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Genetic Diversity in Iranian Fennel (*Foeniculum vulgare* Mill.) Populations Based on Sequence Related Amplified Polymorphism (SRAP) Markers

H. Maghsoudi Kelardashti[1], M. Rahimmalek[1*], and M. Talebi[2]

ABSTRACT

To evaluate the genetic variations in eleven fennel populations in Iran, 55 accessions were analyzed by SRAP markers. Twelve primer combinations produced 171 polymorphic bands. The cumulative dendrogram delineated fennel accessions into five major groups in accordance to the geographical regions from which the populations were originated. About 43.57% of total genetic variation was detected among the populations, while 56.43% of total variation were observed within the populations. The studied populations showed high genetic differentiation (Gst= 0.52) and low gene flow (Nm= 0.46). Among the studied populations, Yazd and Kerman accounted for the highest values of PPB (%), Shanon index (I), and heterozygosity. The self-pollinated seeds were in the range of 2.01% in Paveh-2 to 9.24% in Isfahan-2 accessions. The essential oil content ranged from 0.62% in Isfahan-3 to 2.21% in Tabriz-3. Generaly, the dwarf populations *viz*., Tabriz and Paveh, had higher essential oil yield and their pollination was less affected by environmental factors than the average.

Keywords: Apiaceae, Genetic differentiation, Molecular marker, Pollination, Variation.

INTRODUCTION

Fennel is a biennial medicinal and aromatic plant belonging to the family Apiaceae. It is a hardy, perennial umbelliferous herb with yellow flowers and feathery leaves [9]. It is generally considered indigenous to the shores of Mediterranean Sea, but has become widely domesticated in many parts of the world, especially on dry soils near the coastal area and on the river banks [9]. Some researchers have distinguished two sub-species of fennel, piperitum and vulgare: the former has bitter seeds, while the latter is characterized by sweet seeds mainly used as flavoring agents in baked foods, meat and fish dishes, ice creams, and alcoholic beverages due to their characteristic anise odour [9]. Herbal medicine and essential oils extracted from fennel are known for their diuretic, anti-inflammatory, analgesic, stomachic and galactogogue properties and antioxidant activities among many others[8, 26].

Plants breeders indebt their success in the past, present, and future to genetic variations in crops and their wild relatives. Landraces widely characterized with unique traits are promising for breeding programs [31]. The main objective to study genetic variation and interrelationships among germplasm collections is to apply such information to develop and release much productive cultivars among cultivated species [2]. Furthermore, genetic variation pattern can

[1] Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan 84156 83111, Islamic Republic of Iran.
[*] Corresponding author; e-mail: mrahimmalek@cc.iut.ac.ir
[2] Department of Agricultural Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, 8415683111, Islamic Republic of Iran.

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provide new insights for developing gene-pool collections.

Recent breakthroughs in DNA-based markers technology have led to breeders overcome wide varieties of issues on classification and conservation of plant genetic sources and to screen repetitive specimens in gene banks. SRAP (Sequence Related Amplified Polymorphism) is a Polymerase Chain Reaction (PCR)-based marker system which preferentially amplifies Open Reading Frames (ORFs) [18]. It has been employed to detect polymorphism among individuals in various crop species [18], considering advantages such as reasonable throughput rate, disclosing numerous codominant markers such as ISSR and easy isolation of bands for sequencing [19, 34].

It is possible to evaluate genetic relationships of the fennel ecotypes to introduce them for further breeding programs. In spite of its medicinal potential, limited number of molecular and breeding studies on Iranian fennels in literatures is available. Furthermore, there are no reports concerning the genetic diversity among and within Iranian fennel populations. Zahid *et al*. (2009) assessed the genetic diversity of Pakistan fennel germplasm using 16 RAPD primers [35]. Genetic diversity among Indian varieties of *Foeniculum vulgare* was also evaluated using nuclear ribosomal DNA and RAPD markers [32]. Bahmani *et al*. (2013) evaluated the genetic diversity of 25 Iranian ecotypes based on 72 RAPD polymorphic bands [5]. Torabi *et al*. (2012) dealt with the genetic diversity of 30 fennel accession using AFLP markers [33].

The present research aimed to: (i) determine the level and the patterns of genetic variation and differentiation among and within fennel ecotypes using SRAP markers and (ii) assess variation in some morphological traits, self-pollinated seed production and its relationship with essential oil yield.

MATERIALS AND METHODS

Plant Materials

Young leaf samples of 55 accessions from 11 populations of Iranian fennels were used as starting material to carry out a SRAP marker analysis (Table 1). Sampling was performed based on different geographical origins of the country (Figure 1).

Figure 1. Origin of the fennel population collected from different geographical regions of Iran.

Table 1. The geographical location, morphological traits, and essential oil yield of 55 Iranian fennel accessions.

No	Population	Geographical region	Dry weight per plant (gr)	No. of lateral shoots	Flower diameter	Day to 100% flowering	Flowering date	Essential Oil yield (%)	Plant height (cm)	Self-pollination (%)	Seed yield (gr)
1	Shiraz1	Shiraz, Fars	182.33	28	7.32	98	Medium	0.89	182.24	4.23	10.26
2	Shiraz2	Shiraz, Fars	180.17	28	7.54	98	Medium	0.91	170.56	4.72	12.35
3	Shiraz3	Shiraz, Fars	192.17	31	7.33	97	Medium	0.88	167.25	3.96	13.24
4	Shiraz4	Shiraz, Fars	178.14	32	6.98	99	Medium	0.95	180.43	4.05	9.84
5	Shiraz5	Shiraz, Fars	188.74	30	7.87	98	Medium	0.92	172.64	4.86	11.96
6	Hamedan1	Hamedan, Hamedan	208.14	22	7.85	101	Medium	0.88	170.86	6.56	32.14
7	Hamedan2	Hamedan, Hamedan	217.12	23	8.54	101	Medium	0.86	179	5.68	30.46
8	Hamedan3	Hamedan, Hamedan	185.14	20	7.67	103	Medium	0.84	169	5.26	26.42
9	Hamedan4	Hamedan, Hamedan	207.52	21	7.24	100	Medium	0.92	180.32	4.86	25.46
10	Hamedan5	Hamedan, Hamedan	211.45	23	7.87	102	Medium	0.83	173.26	4.67	29.75
11	Kerman1	Kerman, Kerman	208.25	18	4.32	131	Very late	0.89	136.25	4.32	8.35
12	Kerman2	Kerman, Kerman	222.45	19	4.56	132	Very late	0.85	137.62	4.47	9.23
13	Kerman3	Kerman, Kerman	219.68	17	5.22	130	Very late	0.83	130.72	3.98	8.23
14	Kerman4	Kerman, Kerman	232.75	18	4.46	132	Very late	0.81	140	5.2	7.86
15	Kerman5	Kerman, Kerman	215.94	20	3.98	129	Very late	0.91	132	4.62	8.24
16	Isfahan1	Isfahan, Isfahan	172.15	14	7.35	102	Medium	0.64	118	9.1	27.56
17	Isfahan2	Isfahan, Isfahan	173.85	14	7.59	102	Medium	0.71	124	9.24	23.45
18	Isfahan3	Isfahan, Isfahan	182.17	15	7.45	103	Medium	0.62	111.56	8.56	24.32
19	Isfahan4	Isfahan, Isfahan	180.75	16	8.01	103	Medium	0.67	110.85	8.46	30.21
20	Isfahan5	Isfahan, Isfahan	175.46	15	6.88	103	Medium	0.69	115	7.98	20.86
21	Boushehr1	Boushehr, Boushehr	185.35	16	6.33	110	Late	0.86	132.24	3.56	35.12
22	Boushehr2	Boushehr, Boushehr	182.17	13	6.52	109	Late	0.83	130.58	4.1	33.7
23	Boushehr3	Boushehr, Boushehr	187.16	13	6	108	Late	0.81	140.23	3.56	30.56
24	Boushehr4	Boushehr, Boushehr	193.15	15	6.88	108	Late	0.87	135.95	4.2	36.48
25	Boushehr5	Boushehr, Boushehr	194.32	14	6.03	107	Late	0.84	129.48	3.46	29.86
26	Paveh1	Paveh, Kermanshah	345.12	14	5.33	83	Very early	0.95	82.36	2.32	35.45
27	Paveh2	Paveh, Kermanshah	346.75	13	5.45	82	Very early	0.93	85	3.16	30.56
28	Paveh3	Paveh, Kermanshah	355.12	15	6.11	82	Very early	0.86	87.42	2.26	29.86
29	Paveh4	Paveh, Kermanshah	333.45	15	5.22	81	Very early	1.56	88.24	2.42	28.83
30	Paveh5	Paveh, Kermanshah	348.17	14	5.48	83	Very early	1.05	84.68	2.01	37.43
31	Tabriz1	Tabriz, Azarbayjan sharghi	127.22	14	8.11	83	Very early	2.14	73.23	3.14	28.32
32	Tabriz2	Tabriz, Azarbayjan sharghi	130.85	14	8.35	83	Very early	1.98	78	3.02	24.23
33	Tabriz3	Tabriz, Azarbayjan sharghi	122.85	13	7.88	84	Very early	2.21	72.46	3.15	20.23
34	Tabriz4	Tabriz, Azarbayjan sharghi	125.63	13	8	82	Very early	1.76	74.32	3.9	24.16
35	Tabriz5	Tabriz, Azarbayjan sharghi	132.87	11	8.22	81	Very early	2.11	81.23	4.21	26.12
36	Mashhad1	Mashhad, Khorasan razavi	188.17	14	8.55	98	Late	0.81	150.23	3.21	21.23
37	Mashhad2	Mashhad, Khorasan razavi	196.75	10	8.78	100	Late	0.83	149.26	3.45	20.18
38	Mashhad3	Mashhad, Khorasan razavi	209.15	11	8.9	98	Late	0.84	158.23	3.64	19.32
39	Mashhad4	Mashhad, Khorasan razavi	220.56	13	7.86	97	Late	0.91	161.56	2.89	16.42
40	Mashhad5	Mashhad, Khorasan razavi	197.94	11	8.66	98	Late	0.82	157.42	2.76	17.23

Table1 continued…

Continued of Table 1.

No	Population	Geographical region	Dry weight per plant (gr)	No. of lateral shoots	Flower diameter	Day to 100% flowering	Flowering date	Essential Oil yield (%)	Plant height (cm)	Self-pollination (%)	Seed yield (gr)
41	Tehran1	Tehran, Tehran	183.17	12	8.01	117	Late	0.95	130.21	5.26	20.32
42	Tehran2	Tehran, Tehran	175.42	11	8.33	116	Late	0.93	130	5.33	18.16
43	Tehran3	Tehran, Tehran	176.14	11	8.54	116	Late	0.91	128.74	4.98	16.32
44	Tehran4	Tehran, Tehran	179.85	11	7.86	118	Late	0.94	135.42	4.78	18.46
45	Tehran5	Tehran, Tehran	173.15	12	8.12	117	Late	0.96	132.21	5.46	19.64
46	Yazd1	Yazd, Yazd	147.23	13	9.11	110	Late	1.16	126.23	4.75	20.18
47	Yazd2	Yazd, Yazd	150.65	9	9.33	108	Late	1.32	128.24	4.82	21.32
48	Yazd3	Yazd, Yazd	155.45	11	8.54	107	Late	0.98	122.45	4.32	18.56
49	Yazd4	Yazd, Yazd	160.17	10	9.75	105	Late	0.97	120.36	3.98	17.42
50	Yazd5	Yazd, Yazd	148.86	12	9.42	108	Late	1.87	122	4.36	15.46
51	Ardebil1	Ardebil, Ardebil	137.56	7	7.52	76	Very early	1.023	90.23	3.45	33.26
52	Ardebil2	Ardebil, Ardebil	134.16	8	7.56	76	Very early	1.47	95	3.26	32.17
53	Ardebil3	Ardebil, Ardebil	130.56	8	7.33	76	Very early	0.95	91.23	3.75	28.46
54	Ardebil4	Ardebil, Ardebil	144.86	9	7.88	77	Very early	0.98	94.23	2.98	27.89
55	Ardebil5	Ardebil, Ardebil	140.75	10	7.01	75	Very early	1.42	97.46	2.86	30.24

DNA Extraction

DNA extraction from young leaves was performed using modified CTAB procedure as described by Murry and Thompson (1980)[13]. The quality and quantity of DNA was estimated spectrophotometrically and electrophoretically.

The ratio of OD260/280 was measured for the quality of DNA and the quantity of DNA was calculated as the following simple formula:

DNA concentration (ng μL^{-1})= OD260×50×Dilution coefficient

Finally, DNA was diluted to operating concentration of 50 ng mL^{-1}.

Protocol for the SRAP Analysis

SRAP analysis was performed according to the protocol described by Li and Quiros [18] with slight modifications. The primers used in the analysis are reported on Table 2. Initially, all SRAP primer [34] combinations were screened using 20 samples. Based on the screening results, 12 primer combinations, which produced scorable polymorphic bands, were used to amplify all accessions (Table 3). PCR reactions were performed in a total volume of 25.0 µL as follows: 2.5 µL of dNTP mix (2.5 mM), 2.5 µL of PCR buffer (10X), 0.4 µL of Taq polymerasease (2.5 U μL^{-1}), 2.5 µL of forward primer and reverse primer (3 Pmol mL^{-1}), 3.0 µL of DNA template, and 9.8 µL of distilled water. The SRAP markers were amplified on following parameters: 4 minutes at 94°C, 5 cycles of 94°C for 1 minute, 35°C for 1 minute, 72°C for 2 minutes, 35 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes, and final extension of 10 minutes at 72°C. The PCR products were run on 6% denaturing polyacrylamide electrophoresis gel. The amplification products were then detected by silver staining followed by allele scoring for more accuracy. The first five cycles are run at 94°C, 1 minute, 35°C, 1 minute, and 72°C, 1 minute, for denaturing, annealing, and extension, respectively. Then, the annealing temperature was raised to 50°C

Table 2. Sequence of forward and reverse SRAP primer combinations for 55 accessions.

	Primer	Sequence (5'→3')	Annealing temp (°C)
Forward	Me1	*5'-TGAGTCCAAACCGGATA-3'*	50
	Me2	*5'-TGAGTCCAAACCGGAGC-3'*	55
	Me3	*5'-TGAGTCCAAACCGGAAT-3'*	50
	Me4	*5'-TGAGTCCAAACCGGACC-3'*	55
	Me5	*5'-TGAGTCCAAACCGGAAG-3'*	52
Reverse	Em1	*5'-GACTGCGTACGAATTAAT-3'*	49
	Em2	*5'-GACTGCGTACGAATTTGC-3'*	54
	Em3	*5'-GACTGCGTACGAATTGAC-3'*	54
	Em4	*5'-GACTGCGTACGAATTTGA-3'*	52
	Em5	*5'-GACTGCGTACGAATTAAC-3'*	52
	Em6	*5'-GACTGCGTACGAATTGCA-3'*	54

Table 3. The number of total and polymorphic fragments per SRAP primer combinations and Polymorphic Information Content (PIC) along with Gene diversity.

No.	Primer combinations	No. total bands	No. PB[a]	PPB[b] (%)	PIC
1	Me2_Em5	16	12	75	0.27
2	Me5_Em2	20	18	90	0.47
3	Me4_Em3	12	11	92	0.35
4	Me3_Em6	15	13	87	0.34
5	Me1_Em6	12	10	83	0.28
6	Me4_Em1	14	13	93	0.43
7	Me1_Em4	22	21	95	0.31
8	Me2_Em4	16	13	81	0.35
9	Me2_Em2	16	15	94	0.34
10	Me4_Em6	11	9	82	0.28
11	Me3_Em3	18	17	94	0.46
12	Me1_Em1	20	19	95	0.46
Total		192	171	-	-
Average		-	14.14	88.41	0.36

[a] Number of Polymorphic Band; [b] Percentage of Polymorphic Band. * Annealing temp 50°C.

for another 35 cycles. The amplicons were separated by denaturing acrylamide gels.

Essential Oil Extraction

Essential oil yield of each population was measured by hydro-distillation of 20 g shade dried leaf samples in three replicates using a clevenger apparatus.

Morphological Analysis

The fennel populations were collected from different geographical regions of Iran. The fennel accessions were cultivated in Randomized Block Design (RCBD) with three replicates. Some of morphological traits such as plant height, flowering date, flower diameter, day to 100% flowering, number of lateral shoots, dry weight per plant, and seed yield were measured in three replicates and the average values were used for analysis (Table 1).

Determination of Self-pollinated seeds

The inflorescence of each population was divided in two parts. Half was used for cross pollination and the other umbels were

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bagged to prevent cross-pollination. The number of self- pollinated seeds was calculated at seeds harvesting time. The percent of self- pollination (%) was measured as the ratio of the number of self-pollinated seeds to cross pollinated ones.

Data Analysis

Distinct polymorphic SRAP bands in each gel were scored as present (1) or absent (0) and the missing bands was shown with number (9). The number of bands, Percentage of Polymorphic Bands (PPB), and Percentage of Special Bands (PSB) were calculated. PPB was calculated as the products amplified by each individual primer across all accessions, i.e. the number of polymorphic bands produced in all accessions, expressed as a percentage of the total number of products amplified by the given primer. PSB was determined as the percentage of unique bands in each accession's profile over the total number of products yielded by all primers. similarity coefficient $S_{ij}= 2a/(2a+b+c)$, after the Nei and Li (1979)[14] was used to calculate genetic distances ($GD_{ij}= 1-S_{ij}$) among populations, where, S_{ij} represents the similarity between two individuals *i* and *j*; *a* is the number of shared bands; b is the number of bands exclusive of *i*, and *c* is the number of bands exclusive of *j*. Cluster analysis and PCoA (Principle Coordinate Analysis) were performed by the Numerical Taxonomy and Multivariate Analysis System (NT-SYS-pc) Version 2.02 [30]. The Polymorphic Information Content (PIC) was calculated using simplified formula [3]. Jaccard's similarity index was used to calculate genetic similarity among all accessions [17]. Dendrogram was generated using the Unweighted PairGroup Method with Arithmetic mean (UPGMA) clustering procedure of NTSYS-pc ver. 2.02 software. Mantel test (1967) was used to determine correlation between two matrices [22]. The Cophenetic correlation coefficient was generated by means of COPH algorithm to check the goodness of fit between the cluster in the dendrogram and the similarity coefficient matrix. Analysis of Molecular Variance (AMOVA) was calculated within each species using Arlequin ver. 3.1 software. POPGENE32 software was used to compute the number of effective loci, the percentage of polymorphic loci, Shannon's information index (I), observed number of alleles (Na), expected heterozygosity (He) and effective number of alleles (Ne).

RESULTS AND DISCUSSION

SRAP Amplification and Levels of Polymorphism

In the present study, 12 primer combinations were selected according to clarity, sharpness, and number of bands produced in a pre-screening assay carried out with 30 primer combinations on 20 accessions. A total number of 192 distinguishable fragments were produced by 12 SRAP primer combinations out of which 171 (88.3%) were polymorphic ranging from 9 to 21 with an average of 14.4 polymorphic bands per combinations (Table 3), in line with previous researchers who reported 10-20 SRAP polymorphic fragments per primer combinations [18, 11, 7]. Polymorphism Information Content (PIC) was calculated with an average of 0.36. The highest (0.47) and the lowest (0.27) PIC belonged to SRAP primer combinations of Me5-Em2 and Me2-Em5, respectively. The pattern of SRAP markers (Me1-Em4 primer) in the studied fennel accessions is illustrated in Figure 2.

Cluster Analysis

The constructed dendrograms were evaluated by the cophenetic correlation coefficient (0.88) based on the similarity matrix to validate the robustness of the obtained tree topology of cluster analysis. The UPGMA clustering algorithm based on

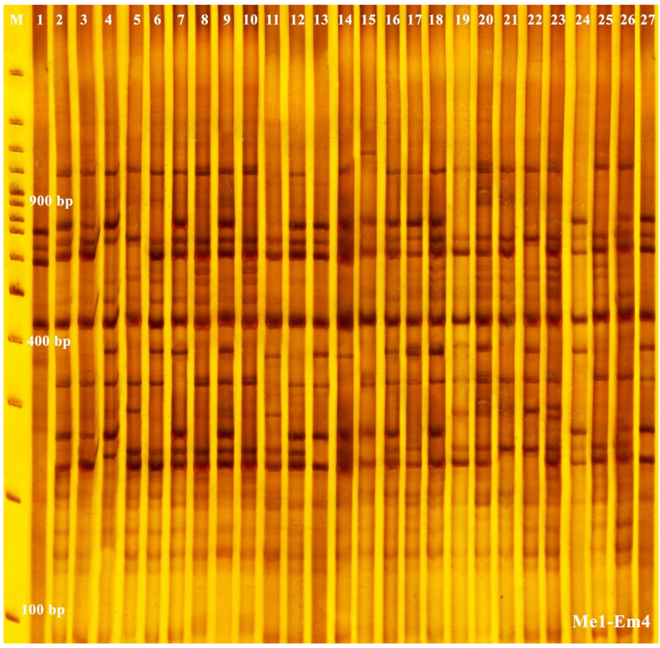

Figure 2. SRAP banding pattern of different fennel accessions using Me1-Em4 primer combination. M is 100 bp ladder and 1-27 includes the half of accessions amplified in one acrylamide gel.

Jaccard's similarity matrix was applied to construct the dendrogram that divided 55 fennel accessions into five groups at the level of similarities of 0.58 (Figure 3). First group involved 25 accessions and this group included populations from Shiraz, Isfahan, Ardabil and Paveh regions. In this group, populations from Shiraz and Isfahan showed more similarity in respect to climate. These populations were located in arid and warm

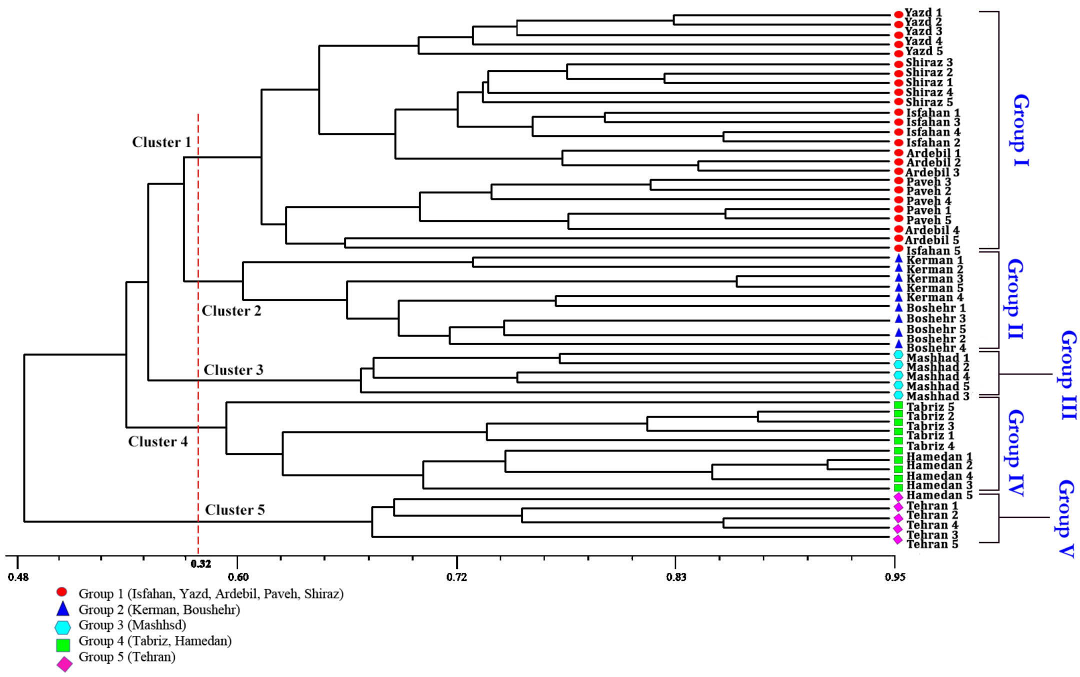

Figure 3. Dendrogram of 55 studied fennel accessions based on SRAP markers according to the Un-weighted Pair Group Mean Algorithm (UPGMA) with the Jaccard similarity index.

climates. Also, populations of Ardabil and Paveh showed some degrees of genetic similarity. These populations, characterized by dwarf, early maturity, light green color, and high essential oil yield, are found commonly in cold climates (Table 1). The second group consisted of two populations (10 accessions). In this group, Kerman and Bushehr were located in warm regions. Furthermore, Kerman and Boshehr populations were similar in morphological and essential oil yield (Table1). The third group involved Mashhad population from North-Eastern part of the country and contained five accessions. It was differed from others groups in climate conditions. The main feature attributed to this population was late-flowering (Table 1). Rahimmalek *et al*. (2009) assessed the essential oil composition of the fennel populations used in this research. In their studies, the lowest Limonene content in essential oil was recorded in Mashhad population compared to other populations [28]. The fourth group included Tabriz and Hamadan populations. Both of them were similar in climate condition. Tabriz and Hamedan populations vary morphologically and phonologically in spite of similar climate conditions. Tabriz is characterized by dwarf plants and light green color leaves, while Hamedan possessed high and late growing plants with dark grey leaves. These accessions revealed high essential oil yield (Table 1). The fifth group included plants from Tehran with moderate ripening and dark green leaves (Figure 3).

Principle Coordinate Analysis (PCoA) of Molecular Data

The first three eigenvectors explained 66.63 % of the total molecular variations, accounting 57.92, 4.78, and 3.93% of the observed variations, respectively. The results of PCoA analysis corresponded to those obtained through cluster analysis (Figure 4). As it is shown in Figures 4, accessions were grouped in similar pattern compared to cluster analysis.

Population Genetic Structure

Using Analysis of Molecular Variance (AMOVA), 43.57% of the total genetic variation was detected among the populations, while 56.43% of total variation was observed within the populations (Table 4).

Genetic distances and similarity coefficients of fennel populations are shown according to Ni and Li approaches in Table 5. The highest and the lowest similarity were obtained between Ardabil and Isfahan (0.910) and Shiraz and Hamedan (0.714) populations, respectively. Since Shiraz and Hamadan showed the highest genetic distance, they might be used as good sources for further breeding purposes. Some other populations indices were calculated using Popgene32 (Table 6). Gene variation, Shannon information index, heterozygosity and polymorphism percent for populations of Yazd and Kerman were found to be the highest, while Hamadan and Tabriz populations had the lowest ones. The highest PPB was recorded in Yazd (45.95) followed by Kerman (42.34) populations. Genetic differentiation coefficient (Gst) was calculated at about 0.52. The average of *Nm* was obtained as 0.46, suggesting relatively low gene flow among populations.

Morphological and Essential Oil Yield Variation

Morphological traits, essential oil yield and percent of self-pollination were measured among the accessions. Analysis of variance showed significant differences among all evaluated traits. The average of each trait is shown in Table 1. High variation was observed among studied morphological traits. Among accessions, Kerman4 and Paveh5 revealed the lowest

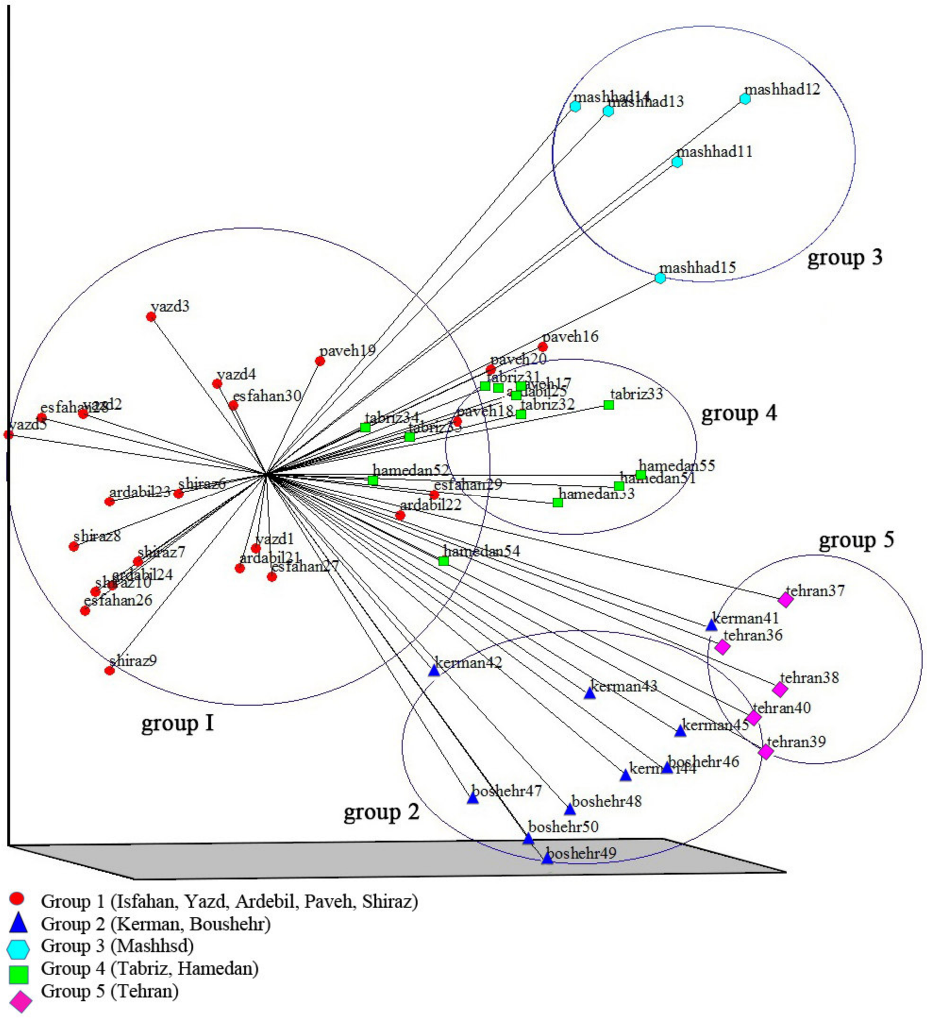

Figure 4. Principle Coordinate Analysis (PCoA) based on first three eigenvectors of 55 fennel accessions used in this study.

Table 4. AMOVA analysis among 11 fennel populations.

Source of variation	*df* [a]	Sum of squares	Mean of squares	Percentage of variation	*P* value	*Fst*
Among groups	10	363.5	5.77	43.57	< 0.001	0.43
Within groups	44	329	7.48	56.43		
Total	54	629.5	13.26			

(8.43 gr) and the highest values (37.43g) for seed yield per accession, respectively. Hamedan, Tabriz, and Paveh populations had very early flowering, while Yazd, Mashahad, Bushehr, Shiraz, and Kerman were late flowering populations. The self-pollinated seeds were in the range from 2.01 to 9.24% in Paveh5 and Isfahan2 accessions, respectively.

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Table 5. Similarity coefficient (above*) and genetic distance (below*) of 11 fennel populations.

Populations	Yazd	Shiraz	Mashhad	Paveh	Ardebil	Isfahan	Tabriz	Tehran	Kerman	Boushehr	Hamedan
Yazd	*	0.886	0.785	0.828	0.849	0.874	0.785	0.780	0.791	0.798	0.729
Shiraz	0.144	*	0.775	0.820	0.858	0.908	0.764	0.800	0.807	0.845	0.714
Mashhad	0.242	0.198	*	0.870	0.801	0.804	0.776	0.810	0.802	0.761	0.769
Paveh	0.188	0.198	0.139	*	0.882	0.874	0.800	0.820	0.842	0.816	0.794
Ardebil	0.164	0.153	0.222	0.126	*	0.910	0.749	0.818	0.816	0.827	0.771
Isfahan	0.135	0.097	0.218	0.135	0.094	*	0.796	0.822	0.832	0.842	0.745
Tabriz	0.243	0.269	0.254	0.223	0.289	0.228	*	0.868	0.776	0.767	0.780
Tehran	0.249	0.223	0.210	0.198	0.201	0.197	0.141	*	0.859	0.836	0.793
Kerman	0.235	0.214	0.221	0.172	0.204	0.184	0.254	0.152	*	0.897	0.841
Boushehr	0.226	0.169	0.273	0.204	0.190	0.172	0.266	0.179	0.109	*	0.851
Hamedan	0.317	0.337	0.263	0.227	0.260	0.295	0.249	0.232	0.173	0.162	*

Table 6. Summary of genetic variation statistics for SRAP markers in 11 fennel populations.

Population No	Population name	Na[a]	Ne[b]	H[c]	*I*[d]	H[e]	% PPB[f]	Nm[g]	Gst[h]
1	Yazd	1.46	1.29	1.17	0.26	0.22	45.95	-	-
2	Esfahan	1.34	1.26	0.14	0.21	0.18	34.23	-	-
3	Mashhad	1.39	1.27	0.15	0.23	0.19	38.74	-	-
4	Hamedan	1.27	1.17	0.10	0.15	0.13	27.03	-	-
5	Paveh	1.31	1.20	0.12	0.17	0.15	30.63	-	-
6	Boushehr	1.30	1.20	0.12	0.17	0.15	29.73	-	-
7	Tehran	1.29	1.19	0.11	0.17	0.14	23.83	-	-
8	Tabriz	1.27	1.17	0.10	0.15	0.13	27.03	-	-
9	Shiraz	1.34	1.23	0.13	0.20	0.17	34.23	-	-
10	Kerman	1.42	1.30	0.17	0.25	0.22	42.34	-	-
11	Ardebil	1.41	1.25	0.15	0.22	0.19	40.54	-	-
	Total	1.86	1.47	0.28	0.43	0.17	86.49	0.46	0.52

[a] Observed number of alleles, [b] Effective number of alleles, [c] Gene diversity, [d] Shannon's information index, [e] Heterozygosity, [f] Percentage of Polymorphic Bands, [g] Gene flow, [h] Genetic differentiation coefficient.

DISCUSSION

A relationship between genetic variability and geographic distribution has been observed in several species of medicinal plants belonging to different families. For instance, *Eryngium alpinum* [12], *Cuminum cyminum*, *Foeniculum vulgare* and *Falcaria vulgaris* [21] and *Changium smyrnioides* [27] were studied in Apiaceae family. The present study revealed high level of polymorphism (88.3 %) for Iranian fennel accessions confirmed by most of the previous reports on Apiaceae family [12, 21, 27]. The level of polymorphism obtained by SRAP primer combinations was higher than those reported by RAPD [5], ISSR [4] and AFLP [33] markers.

The number of polymorphic bands per primer combinations ranged from 9 to 21, on average 14.4, and the average *PIC* value for the amplification products was estimated to be 0.36 (Table 3), smaller than those obtained by Bahmani *et al*. (2011) using ISSR markers in Iranian fennels [4]. Results indicated that the majority of genetic diversity of Iranian fennel populations was obtained within populations, although there was high variation between populations. In the cluster analysis, the accessions from the same region were grouped together. The results were not in agreement with Qiu *et al*. (2004) report in *Changium smyrnioides* from Apiaceae based on RAPD markers [27]. Torabi *et al*. (2012) assessed the genetic similarity of 30 fennel accessions from Iran and some other countries. In their study, the highest genetic similarity among Iranian accessions was reported between Karaj and Kashan (89%), while in this research, the highest similarity was obtained between Ardebil and Esfahan populations [33]. Bahmani *et al*. (2013) studied genetic diversity of 25 Iranian fennel ecotypes using RAPD markers and found the highest and the lowest similarity coefficients between Sari and Kaleibar and between Hamedan and Ardabil populations, respectively [5]. Zahid *et al*. (2009) evaluated the genetic relationships of 50 indigenous fennel germplasm accessions in Pakistan using 30 RAPD primers that generated a total number of 145 bands [35]. In their research, 48% of fragments were polymorphic, that was much lower than those obtained in the present research.

PIC ranged from 0.27 to 0.47 with an average of 0.36. In the previous reports, the range of *PIC* in *F. vulgare* was 0.08 to 0.28 based on RAPD markers [16]. *PIC* determines the degree of polymorphism of marker, i.e. proportion of individuals heterozygous for a marker. In fact, *PIC* is a realistic measure of the heterozygosity [16].

Among the studied populations, Yazd and Kerman revealed the highest values for PPB%, Shanon index (I), and heterozygosity (Table 6), indicating these populations as good candidates for further breeding programs. In this research, the studied populations showed high Gst (0.52) and low *Nm* (0.46). Nei and Li (1979) [24] divided gene differentiation coefficient into three classes of low (Gst< 0.05), moderate (Gst= 0.05-0.15), and high (Gst> 0.15).The relatively high differentiation among populations and low gene flow (Nm= 0.46) observed in this study was in line with the results obtained by Qiu *et al*. [27] in *Changium smyrnioides* from Apiaceae. Low gene flow among populations may result from several factors, such as low seed dispersal, isolation of populations, self-pollination, and population size [27, 13]. Limited gene flow among populations is a plausible reason for the high genetic differentiation observed for this species [27]. In this study, the pollination system was also studied (Table 1). In fennel, flowering phases of the umbels within the same umbel were not simultaneous. Thus, flowering process of fennel assures fertilization among the flowers of each single umbel [25]. In the present study, the self-pollinated seeds in sweet fennel were in the range of 2.01% in Paveh to 9.24% in Isfahan, in agreement with the range (0-10%) obtained for bitter fennel populations[11]. Gross *et al*. (2008) also showed a high (0.7-3.7) index of selfcompatibility, indicative of substantial self-pollination in their studied populations. In the present research, a low gene flow was recorded among the studied fennel populations. Although interbreeding among populations was proven using artificial pollination, geographical isolation and the high likelihood for self-pollination probably restricts gene flow and contributes to the phenotypic diversity observed in wild fennel populations [14]. Furthermore, fennel flowers are strongly protandrous and a series of flowers must be produced to ensure pollination [10].

Different plant heights were observed in fennel populations (Table 1). The tall fennel populations, such as Shiraz and Tehran, are characterized by tall inflorescences and, therefore, withstand winds much stronger than dwarf ones such as Tabriz and Paveh. Therefore, the movement of flying pollinators of fennel, mostly diptera, wasps, or beetles are much smaller than the honeybee, and hence more susceptible to winds. For this, a panmictic pollination either is reduced, or totally eliminated [6]. The pollination also affects the essential oil yield in fennel. Fruit set is a key component of essential oil yield for fennel. Fruit set in tall populations is often low, possibly due to incomplete pollination [10]. Moreover, in fennel, fruit set decreases by a lack of synchrony between pollen production and stigma receptivity [10]. The previous reports also confirmed dwarf fennel populations possessed higher essential oil yield in their leaves than tall ones, while *tans*-anethole content, as the major compound of sweet fennel, was not highly affected by plant height [28]. Furthermore, a reverse correlation between estragole and *trans*-anethole content was reported and the action of a biallelic gene with partial dominance for high estragole content was also inferred [15].

Introducing new variation into plant germplasm is very helpful in the development of new cultivars characterized by either the higher content of important metabolites or better agronomic traits [1, 20]. According to the present data, essential oil yield was highly affected by environmental factors and, in most cases, molecular classification did not confirm the essential oil pattern of the studied accessions (Table 1). High variation was also observed within populations. For example, essential oil yield of Paveh accessions ranged from 0.86 to 1.32%, while Yazd accessions varied from 0.97 to 1.87%. It has been reported that essential oil yield is related to genetic and climatic factors, soil condition, stage of growth (vegetative or flowering), organogenesis, and anatomical part of the plant [29]. Finally, selection of accessions with high essential oil content and high genetic variation can be beneficial for further breeding purposes.

CONCLUSIONS

For the first time, the genetic diversity of different fennel populations was analyzed using SRAP molecular markers. These results showed high efficiency of SRAP markers (83.4%) in distinguishing various individuals from different fennel populations. In this research, the relatively high differentiation and low gene flow observed among populations might be attributed to fennels pollination. Furthermore, it is worthy to suggest that the dwarf populations like Tabriz and Paveh had higher essential oil yield and their pollination was less affected by environmental factors. Consequently, further selection of populations for evaluation of genetic diversity can lead to more insightful results.

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بررسی تنوع ژنتیکی جمعیت های رازیانه های ایرانی با استفاده از نشانگر مولکولی SRAP

ح. مقصودی کلاردشتی، م. رحیم ملک، و م. طالبی

چکیده

به منظور بررسی تنوع ژنتیکی ۵۵ نمونه جمعیتی از ۱۱ جمعیت رازیانه در ایران، نشانگر SRAP مورد استفاده قرار گرفت. ۱۲ ترکیب آغازگری در مجموع ۱۷۱ نوار چند شکل تولید نمود. نوارهای چند شکل با استفاده از ضریب جاکارد و الگوریتم UPGMA آنالیز شدند. دندروگرام حاصل نمونه ها را در پنج گروه بر اساس موقعیت جغرافیایی آن ها تقسیم نمود.بر خلاف نمونه های جنوبی، نمونه های شمال غربی در نواحی خنک تر قرار داشتند و به صورت پاکوتاه، زودرس، رنگ سبز روشن و دارای عملکرد اسانس بالا بودند. در حدود ۴۳/۵۷٪ کل تنوع در بین جمعیت ها آشکار شد در حالی که ۵۶/۴۳٪ تنوع در درون جمعیت مشاهده شد. جمعیت های مورد مطالعه تمایز ژنتیکی بالا (Gst= ۰/۵۲) و جریان ژنی کم (Nm= ۰/۴۶) از خود نشان دادند. در بین جمعیت های مورد مطالعه جمعیت های یزد و کرمان بالاترین تعداد نوارچندشکل، شاخص شانون و هتروزیگوسیتی را نشان دادند. میزان بذور حاصل از خودگشنی از ۲/۰۱٪ در پاوه تا ۹/۲۴٪ در اصفهان-۲ متغیر بودند. عملکرد اسانس نیز از ۰/۶۲٪ در اصفهان-۳ تا ۲/۲۱٪ در تبریز-۳ متغیر بودند. در مجموع جمعیت های پاکوتاه مانند پاوه و تبریز، عملکرد اسانسی بالاتری داشته و گرده افشانی آن ها کمتر تحت تاثیر عوامل محیطی قرار گرفته است.