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ORIGINAL ARTICLES

Genetic variation among three fennel (*Foeniculum vulgare* Mill.) varieties on the basis of morphological characters, essential oil composition and ISSR markers.

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ABSTRACT

Fennel (*Foeniculum vulgare* Mill.) is herbaceous plant, belonging to *Apiaceae* (*Umbelliferae*) family and widely used as spice and medicine. To assess the nature and extent of variability on agronomic, chemical compositions and ISSR markers, a test trial was conducted using three varieties (Balady, Indian and Holland) of fennel arranged in randomized complete blocks design in three replications during two winter seasons. The results of analysis of variance for the selected genotypes showed high significant differences in all the traits except for essential oil percent for Balady variety, high broad sense heritability (> 80%) was noticed for all characters among studied varieties. Maximum values for genetic advance were recorded for fruit yield of Holland followed by Balady and Indian varieties in both seasons. Overall, the highest values of broad sense heritability and genetic advance were obtained for linear growth, plant height, fruit yield and total number of branches among varieties suggesting that phenotypic selection for these traits would be effective. GC analysis of essential oil compositions indicated that, seven different compounds were identified in essential oil of all varieties. Methyl chavicol and anethole were identified as main components in the oil of all fennel varieties. Balady gave the highest methyl chavicol percentage followed by Indian and Holland varieties, respectively. ISSR markers were used in order to assess the genetic relationships among the fennel varieties. By using five primers, DNA fingerprint showed differences in the number of bands among varieties of fennel. The present study provides significant variability based on the quantitative characters, essential oil composition and ISSR markers.

Key words: Fennel varieties, essential oil, ISSR markers and genetic parameters

Introduction

Fennel (*Foeniculum vulgare* Mill.) belongs to the family of *Apiaceae*, and is an annual, biennial, or perennial herbaceous plant, depending on the variety, which grows in good soils from sunny mild climatic regions and is a well-known aromatic plant species. *Foeniculum vulgare* has two commercially important fennel types: bitter fennel, *Foeniculum vulgare* Mill. Subsp. *vulgare* var. *vulgare*, and sweet fennel *Foeniculum vulgare* subsp. *vulgare* var. *dulce*. Several fennel parts are edible (bulbs, leaves, stalks, and fruits). Mature fruit (commonly known as seeds) and essential oil of fennel are used as flavoring agents in food products such as liqueurs, bread, cheese, and an ingredient of cosmetics and pharmaceutical products. Moreover fennel infusions are the classical decoction for nursing babies to prevent flatulence and colic spasms, (Mimica-Duki´c *et al.*, 2003, Oktay *et al.*, 2003, Perry *et al.*, 2011 and Bruyas-Bertholon *et al.*, 2012). Traditionally in Europe and Mediterranean areas fennel is used as antispasmodic, diuretic, anti-inflammatory, analgesic, secretomotor, secretolytic, galactagogue, eye lotion, and antioxidant remedy (Lucinewton *et al*., 2005).

Very few efforts have been made to improve fennel (*Foeniculum vulgare* Mill.) through genetic manipulation. Since most of the yield attributing characters are quantitatively inherited and highly affected by environment, it is difficult to judge whether the observed variability is heritable or not. The primary parameters, namely, genotypic and phenotypic variances, genetic advance and heritability are useful in understanding the nature of inheritance of different traits. (Patel *et al*., 2008)

Essential oil composition depends upon internal and external factors affecting the plant such as genetic structures and ecological conditions; agricultural practices also have critical effects on yield and oil composition in the essential oil crops, although essential oil has some main components that can vitiate significantly according to the maturation period (Telci *et al.*, 2009) .

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The major components of fennel are phenyl propanoid derivatives: *trans*-anethole and estragole (= methyl chavicol), and then alpha-phellandrene, limonene, fenchone, and alpha-pinene (Barazani *et al.,* 2002). Fennel oil have been investigated for its constituents as sweet and bitter oil (Piccaglia and Marotti 2001,D´ıaz *et al.,* 2006 .and Mokhtar *et al.,* 2012)

In recent years, DNA-based molecular markers have been used for assessment of the genetic diversity between germplasm in many plant species. Inter simple sequence repeat (ISSR) markers (Zietkiewicz *et al*., 1994) have been used with success to identify and determine relationships at the species, population and varieties in many plant species, including several aromatic and medicinal plants (Sangwan *et al.,* 1999; Nan *et al.,* 2003). This method is widely applicable because it is rapid, inexpensive, require small amounts of template DNA and, unlike SSR markers, do not require prior knowledge of DNA sequences (Godwin *et al*., 1997). ISSR markers have been efficiently used for study of genetic diversity of different medicinal plant species and crops such as fennel germplasm (Bahmani *et al*., 2012)

Hence, an attempt was made to study molecular diversity, essential oil composition and the genetic variability, by determining the magnitude of genetic variation, heritability estimates and expected genetic advance of different biometric traits in the three fennel varieties.

Materials and Methods

1-Plant material and Field selection experiments:

This investigation was carried out at the farm of Research and Production Station, National Research Centre, Nubaria District, El-Behaira Governorate, Egypt., during two successive winter seasons (2010/11) and (2011/12). Planting dates were at second week of October. Fruits of 10 genotypes from each Balady, Indian and Holland varieties of fennel which were selected from big population were used in the previous seasons. The experimental design was complete randomized blocks with three replicates. At full ripen five individual plants of each three replicates per each selected genotype for three varieties across different seasons were harvested and the plant records were considered as already mentioned. Plant records were considered on individual plants basis. They included eight quantitative characters:

1- Linear growth (cm)
2- plant height (cm)
3- Number of primary branches per plant.
4- Number of total branches per plant.
5- Peduncle length (cm)
6- Fruit yield per plant (g)
7- Oil percent (%)
8- Oil yield (g)

2 -Essential oil extraction:

Twenty five grams of milled fruits were subjected for hydro distillation in micro scale (v/w) return flow distillation apparatus (Guenther 1972) for each genotype. The volume of essential oil was estimated as essential oil content %. Essential oil yield was computed from multiplication of fruit yield per plant with essential oil content for each genotype.

3-Gas liquid chromatography (GLC) analysis:

The volatile oil obtained from the fruits of three fennel varieties which were analyzed using DsChrom 6200 Gas chromatograph equipped with a flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follow: the chromatograph apparatus was fitted with capillary Colum BPX-5, 5% phenyl (equiv.) polysillphenylene- siloxane 30m x 0.25mm IDx0.25 µm film. Temperature program increased with a rate of 10 ^{o}C /min from70 to200 ^{o}C. Flowrates of gases were nitrogen at ml/min, hydrogen at 30ml/min and ml / min or air. Detector and injection temperatures were 300 ^{o}C and 250 ^{o}C, respectively. The obtained chromatogram and report of GC analysis for each sample of variety were analyzed to calculate the percentage of main component of volatile oil.

4-ISSR analysis:

DNA isolation procedure:

Young and fresh leaves samples were collected separately from each variety according to Dellaporta *et al*., (1983).

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Inter simple sequence repeats (ISSRs) markers analysis:

The five used primers in PCR amplification of Inter simple sequence repeat regions are shown in (**Table 1)**. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 57º C and 2 min at 72º C, the reaction was finally stored at 72º C for 10 min.

Genetic distance relationships:

The banding patterns of five ISSR primers were scored. Data were fed to the PC computer as 1 and 0 for the presence and absence of bands, respectively. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to regenerate similarity coefficients, according to (Sneath and Sokal 1973). The similarity coefficients were then used to construct dendrogram, using the un weighted pair group method with arithmetic averages (UPGAMA) employing the SHAN (Sequantial, Agglomerative, Hierachical, and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.1 Program (Rohlf, 2000).

Table 1: ISSR primers code and their sequences used for detection of banding patterns in the three fennel varieties .

No	Name	Sequence	No	Name	Sequence
1	HB09	5` GTG TGT GTG TGT GC 3`	4	HB13	5´ GAG GAG GAG C 3`
2	HB11	5` GTG TGT GTG TGT GTG CC 3`	5	HB15	5´ GTG GTG GTG GC 3`
3	HB12	5`CAC CAC CAC GC 3`			

5-Statistical procedures:

The general statistical procedures were practiced according to standard methods given by Steel and Torrie (1980). The analysis of variance (ANOVA) and the broad sense heritability (h^2_b) were generally assigned according to Robinson *et al*., (1951) and the genetic advance from selection as percentage change from the generation means Δ GA $\times$ 100 was computed according to Johanson *et al*., (1955).

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pair wise difference matrix and plot the phonogram among cultivars (Yang *et al.,* 1996)

Results:

Assessment of morphological variability:

1-Analysis of variance:

Analysis of variance for eight quantitative characters of three varieties (Balady, Indian and Holland) was carried out for ten selected genotypes for all varieties (Table 2). Separated analysis showed that the genotypes had highly significant variations for all studied characters in both seasons except oil percent for Balady variety which was insignificant.

2- Mean performances:

Data in Tables (3, 4 and 5) presented mean values of investigated genotypes in separate seasons.

In the Balady variety genotype 3 had the highest fruit yield (133.33g and 138.00g, respectively) followed by genotype 4 which was 87.67g and 92.67g, respectively in both seasons. In Indian variety, genotypes 9 and 3 had highest mean values for fruit yield which were (65.00g and 70.00g) and (57.67g and 61.00g) in both seasons, respectively.

In the Holland variety genotype 8 had the highest fruit yield (124.67g and 136.67 g, respectively) followed by genotype 1 (122.33g and 131.67g) in both seasons, respectively. Genotypes 5,9 and 7 in Balady variety expressed the highest essential oil percentage (.82 % and 1.83%), (1.61% and 1.64%) and (1.60% and 1.61%) in the first and second season respectively. In Indian variety, genotypes (8, 7 and 9) had the highest essential oil percentage (1.89% and 1.94 %), (1.82 % and 1.86 %) and (1.40% and 1.44%) in both seasons, respectively. In the Holland variety essential oil percentage had the highest values in case of genotypes 5, 7 and 4 (2.90% and 2.92 %), (2.79 % and 2.85 %) and (2.78% and 2.84 %) in both seasons, respectively.

Table 2: Mean squares of eight characters of three fennel varieties in two seasons (2010/11 and 2011/12).

Variety	S.O.V	D.F	linear growth		Plant height		No. of primary branches		Total No. of branches		Peduncle length		Fruit yield		Oil percent		Oil yield	
			1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Balady	Genotypes	9	387.04 **	461.63 **	508.90 **	492.63 **	5.74 **	4.15 **	425.93 **	298.52 **	40.82 **	35.11 **	2020.52 **	2056.89 **	0.127	0.114	0.353 **	0.370 **
	Replicates	2	12.70	18.03	53.77	16.90	0.433	0.133	71.10	12.23	12.63	1.43	3.03	4.43	0.001	0.001	0.002	0.001
	Error	18	6.07	3.03	5.66	1.83	0.248	0.282	16.51	12.23	1.04	0.693	4.77	5.248	0.001	0.001	0.007	0.001
Indian	Genotypes	9	276.00 **	294.16 **	195.42 **	162.46 **	4.80 **	4.31 **	74.43 **	92.87 **	30.48 **	28.92 **	439.89 **	496.90 **	0.457 **	0.484 **	0.093 **	0.116 **
	Replicates	2	5.23	6.53	10.90	5.83	0.133	0.033	3.43	2.53	3.33	0.533	0.400	0.233	0.001	0.001	0.001	0.001
	Error	18	4.60	4.16	4.79	3.46	0.356	0.182	4.73	3.72	1.11	1.42	4.56	7.27	0.002	0.001	0.005	0.001
Holland	Genotypes	9	596.61 **	609.41 **	690.33 **	709.24 **	8.36 **	9.81 **	278.77 **	121.48 **	55.00 **	57.71 **	2722.26 **	2953.63 **	0.287 **	0.306 **	1.274 **	1.358 **
	Replicates	2	14.53	14.63	8.23	13.43	0.533	0.300	10.90	5.23	4.43	9.10	7.90	8.93	0.003	0.001	0.002	0.004
	Error	18	4.83	3.97	3.42	5.66	0.867	0.300	9.863	12.94	1.40	1.40	4.79	5.38	0.002	0.001	0.002	0.003

Table 3: Mean values of eight quantitative studied characters of fennel variety (Balady) in two seasons (2010/11 and 2011/12).

Code No.	linear growth		Plant height		No. of primary branches		Total No. of branches		Peduncle length		Fruit yield		Oil percent		Oil yield	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	94.67	97.67	55.00	57.33	6.33	7.33	47.67	47.67	14.33	15.67	63.33	67.00	1.50	1.50	0.95	1.00
2	82.67	86.33	57.33	59.00	7.67	8.33	33.33	45.67	13.67	16.00	46.33	48.33	1.45	1.46	0.67	0.71
3	92.67	95.33	47.33	55.67	8.33	8.67	75.00	73.33	16.67	18.33	133.33	138.00	1.40	1.42	1.87	1.96
4	122.67	127.33	91.33	96.00	11.67	11.33	48.33	51.67	23.67	16.33	87.67	92.67	1.42	1.44	1.23	1.33
5	102.33	108.33	58.67	65.33	8.33	9.67	38.33	41.67	13.00	24.00	42.67	47.33	1.82	1.83	0.77	0.87
6	107.00	114.67	81.67	84.33	9.00	8.67	54.33	51.67	10.00	14.00	72.00	76.33	1.09	1.14	0.79	0.87
7	87.00	92.67	58.33	60.33	8.00	8.33	41.67	41.67	14.00	11.00	52.67	58.67	1.60	1.61	0.84	0.95
8	104.33	112.67	64.33	70.67	8.67	9.00	57.67	58.33	16.33	15.33	72.33	77.33	1.24	1.28	0.90	0.99
9	94.67	100.33	62.33	67.00	8.33	8.00	45.00	42.33	17.67	18.67	61.33	66.33	1.61	1.64	0.99	1.09
10	102.00	112.33	65.00	71.33	7.33	7.33	57.67	58.33	13.00	15.00	71.67	75.67	1.30	1.36	0.93	1.03
Mean	99.00	104.77	64.13	68.70	8.37	8.67	49.90	51.23	15.23	16.43	70.33	74.77	1.44	1.47	1.00	1.08
L.S.D 0.05	4.23	2.99	4.08	2.32	0.85	0.91	6.97	6.00	1.75	1.43	3.75	3.93	0.00	0.00	0.05	0.06

3-Heritability and genetic advance:

Heritability in broad sense estimates were detected in the studied varieties for all traits in both seasons and shown in (Table 6). The results indicated that heritability estimates were high for fruit yield, essential oil percentage and yield among studied varieties. Heritability values were (99.76 % and 99.74%), (99.92% and 99.95%) and (99.80 % and 99.71 %) for fruit yield, essential oil percentage and essential oil yield in Balady variety across both seasons, respectively. Similar results were also detected in broad sense heritability estimates in the Indian and Holland varieties for the same characters (Table 6). The highest values of genetic advance characterized the fruit yield (61.67 % and 64.23 %), linear growth (28.22% and 28.69%) and plant height (30.71 % and 30.79%) in the Holland variety . While in the Balady variety the same traits gave (53.02% and 53.46%), (22.10% and 24.96 %) and (25.78 % and 26.05 %) in both seasons , respectively. In addition Indian variety gave (24.03% and 25.15%), (18.60 % and 19.38%) and (15.18 % and 14.02%) in the first and second seasons, respectively.

Table 4: Mean values of eight quantitative studied characters of fennel variety (Indian) in two seasons (2010/11 and 2011/12).

Code No.	linear growth		Plant height		No. of primary branches		Total No. of branches		Peduncle length		Fruit yield		Oil percent		Oil yield	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	92.67	96.67	68.00	69.00	6.67	7.00	35.67	39.00	8.00	9.33	52.00	56.33	0.77	0.78	0.40	0.44
2	103.00	106.67	72.67	76.33	7.67	8.00	30.33	43.00	16.00	17.33	31.67	33.67	1.06	1.06	0.33	0.36
3	103.67	105.00	75.00	76.67	8.67	8.67	35.67	48.33	11.00	12.67	57.67	61.00	1.16	1.18	0.67	0.72
4	92.67	96.33	62.33	64.67	7.33	7.33	29.67	40.33	11.33	14.33	42.00	46.00	1.04	1.06	0.44	0.49
5	85.33	87.76	63.00	65.67	6.67	7.00	33.67	39.00	16.00	17.33	44.00	48.33	0.93	0.96	0.41	0.46
6	98.67	103.00	57.67	68.67	7.67	8.00	42.33	42.33	14.00	14.33	55.00	60.00	0.82	0.84	0.45	0.50
7	92.33	97.00	69.67	72.33	7.33	7.33	28.33	40.33	18.33	20.33	31.67	34.67	1.82	1.86	0.58	0.64
8	86.33	88.00	62.00	66.33	6.33	6.00	24.33	31.00	11.33	13.67	32.67	36.00	1.89	1.94	0.62	0.70
9	100.00	102.67	72.33	72.67	10.67	10.33	34.00	51.67	16.00	17.33	65.00	70.33	1.40	1.44	0.91	1.02
10	117.67	121.67	85.33	89.33	8.33	8.67	35.33	42.33	11.67	13.67	35.33	39.00	1.06	1.10	0.38	0.43
Mean	97.23	100.47	68.80	72.17	7.73	7.83	32.93	41.73	13.37	15.03	44.70	48.53.	1.20	1.22	0.52	0.58
L.S.D 0.05	3.68	3.50	3.75	3.19	1.02	0.73	3.73	3.31	1.81	2.05	3.67	4.63	0.02	0.00	0.04	0.05

Table 5: Mean values of eight quantitative studied characters of fennel variety (Holland) in two seasons (2010/11 and 2011/12).

Code No.	linear growth		Plant height		No. of primary branches		Total No. of branches		Peduncle length		Fruit yield		Oil percent		Oil yield	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	143.00	147.33	113.00	117.67	8.67	9.00	48.33	48.33	33.33	35.67	122.33	131.67	2.17	2.20	2.66	2.89
2	152.00	157.33	126.00	128.67	11.33	12.00	45.00	46.33	22.00	24.00	103.67	112.33	2.12	2.14	2.20	2.40
3	113.00	117.33	81.33	83.67	11.00	11.33	48.33	45.67	21.00	24.33	73.33	83.33	2.21	2.24	1.62	1.86
4	121.67	127.33	86.33	90.00	8.00	8.33	41.67	41.67	23.67	28.33	96.33	102.33	2.78	2.84	2.68	2.91
5	122.33	126.67	93.00	95.00	7.00	7.00	32.67	36.00	20.67	23.67	42.00	47.33	2.90	2.92	1.22	1.38
6	112.67	117.67	81.00	83.67	8.67	9.00	42.33	43.00	27.33	31.00	73.33	78.67	2.16	2.19	1.59	1.73
7	127.33	132.67	86.33	90.67	8.33	8.00	32.33	41.00	21.00	23.67	62.33	70.00	2.79	2.85	1.74	1.99
8	132.67	137.33	99.67	103.67	11.62	12.00	62.67	60.00	21.00	24.33	124.67	136.67	2.16	2.19	2.69	3.00
9	124.67	128.33	93.33	97.00	8.67	8.33	51.00	41.67	18.67	20.67	102.33	111.00	2.59	2.66	2.68	2.96
10	106.00	110.33	79.67	82.67	7.33	8.00	32.67	43.00	21.67	25.33	42.67	51.00	2.50	2.55	1.03	1.30
Mean	125.53	130.23	93.97	97.27	9.07	9.30	43.70	44.67	23.03	26.10	84.30	92.43	2.44	2.48	2.01	2.24
L.S.D 0.05	3.77	3.42	3.17	4.08	1.60	0.94	5.39	6.17	2.03	2.03	3.75	3.98	0.03	0.02	0.07	0.09

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Essential oil composition among fennel varieties:

The essential oils of three bitter fennel varieties (Balady, Indian and Holland) were subjected to detailed GC analysis Table (7). Meanwhile 7 compounds were identified in fennel varieties including monoterpene, Hydrocarbon , oxygenated monoterpenes (e.g., 1,8- cineole) and aromatic compounds (e.g., methyl chavicol). The constituents obtained from fennel varieties (Table 7) were characterized by a high content of aromatic compounds, with methyl chavicol as the main constituent. The composition of Holland and Indian varieties appears to be different from Balady in all identified components. Balady gave highest methyl chavicol percentage followed by Indian and Holland varieties, respectively. Meanwhile, the essential oil of Holland variety gave highest value of anthole followed by Indian and Balady, respectively. Holland had lower limonene concentration then Indian and Balady. α-Pinene and myrcene were higher in Balady and Holland by comprising Indian variety. In addition, Indian variety essential oil also contained low fenchon compared to Balady and Holland varieties. Those components compose 99.66 % of the essential oil with the remaining 0.4 % of the oil being made up of the numerous components in trace amounts in the Holland variety.

Table 6: Broad-sense heritability (h^2_b)and genetic advance (GA%) estimates of three varieties in yield its attributes in two seasons (2010/11 and 2011/12).

Characters	h^2_b						GA%					
	Balady		Indian		Holland		Balady		Indian		Holland	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
(X_1) Linear growth	98.43	99.34	98.33	98.58	99.19	99.35	22.10	24.96	18.60	19.38	28.22	28.69
(X_2) Plant height	98.89	99.63	97.55	97.87	99.50	99.20	25.78	26.05	15.18	14.02	30.71	30.79
(X_3) No. of primary branches	95.67	93.21	92.59	95.79	89.63	96.94	2.41	1.83	1.90	2.10	2.12	3.32
(X_4) Total No. of branches	96.12	95.90	93.65	96.00	96.46	89.35	21.15	17.54	7.91	9.83	17.36	7.96
(X_5) Peduncle lenght	97.45	98.03	96.35	95.08	97.46	97.58	6.91	6.56	5.71	5.27	8.03	8.26
(X_6) Fruit yield	99.76	99.74	98.96	98.54	99.82	99.82	53.02	53.46	24.03	25.15	61.67	64.23
(X_7) Oil percent	99.92	99.95	99.96	99.97	99.93	99.95	0.423	0.401	0.802	0.827	0.636	0.657
(X_8) Oil yield	99.80	99.71	99.43	99.13	99.87	99.81	0.701	0.716	0.356	0.393	1.34	1.38

Table 7: Chemical composition (%) of essential oils in three fennel varieties by G.L.C .analysis

RT (min)	Compound	Fennel varieties (Area %)		
		Balady	Indian	Holland
1.60	α-Pinene	1.68	1.04	1.39
1.98	Myrcene	2.49	1.65	2.00
2.48	Limonene	10.30	8.86	8.10
2.76	Fenchone	3.09	1.41	1.99
3.24	1,8 Cineol	4.24	5.74	9.43
4.98	Methy chavicol	74.20	69.03	60.99
6.04	Anthole	4.013	12.27	15.70
Total identified compounds		100	100	99.6
Total unidentified compounds		0.00	0.00	0.4

Genetic characterization of some Fennel varieties by Inter simple sequence repeats (ISSR) markers analysis:

The DNA of the three Fennel varieties Balady (F1), Indian(F2) and Holland (F3) were tested against 5-mer primers banding Pattern for the five primers (HB-09, HB-11, HB-12, HB-13and HB-15) and showed in nucleotide sequences in Table (1) and illustrated in figure (1). The five primers (HB-09, HB-11, HB-12, HB-13 and HB-15) were relatively distinguished by one or more unique bands from each primer as shown in fig(2) and Tables (8 and 9). The banding patterns of ISSR fragments using the five specific primers with the three fennel varieties (Fig. 1) revealed 31 amplified fragments; 17 of them were polymorphic (45 %). The total number of amplified and polymorphic fragments obtained with each primer is shown in Table (8).

The ISSR data revealed that the genetic similarity indices ranged from 0.58 % - 0.77% (Table 8 and Fig. 1). The nearest relationship was detected between Indian and Holland varieties (77.00 %), followed by between (Balady and Holland) and (Balady and Indian), respectively. The dendrogram based on the similarity matrices of ISSR-PCR banding patterns separated the three fennel varieties into one cluster (including Indian and Holland varieties) and Balady alone. ISSR amplification does not require genome sequence information but produce highly polymorphic patterns.

Table 8: Similarity index (Pair wise comparison) among the three fennel varieties, based on ISSR data.

varieties	Balady (F1)	Indian (F2)	Holland (F3)
Balady (F1)	1		
Indian (F2)	0.58	1	
Holland (F3)	0.60	0.773	1

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Table 9: Polymorphisms were revealed by the five primers that used for identification the fennel varieties.

Primers	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism percentage
HB-09	7	4	3	43%
HB-11	4	3	1	25%
HB-12	7	5	2	29%
HB-13	6	2	4	67%
HB-15	7	3	4	57%
All primers	31	17	14	45%

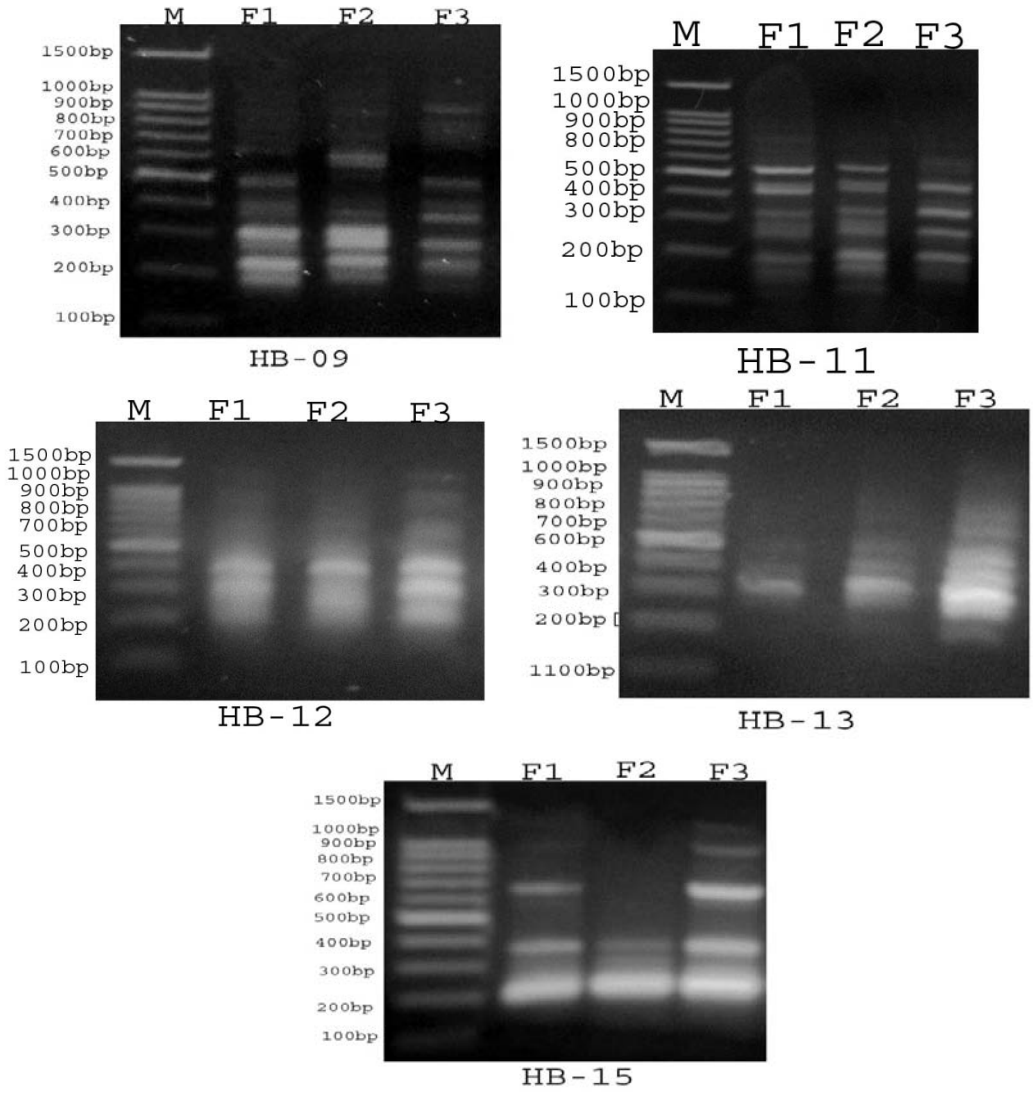

Fig. 1: ISSR banding patterns of the three fennel varieties with five arbitrary primers. (F1=Balady, F2 = Indian, F3 = Holland and M= marker)

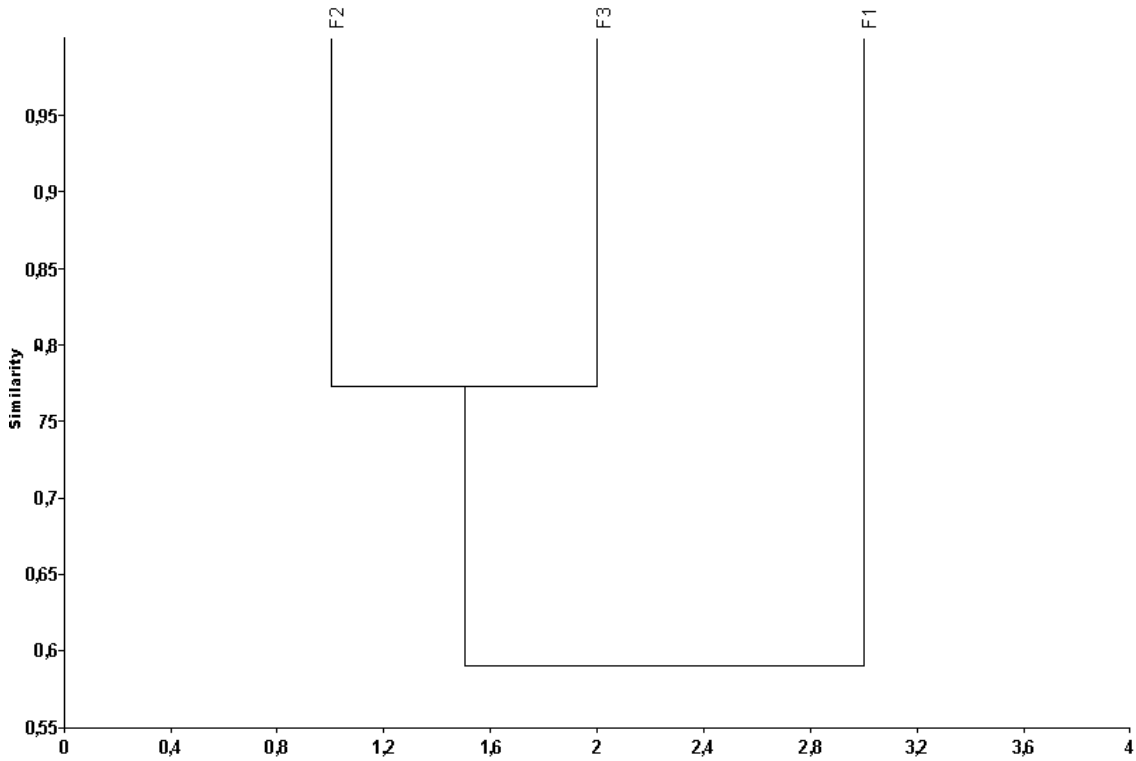

Fig. 2: Dendrogram represented the genetic relationships among the studied fennel varieties (F1= Balady, F2 = Indian and F3 = Holland) based on ISSR markers

Discussion:

One of the main objectives of any breeding program is to produce high yielding and better quality lines for release as cultivars to farmers. The prerequisite to achieve this goal is the presence of sufficient amount of variability to be selected for further manipulation to achieve the target. Introduction of new populations can be made from one region to the other easily and may be used for further manipulation to develop breeding lines. The present study was conducted to evaluate the performance of ten selected genotypes of three fennel varieties (Balady, Indian and Holland) in order to assess the presence of variability for desired traits and a significant amount of variation for different parameters was observed. In addition, essential oil composition and genetic variability using ISSR markers was conducted.

The analysis of variance revealed highly significant difference among the genotypes for all the characters studied in all studied varieties across both seasons except essential oil percent in Balady variety, indicating the presence of considerable amount of genetic variability for these traits. Whereas, essential oil percent showed non significant differences among the genotypes. Non significant differences among genotypes of Balady variety suggested a uniform of genetic and genetic x environment back ground in these genotypes for this particular trait. The variations in the essential oil percent might be due to the environment. These results confirmed the differences in the genotypes genetics potential, and indicated that certain genotypes carried alleles with different additive and additive effects and these effects were constant from season to another (Abou El-Nasr *et al*., 2004 , Curioni *et al*., 2003 and Patel *et al.,* 2008) . The difference between traits could be influenced by genotype, environment and interaction among them (Lopez *et al*., 2008).

The mean values varied in all traits among genotypes in both seasons among studied varieties confirm the results of analysis of variance and reflect the different genetic background of genotypes and their interaction with the seasonal variations. The same findings were reported in other *Apiaceae* plants like coriander and fennel, (Singh *et al.,* 2002, Abou El- Nasr *et al.,* 2004., and Telci *et al.,* 2009).

Three characterized genotypes (3,5), (3,8) and (8,5) attracted more attention according to their owing the highest mean valuesfor fruit yield and essential oil characters in Balady followed by Indian and Holland varieties, respectively reflecting the different genetic background of genotypes and their interaction with the seasonal variations. Heritability is useful in predicting the expected progress to be achieved through selection (Burton and DeVane, 1953). High heritability (broad sense) estimates among studied varieties were found for all traits in both seasons and ranged from 89.35 to 99.97% in Holland and Indian varieties, respectively, which indicate that these characters were less influenced by the environment and direct selection for these traits would be effective for further improvement (Table 6). These findings are in agreement with the results obtained by (Mehta and Patel . 1983; Jindal and Allah-Rang 1986; and Rajput *et al*., 2004).

High genetic advance was observed for fruit yield in Holland followed by Balady and Indian varieties. The fruit yield, plant height, linear growth and total number of branches had the highest genetic advance in all varieties. The characters namely, linear growth, plant height, fruit yield and total number of branches displayed high heritability estimates along with high genetic advance among three varieties. High heritability with high genetic advance indicate the control of additive gene and selection may be effective for those characters. Johnson *et al*., (1955) suggested that heritability together with genetic advance is a more useful parameter in choice of the best genotype by selection. Comparing with all traits, essential oil yield presented more heterogeneity and the highest heritability, but it presented the lowest genetic advance. Therefore, this trait is an important trait for fennel and can be good criteria for selection at the segregating generations in the future plant breeding programs. This result was in agreement with Abou El–Nasr *el al.,* (2004), while Singh *et al.,* (2002) suggested that the improvement would be possible by exercising selection for oil yield and fruit yield.

The essential oil compositions of fennel fruits varieties contains 7 identified compounds and the major compounds are methyl chavicol, anethole, and limonene in all oils. Essential oils obtained from Bitter fennel varieties Balady, Indian and Holland showed significant variability in their chemical compositions . Balady variety has the highest percentage of methyl chavicol followed by Indian and Holland .Also Balady has the lowest values of anethole followed by Indian and Holland varieties.

However, (Ana Clara *et al.*, 2010, and Abdelaaty *et al*., 2011) showed that variations between chemical compositions depend on seasonal variation, varieties and stages of development.

ISSR bands of genomic DNA using five primers confirmed the differences among fennel varieties. ISSR markers have been efficiently used for study of genetic diversity of different medicinal plant species and crops such as *Phaseolus vulgaris L.* (Galvan *et al*., 2003), barley (Yong-Cui *et al*, 2005), *Artemisia capillaries* (Shafie *et al*, 2009), *Lippia alba Mill.* (Manica-Cattani *et al*, 2009) and *Achillea millefolium* (Farajpour *et al.,* 2012). Inter simple sequence repeat (ISSR) markers have been used with success to identify and determine relationships at the species, population and cultivar levels in many plant species, including several aromatic and medicinal plants (Zietkiewicz *et al.,* 1994 , Sangwan *et al.,* 1999 and Nan *et al*., 2003). It can be concluded that, PCR-ISSR technique could be a useful tool for the identification and characterization of the three fennel varieties. These results are in agreement with those obtained by Bahmani *et al.,* 2012. They indicated that PCR-ISSR technique can be used as a tool for determining the extent of genetic diversity among fennel varieties. These results may help to understand the genetic diversity and structure of fennel with possible applications in breeding programs.

Conclusion:

Morphological traits, essential oil compositions and ISSR markers show a high degree of variation among the fennel varieties. The essential oil of fennel fruits showed a characteristic chemical profile among varieties. While methyl chavicol, anethole, fenchone and limonene were the main components in all oils, qualitative and quantitative differences in composition were observed. In the present study, ISSR markers provided a comprehensive insight allowing the characterization of the fennel varieties under study.

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