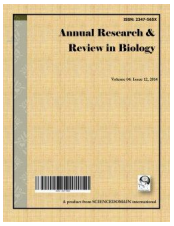

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Variations in Phytophenol Compounds in Association with Morphological Traits in *Trigonella spp*. Accessions

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Authors' contributions

This work was carried out in collaboration between all authors. The author MR performed the field evaluations, HPLC analysis and wrote the first draft of the manuscript. The authors HP and BH designed the study, evaluated the statistical analysis, wrote the protocol and edited the draft of the manuscript. The author AAJ provided the seeds of the plants. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the interrelationship between morphological traits and several phytophenol compounds as important essential oils in 23 fenugreek (*Trigonella spp*) accessions.
Study Design: The experimental design was a Randomized Complete Block Design (RCBD) replicated in two growing seasons.
Place and Duration of Study: Department of Natural Resources, Fars Agricultural and Natural Resources Research Center, Shiraz, Iran in the years 2015 and 2016.

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Methodology: Several agronomic and morphological traits were measured in the field. Leaf tissue samples were used to extract phytophenol compositions by high performance liquid chromatography (HPLC) method.
Results: The results of analysis of variance revealed that the year × accession interaction was not significant for phytophenol compounds whereas morphological traits were affected by this variance component. Great variations were found for phytophenols. Simple correlation analysis revealed that except quercetin most of phytophenols were not associated with morphological traits but multivariate statistical analysis techniques demonstrated association of these two sets of variables. Trans-ferulic acid, p-Coumaric acid, rutin and hesperidin were the most important phytophenol compounds associated with shoot fresh weight. Several phytophenols had direct and positive relation with shoot fresh weight whereas several others decreased as shoot fresh weight were increased. Quercetin was significantly associated with most of morphological attributes.
Conclusion: The outcome of this study provides more options to breeders in terms of several unresolved issues in fenugreek with respect to phytophenol compositions and their associations with important morphological traits.

Keywords: Trigonella foenum-graecum; accession; polyphenols; morphological; flavonoid; quercetin; Iran.

1. INTRODUCTION

The fenugreek (*Trigonella foenum-graecum L.*) is an annual plant belonging to the *Fabaceae* family. It is a multi-use and commercially important spice grown for its seeds, leaves and tender shoots. The cultivation of fenugreek is confined to areas with moderate or low rainfall and cool growing seasons [1]. The essential oils extracted from different organs, i.e. seed and leaves are used in medicine, food processing and several industrial food products [2]. Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fibre (galactomannans), 20-30% lysine and tryptophan rich proteins and 5-10% fixed oils [3,4]. Fenugreek seeds also contain lysine and tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents consisting of saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline. These compounds are thought to account for many therapeutic effects. The multi-functional activities of the phytophenols of fenugreek have been attributed to diverse chemical and phytochemicals constituents of its stem, leaf and seeds [5]. Epidemiological studies support a close relationship between the consumption of phenolic rich food products and low chance of coronary heart incidence, atherosclerosis, certain forms of cancer and stroke. The biological and pharmacological properties of fenugreek essential oils are attributed to the variety of its constituents, i.e. steroids, N-compounds, polyphenolic substances, volatile constituents and amino acids [3,6]. Considering the high commercial merits of fenugreek species, and the importance of phytophenol production, the demand for raw material in processing industries and medicinal sciences is increasing. Accordingly, biomass and morphological traits are important in enhancement of raw materials for production of phytophenols in industrial and medicinal plants. In most of studies with fenugreek, simultaneous consideration of morphological and field evaluated traits variations and phytophenol compositions is missing. Literature shows that great morphological and phytochemical variations exist in fenugreek germplasm [7]. In a study, Riasat et al. [1] reported that the main compounds of essential oil of *Trigonella foenum graecum* leaves were predominantly (2E)-hexenal (26.61%), n-hexadecanoic acid (10.14%), (E)-b-Ionone (7.99%), thymol (4.79%), 6,10,14-trimethyl-2-Pentadecanone (4.59%), Carvacrol (3.40%), (E)-Nerolidol (3.32%) and (2E,6Z) nonadienal (3.30%). In the Marzougui et al. [8] study, Tunisian fenugreek cultivars were assessed for vegetative and reproductive characteristics and chemical markers and significant variations were found for these traits except flowers' standard colour. Avtar et al. [9] found a positive and significant correlation between catalase activity, total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, total phenols, and orthodihydric phenols in fenugreek. In another study with fenugreek, high level of total phenols (0.62%), orthodihydroxy phenols (0.80%), and flavonoids (2.13%) were recognized in the essential oils extracted from leaf tissue [10]. Results of a study revealed that, the total phenolic contents in fenugreek seeds ranged from 38 to 41 mg/g GAE (GAE=Gallic

Acid Equivalent), and flavonoid contents ranged from 1.2 to 2.3 mg/g QE (QE=Quercetin Equivalent) [11]. Germplasm characterization is crucial in genetic resource preservation and breeding for cultivar release. Despite all the merits mentioned above, the interrelationship of morphological traits and phytophenol compositions of fenugreek is missing in the literature. Accordingly, the present study aimed to investigate variations in phytophenol compositions and the interrelationship between several morphological traits and phytophenol compositions in fenugreek (*Trigonella spp*) accessions. The outcome of this study provides more options to medicinal plant research community to better programming fenugreek improvement for the development of new varieties with better field establishment and specific phytophenol compositions.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

Twenty-three accessions of fenugreek were collected from different ecological regions of Iran (Table 1). The accessions were provided by the Gen Bank Center for Forests and Pastures Researches of Fars and Hamadan provinces. The seeds had been collected based on morphological variability under natural habitats where this species is normally growing. Discrimination of accessions was followed based on the standard procedures of the herbarium samples of Iranica. The seeds were sown in an experimental field located in Zarghan Researches Station of Fars Agricultural and Natural Resources Research Center, Shiraz, Iran (29° 46' 12", 52° 42' 48", and 1500 m).

2.2 Data Collection

The experimental design was a Randomized Complete Block Design (RCBD) replicated in two growing seasons, 2015 and 2016 years. The sowing dates were 9th April 2015 and 18th April 2016. Each experimental plot consisted of four 3-m long rows spaced 1 m. The individual plants spaced 50 cm apart on each row. Weeds were manually controlled and standard irrigation practices were followed throughout each growing season. Morphological traits including plant establishment, stem length, number of stems per plant, canopy cover, shoot fresh weight, shoot dry weight, stem appearing time and flowering time were measured. After the harvesting on

Table 1. Geographical locations of the collected fenugreek accessions and their herbarium numbers

Number	Location (Province; City)	Scientific name	Herbarium number	Code
1	Europe	*T. foenum graecum*	31137	T. f.g[H-31137]
2	Alborz; Karaj	*T. foenum graecum*	915	T. f.g[H-915]
3	Esfahan; Kashan	*T. persica*	10110	T. p[H-10110]
4	Hormozgan; Haji Abad	*T. uncata*	32571	T. u[H-32571]
5	Hormozgan; Haji Abad	*T. uncata*	32617	T. u[H-32617]
6	Hormozgan; Minab	*T. uncata*	22864	T. u[H-22864]
7	Kerman; Rafsenjan	*T. foenum graecum*	23017	T. f.g[H-23017]
8	Kermanshah; Javanrood	*T. monantha*	24556	T. m.[H-24556]
9	Kermanshah; Javanrood	*T. monantha*	24564	T. m.[H-24564]
10	Kermanshah; Kermanshah	*T. persica*	24607	T. p[H-24607]
11	Kermanshah; Kermanshah	*T. monantha*	24609	T. m.[H-24609]
12	Kermanshah; Kermanshah	*T. monantha*	24614	T. m.[H-24614]
13	Kermanshah; Kermanshah	*T. monantha*	24638	T. m.[H-24638]
14	Lorestan; Azna	*T. elliptica*	27893	T. e[H-27893]
15	Markazi; Arak	*T. persica*	2890	T. p[H-2890]
16	Southern Khorasan; Boshroi	*T. foenum graecum*	38910	T. f.g[H-38910]
17	Southern Khorasan; Boshroi	*T. foenum graecum*	38919	T. f.g[H-38919]
18	Southern Khorasan; Boshroi	*T. foenum graecum*	38949	T. f.g[H-38949]
19	Southern Khorasan; Qaen	*T. foenum graecum*	37527	T. f.g[H-37527]
20	Southern Khorasan; Qaen	*T. foenum graecum*	38973	T. f.g[H-38973]
21	Western Azarbaijan; Miandoab	*T. coerulescens*	38420	T. c[H-38420]
22	Yazd; Taft	*T. elliptica*	15965	T. e[H-15965]
23	Zanjan; Eejrood	*T. monantha*	35844	T. m.[H-35844]

10th June 2015 and 20th June 2016, leaf samples were used for extraction of phytophenol compositions. Leaf samples were finely crushed, grinded and were then completely drenched in methanol (70%) for 24 h. afterward, the mixtures were passed through filter papers and placed in an instrument to being rotated and concentrated under vacuum condition. The extracts were mixed with methanol (70%) and passed through filter paper (0.22 micro) and stored in refrigerator (20°C) till measuring polyphenol contents [12]. The HPLC system which was used for phytophenol analysis was Agilent 1200 series HPLC apparatus (Agilent Technologies, USA) including high-pressure quaternary-gradient solvent-delivery pump, DAD (diode-array detector), autosampler and chemstation (B.04.02 version) software. Zorbax XDB-C18 column (4.6 × 150 mm, 5 µm) was used to analyze the samples. The mobile phase was of a mixture of methanol: formic acid (1%) with the flow rate of 1 ml min^{-1}. The linear gradient started with the ratio of 10:90 that was turned to 25:75, 60:40 and 70:30 after 10, 20 and 30 min, respectively. Subsequently, 40-min isocratic hold was inserted for higher resolution of eluting polyphenols. The wavelengths were 280 and 320 nm, and the temperature of the column oven was 30°C. The injection volume was 20 µL. The standard concentrations ranged 1-1000 mg L^{-1} methanol. The quantitative determination was performed using the external standard method. The identification of the compounds was carried out by separate injection of each standard solution and also with the injection of the stock solution containing all standards. Thus, for each compound the resolution peak and the run time were determined. Several polyphenols including hesperetin, hesperidin, trans-ferulic acid, coumarin, quercetin, catechin, p-Coumaric acid, rutin, chlorogenic acid, caffeic acid, vanillin, carvacrol, eugenol, ellagic acid, rosmarinic acid, gallic acid, and sinapic acid were analyzed using chromatogram peaks identified in HPLC.

2.3 Statistical Analyses

The Pearson correlation coefficients were calculated to identify simple relationships of traits. Several multivariate statistical techniques including principal components analysis (PCA), factor analysis (FA) and cluster analysis methods were performed to better understanding the interrelationships between traits. Hierarchical cluster analysis method was used to examine the interrelationship between variables contributed to variations of fenugreek accessions and to classify accessions based on similarity coefficients. Multivariate analysis of variance (MANOVA) method was used to compare differences among classified groups in the cluster analysis. Discriminant analysis method was also performed to determine the best possible cut-off line on the tree dendrogram of accessions. Shoot fresh weight as dependent variable was subjected to multiple regression analysis. Stepwise regression was performed to identify the most important polyphenolic compounds explaining shoot fresh weight variation.

3. RESULTS

The results of ANOVA for polyphenols compositions and morphological traits are displayed in the Supplementary Table S1. The main effect of accession was significant ($p < 0.01$) demonstrating the existence of great variations among fenugreek accessions. The effect of year on polyphenols and morphological traits were not significant. The year × accession interactions were not significant for polyphenols whereas this component was significant for morphological traits. A representative HPLC elution profile of different phytophenol compounds in one of accessions (*T. persica* number 10110) is shown in Fig. 1. The HPLC profile for this accession shows variations in peak and retention time of different phytophenols in fenugreek. The peak of most of phytophenols was indentified between 10-25 min after sample injection. Pearson correlation coefficients of morphological traits and phytophenols are presented in Table 2. Among morphological traits, the highest correlations were observed between number of stems per plant and canopy cover (0.78), and between days to stem appearing and days to flowering (0.93).Weak correlations were found between most of polyphenols variations with the exception of correlations between trans-ferulic acid and catechin (0.87), coumarin and p-Coumaric acid (0.99), coumarin and chlorogenic acid (0.94), and between p-Coumaric acidand chlorogenic acid (0.94). Cross-correlations between morphological traits and polyphenol compositions were rather weak. The first two principal components described 90% of total variation in phytophenol compositions and morphological traits (Table 3). In factor analysis, the first two factors captured 85% of total variations associated with phytophenols and morphological traits.

Table 2. The Pearson correlation between morphological traits and phytophenol compounds in fenugreek

	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11	x12	x13	x14	x15	x16	x17	x18
x1	1																	
x2	0.42	1																
x3	-0.08	0.15	1															
x4	0.44	0.08	-0.29	1														
x5	-0.06	-0.12	-0.01	0.01	1													
x6	0.01	0.16	0.87	-0.48	-0.01	1												
x7	0.39	0.01	-0.35	0.99	-0.01	-0.53	1											
x8	0.01	-0.13	0.78	-0.17	-0.09	0.77	-0.2	1										
x9	0.49	-0.02	-0.25	0.94	-0.01	-0.34	0.94	0.01	1									
x10	0.24	0.26	0.33	-0.01	-0.01	0.43	-0.07	0.35	0.07	1								
x11	0.34	0.08	0.13	0.11	0.1	0.21	0.04	0.26	0.14	0.75	1							
x12	-0.05	0.05	-0.22	-0.26	0.04	-0.11	-0.25	-0.15	-0.3	-0.14	0.1	1						
x13	-0.03	-0.02	-0.44	-0.01	0.01	-0.31	0.01	-0.37	-0.05	0.02	0.18	0.78	1					
x14	0.01	-0.07	0.36	0.2	-0.05	0.18	0.17	0.08	0.15	-0.01	-0.01	-0.22	-0.1	1				
x15	-0.18	-0.31	0.04	0.02	-0.04	-0.14	0.02	-0.02	-0.01	-0.12	-0.14	-0.04	0.17	0.26	1			
x16	-0.04	0.02	-0.18	0.17	-0.03	-0.33	0.21	-0.3	0.05	-0.75	-0.67	0.24	0.05	0.03	-0.05	1		
x17	-0.04	-0.03	-0.12	0.21	0.12	-0.3	0.26	-0.26	0.09	-0.61	-0.57	0.18	0.02	-0.02	-0.09	0.93	1	
x18	0.08	-0.04	-0.23	0.2	0.09	-0.15	0.25	-0.11	0.26	-0.31	-0.41	0.19	0.04	-0.29	-0.36	0.58	0.61	1

Correlations between -0.25 and +0.25 are not significant.

x1: Hesperetin, x2: Hesperidin, x3: Trans-ferulic acid, x4: Coumarin, x5: Quercetin, x6: Catechin, x7: p-Coumaric acid, x8: Rutin, x9: Chlorogenic acid, x10: Establishment, x11: Stem length, x12: Stem number, x13: Canopy, x14: Shoot fresh weight, x15: Shoot dry weight, x16: Days to stem appearing, x17: Days to flowering, x18: Days to seeding.

Table 3. Description of the first three principal components/factors for phytophenols and morphological traits in fenugreek

	Component-1		Component-2		Component-3	
	PC-1	FL-1	PC-2	FL-2	PC-3	FL-3
Proportion	0.58	0.52	0.32	0.32	0.09	0.05
Cumulative	0.58	0.52	0.90	0.85	0.99	0.91

PC: principal component, FL: factor loading

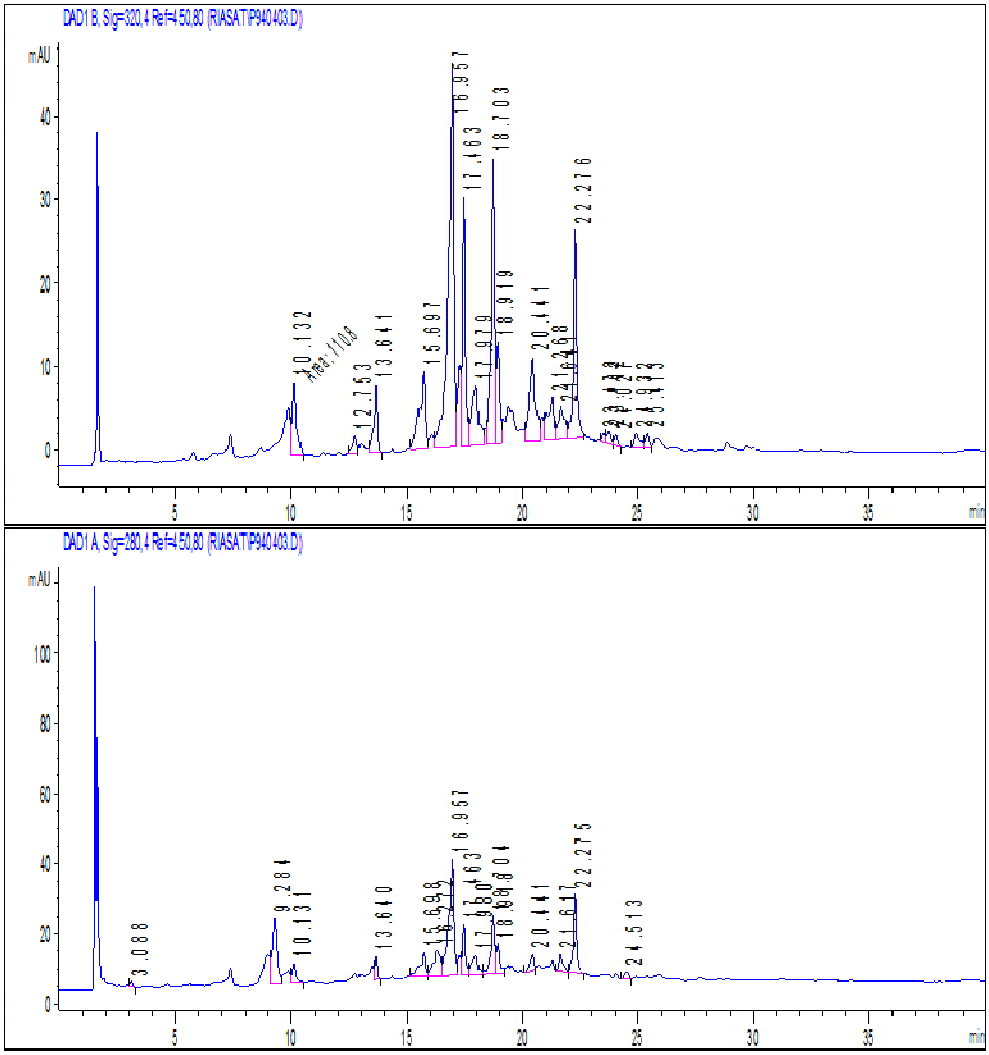

Fig. 1. High-performance liquid chromatography (HPLC) elution profile of different phytophenol compounds in *T. persica* (10110). Upper chromatogram was identified in 320 nm wavelength whereas the lower was found in 280 nm.

The PC derived bi-plot accommodated with the vectors for traits represented the interrelationships between polyphenols and morphological traits (Fig. 2). The traits were represented as vectors whose length revealed the degree of association with a direction in orientation space. Acute angles among the vectors associated with hesperetin, coumarin, p-Coumaric acid and chlorogenic acid showed their strong interrelationships. Among morphological characteristics, the vectors for plant establishment, stem length and shoot fresh weight had acute angels with the vectors represented rutin, trans-ferulic acid and catechin compounds. The orientation of the vectors showed these three phytophenols were not

associated with days to flowering, canopy cover, days to stem appearance and days to seeding. The vectors for hesperidin and shoot fresh weight overlapped showing their strong associations in fenugreek. Shoot dry weight had strong but negative correlations with polyphenols hesperetin, coumarin, p-Coumaric acid and chlorogenic acid. Hesperidin also showed direct and high association with plant establishment and stem length. Strong associations were identified between quercetin and several morphological traits including number of stems per plant, canopy cover, days to stem appearing, days to flowering and days to seeding. The length of the vectors for hesperidin, quercetin, shoot fresh weight and shoot dry weight showed that these characters had lowest variations in fenugreek.

Projection of factor loadings for the first two components of factor analysis demonstrated the results of principal component analysis indicating the interrelationships of morphological traits and phytophenols (Fig. 3). The results of cluster analysis showed that phytophenol compositions and morphological traits were classified in almost three main groups. These three groups were similar to those categories identified in the bi-plot of PC analysis.

Results of multiple and stepwise regression analyses for selection of polyphenolic compounds in a model with shoot fresh weight as dependent variable are presented in Tables 4 and 5. The effect of hesperidin, trans-ferulic acid, quercetin and rutin on shoot dry weight was significant in the multiple regression models. Trans-ferulic acid had increasing effect on shoot fresh weight whereas others reduced this trait. When variations of aforementioned independent variables were subjected to stepwise regression, trans-ferulic acid, p-Coumaric acid, rutin and hesperidin were entered to the final regression model, only. In such cases with contrasting results found in multiple regression and stepwise regression analyses, interpretation of the results of stepwise regression analysis is more reliable. This is because the interrelationships of independent variables in response to dependent variable is tested when new variable enters into the model in each step of variable selection and accordingly the results of stepwise regression has more power statistically. The results of stepwise regression revealed that quercetin, rutin and hesperidin significantly reduced shoot fresh weight whereas this trait was increased as trans-ferulic acid and p-Coumaric acid increased.

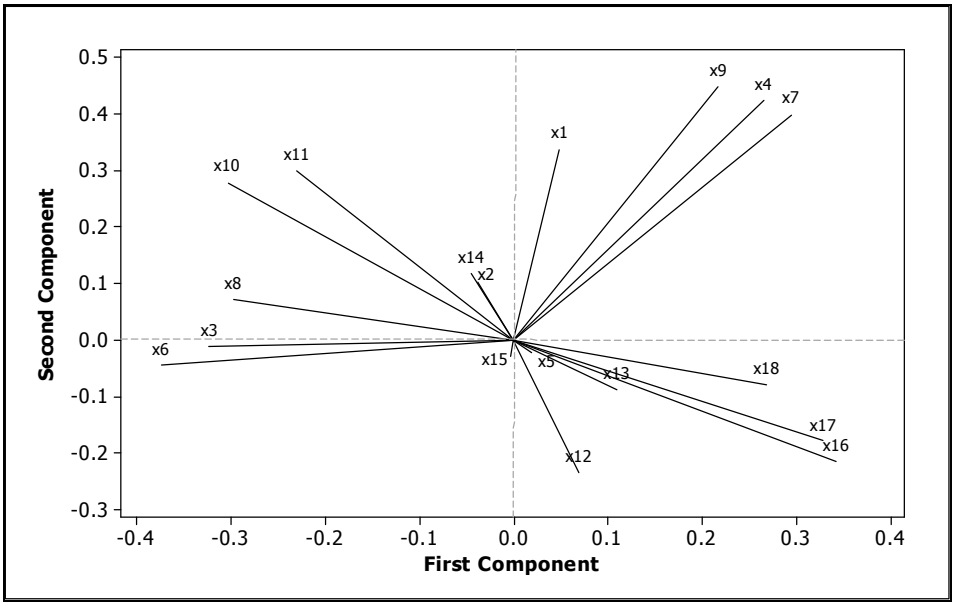

Fig. 2. The bi-plot of the first two principal components representing the interrelationship of morphological traits and polyphenolics. x1: Hesperetin, x2: Hesperidin, x3: Trans-ferulic acid, x4: Coumarin, x5: Quercetin, x6: Catechin, x7: p-Coumaricacid, x8: Rutin, x9: Chlorogenic acid, x10: Establishment, x11: Stem length, x12: Stem number, x13: Canopy, x14: Shoot fresh weight, x15: Shoot dry weight, x16: Days to stem appearing, x17: Days to flowering, x18: Days to seeding

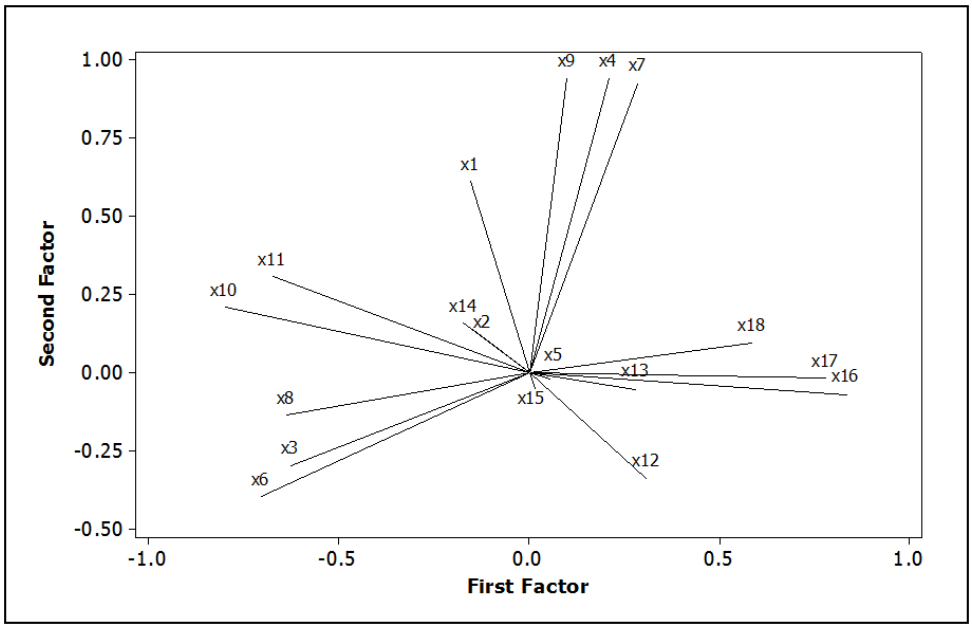

Fig. 3. The bi-plot of the first two factor loadings representing the interrelationship of morphological traits and polyphenolics. x1: Hesperetin, x2: Hesperidin, x3: Trans-ferulic acid, x4: Coumarin, x5: Quercetin, x6: Catechin, x7: p-Coumaric acid, x8: Rutin, x9: Chlorogenic acid, x10: Establishment, x11: Stem length, x12: Stem number, x13: Canopy, x14: Shoot fresh weight, x15: Shoot dry weight, x16: Days to stem appearing, x17: Days to flowering, x18: Days to seeding

Table 4. Results of multiple regression analysis for shoot fresh weight as dependent variable and polyphenols as independent variables

Independent variable	Coefficient in the model	Standard error	*t-value*	*p-value*
Intercept	-13.71	26.63	-0.51	0.615
Hesperetin (x1)	0.213	2.118	0.1	0.921
Hesperidin (x2)	-2.455	1.427	-1.72	0.010
Trans-ferulic acid (x3)	8.139	3.712	2.19	0.047
Coumarin (x4)	0.349	8.206	0.04	0.967
Quercetin (x5)	-3.406	3.612	-0.94	0.036
Catechin (x6)	-0.069	1.396	-0.05	0.961
p-Coumaric acid(x7)	-1.834	5.235	-0.35	0.732
Rutin (x8)	-3.996	1.567	-2.55	0.024
Chlorogenic acid (x9)	2.654	3.274	0.81	0.432

Morphological traits and phytophenols were classified in several groups based on similarities between accessions (Fig. 4). The results revealed that coumarin, p-Coumaric acid and chlorogenic acid were classified in same group. Another group of traits consisted of hesperetin, hesperidin, trans-ferulic acid, catechin, rutin, stem length and plant establishment. The remaining phytophenol compositions and morphological traits were classified in the third group. The tree dendrogram constructed based on the similarities of fenugreek accessions for polyphemolics and morphological traits indicated that accessions were discriminated in three groups (Fig. 5).

The results of MANOVA for comparison of differences among the three groups identified by cluster analysis showed highly significant between groups differences (Table 6). The first group of accessions consisting of T. m [H-24609], T.c [H-38420] and T. f.g [H-38910] had

highest hesperetin, coumarin, p-Coumaric acid and chlorogenic acid. This group had the highest mean for several morphological traits, i.e. plant establishment, stem length, and shoot fresh weigh and phonological traits (Table 7). Eight accessions were classified into the second group. The second group had the lowest values for most of phytophenol compositions although it had the highest stem number and shoot dry weight. The third group of accessions had the highest hesperidin, catechin and rutin. Accessions in this group were early flowered and had the highest shoot fresh weight, stem length and plant establishment.

Table 5. Results of stepwise regression analysis for selection of the most important polyphenols associated with shoot fresh weight in fenugreek

Parameters	Step			
	First	Second	Third	Fourth
Intercept	21.306	9.671	-5.392	-10.427
Trans-ferulic acid (x3)	2	2.6	5.1	6.5
t-Value	1.76	2.27	3	3.67
p-Value	0.093	0.034	0.007	0.002
p-Coumaric acid(x7)		1.06	1.21	1.35
t-Value		1.61	1.93	2.27
p-Value		0.124	0.069	0.036
Rutin (x8)			-1.82	-2.58
t-Value			-1.9	-2.6
p-Value			0.072	0.018
Hesperidin (x2)				-1.9
t-Value				-1.85
p-Value				0.081
R-Square	12.84	22.79	35.14	45.49
Adjusted R-Square	8.69	15.07	24.9	33.38
Mallows Cp	5.1	4.3	2.9	2.1

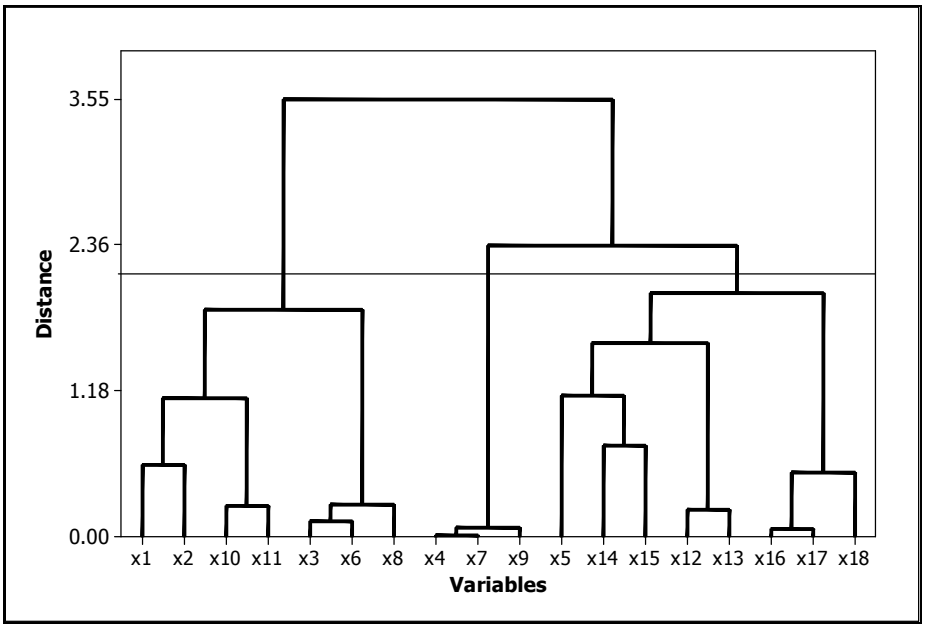

Fig. 4. The tree dendrogram for similarities of different morphological and polyphenolics in fenugreek. x1: Hesperetin, x2: Hesperidin, x3: Trans-ferulic acid, x4: Coumarin, x5: Quercetin, x6: Catechin, x7: p-Coumaric acid, x8: Rutin, x9: Chlorogenic acid, x10: Establishment, x11: Stem length, x12: Stem number, x13: Canopy, x14: Shoot fresh weight, x15: Shoot dry weight, x16: Days to stem appearing, x17: Days to flowering, x18: Days to seeding

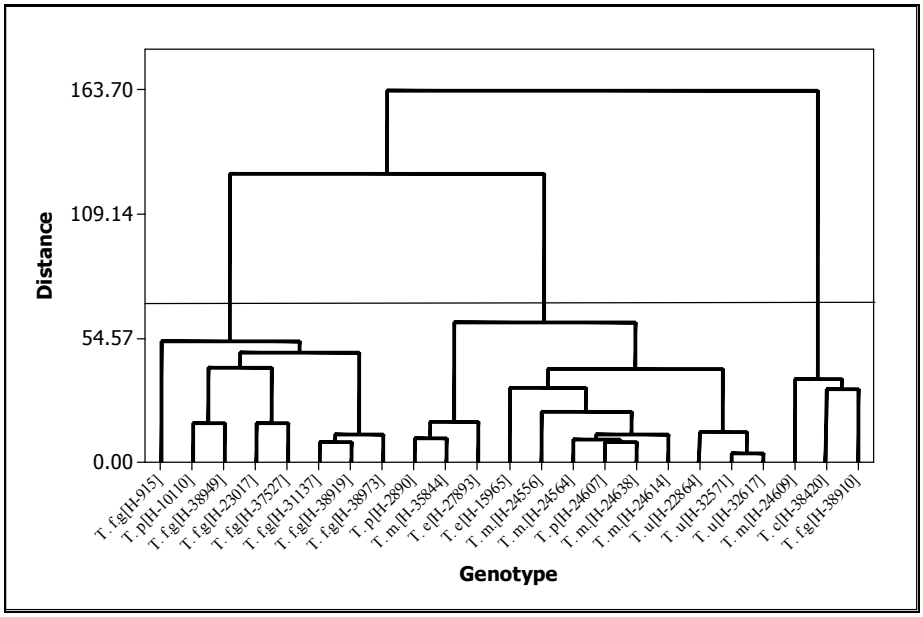

Fig. 5. The similarities of fenugreek accessions with respect to morphological traits and polyphenolics

Table 6. Multivariate analysis (MANOVA) for groups of fenugreek accessions identified in cluster analysis

Criterion	Statistic	F	DF	*p-value*
Wilks'	0.00002	33.767	6	0.001
Lawley-Hotelling	797.6708	44.315	4	0.001
Pillai's	1.98071	22.817	8	0.001

DF: degree of freedom

The results of validation test for clustering of genotypes based on principal components and factor loadings are presented in Fig. 6 and 7, respectively. Both methods demonstrated the results of cluster analysis. Accessions that were joined together in the tree dendrogram of cluster analysis were placed in the vicinity of each other in PCA and factor analysis derived bi-plots whereas non-similar accessions were far away.

4. DISCUSSION

To the best of our knowledge, little or no information is available with respect to the interrelationship of phytophenols tested in this study and the quantitative and morphological traits under field conditions. Simultaneous consideration of morphological variations and phytophenol compositions helps breeders to produce commercial fenugreek cultivars. Literature shows that no attempts have been made on joint analysis of morphological traits and phytophenol compounds. In a study, Singh et al. [13] assessed variations in some of chemical constitutes, micronutrients and total phytophenol content of the seed in fenugreek, but phytophenol compositions were not measured. In another study with fenugreek, McComarik et al. [14] focused on quantitative and morphological traits only. The novelty of the present study is simultaneous consideration of the data derived from morphological traits and phytophenol compositions assisting genotype selection. The results of the current study implied great variations among fenugreek accessions with respect to quantitative, morphological traits and phytophenol compositions. Although simple correlation coefficients indicated weak association between morphological traits and phytopheol compositions, multivariate statistical analysis methods demonstrated the interrelationship of these variables. In the current situation information form multivariate analysis techniques (herein PCA and factor analysis) bear more accurate gain because multivariate techniques combined variations of several traits and therefore contains greater patterns portion compared to simple statistics.

Table 7. Mean comparison for phytophenols and quantitative and morphological traits in the three clusters of fenugreek accessions

Cluster	Hesperetin	Hesperidin	Trans-ferulic acid	Coumarin	Quercetin	Catechin	p-Coumaric acid	Rutin	Chlorogenic acid
1	14.5^{a}	2.34^{a}	3.01^{b}	16.58^{a}	2.95^{a}	0^{c}	29.26^{a}	0^{a}	35.63^{a}
2	8.2^{b}	1.82^{a}	10.62^{a}	0.74^{b}	2.78^{a}	25.22^{b}	0^{b}	5.07^{a}	0.63^{c}
3	10.41^{ab}	4.68^{a}	14.78^{a}	1.95^{b}	2.69^{a}	38.93^{a}	1.16^{b}	13.08^{a}	5.39^{b}
Cluster	**Establishment**	**Stem length**	**Stem number**	**Canopy**	**Shoot fresh weight**	**Shoot dry weight**	**Days to stem appearing**	**Days to flowering**	**Days to seeding**
1	7.33^{b}	11^{b}	6^{a}	11.67^{a}	41.08^{c}	25.93^{bc}	56^{a}	76.67^{a}	94.33^{a}
2	4.17^{b}	8.08^{c}	8.75^{a}	11.17^{a}	43.36^{ab}	29.23^{ab}	55.58^{a}	73.08^{a}	86.17^{b}
3	15.75^{a}	14.38^{a}	7.13^{a}	10.13^{a}	42.91^{bc}	20.24^{c}	47.38^{b}	61.63^{b}	81.5^{c}

For each trait/compound, means with the same letters are not significantly different ($LSD_{5\%}$).

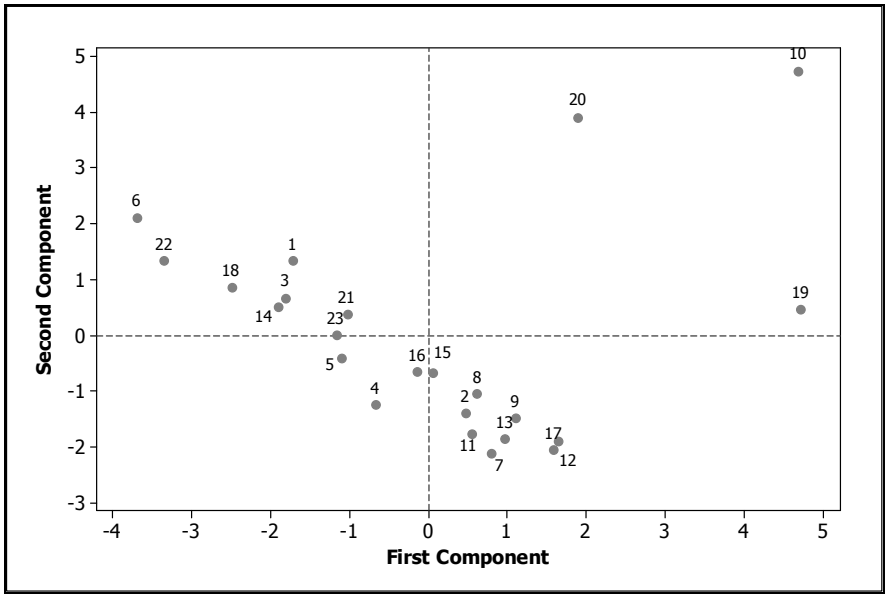

Fig. 6. The bi-plot of the first pair of principal components representing distribution of fenugreek accessions with respect to morphological traits and polyphenolics. 1: T. f.g[H-915], 2: T. p[H-2890], 3: T. p[H-10110], 4: T. e[H-15965], 5: T. u[H-22864], 6: T. f.g[H-23017], 7: T. m.[H-24556], 8: T. m.[H-24564], 9: T. p[H-24607], 10: T. m.[H-24609], 11: T. m.[H-24614], 12: T. m.[H-24638], 13: T. e[H-27893], 14: T. f.g[H-31137], 15: T. u[H-32571], 16: T. u[H-32617], 17: T. m.[H-35844], 18: T. f.g[H-37527], 19: T. c[H-38420], 20: T. f.g[H-38910], 21: T. f.g[H-38919], 22: T. f.g[H-38949], 23: T. f.g[H-38973]

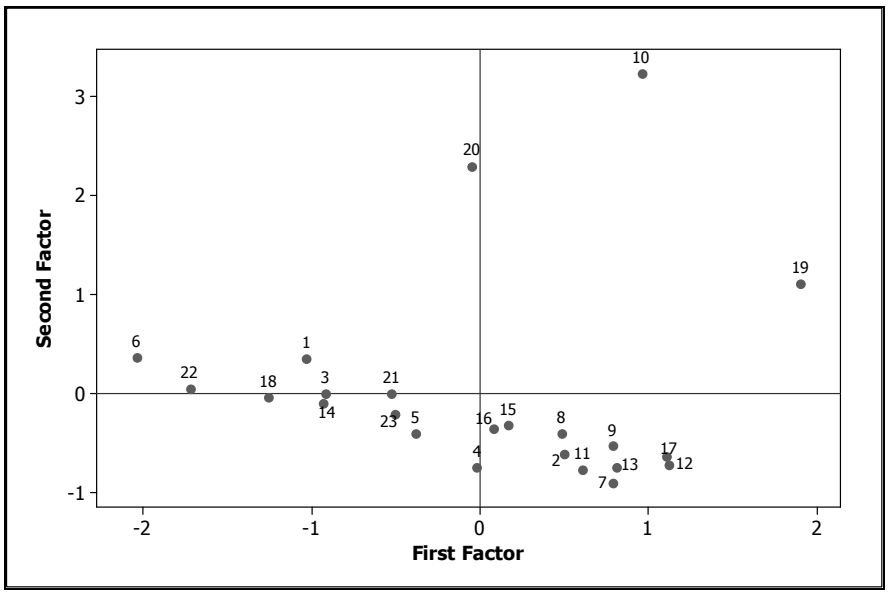

Fig. 7. The bi-plot of the first pair of factor loading vectors representing distribution of fenugreek accessions with respect to morphological traits and polyphenolics. 1: T. f.g[H-915], 2: T. p[H-2890], 3: T. p[H-10110], 4: T. e[H-15965], 5: T. u[H-22864], 6: T. f.g[H-23017], 7: T. m.[H-24556], 8: T. m.[H-24564], 9: T. p[H-24607], 10: T. m.[H-24609], 11: T. m.[H-24614], 12: T. m.[H-24638], 13: T. e[H-27893], 14: T. f.g[H-31137], 15: T. u[H-32571], 16: T. u[H-32617], 17: T. m.[H-35844], 18: T. f.g[H-37527], 19: T. c[H-38420], 20: T. f.g[H-38910], 21: T. f.g[H-38919], 22: T. f.g[H-38949], 23: T. f.g[H-38973].

Results of our study revealed that quercetin had strong association with variations in most of morphological traits. This shows breeding for higher quercetin should be accompanied by genotype selection for appropriate quantitative and morphological traits in fenugreek. Also, plant establishment and stem weight were associated with several phytophenol compounds. Plant establishment is associated with early vigour attribute and consequently end use seed yield. In a study on fenugreek, McCormick et al. [7] demonstrated that early vigour, biomass at late flowering and flowering date are the most important traits for high seed yield. Such associations were identified in other studies with fenugreek [15-21]. Randhawa et al. [22] assessed genetic relatedness among 49 accessions of fenugreek (*Trigonella-foenum-graecum* L.) and the results indicated significant differences within the accessions for all the quantitative descriptors. Results of a study [14] revealed that significant variation were identified for phenotypic traits including growth habit, flowering time, seed colour, seed size, biomass and seed yield in fenugreek accessions in south-eastern Australia. .

In the present study, several phytophenol compounds had significant relations with shoot fresh weight in fenugreek. Accordingly, selection of superior genotypes for higher shoot fresh weight might expedite cultivar release accumulating higher desirable phytopheols. The results of regression analysis indicated that phytophenols increased with fresh weight increase. This shows that the interrelationship of phytophenols and morphological traits should be of the main focus in breeding schemes of fenugreek. The results of stepwise regression for polyphenol selection demonstrated that trans-ferulic acid, p-Coumaric acid, rutin and hesperidin were the most important phytophenol compounds associated with shoot fresh weight. This was in accord with the results of other statistical methods used; indicating the importance of relations among morphological traits and phytopheols in fenugreek. In a study, the relation of agronomic practices, plant yield and genetic variation with polyphenol biosynthesis in *Hypericum perforatum* L. was documented [23]. Assessment of variations in quantitative traits in Ferula species revealed the effects of several morphological traits i.e. thousand kernel weight and fruit length/width ratio on variation in bioactive compounds [24]. As a consequence, great effort should be directed towards the management of field practices and traits, in order to maximize gross yield and quantity of phytochemicals in medicinal plants [23,25]. In the present study, the selected polyphenols in the final model of stepwise regression have industrial, pharmaceutical and medical uses demonstrating the importance of screening fenugreek genotypes with respect to phytophenols in breeding programs. For instance, p-Coumaric acid is used as an important component in flavours, perfumes, synthetic indigo and pharmaceuticals. It has antioxidant activities and reduces the risk of stomach cancer [26]. Trans-ferulic acid has proven to be a very effective antioxidant being used by the human body to control free radicals. Rutin is the glycoside combining the flavonol quercetin and the disaccharide rutinose and it could be a potential multitargeted polypharmacological agent in prevention and treatment of diseases in human beings [27]. Hesperidin is a compound providing the flavonoid hesperitin to the human body, and this flavonoid mediates most benefits of hesperidin and increase circulation and possible brain protective effects [28].

Herein in this study, the results of cluster analysis for morphological and phytophenol compounds revealed that rutin, hesperidin, trans-ferulic acid and plant establishment were classified in the same group. Therefore, assessment of these phytophenlos might be useful in identifying common chemical pathways responsible for the production of these compounds in fenugreek and possible gene networks controlling both polyphenols and field traits. Fenugreek accessions tested in this study were classified based on variations in polyphenols and field measured traits using cluster analysis, principal component analysis and loading factors methods. The results of these techniques were in accord with each other. Cluster analysis and mean comparison of groups indicated that a group of accessions had higher phytophenol compounds compared with the two others. Several accessions that were classified in same group had better plant establishment and higher shoot dry weight. Accordingly, genotype selection from the group with high phytophenol for cross hybridization with those having appropriate morphological attributes might expedite the improvement of fenugreek for commercial uses.

The results of our study indicated that the year component was not significant for polyphenols and morphological traits. This was against those

reported by Ganopadhyay et al. [17]; indicating significant effect of year on morphological traits of diverse accessions of fenugreek. In another study, significant differences were found among twenty fenugreek ecotypes for morphological traits under rain-fed and irrigated conditions [29]. Significant accession × season interactions were identified for morphological traits in other studies with fenugreek [30,31,21]. Investigation for genotype by environment (year and location) interactions might help breeders to select suitable strategy for breeding crop plants. Non-significant year × genotype interaction exhibited in the present study demonstrated the possibility of genotype selection with general adaptability to environmental conditions with respect to both morphological traits and phytophenol compositions.

5. CONCLUSION

The results showed that there was low correlation between polyphenols compounds. However, high correlations were observed between trans-ferulic and several morphological traits. Cross-correlations between morphological traits and polyphenols compositions were almost low although several significant correlations were observed between them. The results of factor analysis revealed that polyphenols and morphological traits were classified into separate categories, hesperetin, coumarin, p-Coumaric acid and chlorogenic acid placed in one group and other polyphenols compositions were classified as another group. The results of multivariate analyses demonstrated strong correlation of quercetin with most of morphological traits. The effects of hesperidin, trans-ferulic acid, quercetin and rutin on shoot fresh weight were significant. The results of stepwise regression showed that tran-ferulic acid, p-Coumaric acid, rutin and hesperidin were the most important phytophenol compounds influencing shoot fresh weight. Phytophenols were not affected by genotype × year interaction demonstrating environmentally stable phytophenol production is possible in fenugreek genotypes. In general, it can be concluded that the diversity exhibited for phytophenol compositions and morphological traits provides a promising basis for a genetic improvement of these variables which is important in further fenugreek breeding programmes for the production of commercial cultivars. In addition, the results indicated that, an ideal strategy in this direction should include the definition of optimum trait management and the availability of specific agronomic practices centred on secondary metabolism enhancement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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