J. Agr. Sci. Tech. (2017) Vol. 19: 157-172

Genetic Variability of Outcross and Selfed Fennel Based on Morphological and ISSR Markers

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ABSTRACT

Inter Simple Sequence Repeat (ISSR) markers were used to assess the genetic diversity among 23 outcross and self pollinated populations of fennel collected from different geographical regions of Iran and some European countries. The experiment was carried out to determine the effect of self-pollination on morphological traits and genetic diversity in the next generation. Fifteen primers produced 248 polymorphic bands with an average of 16.53 fragments per primer in outcross populations, while 217 polymorphic fragments with an average of 14.46 fragments per primer were generated in self-pollinated populations. UPGMA dendrogram using Jaccard's similarity coefficients placed outcross populations in five major groups. The maximum and minimum gene diversity over loci was observed in Albania (0.53) and Poland (0.42) populations, respectively. In general, European fennel populations revealed higher expected heterozygosity (0.47) in comparison with Iranian ones (0.35). Polymorphism Information Content (PIC) ranged from 0.37 to 0.49 in self-pollinated populations, while it varied from 0.39 to 0.46 in out-cross ones. The classification based on morphological data did not confirm the molecular ones in most cases. Self-pollination led to decline in plant height in most of the studied populations. In overall, plant height of the European populations (54-66.02 cm) was less than that of Iranian ones (55-109.54 cm). Self-pollination elevated the yield of essential oil in studied fennels through its influence on fruit set. In conclusion, Albania population had the highest oil content affected by self-pollination; hence, it can be introduced as one of the valued sources in fennel breeding programs aimed for oil yield improvement.

Keywords: Apiaceae, Genetic diversity, Inbreeding depression, Molecular markers.

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill) is an annual herb belonging to family Umbelliferae (Apiaceae). Fennel is used in folk medicine for its balasimic, cardiotonic, digestive, lactogogue and tonic properties (Patra *et al.*, 2002; Saleha, 2011; Saravanaperumal and Terza, 2012). Its essential oil has been used in cosmetics and pharmaceutical products. Fennel contains volatile oils, phenolic glycosides, flavonoids, phytosterols, triterpines and saponins (Ebeed *et al.*, 2010) and is useful as an estrogenic, lactagouge, diuretic and immune booster. It has also bronchodilatory effects (Boskabady *et al.*, 2004).

In recent years, the application of molecular markers for evaluation of the genetic variability has become an important tool in various plant species including *Codonopsis lanceolata* (Guo *et al.*, 2006), *Cunila* (Agostini *et al.*, 2008), *Tribulus terrestris* (Sarwat *et al.*, 2008) and *Achillea* species (Rahimmalek *et al.*, 2009a). Among molecular markers, Inter-Simple Sequence Repeats (ISSRs) markers have gained attention recently as an alternative means for characterizing complex genomes. ISSR amplification utilizes anchored SSR motifs as primers complement to genomic

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microsatellites and target numerous, highly variable loci (Josh *et al.*, 2000; Sedighi and Rahimmalek, 2015). As a consequence, ISSR markers can amplify more polymorphic fragments per primer than RAPDs in *Oryza granulata* (Qian *et al.*, 2001).

The production of self-pollinated plants can help fix some morphological as well as phytochemical properties of medicinal plants in next generations. Since the seed of Apiaceae plants have been considered as the most important part in respect to their medicinal properties, the study of pollination in this family can reveal phytochemical as well as anatomical variations in next generations (Chu and Liu, 2007). Assessment of pollination system and outcrossing has been reported in some Apiaceae plants such as *Eryngium alpinum* (Gaudeul *et al.*, 2000), *Trachymene incisa* (Yvonne-Davila and Glenda-Wardle, 2007) and *Heracleum mantegazzianum* (Perglová *et al.*, 2006). In fennel and many Apiaceae plants, geitonogamous mode has been reported. Selfing can be promoted by geitonogamous mode that highly depends on insects and wind pollination (Koul *et al*., 1986). Previous reports evaluated flowering dynamics and cross ability of different populations of bitter fennel (Gross *et al.*, 2008). Moreover, there are some reports regarding the genetic diversity of fennel populations. Patel *et al.* (2008) studied genotypic and phenotypic variation of different fennel populations in Egypt. Lopes *et al.* (2009) evaluated nine Portuguese fennel accessions from wild populations for morphological and essential oil evaluation. Shukla *et al.* (2003) studied the coefficient of variability and genetic advance for seed yield and five major contributing traits in 30 genotypes of fennel in Egypt. Zahid *et al.* (2009) assessed the genetic diversity of Pakistan fennel germplasm using 16 RAPD primers. Genetic diversity among Indian varieties of fennel was also evaluated using nuclear ribosomal DNA and RAPD markers (Singh *et al.*, 2012). Bahmani *et al.* (2012) evaluated the genetic diversity of 25 Iranian populations based on 72 RAPD polymorphic bands. Torabi *et al.* (2012) assessed the genetic diversity of 30 Iranian fennel accessions using AFLP markers. The ISSR markers also showed high level of polymorphism in Apiaceae plants (El-Nasr *et al*., 2013; Tomar-Rukam *et al*., 2014). However, most of the previous studies assessed the genetic diversity of outcross fennel accessions, but the effect of selfing on genetic diversity of Iranian and some European fennels was not yet evaluated using molecular tools.

Therefore, the aims of this study were: (1) To determine the level and patterns of genetic variation among outcross and self-pollinated fennel populations using ISSR markers and morphological characters, and (2) To evaluate the effect of self-pollination on morphological variation and genetic diversity in next generation.

MATERIALS AND METHODS

Plant Material

Nineteen fennel populations were collected from different geographical regions of Iran. Four other European populations were also included from Spain, England, Albania and Poland (Table 1). The seeds of the collected samples were sown in Randomized Complementary Block Design (RCBD) in three replicates. Ten plants from each population were selected for experiment. Each plant inflorescence was divided in two parts. The half (five umbels) was bagged and the remaining was permitted for out crossing. The seeds were harvested at full maturity stage when the seeds were completely dried. The self-fertilized seeds were sown in a new RCBD design with three replicates.

The index of self pollination (%) was measured as the ratio of the number of self-fertilized seeds to outcross fertilized ones. Inbreeding Depression (ID) for each trait was also evaluated using the following formula:

Table 1. Collection site and geographical characteristics of the fennel populations used in the current study.

No.	Accession name	Collection site	Altitude (m asl)	Latitude	Longitude
1	Al1	Karaj, Alborz, Iran	1300	35° 48′ N	51° 00′ E
2	Ya	Yazd, Yazd, Iran	1230	31° 41′ N	53° 49′ E
3	Is1	Isfahan, Isfahan, Iran	1570	32° 39′ N	51° 43′ E
4	Ha1	Nahavand, Hamadan , Iran	1644	34° 52′ N	50° 10′ E
5	Te1	Tehran, Tehran, Iran	1190	36° 52′ N	53° 10′ E
6	Ha2	Hamedan, Hamedan, Iran	1900	34° 52′ N	48° 32′ E
7	Is2	Kashan, Isfahan, Iran	982	51° 35′ N	33° 59′ E
8	Ke1	Pave, Kermanshah, Iran	1530	46° 22′ N	35° 03′ E
9	Az1	Tabriz, Azarbayjan Sharghi, Iran	1561	51° 17′ N	38° 04′ E
10	Te2	Varamin, Tehran, Iran	918	51° 12′ N	34° 12′ E
11	Ke2	Kerman, Kerman, Iran	800	59° 00′ N	30° 16′ E
12	Fa	Shiraz, Fars, Iran	1486	52° 33′ N	29° 36′ E
13	Kh1	Shirvan, Khorasan Shomali, Iran	1160	56° 03′ N	36° 42′ E
14	Kh2	Yasuj, Khozestan, Iran	1870	51° 35′ N	30° 39′ E
15	Is3	Semirom, Isfahan, Iran	2500	51° 34′ N	31° 25′ E
16	Bu	Bushehr, Bushehr, Iran	5	50° 08′ N	27° 17′ E
17	Az2	Ardebil, Azarbayjan Gharbi, Iran	1354	48° 55′ N	37° 45′ E
18	Kh3	Gonabad, ,Khorasan Razavi Iran	1150	58° 45′ N	34° 15′ E
19	Kh4	Mashhad, Khorasan Razavi, Iran	979	59° 34′ N	36° 16′ E
20	Sp	Madrid, Spain, Europe	654	40° 40′ N	3° 68′ W
21	Al2	Tirana, Albani, Europe	316	41° 32′ N	19° 81′ E
22	Po	Wroclaw, Poland, Europe,	110	51° 11′ N	17° 03′ E
23	En	London, England, Europe	360	51° 30′ N	00° 10′ W

ID (%)= [(Mop–Ms)/Mop]×100

Where, *Mop*: Mean values of cross pollinated population, *Ms*: Mean value of self pollinated seeds.

DNA Extraction and ISSR Analysis

Ten plants from each population were used for DNA extraction. DNA from young leaves of self and outcross pollinated samples were extracted using the modified CTAB method (Murray and Thompson, 1980).Then, the extracted DNAs from each self and out-cross populations were mixed. DNA concentration and quality was measured using a spectrophotometer U-1800 (Hitachi, Japan) and agarose gel electrophoresis. The DNA was diluted to a working concentration of 10 ng mL^{-1}.

Out of the 20 ISSR primers screened, 15 primers amplified reproducible and scorable banding pattern. PCR reactions were carried out in a volume of 15 µL containing 10 ng total DNA, 10X PCR buffer, 2% formamide, 0.25 mM each dNTP, 10 pM each primer, 4 mM $MgCl_2$, 1 U *Taq* DNA polymerase. PCR cycling conditions for all accessions were 2 minutes of initial denaturation (94˚C); followed by 40 cycles of 1 minute at 94˚C, 1 minute at the specific annealing temperature, and 2 minutes at 72˚C; ending with a final extension step of 10 minutes at 72˚C. DNA amplification fragments were separated in a 2% agarose gel at 100 Watt for 3 hours in 1X TBE buffer (100 mM Tris–Borate, pH 8.0, 2 mM EDTA) and stained with ethidium bromide.

Morphological Data Analysis

Percentage of germination, plant height, number of lateral branches, florescence diameter, canopy diameter, plant wet and dry weights, ratio of canopy diameter to

plant height, ratio of plant height to number of lateral branches, day to 50% flowering, day to 50% germination, 1,000 seed weight, number of seed per umbel, seed yield, and percentage of self-pollination were measured in three replicates and the mean value was used for the analysis (El-Nasr *et al*., 2013). The dendrogram of morphological characters was constructed using SPSS ver17. The Clevenger-type apparatus was applied to extract essential oils. For each hydro-distillation run, 50 g of powdered seeds were placed in a round bottom flask. An aliquot of 400 mL distilled water was added and boiled for 5 hours. Then, the essential oil was collected in a container. The essential oil content was determined on the base of dry matter and measures were done in triplicates (Rahimmalek *et al*., 2009b).

Data Analysis

Polymorphic ISSR bands of each gel were scored as present (1) or absent (0). Cluster analysis was conducted by the software NTSYSpc version 2.02 (Rohlf, 1998). Polymorphic Information Content (PIC) was calculated by applying the simplified formula: *PICi= 2fi (1-fi),* where *fi* is the percentage of the i^{th} amplified band present (Anderson *et al.*, 1993).

Genetic similarity among all accessions was calculated according to Jaccard's similarity index (Jaccard, 1908) using the similarity of qualitative data (Simqual) routine. The dendrogram was constructed using the Unweighted Pair Group Method Average (UPGMA) clustering procedure. A Mantel test (Mantel, 1967) was used to detect the correlation between Cophenetic and similarity matrices. The Cophenetic correlation coefficient was generated by means of the COPH routine in order to check the goodness of fit between the cluster in the dendrogram and the similarity coefficient matrix. Analysis Of Molecular Variance (AMOVA), gene diversity over loci and expected heterozygosity among assumed groups (Iranian and European fennel populations) were calculated using Arlequin version 3 software. The dendrogram of morphological characters was constructed using SPSS ver17 based on Ward's method.

RESULTS AND DISCUSSION

ISSR-Based Genetic Diversity in Outcross and Self-pollinated Populations

Fifteen primers were selected for the assessment of genetic relationships based on the number of amplification products, the quality of the profiles, the level of polymorphism and the reproducibility of bands (Table 2). Selected primers generated 248 polymorphic bands, with an average of 16.53 fragments per primer in the outcross populations (Table 2), while 217 polymorphic fragments with an average of 14.46 fragments per primer were generated in the self-pollinated populations. The size range of the amplified products was 100 to 1000 bp and the number of products per primer varied from 10 in P9 [$(GA)_8$ YG)] to 27 in P6 [$(CA)_8$ VT)]. The ISSR profile obtained by P13 [$(AG)_8$ T)] primer is illustrated in Figure 1.

Percentage of polymorphic bands averaged 88.32% in outcross and 82.33%, in self-pollinated populations. PIC values averaged 0.43 and 0.42 in outcross and self-pollinated populations, respectively (Table 2). In the present study, the number of polymorphic bands per primer ranged from 10 to 27, with an average of 16.53 and the average *PIC* value for the amplification products was 0.43, more than self-pollinated populations and those obtained by Okon *et al.* (2013) in wild and cultivated germplasm of fennel using ISSR markers. In the present research, high level of polymorphism was recorded that was comparable with the data obtained in some other Apiaceae plants including *Coriandrum sativum* (El-Nasr *et al.*, 2013; Tomar-Rukam *et al.*, 2014), *Daucus carota* (Iorizzo *et al.*, 2013) and

Table 2. Characteristics of the ISSR primer used to analyze genetic diversity in outcross and self pollinated fennel accessions.

Primer	Motif [a]	Scorable bands		Polymorphic bands		Polymorphism (%)		*PIC*/Primer	
		Outcross	Self	Outcross	Self	Outcross	Self	Outcross	Self
P1	5'-$(CT)_8$ G-3'	26	21	23	20	88.46	95.23	0.44	0.43
P2	5'-$(CA)_8$ G-3'	16	20	15	18	93.75	90	0.41	0.42
P3	5'-$(TC)_8$ C-3'	17	17	13	16	76.47	94.11	0.39	0.37
P4	5'-$(AC)_8$ G-3'	13	18	11	16	84.61	88.88	0.41	0.39
P5	5'-$(AC)_8$ YG-3'	20	19	19	17	95	89.47	0.44	0.43
P6	5'-$(CA)_8$ VT-3'	27	15	25	11	92.59	73.33	0.43	0.45
P7	5'-$(CA)_8$ RT-3'	13	11	11	10	84.61	90.9	0.44	0.39
P8	5'-$(GA)_8$ T-3'	18	18	17	17	94.44	94.44	0.43	0.49
P9	5'-$(GA)_8$ YG-3'	26	10	23	8	88.46	80	0.44	0.4
P10	5'-HVH $(TCC)_7$-3'	17	14	14	11	82.35	78.57	0.4	0.45
P11	5'-CCA $(CT)_8$-3'	16	23	15	21	93.75	91.3	0.45	0.39
P12	5'-BDB $(TCC)_7$-3'	14	17	13	15	92.85	88.23	0.46	0.42
P13	5'-$(AG)_8$ T -3'	20	19	19	18	95	94.73	0.43	0.44
P14	5'-$(TCC)_5$ YR-3'	12	12	9	9	75	75	0.45	0.41
P15	5'-T $(AG)_9$-3′	24	11	21	10	87.5	90.9	0.44	0.42
Total		279	245	248	217	-	-	-	-
Average		18.6	15	16.53	14.46	88.32	82.33	0.43	0.42

[a] Type of degenerate nucleotide: R = A/T, Y = G/C, B = T/G/C; D = A/T/G, H = A/T/C, V = 3A/G/C.

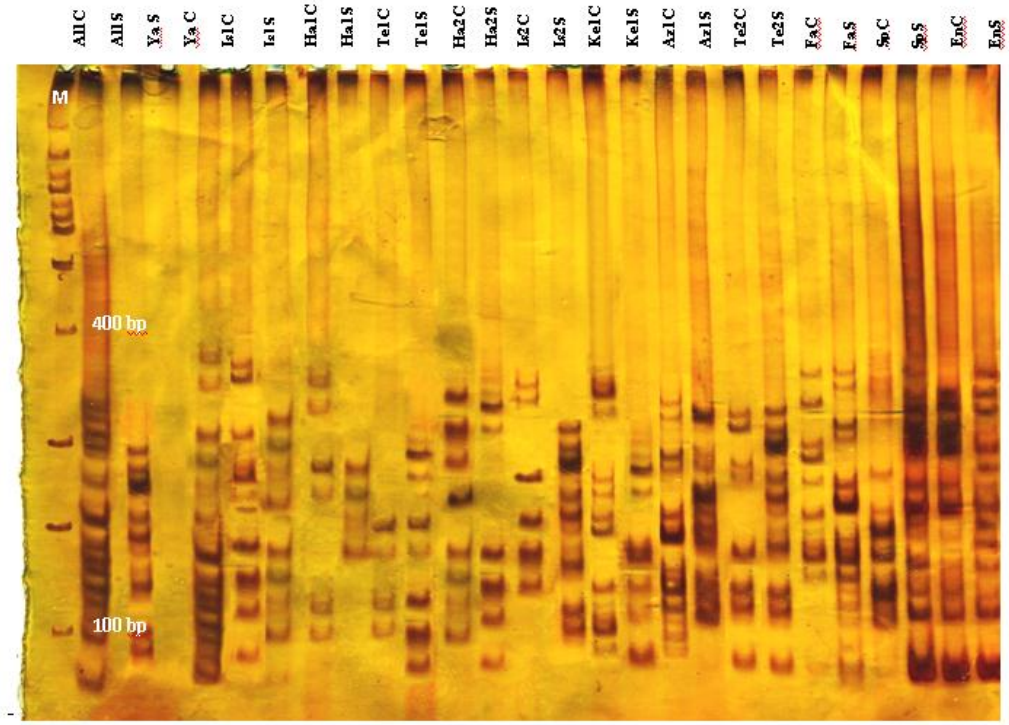

Figure 1. ISSR banding pattern of P13 [$(AG)_8$ T)] primer in different outcross (C) (Al1c, Yac, Is1c, Ha1c, Te1c, Ha2c, Is2c, Ke1c, Az1c, Te2c, Fac, Spc, Enc) and Self (S) (Al1s, Yas, Is1s, Ha1s, Te1s, Ha2s, Is2s, Ke1s, Az1s, Te2s, Fas, Sps, Ens) pollinated fennel populations. M is 100 bp ladder.

Foeniculum vulgare and *Cuminum cyminum* (Singh *et al.*, 2012).

Cluster analysis revealed five groups in out cross populations (Figure 2). A high Cophenetic correlation coefficient of 0.89 indicates the goodness of fit in the dendrogram. Karaj population was classified in group I, while group II comprised two sub-groups. Pave and Yasuj populations were classified in subgroup I, while Isfahan, Kashan, Semirom and Yazd populations were grouped in sub-group II. They belonged to central regions of the country. Group III consisted of five populations including Shiravan and Mashhad, from Northern regions, and Shiraz, Nahavand and Hamadan, from central regions of the country. Bushehr, Kerman, Gonabad, Tabriz, Ardebil, Tehran and Varamin were grouped in cluster IV. Group V included European populations, namely, Poland, Albania, England, and Spain. Most of the populations used in the present study were classified according to their geographical distribution. Similar trend was also reported in other Apiaceae plants using ISSR markers such as coriander (Ahmadi *et al*., 2014) and *Cuminum cyminum* (Rostami-Ahmadvandi *et al*., 2013).

To visualize the association among self-pollinated populations of fennel, cluster analysis was constructed using UPGMA algoritm and Jaccard's similarity coefficients (Figure 3). As a result of self-pollination, the pattern of classification was not remarkably changed. For instance, Karaj population was classified in group III near the Central populations. Gonabad was separated from Southern populations and classified in group III along with North-eastern populations.

AMOVA with 1,000 permutations was

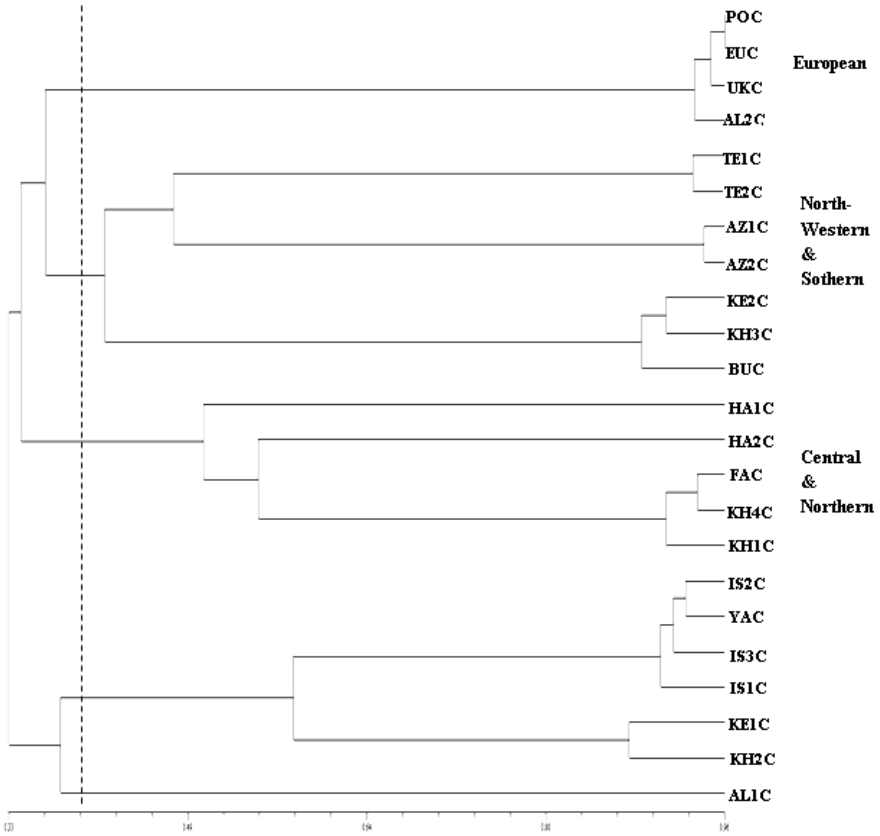

Figure 2. UPGMA dendrogram based on Jaccard's similarity coefficient among outcross (C) pollinated fennel populations collected from Iran and Europe. *The codes used in dendrograms were explained in Table 1.

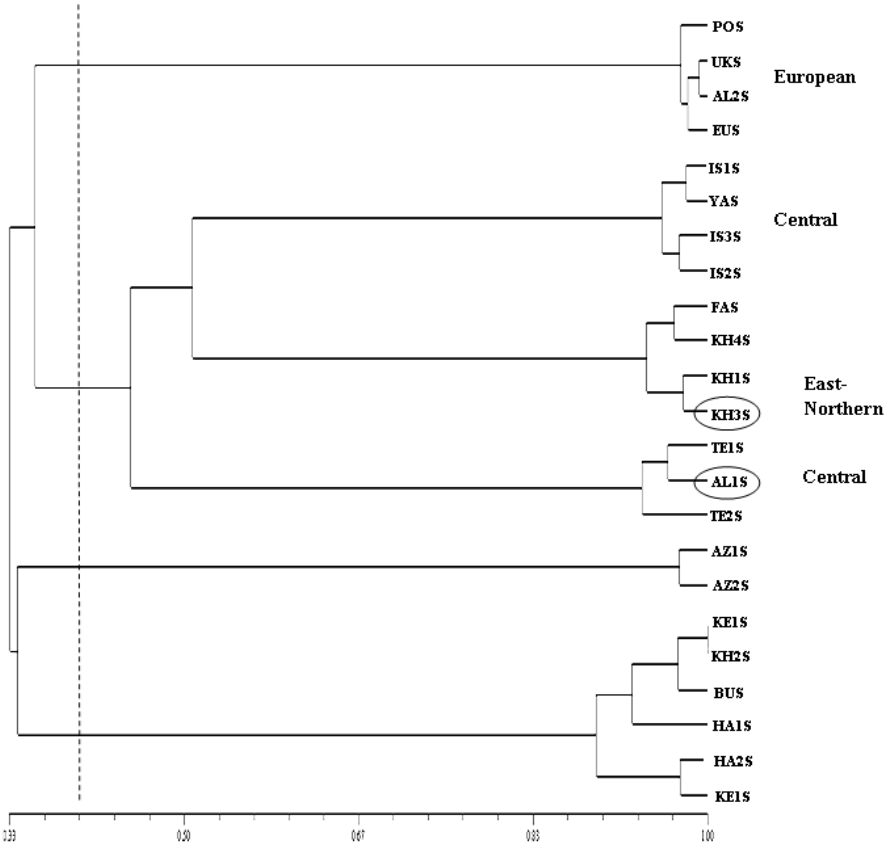

Figure 3. UPGMA dendrogram based on Jaccard's similarity coefficient among Selfed (S) fennel populations collected from Iran and Europe. *The codes used in dendrograms were explained in Table 1.

performed for outcross populations assuming two groups; Iranian (FI) and European (FE) populations. Analysis of molecular variance (AMOVA) ($P< 0.05$) with 1,000 permutations for outcross fennel populations was conducted to divide the total genetic variation into separate components including among (41.21 %) and within (58.79 %) groups, while for selfed ones, 44.68 and 55.32% of variations belonged to among and within populations, respectively.

The AMOVA results showed that the most significant variation was attributed to within-population contribution (Fst= 0.44) ($P< 0.001$). A relatively high differentiation among populations (Fst= 0.44) ($P< 0.001$) was observed in the present research. Comparisons of outcross and self-pollinated fennel population revealed that the genetic diversity decreased as a result of selfing and the Iranian ones were more affected by selfing than the European ones. Estimates of inbreeding and outcrossing were also previously studied using molecular markers (Jolivet *et al.*, 2013; Robinson *et al.*, 2013). The genetic variation among fennel populations decreased 3% as a result of selfing. This suggests that inbreeding can decrease genetic diversity among fennel populations in subsequent generations. In general, European fennel populations revealed higher expected heterozygosity (0.47) in comparison with the Iranian ones (0.35). As most of the relevant fennels originated from European countries, higher variation in their populations than the Asian ones might be attributed to their origin.

Morphological Results

Several morphological and phonological characteristics were measured in the studied populations. Analysis of variance showed significant differences among population for all traits studied (P< 0.05). The mean, maximum, and minimum values of each trait are shown in Table 3. Percentage of germination had the maximum value of Coefficients of Variability (CV) (10.57%), while the minimum was observed for the number of lateral branches (0.98%). The results of mean comparison, based on *LSD* test, are also shown in Table 4. For most of the morphological traits, the Iranian populations showed higher values compared to the European ones. Among the Iranian populations, Gonabad possessed the highest 1,000 seed weight and seed yield in selfed and outcross pollinations compared with other populations (Table 4). In general, self-pollination ranged from 2.25% in Shiravan to 6.58% in Pave populations, respectively. Essential oil yield obtained from Iranian populations showed significant differences from 2.4 to 6.4%, while the European ones ranged from 3.2% in Albania to 6.2% in Poland. In medicinal plants, selection of suitable parents for breeding purposes is highly related to obtaining useful morphological traits as well as high essential oil yield. In fennel, seed yield is considered as the most important trait in order to improve desirable medicinal properties (Mahfouz and Sharaf-Eldin, 2007). Moreover, essential oil yield has been considered as the major economically important trait in breeding programs of medicinal plants. Among the populations, Yazd, Pave, Tabriz, Yasuj, Varamin and Kerman indicated acceptable seed yield as influenced by self-pollination (Table 4). Essential oil yield in many medicinal plants may be influenced by the ploidy level (Nemeth, 2005), plant phonological stage (Rahimmalek *et al.*, 2009b) and bio-regulators. In the present research, European populations showed higher essential oil yield compared to Iranian ones .

Inbreeding increased the inflorescence diameter (14.67%), the ratio of canopy diameter to plant height (16.85%), the ratio of plant height to the number of lateral branches (7.32%), day to 50% flowering (7.74%), 1,000 seed weight (16.23%) and essential oil yield (25.61%), while other morphological traits such as number of seed per umbel and seed yield decreased (Table 4). The highest effect of Inbreeding Depression (ID) on seed yield was obtained in England and Gonabad populations, while the lowest *ID* was observed in Yazd and Pave populations (Table 4). Tehran, Kerman and Shiraz populations showed the highest *ID* in respect to the number of seed per umbel.

In the present research, the plant height of the European populations (54-66.02 cm) was shorter than that of Iranian ones (55-109.5 cm). In respect to this trait, Tabriz and Varamin populations showed higher similarity to European ones (Table 4). Pollination affected the essential oil yield in fennel via its influence on fruit set (Maghsoudi-kelardashti *et al.*, 2015). Fruit set in tall populations is often low, possibly due to the incomplete pollination (Falzari *et al.*, 2005). Furthermore, in fennel, fruit set is reduced by a lack of synchrony between pollen production and stigma receptivity (Maghsoudi-kelardashti *et al.*, 2015). As a result, self-pollination can increase essential oil yield by decreasing plant height and elevating fruit set. According to Maghso udi-kelardashti *et al.* (2015) climatic condition can influence the yield components as well as essential oil content of fennel seeds.

Most of the morphological traits revealed high levels of variation in self and outcross pollinated populations which were in agreement with those reported by Knight and Winters (1963). Positive and negative effects of inbreeding were also reported by Saeidi *et al.* (2007) in *Petunia hybrid* on seed yield components. The mean of inflorescence diameter of the studied fennels showed high variation among Iranian and

Table 3. The mean, maximum, minimum and coefficients of variability for morphological and phonological traits measured in self and outcross pollinated populations of fennel.

Traits	Mean		Minimum		Maximum		*CV* (%)
	Outcross	Self	Outcross	Self	Outcross	Self	
1. Percentage of germination	0.532	0.354	0.3	0.14	0.7	0.61	10.57
2. Plant height	78.70	68.7	53	40	110.75	115.32	5.02
3. The number of lateral branches	12.26	10.26	6	4	32	30	0.989
4. florescence diameter	18.63	5.68	2.23	3.21	15.12	6.72	3.79
5. Canopy diameter	74.04	71.74	53	43.32	115.12	98.87	2.79
6. Plant wet weight	195.74	157.43	97.33	80.32	454.44	324.7	7.72
7. Plant dry weight	90.26	71.56	30.43	22.2	252.08	187.5	6.51
8. The ratio of canopy diameter to plant height	0.956	1.12	0.613	0.528	1.37	1.76	6.78
9. The ratio of plant height to number of lateral branches	7.06	7.58	3.04	2.86	12.9	16.05	5.44
10. Day to 50% flowering	89.23	94.63	62.44	65	132.34	187	4.41
11. Day to 50% germination	42.21	58.68	24.53	41	65.66	84	3.65
12. One thousand per seed weight	3.41	3.67	1.97	2.21	5.89	6.38	9.71
13. Number of seed per umbel	12.25	7.27	4.21	4	18.34	15	4.31
14. Seed yield	20.25	17.23	7.21	6.54	40.99	40.31	6.43
15. Percentage of self pollination	4.15		2.25		6.58		3.34

European populations. It might be due to the intensive selection pressure combined with geographical isolation and differences in pollination (Ahmadi *et al.*, 2014). As a result of inbreeding, this trait was elevated in most of the populations. Moreover, number of seeds per umbel decreased, while one thousand seed weight increased as a result of inbreeding. Sharma and Meena (2013) reported higher number of seed per umbel in Indian fennel populations compared with Iranian ones. Singh and Sastry (2005) investigated the correlation between the yield components of 100 bi-parental progenies of fennel genotypes. Their results suggested that the seed yield of fennel can be improved through selection for the number of branches per plant.

A dendrogram based on morphological data was generated using Ward's method to reveal the relationships among populations. Outcross pollinated populations were clustered into five main groups (Figure 4). Group I consisted of seven populations including Tabriz, Varamin, Semirom, Poland, England, Spain and Albania from Iran and European populations. Group II was comprised of 11 populations including Yasuj, Kerman, Shiraz, Hamedan, Nahavand, Shiravan, Ardebil, Karaj, Yazd, Bushehr and Pave. Kashan classified in group III and showed the highest plant height and canopy diameter. Group IV included three populations including Tehran, Mashhad and Isfahan. Gonabad was clustered in group V. It had the largest number of lateral branches, one thousand seed weight and seed yield.

Self-pollinated populations were categorized into six groups (Figure 4). According to dendrograms, the classification of some populations such as Tabriz and Spain were changed as affected by inbreeding.

Hence, considering the importance of fennel as a valuable medicinal plant and the future need to increase its yield, it is necessary to strengthen the available gene pool of the fennel by introducing more variation to develop high yielding genotypes (Bahmani *et al*., 2012). So, the application of morphological markers for selecting of these elite populations can provide new insights for further breeding programs. On

Table 4. *LSD* test for comparisons of the morphological and phonological traits among different outcross and self pollinated populations of fennel.

Accession	Traits													
	Ratio of canopy diameter to plant height (%)		Ratio of plant height to number of lateral branches (%)		Day to 50% germination		Day to 50% flowering		Weight of 1000 seed (g)		Number of seed per umbel		Yield of seed (%)	
	outcross	self	outcross	self	outcross	self	outcross	self	outcross	self	outcross	self	outcross	self
Tabriz	0.979^{ab}	0.612^{c}	5.03^{f}	8.17^{c}	64.01^{a}	83.66^{a}	63.32^{de}	74^{e}	3.70^{b}	4.50^{b}	6.22^{g}	6.66^{e}	23.76^{b}	19.51^{b}
Yasuj	0.867^{b}	1.14^{ab}	7.86^{d}	7.30^{d}	61.92^{a}	81.66^{a}	127.2^{ab}	121.66^{b}	3.49^{b}	2.84^{cd}	4.28^{h}	4.64^{g}	16.12^{d}	13.26^{e}
Kashan	1.04^{ab}	1.06^{b}	9.4^{b}	9.51^{b}	26.32^{d}	46^{e}	74.19^{c}	87^{d}	3.47^{b}	3.83^{c}	10.66^{e}	8.33^{c}	22.21^{b}	18.15^{c}
Semirom	1.09^{ab}	1.38^{ab}	4.69^{g}	4.85^{g}	41.29^{c}	61^{c}	128.6^{ab}	95^{c}	2.16^{cd}	3.20^{c}	10.93^{e}	4.66^{g}	19.14^{c}	15.31^{d}
Kerman	1^{ab}	1.39^{ab}	6.96^{e}	5.91^{f}	45.39^{b}	71^{b}	112.7^{ab}	89^{d}	2.50^{c}	4.46^{b}	13.13^{c}	4.66^{g}	9.89^{h}	6.98^{h}
Hamedan	0.961^{ab}	1.27^{ab}	6.71^{e}	5.67^{f}	25.57^{cd}	55^{d}	122.8^{ab}	90.66^{c}	2.33^{c}	3.44^{c}	16.9^{ab}	8.33^{c}	28.48^{b}	22.50^{b}
Tehran	0.999^{ab}	1.09^{b}	4.73^{g}	4.69^{g}	44.2^{b}	58.66^{d}	74.62^{c}	66^{f}	3.31^{b}	4.18^{b}	16.3^{ab}	7^{d}	18.70^{c}	14.39^{d}
Bushehr	0.993^{ab}	1.03^{b}	5.64^{f}	5.50^{f}	43.28^{b}	51.33^{d}	77.79^{c}	75^{e}	3.8^{b}	3.47^{c}	9.8^{f}	8^{c}	32.88^{ab}	29.43^{a}
Nahavand	1.01^{ab}	1.40^{ab}	7.79^{d}	8.15^{c}	30.91^{c}	43.33^{ef}	78.36^{c}	89.33^{d}	2.24^{c}	2.38^{cd}	14.88^{b}	10^{b}	18.20^{c}	15.97^{d}
Shiraz	0.997^{ab}	1.16^{ab}	7.62^{d}	7.79^{d}	62.96^{a}	78^{b}	101.5^{ab}	131^{ab}	3.32^{b}	2.21^{d}	13.81^{c}	6^{e}	11.64^{g}	9.51^{g}
Ardebil	0.674^{bc}	0.735^{c}	12.17^{a}	14.20^{a}	26.39^{d}	46.33^{e}	77.39^{c}	85.66^{c}	3.37^{b}	2.59^{cd}	13.9^{c}	7.66^{d}	31.60^{ab}	27.62^{ab}
Karaj	0.998^{ab}	1.24^{ab}	11.04^{ab}	11.11^{a}	39.5^{c}	47.33^{e}	69.67^{d}	91.66^{c}	2.22^{c}	2.70^{cd}	9.64^{f}	5.66^{f}	16.91^{d}	15.27^{d}
Pave	1.36^{a}	0.977^{b}	6.01^{e}	8.87^{c}	39.7^{c}	54.66^{d}	81.99^{b}	91.66^{c}	3.71^{b}	3.76^{c}	17.67^{a}	15.66^{a}	31.49^{ab}	29.04^{a}
Varamin	0.989^{ab}	1.64^{a}	6.22^{e}	5.93^{f}	29.26^{d}	46^{e}	80.62^{b}	96.33^{c}	2.75^{c}	4.81^{b}	16.1^{ab}	7^{d}	20.96^{b}	17.92^{c}
Isfahan	1^{ab}	0.765^{c}	4.64^{g}	6.29^{e}	45.47^{b}	58.33^{d}	75.65^{c}	83.66^{d}	3.26^{b}	4.37^{b}	11.5^{de}	10.66^{b}	24.50^{b}	21.15^{b}
Spain	0.996^{ab}	1.43^{ab}	5.52^{f}	4.19^{g}	64.48^{a}	72.66^{b}	131.37^{a}	169.33^{a}	2.16^{cd}	3.04^{c}	12.28^{d}	9^{b}	14.38^{e}	12.73^{e}
Shirvan	0.934^{ab}	0.897^{c}	5.96^{f}	6.84^{e}	29.41^{d}	47.33^{e}	84.95^{b}	95.66^{c}	3.23^{b}	3.64^{c}	5.7^{g}	4.66^{g}	18.20^{c}	17.03^{c}
Yazd	0.699^{bd}	0.678^{c}	11.15^{ab}	13.87^{ab}	40.81^{c}	53.33^{d}	70.77^{c}	86^{d}	2.38^{c}	3.59^{c}	14.73^{b}	7^{d}	20.89^{b}	19.33^{b}
Poland	1^{ab}	1.21^{ab}	8.61^{c}	9.57^{b}	26.43^{d}	47^{e}	72.66^{c}	83^{d}	4.42^{ab}	3^{c}	14.43^{b}	10.66^{b}	11.93^{g}	10.77^{f}
England	0.996^{ab}	1.59^{a}	7.61^{d}	8.63^{c}	31.3^{c}	56.66^{d}	69.73^{d}	80.33^{d}	4.27^{ab}	3.17^{c}	13.15^{c}	8.66^{c}	9.95^{h}	6.95^{h}
Gonabad	0.769^{b}	0.835^{c}	3.12^{h}	2.94^{h}	29.64^{d}	53.33^{d}	73.87^{c}	79.33^{d}	4.69^{a}	6.38^{a}	10.51^{e}	6.33^{e}	40.75^{a}	27.86^{ab}
Mashhad	0.624^{bd}	0.585^{cd}	6.62^{e}	6.44^{e}	61.72^{a}	74^{b}	97.78^{b}	101.78^{b}	4.37^{ab}	4.58^{b}	11 ^{de}	5.33^{f}	18.14^{c}	15.72^{d}
Albani	1^{ab}	1.60^{a}	7.39^{d}	8.03^{c}	64.03^{a}	63^{c}	109.5^{ab}	113.59^{b}	3.29^{a}	2.91^{cd}	13.7^{c}	8^{c}	12.36^{f}	10.01^{f}

Table 4. (continued)

Continued of Table 4.

Accession	Traits													
	Percentage of emergence (%)		Plant height (cm)		The number of lateral branches		Inflorescence diameter (cm)		Canopy diameter (cm)		Plant wet weight (g)		Plant dry weight (g)	
	Outcross	Self	Outcross	Self	Outcross	Self	Outcross	Self	Outcross	Self	Outcross	Self	Outcross	Self
Tabriz	0.560^{ab}	0.350^{c}	55^{f}	73^{d}	11^{c}	9^{e}	5.17^{d}	6.29^{a}	53.86^{e}	44.65^{e}	134.43^{d}	114.43^{cd}	31.61^{e}	22.27^{e}
Yasuj	0.333^{d}	0.146^{e}	86.12^{c}	65.22e	11^{c}	9^{e}	5.09^{d}	6.31^{a}	74.7^{c}	74.83^{b}	168.06^{d}	150.69^{cd}	45.34^{d}	35.57d^{e}
Kashan	0.483^{c}	0.283^{d}	109.54^{a}	91.92^{b}	11.66^{c}	9.66^{e}	4.01^{e}	6.04^{a}	114.3^{a}	97.70^{a}	272.14^{c}	144.69^{cd}	135.19^{b}	85.40^{b}
Semirom	0.346^{d}	0.216^{d}	59.44^{f}	51.87^{f}	12.66^{c}	10.66^{d}	3.32^{f}	4.36^{c}	64.93^{d}	71.13^{b}	155.27^{d}	136.20^{cd}	79.88^{c}	65.15^{c}
Kerman	0.330^{cd}	0.233^{d}	81.15^{c}	57.24^{f}	11.66^{c}	9.66^{e}	7.40^{b}	6.25^{b}	81.47^{c}	79.01^{b}	180.06^{d}	151.87^{c}	51.31^{d}	45.10^{d}
Hamedan	0.616^{b}	0.403^{b}	80.17^{c}	55.91^{f}	12^{c}	10^{d}	4.03^{e}	5.18^{b}	77.12^{c}	70.38^{b}	190.33^{d}	178.11^{c}	86.25^{c}	64.39^{c}
Tehran	0.616^{b}	0.336^{c}	91.48^{b}	81.42^{c}	19.33^{b}	17.33^{b}	4.47^{e}	5.62^{b}	91.48^{b}	89.17^{ab}	322.33^{b}	158.20^{c}	209.16^{b}	143.96^{ab}
Bushehr	0.383^{d}	0.226^{d}	77.05^{d}	64.05^{e}	13.66^{c}	11.66^{d}	6.20^{c}	5.47^{b}	76.55^{c}	66.46 ^{c}	227.46^{c}	156.17^{c}	88.48^{c}	76.04^{bc}
Nahavand	0.586^{b}	0.373^{c}	75.17^{d}	62.35^{e}	9.66^{cd}	7.66^{r}	4.89^{e}	5.28^{b}	76.02^{c}	87.75^{ab}	156.7^{d}	144.17^{cd}	40.7^{d}	35.56^{de}
Shiraz	0.655^{ab}	0.483^{b}	81.17^{c}	67.32^{e}	10.66^{cd}	8.66^{r}	3.83^{f}	5.63^{b}	80.94^{c}	78.25^{b}	170.67^{d}	160.55^{c}	49.67^{d}	43.53^{d}
Ardebil	0.613^{b}	0.410^{b}	105.23^{a}	94.05^{b}	8.66^{d}	6.66^{g}	4.56^{e}	6.38^{a}	70.99^{c}	69.12^{c}	161.82^{d}	122.03^{cd}	42.59^{d}	32.91^{de}
Karaj	0.480^{c}	0.280^{d}	84.37^{c}	62.59^{e}	7.66^{d}	5.66^{g}	3.93^{f}	5.11^{b}	84.22^{c}	78.01^{b}	148.73^{d}	149.20^{cd}	84.87^{c}	76.49^{bc}
Pave	0.416^{b}	0.280^{c}	76.04^{d}	94.43^{b}	12.66^{c}	10.66^{d}	10.56^{a}	5.70^{b}	103.8^{a}	92.28^{a}	197.03^{d}	189.03^{c}	74.18^{c}	69.43^{c}
Varamin	0.583^{b}	0.323^{c}	55.58^{f}	40.96^{gh}	9^{cd}	7^{f}	3.78^{f}	6.37^{a}	54.96^{e}	67.53^{c}	132.61^{d}	143.51^{cd}	33^{e}	28.94^{e}
Isfahan	0.683^{a}	0.496^{b}	92.73^{b}	113.03^{a}	20^{b}	18^{b}	5.41^{d}	6.22^{a}	92.89^{b}	85.51^{ab}	358.28^{b}	223.09^{b}	153.20^{b}	97.60^{b}
Spain	0.666^{ab}	0.480^{b}	66.02^{e}	41.71^{g}	12^{c}	10^{d}	4.89^{e}	6.21^{a}	65.81^{d}	59.78^{d}	110.03^{d}	95.33^{e}	71.23^{c}	65.51^{c}
Shirvan	0.650^{ab}	0.493^{b}	69.53^{e}	65.99^{e}	11.66^{c}	9.66^{e}	4.32^{e}	9.66^{e}	65.01^{d}	59.24^{d}	172.11^{d}	183.83^{c}	53.10^{d}	44.41^{d}
Yazd	0.550^{b}	0.323^{c}	92.72^{b}	87.40^{c}	8.33^{d}	6.33^{g}	6.95^{c}	6.33^{g}	64.87^{d}	59.29^{d}	156.34^{d}	166.90^{c}	76.26^{c}	65.40^{c}
Poland	0.466^{c}	0.346^{d}	57.11^{f}	44.41^{g}	6.66^{e}	4.66^{h}	4.81^{e}	4.66^{h}	57.38^{e}	53.87^{d}	110.08^{d}	97.55^{e}	83.40^{c}	78.35^{bc}
England	0.363^{d}	0.216^{b}	55.66^{f}	45.59^{g}	7.33^{d}	5.33^{g}	5.21^{d}	5.33^{g}	55.45^{e}	72.61^{b}	98.37^{e}	82.71^{ef}	72.65^{c}	68.37^{c}
Gonabad	0.570^{b}	0.433^{a}	96.73^{b}	85.34^{c}	31^{a}	29^{a}	5.87^{d}	29^{a}	74.41^{c}	71.21^{b}	446.69^{a}	319.90^{a}	251.35^{a}	152.13^{ab}
Mashhad	0.633^{ab}	0.533^{b}	108.07^{a}	92.37^{b}	16.33^{b}	14.33^{c}	7.76^{b}	14.33^{c}	67.51^{d}	54.04^{d}	310.77^{b}	212.13^{b}	186.45^{b}	161.68^{a}
Albani	0.616^{b}	0.480^{b}	54^{f}	42.57^{g}	7.33^{d}	5.33^{g}	5.04^{d}	5.33^{g}	54.04^{e}	68.16^{c}	121.67^{d}	140.80^{cd}	94.20^{c}	87.76^{b}

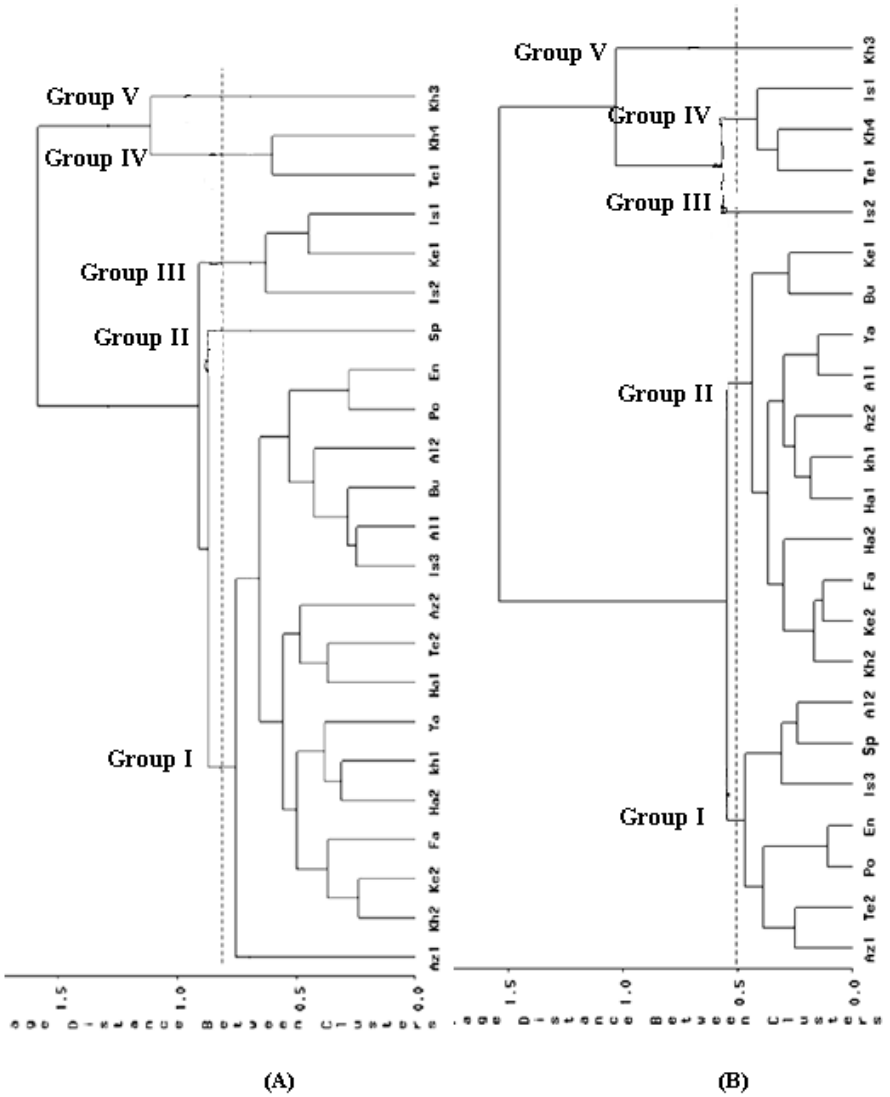

Figure 4. Morphological dendrogram based on Ward's method: (A) Selfed and (B) Outcross fennel populations. *The codes used in dendrograms were explained in Table 1.

the other hand, molecular markers can amplify some parts of the genome. ISSR is considered as a dominant marker and its distribution in genome is much lower than co-dominant ones, while most of the morphological traits can be controlled by quantitative genes that are more evenly distributed throughout the genome. So, in the present research, inbreeding might be probably more effective in altering the morphological classification than molecular ones (Ahmadi *et al*., 2014).

CONCLUSIONS

In fennel, selection of populations with high essential oil yield and genetic distance can be beneficial for breeding purposes. Among the studied outcross populations, Semirom (Is3) from the central part of Iran and Poland (Po) from Europe showed a relatively high genetic distance (0.42) and possessed low (3.1%) and the highest essential oil yield (6.2%), respectively. In self-pollinated populations, Yasuj (Kh2)

from the southwest of Iran and Albania (Al2) from Europe showed a relatively high genetic distance (0.53) and possessed low (3.5%) and the highest essential oil yield (5.8%), respectively. These populations can be introduced as appropriate candidates for future breeding programs aimed for increasing oil yield in fennel. Overall, inbreeding increased the essential oil yield of most of the studied populations. So, in respect to essential oil yield of fennel, selecting of self pollinated populations can produce more insightful results. Finally, according to the results of the present study, it is suggested to assess the next generations of the studied fennel populations in respect to the secondary metabolites variation as well as genetic diversity, using other polymorphic markers.

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تنوع ژنتیکی در جمعیت های آزادگرده افشان و خودگرده افشان رازیانه با استفاده از نشانگرهای مورفولوژیک و مولکولی ISSR

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چکیده

نشانگر ISSR برای ارزیابی تنوع ژنتیکی ۲۳ جمعیت آزادگرده افشان و خود گرده افشان رازیانه جمع‌آوری شده از مناطق جغرافیایی مختلف ایران و برخی مناطق اروپا استفاده گردید. تحقیق حاضر به منظور ارزیابی تاثیر خویش آمیزی بر صفات مورفولوژی و تنوع ژنتیکی در طی نسل بعد انجام شد. پانزده ترکیب نشانگری ISSR در توده‌های آزادگرده افشان در مجموع، ۲۴۸ نوار چند شکل تولید نمودند که به طور متوسط ۱۶/۵۳ نوار به ازای هر آغازگر تکثیر شد در حالی که در جمعیت‌های خودگرده افشان در مجموع ۲۱۷ نوار چند شکل تولید نمودند و به طور متوسط ۱۴/۴۶ نوار به ازای هر آغازگر تکثیر شد. طبق ضریب تشابه جاکارد و الگوریتم UPGMA برای خوشه‌بندی، توده‌های دگرگرده افشان رازیانه را بر اساس مناطق جغرافیایی به پنج گروه تقسیم‌بندی شدند. بیشترین و کمترین

تنوع ژنتیکی در هر مکان ژنی، به ترتیب در جمعیت آلبانی (۰/۵۳) و لهستان (۰/۴۲) مشاهده شد. به طور کلی، جمعیت‌های اروپایی میزان هتروزیگوسیتی مورد انتظار بیشتری را (۰/۴۷) در مقایسه با جمعیت‌های ایرانی نشان دادند(۰/۳۵). میزان چندشکلی (PIC) در جمعیت‌های خودگرده افشان از ۰/۳۷ تا ۰/۴۹ متغیر بود در حالی که این مقدار در جمعیت‌های آزادگرده افشان برابر با ۰/۳۹ تا ۰/۴۶ بود. نمودار خوشه‌ای حاصل از داده‌های مورفولوژیک نتایج نمودار خوشه‌ای حاصل از داده‌های مولکولی را در بیشتر موارد مورد تایید قرار نداد. خود گرده افشانی سبب کاهش ارتفاع دراکثر جمعیت‌های مورد مطالعه شد. در مجموع، جمعیت‌های اروپایی (۵۴-۶۶/۰۲ سانتی‌متر) کوتاه‌تر از جمعیت‌های ایرانی (۵۵-۱۰۹/۵۴سانتی‌متر) بودند. گرده افشانی عملکرد اسانس را در جمعیت‌های مورد مطالعه با تأثیرگذاری بر روند تشکیل میوه، افزایش داد. می‌توان نتیجه گرفت که جمعیت آلبانی با داشتن بیشترین میزان اسانس تحت تاثیر خویش‌آمیزی، در برنامه‌های اصلاحی در جهت بهبود میزان اسانس مورد استفاده قرار گیرد.