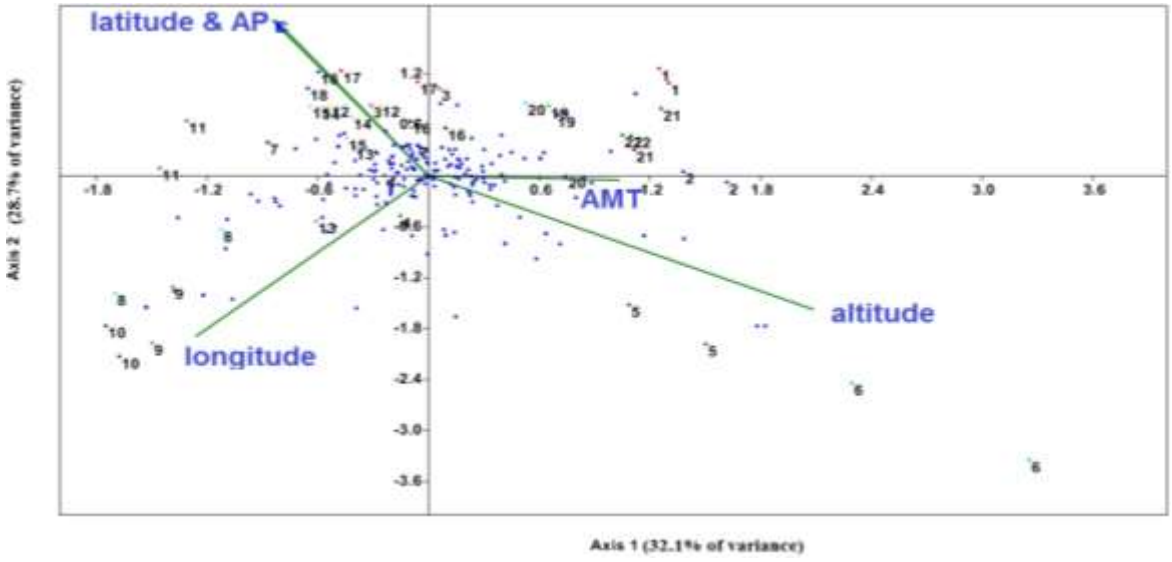

Fig. 7 Canonical correspondence analysis (CCA) biplot of the genetic features with ecological factors of the origin habitats of the studied accessions. AMT: annual mean temperature; AP: annual precipitation.

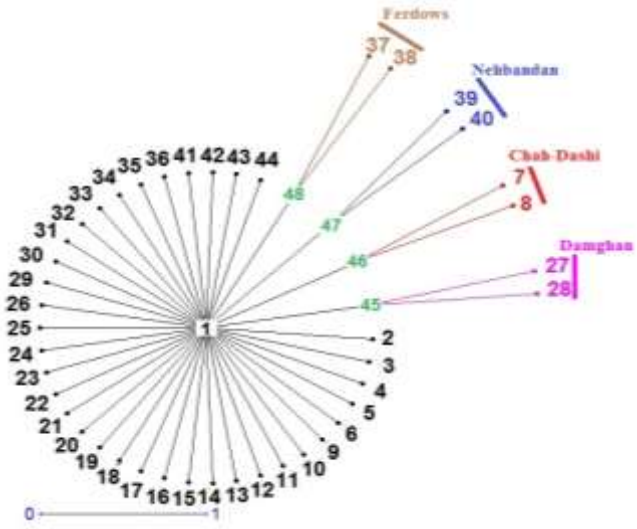

Fig. 8 Consensus tree of molecular and agro-morphological characteristics; Khaf: 1, 2; Khoshab: 3, 4; Chah-Dashi: 5, 6; Ayesk: 7, 8; Ilkhchi: 9, 10; Shabistar: 11, 12; Khur: 13, 14; Mobarakeh: 15, 16; Rig: 17, 18; Kuhbanan: 19, 20; Yazd: 21, 22; Dlazyan: 23, 24; Damghan: 25, 26; Sorkheh: 27, 28; Birjand 1: 29, 30; Birjand 3: 31, 32; Boshruyah: 33, 34; Ferdows: 35, 36; Nehbandan: 37, 38; Faizabad: 39, 40; Tehran: 41, 42; Bojnord. 43, 44

Discussion

Generally, in plant breeding and genotype conservation programs, the population structure and genetic diversity information are valuable keys. Agro-morphological traits are essential for classification and description of the genotype diversity [29]. In the current study, all agro-morphological traits (other than the number of branches) showed significant differences between the studied ecotypes. The results of the present study agree with some previous studies on genetic diversity of Iranian cumin ecotypes [19,22,30]. There are obvious differentiations between the studied accessions, on the basis of agro-morphological traits. As it was mentioned previously, the studied accessions were divided into two main clusters, which this grouping does not thoroughly match the geographic origin of the seeds. It seems that planting all ecotypes in the Semnan area and different cultivation conditions have influenced the agro-morphological traits. Despite the results of the present study, Bahraminrjad *et al*. [21] divided 49 cumin ecotypes into three groups, indicating geographical distances of the origin provinces. Generally, because of interaction between genotype and environment, and large unbeknown genetic control of polygenic morphological and agronomic traits, morphological variation does not always indicate true genetic variation [17]. However, morphological traits are efficient tools for primary assessment, because they have fast and simple application and can be used for estimation of genetic diversities among morphologically differentiable populations [22]. In this study, the most important traits in discriminated accessions were seed weight per plant, grain yield and number of mini-umbel, which have the best potential for increasing seed yield in cumin breeding programs.

A detailed information on genetic diversities and polymorphisms of the available cultivars, is necessary for planning of breeding programs. Average numbers of polymorphic loci (ISSR=25.74 and SCoT=29.67) in the present study were lower than the reported values in Iranian cumin accessions, collected from Kerman, Esfahan and Khorasan provinces of Iran, which ISSR and RAPD primers, revealed 67.3% and 54.9% polymorphic bands, respectively [22]. Moreover, Parashar *et al*. (2014) showed 79.8% polymorphic bands for SCoT markers in cumin plant.

Genetic similarity, marker technique, ecotypes, and their origins are noticeable parameters affecting the observed genetic polymorphism in the plants [30], which may explain low polymorphism percentages, detected in the present study. Clustering of accessions, on the basis of maximum genetic distance, would be useful in hybrid breeding programs [31]. Two main clusters were observed in both molecular and agro-morphological-based procedures. Whereby, the accessions Ferdows, Nehbandan and Faizabad were nested in the group I and the accessions Rig, Khur, Ilkhchi, Dlazyan and Damghan were nested in the group II. This conformity could be remarkable in breeding programs. However, considering agro-morphological and molecular traits in the consensus tree indicated that definition of ecotypes is suitable for the accessions Chah-Dashi, Damghan, Ferdows and Nehbandan. Thus, these results indicated that different agro-morphological traits in the studied accessions are related to different genetic backgrounds. Comparing of the accession groups, based on the agro-morphological and genetic data, indicated that the accessions with the highest and lowest performances (Boshruyah and Faizabad) discriminate in agro-morphological clustering, but not by genetic-based clustering. The accession 20 (Feyzabad) was completely separated from other accessions, and because of the lowest quantitative agro-morphological traits was nested in the cluster I. The accessions Birjand 3, Boshruyah (south Khorasan), and Mobarakeh (Esfahan) showed the best performances, based on the phenotypic data, while then accession Feyzabad (south Khorasan) had almost the lowest traits. Chah-Dashi, Damghan, Ferdows and Nehbandan accessions could be defined as ecotypes, based on both agro-morphological and molecular differentiation. Bahraminejad *et al*. [20] reported high variations between and within Iranian cumin accessions, using phenotypic traits and RAPD markers.

Table 6 Genetic diversity parameters of SCoT and ISSR markers in 22 cumin accessions.

GP	PPB%			Na			Ne			I			He			UHe		
Eco	SCoT	ISSR	S&I	SCoT	ISSR	S&I	SCoT	ISSR	S&I	SCoT	ISSR	S&I	SCoT	ISSR	S&I	SCoT	ISSR	S&I
1	18.68	10.89	14.58	0.63	0.46	0.54	1.13	1.07	1.1	0.11	0.06	0.08	0.07	0.04	0.06	0.1	0.06	0.08
2	21.98	24.75	23.44	0.65	0.69	0.67	1.15	1.17	1.16	0.13	0.15	0.14	0.09	0.1	0.09	0.12	0.13	0.12
3	23.08	23.76	23.44	0.69	0.7	0.69	1.16	1.16	1.16	0.14	0.14	0.09	0.09	0.09	0.09	0.12	0.13	0.12
4	23.08	12.87	17.71	0.61	0.48	0.54	1.16	1.09	1.12	0.14	0.07	0.1	0.09	0.05	0.07	0.12	0.07	0.09
5	21.98	12.87	17.19	0.68	0.6	0.64	1.15	1.09	1.1	0.13	0.07	0.1	0.09	0.05	0.07	0.12	0.07	0.09
6	23.08	10.89	12.50	0.42	0.46	0.44	1.1	1.07	1.08	0.08	0.06	0.07	0.05	0.04	0.05	0.07	0.06	0.06
7	21.98	9.9	14.58	0.59	0.47	0.53	1.1	1.07	1.1	0.12	0.06	0.08	0.08	0.04	0.06	0.1	0.05	0.08
8	14.29	22.77	18.75	0.57	0.68	0.63	1.1	1.16	1.13	0.08	0.13	0.11	0.05	0.09	0.07	0.07	0.12	0.1
9	23.08	19.8	21.35	0.69	0.64	0.66	1.16	1.14	1.15	0.14	0.12	0.12	0.09	0.08	0.08	0.12	0.1	0.11
10	29.67	24.75	27.08	0.82	0.77	0.79	1.21	1.17	1.19	0.17	0.15	0.16	0.12	0.1	0.11	0.16	0.13	0.15
11	16.48	17.82	17.19	0.59	0.6	0.58	1.11	1.12	1.12	0.1	0.1	0.1	0.06	0.07	0.07	0.09	0.09	0.09
12	23.08	25.74	24.48	0.61	0.74	0.68	1.16	1.18	1.17	0.14	0.15	0.14	0.09	0.1	0.1	0.12	0.14	0.13
13	24.18	11.88	17.71	0.6	0.57	0.58	1.17	1.08	1.12	0.14	0.07	0.1	0.1	0.04	0.07	0.13	0.06	0.09
14	19.78	20.79	20.31	0.57	0.72	0.65	1.14	1.14	1.14	0.12	0.12	0.12	0.08	0.08	0.08	0.1	0.11	0.11
15	3.3	4.95	4.71	0.33	0.44	0.39	1.02	1.03	1.02	0.02	0.03	0.02	0.01	0.02	0.01	0.01	0.02	0.02
16	0	9.9	5.21	0.2	0.52	0.37	1	1.07	1.03	0	0.06	0.03	0	0.04	0.02	0	0.05	0.02
17	1.1	8.91	5.21	0.33	0.4	0.37	1	1.06	1.03	0.007	0.05	0.03	0.005	0.03	0.02	0.006	0.04	0.02
18	2.2	12.87	7.81	0.37	0.5	0.44	1.01	1.09	1.05	0.01	0.07	0.04	0.009	0.05	0.03	0.01	0.07	0.04
19	8.79	9.9	9.38	0.41	0.38	0.4	1.06	1.07	1.06	0.05	0.06	0.05	0.03	0.04	0.03	0.04	0.05	0.05
20	4.4	20.79	13.02	0.42	0.62	0.53	1.03	1.14	1.09	0.02	0.12	0.07	0.01	0.08	0.05	0.02	0.11	0.07
21	7.69	18.81	13.54	0.39	0.59	0.5	1.05	1.13	1.09	0.04	0.11	0.08	0.03	0.07	0.05	0.04	0.1	0.07
22	4.4	7.92	6.25	0.39	0.5	0.45	1.03	1.05	1.04	0.02	0.04	0.03	0.01	0.03	0.02	0.02	0.04	0.03
Total	14.79	15.62	15.22	0.52	0.57	0.55	1.1	1.1	1.1	0.08	0.09	0.09	0.06	0.06	0.06	0.08	0.08	0.08

PPB: genetic polymorphism percentage; Na: the mean number of different alleles over all loci; Ne: the highest effective number of the alleles; I: Shannon Information Index; He: expected heterozygosity; UHe: unbiased expected heterozygosity; S & I: SCoT and ISSR.

Moreover, use of ISSR and RAPD markers indicated different molecular and morphological groups in Iranian cumin accessions [22].

In AMOVA, 57 % of total variation was due to within population genetic variability. Similar results have been reported in different plant species and should be related to outcrossing nature of these studied species. High within-population genetic variation is a useful tool for adapting to local environmental changes [33]. The observed between-population differentiation in molecular variation (43%) could be attributed to different factors, including isolation, drift, founder effects and local selection [34].

In Iran, cumin accessions are distributed in various different regions, including eastern, south-eastern, central and western geographical regions, with different climates, such as arid and semi-arid area, which results in adaptation to ecological factors and genetic variation [3]. In the present study, the Mantel test showed no significant correlation between geographical and genetic distances. Thus, despite genetic differentiation among the studied cumin accessions, they are not isolated and hence, some genetic exchanges are probably occurred among them. The relationships within infra-species specimens sometimes are consistent with their geographical distance, while in species with a wide distribution region, sometimes do not consistent with one [35-37].

Investigation of relationships between genetic diversity and environmental factors revealed impact of ecological conditions on adaptation and differentiation of cumin accessions. Also, genetic diversity of the studied accession was assessed in relation to local ecological parameters. The relationship between accession genetic diversity was not well accorded with ecological conditions of the seed origin, including altitude, latitude, longitude, annual mean temperature and precipitation. However, based on the CCA results, altitude and latitude affected genetic variations of the studied accessions.

Climate has an important role in local adaptation of the plant species and thus, genetic diversity of the plant populations [38,39]. In other words, increasing of genetic differentiation is a possible consequence of the environmental changes [40]. The results of the present study agree with the observed relationship between genetic diversity and ecological parameters in *Stipa grandis* from Inner Mongolia [41]. Similarly, Huang *et al*. [42] reported influence of some climatic factors on genetic diversity of *Caragana microphylla* (Fabaceae). Similar results in *Artemisia halodendron* (Asteraceae) were also reported by Huang *et al*. [43].

Conclusions

In the present study, molecular analyses indicated high genetic differentiation between the studied cumin accessions. A significant variation was also observed between the accessions in terms of genetic and agro-morphological data. Evaluation of effective ecological parameters on genetic differentiation showed influences of altitude and latitude on genetic variation of the cumin accessions. In spite of genetic differentiation among the studied cumin accessions, they are not totally isolated and hence some amount of gene flow has been occurred between them. Therefore, no isolation by distance exists between them.

Consent for Publication

Author agree to send this manuscript to Hereditas, are in agreement with its content, and do not have any restriction in order to publish the obtained results.

Availability of Data and Materials

This manuscript contains original data and it is not under editorial consideration elsewhere. We have adhered to the ethical guidelines of your journal.

Conflict of Interest

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