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Genetic diversity of Iranian indigenous fennel (*Foeniculum vulgare* Mill.) populations using agronomic traits and essential oil

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is one of the most important medicinal plants. It has a variety of secondary metabolites which are used in treatment of heart, nerves, digestive disorders and particularly alzheimer's disease. In this research, the genetic diversity of fifteen Iranian populations and four foreign populations of fennel from Germany and Turkey were evaluated in a field experiment based on randomized complete block design with three replications. Nineteen morphological traits and essential oil of fennel were evaluated. Analysis of variance revealed significant differences among populations for all studied traits. The highest values of phenotypic and genetic coefficient of variation were shown by harvest index, grain yield and umbels per plant. The heritability estimates ranged from 30% for length of last internode to 99% for days to germination and to 50% flowering. Cluster analysis was performed based on all the traits and 19 populations were located in two main groups. Second group was detected superior in terms of essential oil, yield and its related traits.

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Introduction

Fennel (*Foeniculum vulgare* Miller) is an open pollinated species belongs to the *Apiaceae* family and originated in the Mediterranean region, where it is possible to observe a high genetic variability (Tutin, 1976, Miraldi, 1999). Fennel is important for its seeds and essential oil. Seeds are used to flavor liqueurs, vinegars, breads, pastries, candies and pickles. Leaves and stems serve as vegetable, salad or potherb. Fennel has a variety of secondary metabolites which is used in treatment of cancer, digestive irregularities, respiratory and skin ulcers (lten and Saller 2004, Lucinewton *et al.* 2005, Ebeed *et al.* 2010). Recent studies have shown that essential oil of fennel has valuable antioxidant, antidiabetic, antibacterial and antifungal properties (Lucinewton *et al.* 2005, El-Soud *et al.* 2011).

In the last few years the interest for a possible industrial use of fennel is growing. Nevertheless, this utilization would allow a diversification of the supply and the introduction of new products. Recently, fennel has become attractive for main international seed companies, which have improved research breeding programs (Fiore *et al.* 2008).

In the case of medicinal plants cultivated in Iran, the main agents of low yield are low genetic diversity, lack of appropriate genotypes, dysfunctional system of planting, low harvest index and susceptibility to the disease. (Rahimmalek *et al.* 2009).

Considering the outcrossing rate and consequently the high genetic variability of this plant more attention should be placed on the characterization of germplasm. 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, genetic and phenotypic variances, genetic advance and heritability are useful in understanding the nature of inheritance of different traits (Patel *et al.* 2008).

Kirici *et al.* (2010) studied agronomic traits and essential oil content of four wild fennel populations from Turkey. Results showed extremely high genetic diversity among all traits except of plant height and number of branches per umbels. Bernath *et al.* (1996) evaluated the morphological and physiological traits in the 34 populations of fennel and the results indicate a wide diversity with respect to grain yield and essential oil content.

The objectives of this study were to capture the potential genetic diversity of 15 Iranian and four foreign indigenous fennel populations with respect to some morphological traits and essential oil and to estimate of heritability of these characters.

Materials and methods

Plant materials and experimental design

In this research, the genetic diversity of 15 populations of fennel from Iran and four foreign populations from Germany and Turkey (Table 1) were evaluated in a randomized complete block design with three replications at the research station of Faculty of Agriculture, University of Tabriz.

Each plot consisted of three rows 4m long. The interrow and interplant spacings were 50 and 25cm, respectively. Nitrogen fertilizer was applied at a rate of 100 kg ha^{-1} at pre-planting, Stem elongation and flowering stages. Plots were irrigated as needed to keep soil moisture optimal for plant growth.

Measured traits

Observation were recorded on 20 traits, namely days to germination, days to 50% flowering, days to 100% flowering, days to maturity, Plant height in 50% flowering stage, total plant height, shoot biomass per m^2, number of internodes, length of the first, longest and the last internodes, Number of stems per plant, stem diameter, umbels per plant, peduncle length, number of grains per umbel, 1000 grain weight, grain

yield per m², essential oil content (in 100 gr of dry seed) Harvest index was determined from the ratio of grain yield to shoot biomass. Twenty plants per plot were collected and the mean data points were used for statistical analysis.

Essential oil extraction procedure

The volatile oil was obtained from 100 grams of mature seeds by hydrodistillation in Clevenger apparatus (3 hours). The oil was separated, dried over anhydrous sodium sulfate and kept in a dark glass bottle at 4°C (Aprotosoaio *et al.*, 2010, Piccaglia and Marotti, 2001).

Table 1. List of the studied fennel populations in this research.

Row	Location	Row	Location
1	Germany (1182)	11	Khorooslari (Moghan -Ardabil)
2	Germany (11486)	12	Zeeyar (Esfahan)
3	Turky (Gaziantep)	13	Shirvan (North Khorasan)
4	Turky (Izmir)	14	Karaj (Alborz)
Iranian Indigenous populations		15	Kerman (kerman)
5	Bonab (East Azarbaijan)	16	Khorramabad (Lorestan)
6	Birjand (South Khorasan)	17	Gharineh(Neyshabur- Razavi Khorasan)
7	Tatmaj (Kashan)	18	Varamin (Tehran)
8	Torbat-e jam (Razavi Khorasan)	19	Hamedan (Hamedan)
9	Khorshidabad (Meshkinshahr -Ardabil)		
10	Khur and Biabanak (Esfahan)		

Statistical analysis

The performed statistical analysis including Shapiro-Wilk normality test, analysis of variance, cluster analysis based on all measured traits using Ward's algorithm and standardized means, Discriminant analysis was performed to identification of the clustering point but because of the similarity in the significance results, dendrogram was characterized based on maximum distance among groups. Due to the abnormality of input data for days to 50% flowering and days to maturity, the inverse and logarithmic transforms was utilized respectively. For statistical analysis Excel, MSTATC and SPSS software were used. The variance components, genetic and phenotypic coefficient of variation and broad sense heritability were determined as suggested by Burton and De Vane (1953) and Johnson *et al.* (1955).

Results and discussion

Analysis of variance

The analysis of variance for all the traits showed highly significant differences among the genotypes, indicating sufficient amount of variability in the materials (Table2). A wide range of variability for different characters was also observed by Agnihotri *et al.* (1997), Rajput *et al.* (2004), Patel *et al.* (2008) and Meena *et al.* (2010) in fennel. The highest coefficients of variation (CV) were shown by grain yield, followed by essential oil content and shoot biomass. The least values were shown by developmental characters such as days to germination, to 50% and 100% flowering.

Table 2. Analysis of variance of various characters in 19 fennel populations.

character	Replication MS(df=2)	Population MS(df=18)	Error MS(df=36)	CV (%)
days to germination	20.018^{**}	14.889^{**}	0.018	0.97
days to 50% flowering	5.737	720.754^{**}	7.348	3.30
days to 100% flowering,	2.48×10^{-4ns}	0.018^{**}	3.38×10^{-4}	0.94
days to maturity	2.04×10^{-7ns}	$5.21\times10^{-6**}$	3.84×10^{-7}	8.85
plant height (cm)	80.864^{ns}	306.927^{**}	33.218	10.67
total plant height (cm)	96.470^{ns}	1429.673^{**}	49.801	6.98
shoot biomass (gr/ m²)	1236.776^{ns}	3448.135^{**}	687.854	22.48
number of internodes	1.135^{ns}	20.543^{**}	0.737	8.528
length of the first internode (cm)	0.791^{ns}	4.056^{**}	0.278	11.371
length of the longest internode (cm)	$1.498^{ns.}$	9.688^{**}	0.624	6.559
length of the last internode (cm)	0.052^{ns}	2.238^{*}	1.571	1.571
No. stems per plant	0.039^{ns}	0.737^{**}	0.094	12.66
stem diameter(cm)	0.282^{ns}	11.877^{**}	0.430	6.717
umbels per plant	21.88^{ns}	441.732^{**}	35.693	17.45
peduncle length (cm)	0.17^{ns}	4.617^{**}	0.282	5.90
No. grains per umbel	1442.880^{ns}	9653.427^{**}	1791.254	19.80
1000 grain weight (gr)	0.145^{ns}	2.983^{**}	0.182	8.816
grain yield (gr/ m²)	73.058^{ns}	670.451^{**}	116.94	30.30
essential oil content (cc)	0.183^{ns}	0.522^{**}	0.35	25.71
harvest index	16.424^{ns}	476.357^{**}	14.326	11.92

ns, *, ** non-significant and significant at P=0.05 and 0.01 respectively

Genetic and phenotypic coefficient of variations

The phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV), estimates of the components of variance and broad-sense heritability are shown in Table3. The PCV was higher than the GCV for all of the characters, but in many cases, the two values differed only slightly. The lowest values were shown by days to maturity and days to 100% flowering, followed by length of the last internode, number of stems per plant and essential oil content. The highest values were shown by harvest index, grain yield and umbels per plant. High genetic and phenotypic coefficient of variation for umbels plant^{-1} and seed yield plant^{-1} were reported by Rajput *et al.* (2004) in fennel. The results suggested that characters showing high values of genetic and phenotypic coefficient of variation can easily be improved by careful selection.

Table 3. Phenotypic coefficient of variation (PCV) and genetic coefficient of variation (GCV), components of variance and broad sense heritability (h_b^2) of 20 characters of fennel.

character	PCV (%)	GCV (%)	σ^2ph	σ^2g	σ^2_e/r	h_b^2
days to germination	16.320	16.29	4.97	4.96	0.006	0.99
days to 50% flowering	18.870	18.77	240.25	237.80	2.45	0.99
days to 100% flowering,	0.085	0.084	6×10^{-3}	59×10^{-4}	1.13×10^{-4}	0.98
days to maturity	0.003	0.0008	1.73×10^{-6}	16×10^{-7}	1.28×10^{-7}	0.92
plant height (cm)	18.725	17.68	102.31	91.24	11.073	0.89
total plant height (cm)	21.580	21.20	476.56	459.96	16.60	0.96
shoot biomass (gr/ m²)	29.062	26.002	1149.38	920.09	229.285	0.80
number of internodes	21.754	21.37	6.85	6.602	0.246	0.96
length of the first internode (cm)	17.378	16.78	1.35	1.259	0.093	0.93
length of the longest internode (cm)	12.981	12.55	3.23	3.021	0.208	0.94
length of the last internode (cm)	8.872	4.83	0.75	0.222	0.524	0.30
No. stems per plant	10.755	9.95	0.25	0.214	0.031	0.87
stem diameter(cm)	17.048	16.73	3.96	3.816	0.143	0.96
umbels per plant	35.449	33.99	147.24	135.35	11.90	0.92
peduncle length (cm)	13.808	13.40	1.54	1.45	0.094	0.94
grain yield (gr/ m²)	41.886	38.059	223.48	184.5	597.085	0.83
1000 grain weight (gr)	19.309	18.745	0.99	0.933	0.061	0.94
essential oil content (cc)	18.128	10.376	0.174	0.057	38.98	0.33
harvest index	39.673	39.071	158.79	154.01	0.117	0.97
No. grains per umbel	26.532	23.944	3217.81	2620.72	4.78	0.81

σ^2_{ph}, σ^2_g and σ^2_e/r are phenotypic, genetic, and error variance of genotype means, respectively.

Estimates of broad sense heritability

The heritability estimate ranged from 30% for length of last internode to 99% for days to germination and to 50% flowering (Table3). Heritability of all traits was above 80% except of essential oil content and length of last internode, indicating that these characters were less influenced by the environment and direct selection for these traits would be effective for further improvement.

Petal *et al* (2008) reported high heritability estimates for grain yield plant^{-1}, days to 50% flowering, number of primary branches plant^{-1}, total branches plant^{-1}, test weight and volatile oil content.

Cluster analysis

Dendrogram was achieved from cluster analysis of 19 populations on the basis of 20 measured traits (Fig. 1).

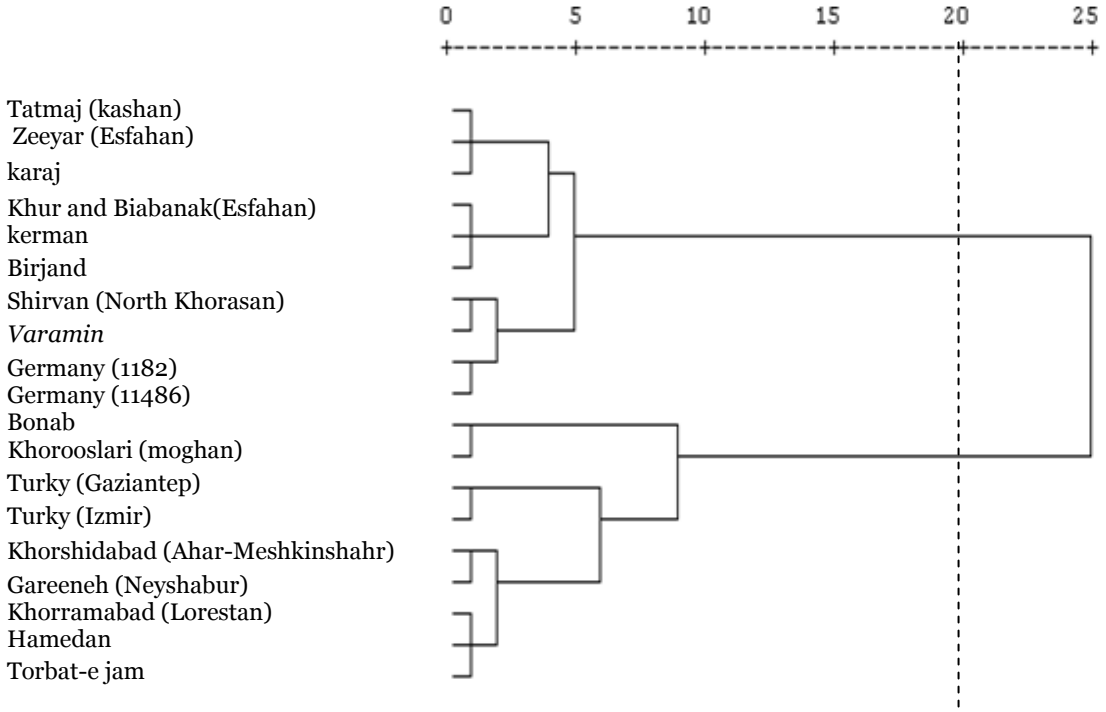

Fig. 1. Dendrogram of cluster analysis for 19 populations of fennel based on all measured traits.

According to this grouping under- study fennel populations were divided into two groups. The average of traits for each cluster and the percent of their deviation from total mean are shown in Table4. In first cluster 10 populations were classified including 52.63% of total populations. The average values of populations of this cluster for days to maturity, length of first and last internode, number of umbels per plant, grains per umbel, grain yield, essential oil content, harvest index were less than the total mean of all populations and for other traits were higher than the total mean. Populations of this group had the lowest mean in terms of all the above mentioned traits so; they had the lowest values for grain yield, yield components and essential oil. Populations of second group (including nine populations) had the greatest means with respect to days to maturity, first and last internode length, number of umbels, grain yield, essential oil content, harvest index and number of seeds per umbel. Members of this cluster are suitable for breeding programs aimed at improving the grain yield and essential oil content. Results of cluster analysis

revealed that populations with the same geographical origin were not necessarily placed in the same group. Nevertheless, some populations with similar geographical origin (such as Turkish and German populations) were placed in the same sub clusters. This results were in agreement with Meena *et al* (2010, safaei *et al* (2011) and Lopes *et al* (2010) findings.

Table 4. Average of traits for each cluster (above number) and the percent of their deviation from ground mean (below number) in 19 fennel population.

cluster	days to germination	days to 50% flowering	days to 100% flowering	days to maturity	Plant height(cm)	total plant height (cm)	shoot biomass (gr/ m²)	number of internodes	length of the first internode (cm)	length of the longest internode (cm)	length of the last internode (cm)	No. stems per plant	stem diameter (cm)	umbels per plant
1	14.30	95.07	92.57	146.27	51.55	118.27	134.86	12.39	3.71	13.35	7.78	2.67	11.26	27.78
	4.63	15.71	0.94	-3.68	13.93	16.91	15.61	23.04	-19.90	10.87	-0.470	10.42	15.29	-18.86
2	12.96	67.81	90.74	158.07	45.66	82.16	96.43	7.49	5.66	10.59	7.86	2.14	8.11	41.40
	-5.15	-17.46	-1.05	4.09	-15.48	-18.79	-17.34	-25.60	22.11	-12.07	0.52	-11.57	-16.98	20.96
Total mean	13.67	82.16	91.70	151.90	54.02	101.16	116.66	10.07	4.64	12.04	7.82	2.42	9.76	34.23

Table 4. Contd.

cluster	peduncle length (cm)	No. grains per umbel	1000 grain weight (gr)	grain yield (gr/ m²)	essential oil content (cc)	harvest index
1	9.88	211.75	4.92	30.84	2.11	22.12
	9.95	-0.96	1.68	-13.58	-8.15	-30.35
2	7.99	216.08	4.75	41.08	2.51	42.47
	-11.05	1.07	-1.86	15.09	9.06	33.72
Total mean	8.99	213.81	4.84	35.69	2.30	31.76

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