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Original Article

Genetic Variation of Shoot Yield, Essential oil and Yield Components in Four Thyme Species

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Abstract

Thymus daenensis Celak, *Thymus kotschyanus* Boiss. & Hohen., *Thymus transcaspicus* Klokov and *Thymus vulgaris* L. are aromatic and medicinal species which due to hybridization within species and between species has high morphological diversity. A study was carried out in order to evaluate yield and yield components in 10 ecotypes of *Thymus* species collected from Isfahan, Markazi, Qazvin and West Azerbaijan provinces. This experiment was performed in a randomized complete block design with three replications at Research Center for Agriculture and Natural Resources Research and Education of Razavi Khorasan province. Plant height, shoot yield, 1000-seed weight, essential oils (EO) content, EO yield, number of nodes per stem, internode length and days to flowering stage were measured in all ecotypes. *T. vulgaris* was considered as the control. The results of analysis of variance showed that there were significant differences ($p \leq 0.05$) for days to 50 % flowering, number of nodes, internode length, plant height, stem weight, seed weight, EO content and yield among species and ecotypes within *Thymus* species. The highest EO content and yield among species were observed by 2.45% and 1.28 g/plant in *T. vulgaris,* and 2.03% and 0.84 g/plant in *T.daenensis*, respectively. The highest EO content was observed among ecotypes at 1.84% in *T.kotschyanus,* ecotype K54 and 2.32% in *T.daenensis*, ecotype D49. The strong and significant ($P \leq 0.01$) positive correlation of EO yield was observed with EO content ($r_= 0.88^{**}$), seed weight per plant ($r_= 0.78^{**}$), plant height ($r_= 0.70^{*}$), shoot yield ($r_= 0.80^{**}$) and internode length ($r_= 0.89^{**}$). In the principal component analysis in thyme species and ecotypes, the eigenvalues obtained from the first three main components, explain 53%, 15%, 11%, and a total of 80% of the total variance of the variables. It was concluded that the ecotypes of D49 and D72, in *T.daenensis* having higher EO yield were recommended for breeding improved varieties.

Keywords: Thyme, Yield, Essential Oil, Correlation Analysis, Heritability

Introduction

Medicinal plants are very important in modern civilization in order to obtain natural active substances, known as secondary metabolites. Nowadays, the uncontrolled harvesting leads to the extinction of ecotype and species [1]. The genus *Thymus* is a well-known aromatic perennial herb widely used in folk medicine, food preservatives and pharmaceutical preparations originated from Mediterranean region belongs to the Lamiaceae family. This genus is consisting of about 215 species of herbaceous perennials and small shrubs in the world and 14 species in Iran. They are distributed in Iranian flora consist of *T. daenensis, T. carmanicus, T. fallax, T. persicus, T. trautvetteri, T. migricus, T. kotschyanus, T. pubesens, T. nummularius, T. transcaspicus, T. eriocalyx, T. caucasicus, T. transcaucasicus,* and *T. fedtschenkoi* [2]. Thymol and carvacrol are the major compounds in most of the *Thymus* essential oil. The therapeutic potential of thyme rests on contents of thymol, carvacrol, flavonoids, eugenol, aliphatic phenols as well as luteolin, saponins, and tetra methoxylated flavones. The EO of Thyme has antibacte-

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rial, antiseptic, antifungal, anti-parasitic and antioxidant activity [3,4].
The terpene phenols thymol and carvacrol represent the most important compounds in the genus, followed by linalool, p-cymene, γ-terpinene, borneol, terpinen-4-ol and 1, 8-cineole [5]. In traditional medicine, the leaves and flowering parts of *Thymus* species are widely used as tonic, herbal tea, flavoring agents (condiment and spice), antiseptic, antitussive and carminative as well as treating colds [6,7].
Based on the results of different studies, EO content (%, *v/w*) in cultivated samples of thyme varied between 0.32-0.75% and 0.62–1.05% within two examined lineages, whereas the herbs from the natural habitat contained 0.49–1.29% [8]. Twenty-four components in total were identified representing more than 85–98% of the oil composition, with thymol (33.9%–70.3%), carvacrol (4.0%–24.8%), *γ*-terpinene (3.9%–10.4%) and *p*-cymene (4.8%–8.6%) in *T. daenensis*. Thymol (35.5%–44.4%), carvacrol (4.4%–16.1%), *γ*-terpinene (10.5%–11.9%) and *p*-cymene (8.5%–16.1%) in *T. vulgaris* as major constitutes. The EO and their chemical compositions of *Thymus* species are strongly affected by environmental conditions and agronomic management practices. Cultivation of thyme, especially *T. daenensis* proved to be superior in both oil content and quality, in terms of, the phenols *carvacrol* and *thymol* [9]. The EO of leaves in *T. transcaucasicus* Ronniger was distinguished by high amounts of thymol (35.83%), α-terpineol (12.38%), geraniol (10.79%) and p-cymene (5.98%). Thirty-six constituents representing 99.75% of the flower oil were identified of which thymol (62.92%), p-cymene (5.78%), geraniol (5.58%) and carvacrol (4.14%) were major components. The main components of the root oil were thymol (60.61 %), p-cymene (9.32%), carvacrol (4.61%), pentacosane (3.17%) and 1.8-cineole (3.08%). Thymol (46.07%) and geraniol (9.20%) predominated in the stem oil [10].
Drought and salinity stress influenced to increase the amount of phenol contain and consequently increases its antioxidant properties and it makes it superior to Thyme [11]. This is an evidence that the synthesis of EO in aromatic plants can be influenced by genetics, stage of development and environmental factors such as temperature, nutrition and photoperiod [12]. Previous researches have shown that the EO yield was high in the full flowering stage, the sum of the two compounds is thymol and carvacrol. In another study concluded that suitable time of harvesting of the EO was at flowering stage in summer [13]. The study of the phenological and morphological characteristics of the medicinal plants has leads to a better understanding of the extent and manner of growth and development in different species and ecotypes. Maximum growth rates in any particular stage of fruit growth and secondary metabolites are not similar in early maturing to late maturing of ecotypes or cultivars in the whole growth stages. The amount of active substances could be done based on this feature [14]. This research was aimed to study of genetic variation and relationship of shoot yield, EO content and yield in four thyme species.

Material and Methods

Field experiments were carried out in a randomized complete block design with three replications at Razavi Khorasan Agricultural and Natural Resources Research and Education Center, Mashhad-Iran. The experimental farm located at latitude 36° 13' N, longitude 59° 40' E, and at an altitude of 980 m above the sea level with semi-arid climate, mean annual precipitation of 254 mm, mean annual temperature of 14.5 °C and the average temperature in winter and summer seasons of -1.8 °C and 33 °C, respectively. The analysis of soil chemical and physical properties are presented in Table 1.
Treatments were consisting of 4 species and 10 ecotypes. The study ran for two years in 2018 and 2019. Seeds were provided by national project leader in Research Institute of forests and rangelands (RIFR). The origin of seeds is presented in Table 2. In order to implement the project, seed samples of the plants were first seeded in greenhouse in winter and then transplanted to the farm in early spring. They were planted in the plots of 2x5 meter with 4 rows for each ecotype in late March 2018. Irrigation was performed weekly in a dripping manner. The fertilization was done according to the recommended values using 50 kg of urea fertilizer + 150 kg of triple super phosphate per hectare. Weeds was controlled mechanical by 2-3 times during growth period. At 50% of flowering stage in the first and second year, plant height, number of nodes per stem, internode length, flowers weight, leaves and stem weight was measured in five plants. Plants in each plot were harvested for biomass production. The fresh and dry weight was measured for each sample. Biomass and grain yield and EO content were measured in all thyme species and ecotypes. EO were extracted by hydro distillation method using 30-gram sample and the Clevenger-type apparatus. Statistical analysis, including correlation, stepwise regression and PCA analysis were performed using SPSS24 and Minitab software.

Results and Discussion

The analysis of variance showed that there were significant differences ($p \leq 0.05$) for days to 50 % flowering, number of nodes, internode length, plant height, stem weight, seed weight, EO content and EO yield, in species and ecotypes within species of *Thymus*.

Table 1 Physical and chemical properties of the field soil

Soil depth (Cm)	(%)								
	WP	FC	Organic Carbon	TNV	SP	Clay	Silt	Sand	N
0-30	8.4	16.4	0.45	17	34	23	36	41	0.092
30-60	9.3	17.8	0.19	23	39	23	34	43	0.038

Soil depth (Cm)	(ppm)						EC (ds/m)	pH
	Cu	Zn	Mn	Fe	K	P		
0-30	0.96	0.56	5.40	3.76	219	10	1.63	8.0
30-60	0.54	0.12	3.32	5.86	69	4.4	1.44	8.0

Table 2 List of used *Thymus* species and ecotypes received from the gene bank resources in Iran.

Species Name	Ecotype code	Gene bank code	Origin	Treatment
	D49	1149	Isfahan	1
T. daenenesis	D60	1160	Markazi	2
	D68	1168	Isfahan	3
	D72	1172	Markazi	4
	K22	1122	Qazvin	5
T. kotschyanus	K5	1105	Qazvin	6
	K54	1154	West Azerbaijan	7
	K70	1170	West Azerbaijan	8
T. transcaspicus	T	1124	Unknown	9
T. vulgaris	V	control		10

The results also showed significant differences among species for all traits except flowers weight, flowers and leaves weight and 1000 seeds weight and also significant differences among ecotypes within species for all of traits except flowers and leaves weight and shoot yield (Table 3).

Essential Oils Content and Yield (EO)

The results of the analysis of the EO of *Thymus* species grown under the same condition showed a significant difference (P < 0.05, Table 3) among ecotypes and species. The EO content and yield per plant of *T. daenensis* was the same with the control *(T. vulgaris)* but significantly higher than others (P<0.05, Table 4). The EO yield for the control was 1.28 g/plant and 0.84 g/plant for *T. daenensis*. The EO production of *T. daenensis* as a native species in this experiment was high and commercially acceptable in comparing with the control. The highest EO content among species was observed 2.45% in *T. vulgaris* and 2.03 % in *T. daenensis* (Table 4). Alavi Samani *et al*. [15] also reported no significant difference between *T. daenensis* and *T. vulgaris* for EO yield. There was a significant difference for the EO content and yield at P< 0.01 among ecotypes (Table 3). The EO content of *T. daenensis* and *T. kotschyanus* ecotypes varied between 0.62 and 2.32%. The highest EO content (1.84%) was observed in *T. kotschyanus*, ecotype K54 (West Azerbaijan) and 2.32% in *T. daenensis*, ecotype D49 (Isfahan) (Table 5). Kaveh *et al*. [16] also showed that the EO content among *T.kotschyanus* was from 0.42 to 2.17%. Aflakian *et al*. [17] concluded that the highest total dry weight, EO percentage and EO yield among different ecotypes of *T. daenensis* was observed in ecotype Khoramabad.

Shoot Yield

Analysis variance showed that the shoot yield among *Thymus* species was significantly different at P<0.05 and the highest shoot yield was observed in *T. vulgaris* (49.10 g/plant) and in *T. daenensis* (39.66 g/plant) but there was no difference observed among ecotypes within species (Tables 3, 4). However, other reports have shown that *T. daenensis* had highest total dry mater, EO percent and yield than *T. vulgaris* [15, 18]. Kaveh *et al*. [16] also showed that there is genetically variation for all traits in thyme genotypes, so that the highest dry matter yield of 2200 and 1640 kg/ha was obtained from of *T. vulgaris*, ecotype Isfahan and *T. kotschyanus*, ecotype Western Azerbaijan, respectively.

The results of analysis of variance were showed there is a significant difference for the stem weight among species and ecotypes within species (P<0.05). *T. transcaspicus* with 2.38 g/plant and *T. daenensis* by 20.69 g/plant had the lowest and highest stem weight, respectively. Stem weight was 17.56 g/plant in ecotype K54 and 24.73 g/plant in ecotype D68 (Table 5, P<0.05).

Weight of leaves and flowers as total aerial parts coupled with high oil percentage in *Thymus* are economically important and led to increasing the yield of the EO. Leaves and flowers weight were no difference among species and ecotypes within species (Tables 3, 4). Flowers weight was significantly different between ecotypes within species. The highest and lowest one was observed in *T. vulgaris* (2.03 g/plant) and *T. transcaspicus* (1.17 g/plant) (Tables 3, 4).

Morphological and Phenological Characters

Variance analysis of the morphological and phenological traits showed that some measured traits like days to 50% flowering stage, number of nodes per stem, internode length and plant height were significantly difference among species and ecotypes within species (Tables 3, 4). *T. daenensis* was the same to the control (*T. vulgaris*) for number of nodes, internode length and plant height, but 50% flowering stage occurred by 52 days after the control. The mean values of plant height in *T. daenensis* were significantly higher than the control.

Seed Weight

Means comparison showed that *T. vulgaris* had the lowest 1000 seed weight, 0.26 g (Table 4) and the native species had higher 1000 seed weight. Within species, ecotype D68 with 0.51 g had the highest 1000 seed weight among the ecotypes (Table 5). The highest seed weight was observed in *T. vulgaris* (2.48 g/plant) and within species in *T. daenensis* ecotype D49 (2.35 g/plant) (Table 5).

Table 3 Nested analysis of variance between for different characters of *Thymus* species and ecotypes

Source	df	Days to 50% Flowering	Stem weight	Leaves and Flowers weight	No. nodes per stem	Internode length	Plant height
Species (A)	3	2663.8**	313.02*	309.4	26.44*	1.2416**	350.25*
Ecotypes (B) within A	6	391.7*	110.53*	135.3	6.639**	0.0946*	63.78**
Error	20	164.2	59.93	121.7	1.776	0.0428	11.79

Continued **Table 3**

Source	df	Shoot yield	Flowers weight	Seed weight	1000 Seed weight	EO yield	EO content
Species (A)	3	963.2*	0.390	4.494*	0.941	0.967*	1.920*
Ecotypes (B) within A	6	185.1	1.243*	1.704**	0.996**	0.220**	0.560**
Error	20	140.5	0.480	0.202	0.118	0.050	0.133*

*, ** are significant at the 0.05 and 0.01 significant levels, respectively.

Table 4 Comparison of different traits measured among *Thymus* species

Species	Days to 50% Flowering	Stem weight (g/plant)	Leaves and Flowers weight (g/plant)	No. nodes per stem	Internode length (cm)	Plant height (cm)
T. kotschyanus	572.8 ab	12.55 b	40.41 a	6.89 b	1.36 b	23.50 b
T. daenensis	584.3 a	20.69 a	41.50 a	9.60 a	1.80 a	32.74 a
T. transcaspicus	550.3 bc	2.38 c	23.14 a	4.93 b	0.78 c	15.69 c
T. vulgaris	532.0 c	14.36 b	33.69 a	9.33 a	2.05 a	33.58 a

Continued **Table 4**

Species	Shoot yield (g. plant -1)	Flowers weight (g.)	Seed weight (g. plant -1)	1000 seed Weight (g)	EO yield (g. plant -1)	EO content (%)
T. kotschyanus	36.36 a	1.49 ab	1.74 b	0.36 a	0.47 b	1.40 b
T. daenensis	39.66 a	1.56 ab	1.41 ab	0.36 a	0.84 a	2.03 a
T. transcaspicus	8.83 b	1.17 b	0.37 c	0.34 a	0.14 b	0.94 b
T. vulgaris	49.10 a	2.03 a	2.48 a	0.26 b	1.28 a	2.45 a

Means with the same letter are not significantly different using Duncan's multiple range tests

Table 5 Comparison of different traits among ecotypes within *Thymus* species

Species	Ecotypes	Days to 50% Flowering		Stem weight (g/plant)		No. nodes per stem		Internode length (cm)		Plant Height (cm)	
T. kotschyanus	K22	592.3	a	8.26	b	5.96	bc	1.16	cd	21.67	ef
	K5	562.3	b	8.85	b	5.43	c	1.49	b	22.00	ef
	K54	569.7	ab	17.56	a	7.57	b	1.52	b	23.33	def
	K70	567.0	b	15.55	ab	8.58	b	1.27	c	27.00	cd
T. daenensis	D49	583.7	ab	23.04	a	8.00	b	1.83	ab	32.10	bc
	D60	582.7	ab	14.99	ab	11.25	a	1.70	b	38.33	a
	D68	580.7	ab	24.73	a	8.67	b	1.63	b	24.53	de
	D72	590.0	a	20.00	a	10.50	a	2.05	a	36.00	ab
T. transcaspicus	T	550.3	bc	2.38	c	4.93	c	0.78	d	15.69	f
T. vulgaris	V	532.0	c	14.36	ab	9.33	ab	2.05	a	33.58	bc

Continued **Table 5**

Species	Ecotypes	Shoot yield (g/plant)		Flowers weight (g/plant)		Seed weight (g/plant)		1000 seed weight (gr)		EO yield (g/plant)		EO content (%)	
T. kotschyanus	K22	33.53	ab	2.00	ab	2.16	abc	0.36	bcd	0.52	cd	1.44	abc
	K5	38.82	ab	1.19	ab	1.16	bcd	0.41	b	0.65	bc	1.70	ab
	K54	32.38	ab	1.92	ab	1.35	bc	0.39	bc	0.48	d	1.84	ab
	K70	40.72	ab	0.83	b	0.98	cd	0.29	de	0.25	d	0.62	c
T. daenensis	D49	46.47	a	1.48	ab	2.35	ab	0.31	cd	1.19	ab	2.32	a
	D60	31.81	ab	2.57	a	2.08	abc	0.32	cd	0.61	bcd	2.03	a
	D68	32.04	ab	1.29	ab	0.36	d	0.51	a	0.54	cd	1.65	abc
	D72	48.32	a	0.91	b	2.19	abc	0.29	cde	1.15	abc	2.10	a
T. transcaspicus	T	8.83	b	1.17	b	0.37	d	0.34	cd	0.14	d	0.94	bc
T. vulgaris	V	49.10	a	2.03	ab	2.48	a	0.26	e	1.28	a	2.45	a

Means with the same letter are not significantly different using Duncan's multiple range tests

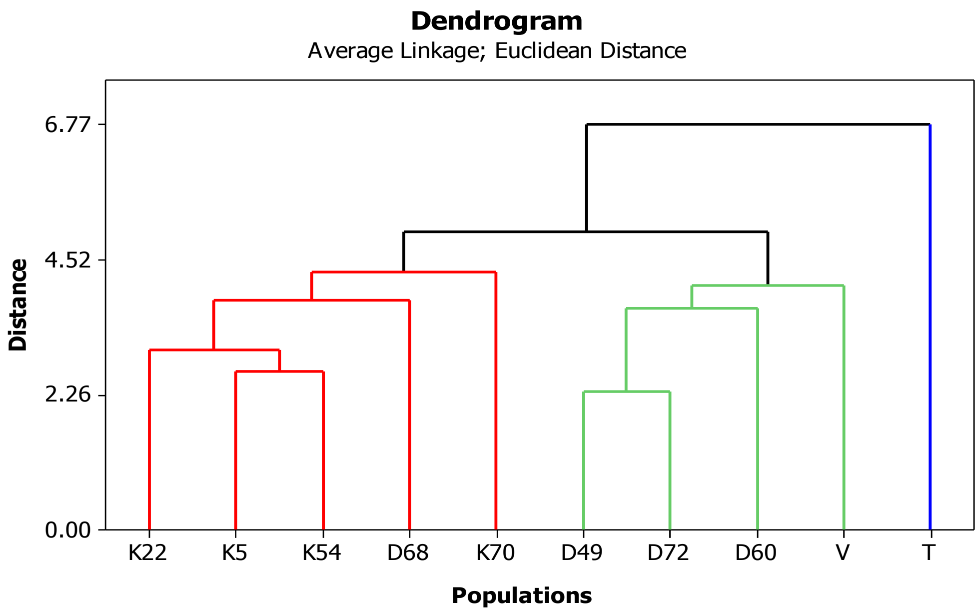

Fig. 1 Dendrogram generated from the genetic similarity across the 10 *Thymus* species and ecotypes using different agronomic and morphological traits. Numbers of ecotypes in different colors indicate ecotypes of *T. kotschyanus* (red), *T. daenensis* and *T. vulgaris* (green), and *T. transcaspicus* (blue). Different branch colors represent the 3 different groups.

Table 6 Pearson's correlation coefficients (r) between phenological, morphological, yield and yield components of *Thymus*

Traits	1	2	3	4	5	6	7	8	9	10	11
Days to 50% Flowering	1	-	-	-	-	-	-	-	-	-	-
Stem weight	0.40	1	-	-	-	-	-	-	-	-	-
Leaves and flowers weight	0.43	0.47	1	-	-	-	-	-	-	-	-
No. nodes per stem	0.22	0.65*	0.43	1	-	-	-	-	-	-	-
Internode length	0.08	0.71*	0.42	0.75**	1	-	-	-	-	-	-
Plant height	0.21	0.59*	0.53	0.92**	0.84**	1	-	-	-	-	-
Shoot yield	0.12	0.59*	0.72*	0.53	0.83**	0.69*	1	-	-	-	-
Flowers weight	0.01	-0.06	-0.31	0.26	0.18	0.31	-0.03	1	-	-	-
Seed weight	0.17	0.20	0.34	0.45	0.64*	0.71*	0.68*	0.49	1	-	-
. 1000 seed weight	0.26	0.17	-0.28	-0.33	-0.22	-0.50	-0.36	-0.08	-0.62*	1	-
. EO yield	0.01	0.49	0.31	0.48	0.89**	0.70*	0.80**	0.15	0.78**	-0.38	1
. EO content	0.04	0.47	0.05	0.48	0.85**	0.66*	0.60*	0.48	0.71*	-0.14	0.88**

* and ** Correlations are significant at the 0.05 or 0.01 levels, respectively

Correlation Analysis of Traits in *Thymus*

To find if there are chemical and genetic relationships among some *Thymus* species, The Pearson's correlation coefficients were applied to determine correlation between the EO yield and genetic variability of 10 ecotypes of *Thymus* (Table 6). The strong and significant (P≤0.01) positive correlation of EO yield was observed with EO content (r= 0.88**), seed weight per plant (r=0.78**), plant height (r=0.70*), shoot yield per plant (r=0.80**) and internode length (r=0.89**). The correlation between seed weight per plant and 1000 seeds weight (r=0.62*) was significantly negative (Table 7). The results are at par with the findings of other researchers, for the positive relation of EO with plant biomass and seed yield [19,20]. According to high correlation analysis between shoot yield and EO yield, breeding for these two traits can be consistent in Iranian ecotypes of *Thymus* [14]. Therefore, they showed identical clustering with the volatile-oil profiles. The dendrograms revealed that the 10 ecotypes formed 3 heterotic groups. The dendrogram proofed the different species based on their distinctive genetic background. It revealed that there was a hierarchical relationship or similarity between *T. vulgaris* and *T. daenensis* and close relationship between geographic locations based on agro-ecological similarities. The two species were grouped in the same group due to similarity and affinity (Fig. 1).

Principal Components Analysis (PCA)

The purpose of PCA is to summarize the number of variables and reduce the data volume to identify the most diverse and important traits associated with a particular task. Considering the existing correlation between the data, it is predicted that the higher the correlation between the variables, the better the importance of the changes are justified, and therefore, the first, second, and third variables are determined with more variance [21]. The parameters of PCA, including eigenvalues, percentage of justified variance, and specific vector coefficients for the main components of Thyme species and ecotypes were listed in Table 7. Results indicated that the eigenvalue values obtained from the first three main components, explain 53%, 15%, and 12% and a total of 80% of the total variance of the variables that involved in increasing the EO yield. Relative values of coefficients in the first component showed that number of nodes, internode length, plant height, shoot weight, EO content and EO yield had the highest value and were important than other traits. This component represents the variables responsible for increasing the EO yield. Previously, according to Table 6, correlation coefficients of traits were found to have a high and positive correlation with EO yield for plant height, shoot yield, and EO percentage. In the second component, stem weight and days to 50% flowering with a negative coefficient and flowers weight and seed weight with a positive coefficient were responsible for changes in this component and also the trend of correlation results indicated no effect of the above traits on EO yield. In the third component, leaves and flowers weight had positive effect and 1000 seed weight had negative effect on EO yield, which showed the same trend. The traits mentioned in the above components had the most variations and it seems that the first component is most important (Table 7). The results also showed that the difference among the ecotypes were less genetically and were more because of variability in geographical origin and regions (Fig. 2). The EO content and yield among the ecotypes of *T. daenensis* was lowest in D68 (Table 5). The biplot analysis for the first and second principal components showed that the ecotype of D68 was more similar to *T. kotschyanus*. It is necessary to consider more traits in the analysis to find the most effective traits.

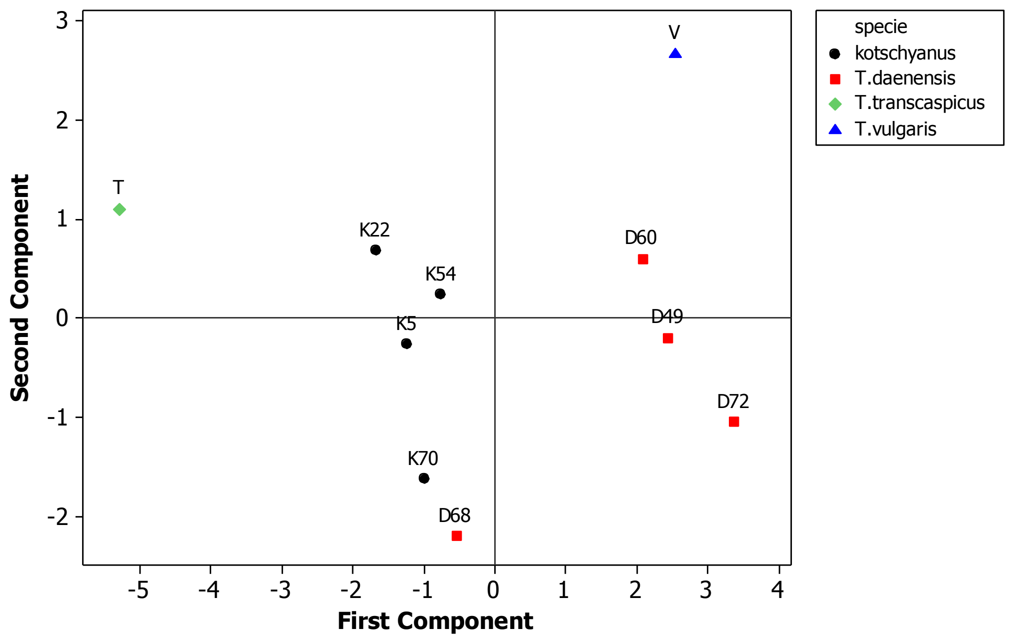

Fig. 2 Biplot of the first and second components for ecotypes of *Thymus kotschyanus* (K), *Thymus daenensis* (D), *Thymus vulgaris* (V), and *Thymus transcaspicus (T)*

Table 7 PCA of measured traits in species and ecotypes of *Thymus*

Variables	Principal Components		
	PC1	PC2	PC3
No. nodes per stem	**0.31**	-0.10	-0.09
Internode length	**0.36**	0.00	-0.10
Plant height	**0.36**	0.03	0.03
Shoot yield	**0.32**	-0.07	0.26
EO yield	**0.33**	0.17	0.04
EO content	**0.31**	0.24	-0.30
Days to 50% Flowering	0.09	**-0.41**	-0.17
Stem weight	0.26	**-0.38**	-0.22
Flowers weight	0.09	**0.43**	-0.43
Seed weight	0.29	**0.32**	0.14
Leaves and Flowers weight	0.21	-0.38	**0.43**
1000 seed weight	-0.15	-0.35	**-0.56**
Eigenvalue	6.89	1.99	1.47
Proportion	0.53	0.15	0.12
Cumulative	0.53	0.68	0.80

The underlined values have significant correlation with the components

The phenotypic variances are always greater than the genotypic variances. The maximum amount of genotypic coefficient of variation (GCV) in percent recorded for EO yield (53.28%) followed by seed yield (49.03%), EO content (30.84%), internode length (26.71%), shoot yield (24.8%), number of nodes (23.16%) and flower weight (22.75%) was found suitable for favorable selection for further genetic improvement. The days to 50% flowering were recorded lowest (3.02%) genotypic coefficient variance and also PCV and ECV. The phenotypic coefficient of variation (PCV) values was higher than their corresponding GCV for all traits, which reflects that the apparent variations depend not only to genotype but also on the environment. The environmental coefficients of variation (ECV) of traits were lower than both genotypic and phenotypic coefficient of variations except plant height, shoot yield, flowers weight and stem weight. This implies that the environmental role was clear for the expression of a few characters (Fig. 3). Although in this experiment the heritability of days to 50% flowering stage is 0.81 but other characters with moderate heritability indicate a high degree of dependence on environmental conditions or genetic control and the success rate depends on the inheritance value and their selection is directly related to the genetic enhancement of the trait, which increases the effectiveness of the chosen selection system and increases its success in different environmental conditions. 50% flowering stage had the lowest coefficient of variation PCV, GCV and ECV (Table 8). This is also reported in other studies on *Ocimum* and *Trachyspermum* ecotypes [22,23].

Assessing variability is fundamental to identify the most important traits in Thyme improvement program. The objective of the present study was to estimate variability, heritability and genetic advance based on some morphological and agronomic characters of Thyme. The higher phenotypic coefficient of variation (PCV) for all traits compared to genotypic coefficient of variation (GCV), indicates that PCV is an expression of both the genetic and environmental effects, while, GCV is expression of the genetic effect [23]. It means that selection based on phenotype may be useful for yield improvement. These results are the same as those reported by Khan *et al*. [24].

Genetics advance was recorded as maximum for days to 50% flowering (24.1) and shoot yield (12.39). Heritability in associated with genetic advance over means (GA%) was more in seed weight, shoot yield and EO content and yield (Table 8). GA% is more effective and reliable in predicting the resultant effect of selection [25] and these traits may be exploited for commercial cultivation.

The selection efficiency for a specific attribute in a population depends largely on genetic and non-genetic factors that contribute to the occurrence of phenotypic differences. Therefore, environmental factors may affect the genetic structure and consequently, inheritance through interaction. Heredity, along with the genetic progress of the selection parameters are important if they are used together, have a very high performance in the development of cultivars. Except some traits such as stem weight, shoot yield, leaves and flowers weight others had a high heritability and it was due to their genetic enhancement effects (Table 8).

Estimates of brood sense heritability (bs) derived from analysis Thyme species were low for flowers weight, leaves and flowers weight and stem weight (0.16 to 0.32), moderate for shoot yield (0.47), and high for other traits (0.47 to 0.89) (Table 8). Our findings were lower than those for plant biomass and some other economic traits in *T. daenensis* that reported by Golestani and Sahhafi [26].

The genetic advances (GA%) were calculated for all of traits. Based on the findings of this study, seed weight and EO yield had higher values of (GA%). It seems selection of ecotypes for traits are effective. These results were corresponding to those reported by Jackson and Hay [27] who found fast progress by selection of seed yield and EO yield in *T. vulgaris*.

Conclusion

Thymus ecotypes were divided into three different clusters, indicating that 10 ecotypes had different origins and the characteristics of the clusters were statistically different. The clusters can be selected with the aim of breeding goal, by separation early and late ecotypes, for simultaneous pollination or in terms of selected other traits. In all the traits, the rate of phenotypic variation is greater than the genetic variation coefficient. Therefore, environmental conditions play a significant and interrelated role on the emergence of traits.

The selection of these traits is influenced by ecotype and phenotype. The heritability of days to 50% flowering, plant height, EO yield, EO content, number of nodes per stem, internode length, 1000 seed weight and seed weight per plant were higher than the other ones. The estimation of genetic advance is effective in understanding the type of activity of the gene involved in the expression of polygenic traits. High levels of genetic advance represent an incremental gene activity, while low levels represent the non-additive activity of the gene. The knowledge of genetic variability in a given crop species for characters under improvement is important in any plant breeding program. Heritability with genetic advance are more helpful in predicting the gain under effective selection. Therefore, high heritability does not necessarily mean that the genetic trait will have a high genetic advance. It occurs when the effects of the gene are additive. While high heritability with low genetic efficiency is observed when there have a static or dominant effect. The characters those exhibit maximum heritability and high GA% could be used as powerful tool in selection process such characters are controlled by the additive genes and less influenced by the environment. For an efficient selection we cannot solely believe on heritability. We must make selection based on the combination of high heritability with high genetic advance that will provide a clear base on the reliability of that particular trait in the selection of variable entries.

Table 8 Estimate of mean, components of variance, heritability (bs) and expected genetic advance in respect of characters in *Thymus*

Traits	Range	Mean	Coefficient of variation			Heritability (bs)%	Genetic advance	GA%
			GCV	PCV	ECV			
Days to 50% Flowering	532-592	570.4	3.022	3.36	2.56	0.81	24.1	4.22
Plant height	16-38	28.42	20.07	24.95	25.66	0.65	7.99	28.1
Internode length	0.78-2.32	1.56	26.71	29.11	20.05	0.84	0.58	37.4
No. nodes per stem	4.93-11.25	7.99	23.16	26.18	21.15	0.78	2.59	32.43
1000 seed weight	0.26-0.51	0.35	20.03	21.28	12.42	0.89	0.99	28.04
Seed weight	0.065-2.48	1.54	49.03	55.2	43.95	0.79	1.06	68.63
Shoot yield	7-52	35.68	24.8	36.13	45.52	0.47	12.39	34.72
EO content	0.62-2.45	1.74	30.84	35.07	28.9	0.77	0.75	43.18
EO yield	0.070-1.1	0.68	53.28	58.48	41.57	0.83	0.5	74.59
Flowers weight	0.83-4.1	2.12	22.75	57.44	91.35	0.16	0.68	31.85
Leaves and Flowers weight	23.1-50.8	38.38	12.56	36.64	34.68	0.28	6.75	17.58
Stem weight	2.38-23	14.97	24.93	43.81	62.4	0.32	5.2	34.9

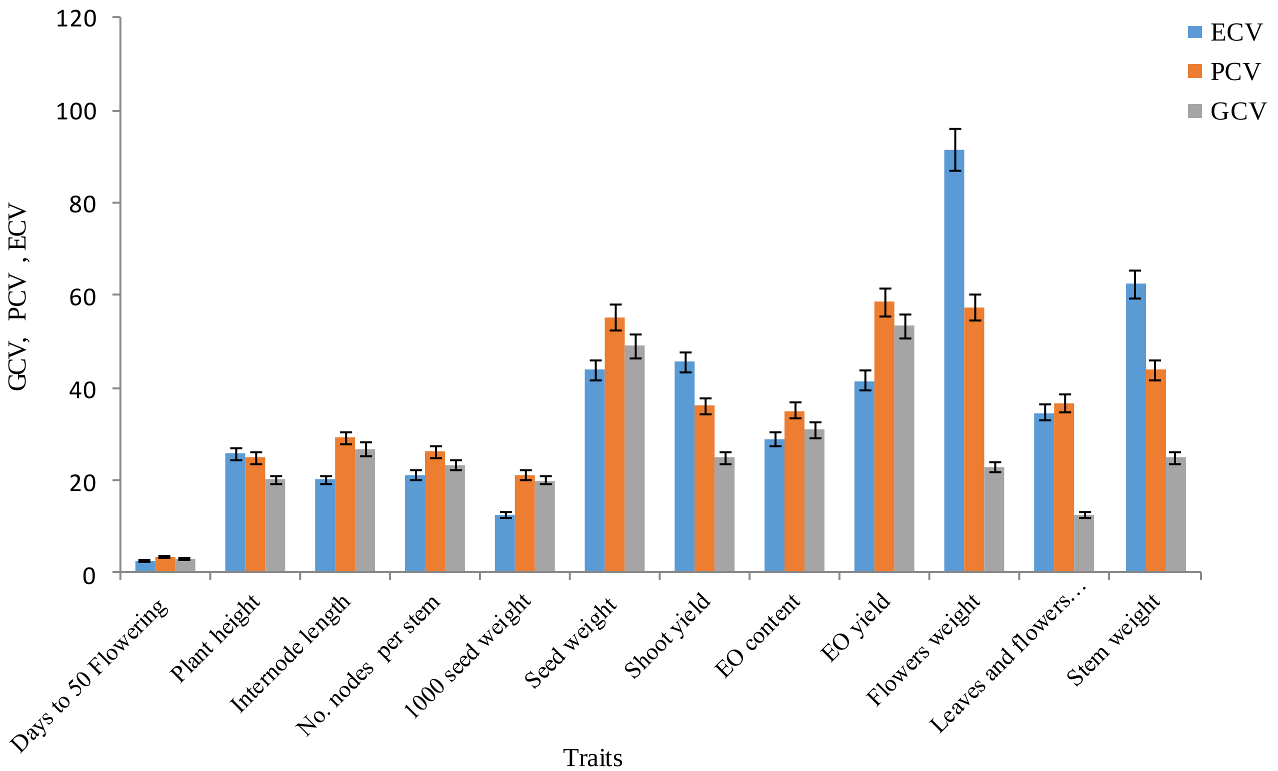

Fig. 3 Graphical presentations of PCV, GCV and ECV for EO yield contributing traits of Thyme ecotypes measured in each plant.

The dendrogram revealed that the 10 thyme ecotypes and species formed 3 heterotic groups and proofed the different species based on their distinctive genetic background. Both EO content and EO yield values in *T. daenensis* were the same as *T. vulgaris* and significantly higher than other ecotypes.

Native species such as *T. daenensis* is less woody and fibrous than the other Thyme species and due to its high EO and suitable yield can be considered in a breeding program.

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