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Heritability Estimates of Agronomic Traits and Essential Oil Content in Iranian Fennels

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ABSTRACT

Fennel (*Foeniculum vulgare* Mill.) benefits from a lot of useful medicinal properties. Iran is known as one of the main producers of fennel. In spite of high medicinal values, fennel's cultivation is not economically feasible, mainly due to its low yield. It grows wild in different areas in Iran from where diverse ecotypes have evolved. Genetic variance and heritability estimates of traits in a plant's primary germplasms are needed before planning of a new breeding program. In the present study the genetic diversity and broad sense heritability for 50 fennel ecotypes were assessed under field conditions for a duration of two years. Seed yield, essential oil content and some morphological traits were recorded during a two experimental years. Through stepwise regression, the yield affecting traits and essential oil content were determined. During the first and second experimental years the most effective traits affecting essential oil content were found to be the number of leaves per plant and days to 50% flowering, respectively. The weight of dry biomass affected seed yield the highest during any of the two years and for each separate year. The broad sense heritability during the two experimental years, for essential oil content and seed yield, were 0.46 and 0.63, respectively. However, some such traits as, days to 50% flowering and length of middle internodes showed higher comparative heritability (0.90 and 0.79, respectively). The high heritability of the studied traits in this germplasm indicates the germplasm's high genetic potential to be made use of in breeding programs.

Keywords: Broad sense heritability, Essential oil Content, Fennel, Seed Yield.

INTRODUCTION

Fennel (*Foeniculum vulgare Mill.*), one of the earliest herbs possesses appealing flavor along with useful medicinal properties. It belongs to the *Apiaceae* family and is native to Mediterranean regions. Fennel is a perennial herb of a height of up to two meters, feathery leaves and golden yellow flowers (Hornok, 1992).

The plant is used for diverse purposes in food, cosmetics, and medicinal industries. The vegetative parts are consumed in green salads, fruit seeds are used as spice, with their essential oil being added to soaps, perfumes, and other cosmetics. Fennel's essential oil and seeds are also used to improve the flavor of prepared foods including meats, ice creams, candies, baked goods and condiments. Recent studies have revealed the antioxidant activity of fennel's essential oil as well as its antibacterial, anticancer and antifungal activities (El-Awadi and Hassan, 2010; Moura *et al.,* 2005; Parejo *et al.,* 2004).

Production of fennel seeds is not economical because of high yielding varieties not being available (Lal, 2007). On the other hand the increasing commercial value of fennel calls for the development of genotypes of high seed yield, high essential oil content as well as other desirable agronomic and market traits. The plant has been cultivated as a medicinal as

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well as spice plant for long times in different parts of Iran and, due to its adaptation to local environments, diverse ecotypes have evolved. The genetic diversity of the plant may include traits that impact seed yield and essential oil content (Heywood, 2002). Knowledge on the level of genetic diversity and the heritability of traits can guide to appropriate selection schemes for breeding programs of the plant. Ecotype, environment as well as ecotype by environment (G×E) interactions are major factors that influence phenotypic diversity in plants (Hadian *et al.,* 2008; Olle *et al.,* 2010, Azizi *et al.,* 2006; Dashti *et al.*, 2010; Khodambashi *et al.*, 2012). Raising plants in different environmental conditions and/or assaying them in different years helps to measure the intensity of the interactions.

Also to improve the performance of cultivated ecotypes' heritability estimates and raise the knowledge related to genetic diversity among wild relatives can be very helpful (Schwartz *et al.,* 2009). In other words the wild relatives are considered as valuable resources in breeding programs of plants, including medicinal plants (Anil *et al.,* 2011). Broad-sense Heritability (Hb) is the proportion of genetic variance relative to total phenotypic variance. The genetic and phenotypic variances can be derived from proper experimental designs. If large and diverse populations of a germplasm are to be studied then *Hb* estimates can be practical for the entire species (Schwartz *et al.,* 2009). Heritability estimates are of very useful information to be used in initiating a plant breeding project, and there exist extensive reports on their assessment (Al-Kordy, 2000; Baghalian *et al.,* 2010a; Baghalian *et al.,* 2010b; Bottignon *et al.,* 2011; Gegas *et al.,* 2010; Gross *et al.,* 2009; Songsri *et al.,* 2008). Gross *et al.* (2009) studied the heritability of volatile phenylpropenes in bitter fennel and reported that this trait is mostly affected by the genetics of the plants. Al-Kordy (2000) reported the heritability of plant height, number of primary branches and fruit yield for fennel to be 0.76, 0.83 and 0.20, respectively. Despite many reports concerning the morphological diversity of the plant fennel existing (Bahmani *et al.,* 2012; Lal, 2007), little information is available on the heritability of the desired traits. The objectives followed in this research were to estimate heritability for seed yield, essential oil content, and as well use of stepwise regression for a determination of characteristics strongly affecting these traits.

MATERIALS AND METHODS

Plant Material

A total of 50 ecotypes of bitter fennel (*Foeniculum vulgare Mill*) from 50 locations of Iran (covering different climate ranges) were collected in fall 2009 and their seeds stored at 4˚C until being utilized in the experiment (Table 1). Seeds were planted at the experimental farm of College of Aburaihan, University of Tehran, Pakdasht in spring 2010. The experiment was performed in the framework of a Randomized Complete Block Design (RCBD), of three replications. Analyses were continued as based on the same design during the other next year as a split-plot (in time) experiment. Soil was a sandy–clay one and was of proper drainage with a pH of 7.2. Weeds were manually controlled. Regular irrigation practices were carried out to maintain soil moisture at its appropriate level. Throughout the growing season, no diseases or pests were detected. Final plant density was 10 plants per m^2, approximately equal to the optimum reported density (Khorshidi *et al.,* 2010).

Agro-morphological traits assessed for a duration of two experimental years (2010 and 2011) included days to 50% flowering, days to 70% seed pasty stage, days to 70% seed dried, essential oil content (cc 100 g^{-1} of dry matter), seed yield (g m^{-2}), plant height (cm), leaf number per plant, number of inflorescences per plot, inflorescence diameter (cm), length of peduncle (cm), number of seeds per largest and smallest inflorescence, number of tillers per plant,

Table 1. Geographical locations of Fennel plant collection sites.

Genotypes	Latitude	Longitude	Altitude (m)	Genotypes	Latitude	Longitude	Altitude (m)
Chahestan	27 13 N	56 22 E	27	Ardakan	30 13 N	52 26 E	1620
Alamot	36 45 N	50 47 E	1500	Bajestan	34 51 N	58 17 E	1265
Arak	34 6 N	49 46 E	1708	Kashan	33 59 N	51 27 E	982
Khash	28 13 N	61 12 E	1394	Abade	31 11 N	52 40 E	2030
Fasa	28 58 N	53 41 E	1288	Sarpol zahab	34 27 N	45 54 E	548
Hasht gerd	35 65 N	50 43 E	1426	Divandare	36 4 N	46 55E	2142
Meshkin shahr	38 23 N	47 40 E	1568	Oromie	37 32 N	45 5 E	1313
Damavand	35 43 N	52 15 E	2000	Sanandaj	35 20 N	47 0 E	1373
Inche boron	37 53 N	55 57 E	460	Kamyaran	34 47 N	46 56 E	1464
Hamedan	34 51 N	48 32 E	1749	Dehgolan	35 10 N	47 30 E	1970
Yazd	31 54 N	54 24 E	1230	Sardasht	36 9 N	45 30 E	1670
Ardabil	38 15 N	48 17 E	1332	Saqez	36 15 N	46 16 E	1522
Haji abad	28 19 N	55 55 E	931	Kerman	30 15 N	56 58 E	1753
Qazvin	36 15 N	50 0 E	1278	Nairiz	29 12 N	54 20 E	1632
Tafresh	34 41 N	50 1 E	1978	Barazjan	29 26 N	51 20 E	80
Fozveh	32 36 N	51 26 E	1612	Tabriz	38 5 N	46 17 E	1361
Sari	36 33 N	53 0 E	23	Shabestar	38 0 N	46 11 E	1350
Rafsanjan	30 25 N	55 54 E	1580	Shiraz	29 36 N	52 32 E	1488
Givi	37 41 N	48 28 E	1682	Qom	34 42 N	50 51 E	877
Kaleibar	38 52 N	47 1 E	1180	Esfahan	32 37 N	51 40 E	1550
Khalkhal	37 38 N	48 31 E	1769	Mahalat	33 91 N	50 45 E	1775
Marvdasht	29 80 N	52 83 E	1502	Razan	35 21 N	49 4 E	1870
Moqan	39 39 N	47 55 E	31	Sabzevar	36 12 N	57 43 E	977
Tehran	35 41 N	51 19 E	1190	Kohin	36 36 N	49 67 E	1527
Ahvaz	31 20 N	48 40 E	22	Aran bidgol	34 70 N	52 30 E	850

number of nodes per plant, germination percentage (%), length of petiole (cm), thousand seed weight (g), weight of dry biomass (g m^{-2}), stem diameter (cm) and the lengths of middle internodes (cm).

Essential oil was collected from green seeds (pasty stage) as other researchers suggest the fennel seeds at this stage to contain their peak level of essential oils (Stefanini *et al.,* 2006). Harvested seeds were dried at 40°C and essential oil extracted through water distillation in a Clevenger and for three hours. For each sample, a 100 g of dried seed was powdered and soaked in one liter of water before each extraction being run.

Statistical Analysis

Analyses of variance for agro-morphological traits and regression analysis were performed by means of IBM SPSS Statistics Version 19 (SPSS Inc., an IBM Company) and SAS 9.0 (SAS Institue Inc.). Analysis of variance was done for each separate year as based on block design and for the combined years as split-plot (in time).

The most effective traits on seed yield and essential oil content were determined through fitting the multiple regression models, using stepwise algorithm (Draper and Smith, 1998). Then colinearity statistics was checked for the validity of the models.

According to ANOVA results of variance components along with their Standard Errors (SE) were obtained through the following formula (Hallauer, 1970):

$$SE\sigma_c^2 = \sqrt{\left(\frac{2}{f^2}\right)\sum_i (M_i^2)/(df_i + 2)} \qquad (1)$$

Where, M_i is the mean squares used to calculate the component of variance, df_i is the degree of freedom of respective mean squares and f the coefficient of component variance.

Broad Sense Heritability Estimates were found out, using variance component estimates by use of the following formula and for traits studied during each year.

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{R}} \quad (2)$$

as for two years:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{yg}^2}{Y} + \frac{\sigma_{gr}^2}{R} + \frac{\sigma_e^2}{RY}} \quad (3)$$

Where, σ_g^2 equals the variance of genotypes, σ_p^2 equals the phenotypic variance, σ_{yg}^2 equaling the variance of years×genotypes, σ_{gr}^2 representing the variance of genotypes×replications, σ_e^2 equaling the error variance, Y standing for the number of years and R representing the number of replications. Standard errors of heritability estimates were calculated for the traits evaluated for each year as:

$$SE(H^2) = \frac{SE(\sigma_g^2)}{\sigma_g^2 + \frac{\sigma_e^2}{R}} \quad (4)$$

and for a two year experiment:

$$SE(H^2) = \frac{SE(\sigma_g^2)}{\sigma_g^2 + \frac{\sigma_{yg}^2}{Y} + \frac{\sigma_{gr}^2}{R} + \frac{\sigma_e^2}{RY}} \quad (5)$$

RESULTS AND DISCUSSION

Analysis of Variance

ANOVA results obtained for each experimental year revealed a high genetic diversity among ecotypes as the ecotype effect was significant for all the traits in either year (P< 0.01). The significant effect of ecotype was repeated when ANOVA was carried out using both years' data combined (P< 0.01) (data not shown). Year effect was significant for all the traits except for days to 70% seed drying stage, weight of dry biomass, and inflorescence diameter. The effect of genotype×year interaction was significant for all the traits. The highly significant effect of genotype×year for all the traits indicated that the ranking of genotypes may not be the same during some time, and yet valid estimates of genetic architecture should be essentially obtained through multiple time evaluations. Within the first year, the five ecotypes that had the highest level of essential oil content were: Fozve (3.4%), Givi (3.2%), Kashan (3.2%), Marvdasht (3.1%) and Qazvin (3%) whilst for the second year the highest levels of essential oil were recorded for another five ecotypes namely: Razan (5.1%), Sari (4.7%), Kaleibar (4.2%), Marvdasht (4.2%) and Arak (4.2%). The number one ecotype for essential oil content during the first year (Fozve) ranked tenth in the second year. Similarly Razan as number one ecotype of the second year ranked 14th in the former year regarding essential oil content. However the high number of ecotypes can amplify the significance of this effect (Schwartz *et al.*, 2009). The ranges of essential oil content for the first year were 1.9% (Tabriz) to 3.4% (Fozve) and for the second experimental year it amounted to 0.56% (Barazjan) to 5.1% (Razan). For seed yield the range in first year was 52 g m^{-2} (Qom) to 395 (Meshkin shahr) and for the second year it was 48 g m^{-2} (Shiraz) to 610 (Moqan). The traits which exerted the highest effects on essential oil content and seed yield were detected using stepwise regression separately for each year (Table 2) and for both years *via* combined analysis (Table 3). During the first and second years of the experiment the most effective traits affecting essential oil content were the number of leaves and days to 50% flowering, respectively. As for the two year combined analysis, days to 50% flowering showed positive effects on essential oil content. The dry weight biomass was the most effective trait affecting seed yield in each separate year and as well within the two years combined. Tables 4 show the variance components and heritability estimates for the traits detected

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Table 2. Stepwise regression regarding the essential oil content and seed yield as dependent variables *vs.* other traits as independent variables for each separate year.

Variables	First year				Second year			
	Essential oil content	Partial R^2	Seed yield	Partial R^2	Essential oil content	Partial R^2	Seed yield	Partial R^2
Number of leaves	0.94**	0.32					0.23**	0.02
Plant height	-0.76**	0.04						
Days to 50% flowering	0.42*	0.04	-0.79**	0.02	0.49**	0.23	-0.17*	0.04
Weight of dry biomass			0.55**	0.55			0.96**	0.79
Number of inflorescence			0.26**	0.12				
Days to 70% seed at pasty stage			0.79**	0.11				
Number of seeds per largest inflorescence					-0.32*	0.06		
Stem diameter							-0.11*	0.01
Length of middle internodes							0.11*	0.01

**: Significant at 1%, and *: Significant at 5% level.

Table 3. Stepwise regression regarding the essential oil content and seed yield as dependent variables *vs.* other traits as independent variables for a two year combined experiment.

Variables	Essential oil content	Partial R^2	Seed yield	Partial R^2
Stem diameter	0.45*	0.07		
Days to 50% flowering	0.64**	0.30		
Length of petiole	-0.56**	0.05		
Number of seeds per largest inflorescence			0.27**	0.06
Weight of dry biomass			0.78**	0.75

**: Significant at 1%, and *: Significant at 5% level.

through regression analysis for their significant roles played on dependent variables. Variance component demonstrates the proportion of ecotype effect on phenotypic variation. For both essential oil content and seed yield, during the second experimental year, the genetic variances were distinctly improved implying that, for the second year the ecotypes more strongly expressed their genetic potential. Selection among accessions of the germplasm set will guarantee the stability within different years, as the estimates of genetic parameters are based on multiple time experiments.

In general, during the second year the broad-sense heritability increased for most of the traits. During the first year, the broad-sense heritability of the essential oil content trait wasn't as high as heritability for the other traits. Therefore, other affecting traits can be considered in the breeding programs. Although during the second year this estimate was very high but its measurement is more difficult than those of the other traits and if a breeder has to do a lot of selections his focus on other traits is suggested. As for the second year the error variance for seed yield is nearly doubled, but because of high increase in genetic variance, the heritability has slightly improved. The error variance change for essential oil during the two years was not very high but genetic variance significantly increased (0.091±0.024 in the first year *vs.* 1.304±0.26 for the second year). This is the main reason for increased heritability from 0.74 (in the first year) to 0.98 (in the second year). Table 5 shows the variance components and heritability estimates for the traits evaluated during the two experimental years.

According to Table 6; the genetic variance and broad sense heritability estimates of combined analysis of variance indicate that the day to 70% seed being at its pasty stage and the essential oil content are of the most *vs.*

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Table 4. Variance component estimates and broad-sense heritability values (H2) for traits assessed during the first and second year. These traits are those which have entered the regression model using stepwise regression.

	Source	Weight of dry biomass	Number of inflorescence	Days to 50% flowering	Days to 70% seed at pasty stage	Number of leaves	Plant height	Seed yield	Essential oil content
First year	Genotype (G)	79474±23080	86±23	283.1333±57	1104.333±220	604±126	645±137	4108±923	0.091±0.024
	Error	103351	84	15.6	28	95	147	1620	0.093
	H2	0.69±0.2	0.75±0.2	0.98±0.19	0.99±0.19	0.95±0.19	0.92±0.19	0.88±0.19	0.74±0.2
Second year	Genotype (G)	330032±70308	1058±226	455±90	2.07±0.6	1.63±0.4	23077±4737	10836±2383	1.304±0.26
	Error	73720	246	9.2	2.78	1.2	2517	3509	0.068
	H2	0.93±0.19	0.92±0.19	0.99±0.19	0.69±0.2	0.80±0.19	0.96±0.19	0.90±0.19	0.98±0.19

Table 5. Variance component estimates and broad-sense heritability values (H2) for traits assessed within a two year priod. These traits are the ones which have entered the regression model using stepwise regression, response variable being seed yield.

Source	Weight of dry biomass	Number of inflorescence	Number of days to 50% flowering	Days to 70% seed at pasty stage	Number of leaves	Length of middle internodes	Stem diameter	Seed yield	Plant height	Number of seeds per largest inflorescence	Essential oil content
Genotype (G)	138910±38071	366±165	296±66	715±156	559±147	2.25±0.53	1.2±0.46	3885±1284	743±172	14454±5963	0.21±0.1
Error	106471	207	14	22	152	1.82	1.04	3109	167	10385	0.09
H2	0.73±0.20	0.47±0.21	0.88±0.19	0.90±0.19	0.76±0.20	0.79±0.18	0.55±0.21	0.63±0.20	0.86±0.19	0.52±0.21	0.46±0.22

least heritability values, respectively. One easy solution for selecting high essential oil content in fennel as based on these results is to use the number of days to 50% flowering as a criterion. This seems rational as not only its heritability is high but also its effect on essential oil content is significant for both years' analyses (Tables 2 and 3). However it should be considered that late flowering ecotypes are not good in seed yield so, compromise between earliness and seed yield should be taken into account. Gross *et al.* (2009) reported the broad-sense heritability of Estragole from among the total volatile Phenylpropenes in fennel to be 0.94 to 0.99. Their estimate is very near to the estimates of essential oil content heritability evaluated for each separate year throughout this experiment. Especially the estimate for essential oil content during the second year being very high (0.98). The difference between a one year estimate and a multiple year one shows the conspicuous effect of genotype×year, as this leads to affect diminished estimates (Formula 3). The broad sense heritability estimates were much higher than those reported by other researchers. Al-Kordy (2000) reported the broad sense heritability of seed yield as; 0.201, while the estimate in the present study amounts to 0.63 for the combined analysis. Altogether the combined estimates are of a more conservative nature and breeding based on these estimates can be more dependable.

Throughout the peresent study the correlation coefficients between these two traits were 0.35, 0.17 *vs.* 0.25 for the first, second *vs.* both years combined, respectively. Then, the screening as based on other related traits rather than yield and essential oil content can be of help in plant improvement. Weight of dry biomass exerted the highest effect on seed yield during the two year period and for each separate year. It should be noted that shorter days to 50% flowering exerted contracting effects on seed yield and on essential oil content making it an unsuitable trait when the target for plant improvement is essential oil yield. Number of leaves per plant positively affected the essential oil content and seed yield during the second experimental year.

CONCLUSIONS

Wide ranges of essential oil content and seed yield among ecotypes as well as high heritability estimates indicated the high genetic diversity of the bitter fennel germplasm in Iran. This variability shows the quantitative nature of these two traits. High values of genetic variance and broad sense heritability were observed throughout the experiment. Researchers have demonstrated that variance estimates and genetic progress may be different in optimum *vs.* stress conditions (Rose *et al.,* 2007). A performance of this experiment in varying ecological locations will help the most suitable ecotypes to be selected. Since the yield of this plant is far from ideal, the genetic component estimates in this experiment can be very helpful, but to attain more progress in the future breeding programs, designs of new experiments for an assessment of narrow sense heritabilities would be indispensable.

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برآورد وراثت پذیری صفات زراعی و محتوای اسانس در رازیانه های ایران

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چکیده

گیاه دارویی رازیانه (*Foeniculum vulgare* Mill.) دارای خواص دارویی بیشماری می باشد و کشور ایران به عنوان یکی از تولید کنندگان عمده آن در جهان شناخته شده است. برخلاف ارزش دارویی بسیار بالای رازیانه، کشت آن به دلیل پایین بودن عملکرد، توجیه اقتصادی کمی دارد. جمعیت های مختلف گیاه رازیانه در اقصی نقاط ایران می روید. محاسبات واریانس ژنتیکی و وراثت پذیری صفات رازیانه قبل از کشت و کار وسیع آن و تعریف برنامه های اصلاحی لازم می باشد. در این تحقیق، ارزیابی تنوع ژنتیکی و وراثت پذیری عمومی برای ۵۰اکوتیپ مختلف رازیانه در شرایط مزرعه‌ای انجام شد. عملکرد دانه، محتوای اسانس و برخی صفات مورفولوژی دیگر در طی دو سال اندازه‌گیری شدند. از روش رگرسیون گام به گام برای شناسایی صفات تاثیرگذار بر عملکرد دانه و محتوای اسانس استفاده شد. تعداد برگ و روز تا ۵۰ درصد گلدهی بیشترین تاثیر را بر محتوای اسانس به ترتیب در سال اول و دوم داشتند و صفت ۵۰ درصد تا گلدهی بیشترین تاثیر را در بین صفات بر میانگین محتوای اسانس داشت. وزن خشک زیست توده بیشترین تاثیر را بر عملکرد دانه در هر دو سال داشت. وراثت پذیری عمومی در دو سال برای محتوای اسانس و عملکرد دانه به ترتیب ۰/۴۶ و ۰/۶۳ بود. برخی صفات مانند تعداد روز تا ۵۰ درصد گلدهی و طول میانگره وسط دارای بیشترین مقادیر وراثت پذیری گردیدند (به ترتیب ۰/۹۰درصد و ۰/۷۹ درصد). وراثت پذیری بالا در صفات مورد مطالعه، پتانسیل ژنتیکی آنها را برای برنامه های اصلاحی تائید کرد.

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