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Variability in Morpho-Physiological and Nitrogen-Fixing Traits of Ethiopian Fenugreek (*Trigonella foenum-graecum* L.) Landraces

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ABSTRACT

The extent and pattern of genetic variability present in a population of fenugreek a given crop is indisputably essential for further improvement. A field experiment was conducted at Ambo and Adadi during the main cropping season to assess the extent and pattern of genetic diversity of morpho-physiological and nitrogen fixation traits in which 143 random samples of fenugreek accessions along with a commercial variety 'Challa' were arranged in a 12 × 12 simple lattice design. Mean squares due to genotypes were highly significant for all traits studied, except for days to maturity (DM), grain-filling period (GFP) and number of secondary branches per plant which were significant indicating the presence of genetic variability for these traits in fenugreek germplasm accessions. The range of GCV observed ranged between 3.02 and 68.95% while genotypic variance ranged between 0.001 and 28.62. Broad sense heritability was 48.63% for DM and 2.92% for GFP. Genetic gains varied between 0.20% and 28.22%. Five of the ten PCAs accounted for more than 78.10% of the total variation. The average linkage technique of clustering produced a more understandable portrayal of the 144 fenugreek accessions by grouping them into five clusters and the maximum distance was found between clusters two and five ($D^2 = 64.07$). The study generally indicated the possibility for genetic improvement of fenugreek through selection and cross breeding.

Keywords: crude protein, cluster, heritability, nodulation, principal component

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a diploid with eight pairs of chromosomes (2n = 16) (Raghuvanshi and Singh 1977) which is well known as traditional medicine for diabetes, indigestion, elevation of lipids and edema (fluid retention) of the legs (Ruveyd 2011). Undoubtedly, as one of the oldest cultivated plants, fenugreek is widely grown today in the Mediterranean countries, Argentina, France, India, North Africa, and the United States for food, condiment, medicine, dye, and forage purposes (Simon *et al.* 1984). In addition to the above mentioned countries, fenugreek is an important field crop also in Ethiopia, Egypt, India and Turkey (Beyene 1965; Westphal 1974).

Fenugreek is a good soil renovator and widely used as a green manure (Abdelgani *et al*. 1999). An experiment conducted in Russia (Provorov *et al.* 1996) showed that fenugreek used in cotton as a green manure increased number of bolls per plant (Hugh and Hailu 1963). It is a host for more than twelve strains of *Rhizobium* species that are effective in the process of biological N-fixation unlike most other legumes with strain-specific character efficiency of fixing nitrogen. Similarly Egrove *et al*. (1982) concluded that ploughing-in fenugreek has significantly increased four-year average fodder yield of maize and three-year average yield of the following spring wheat. The authors also found that when fenugreek was used as bio-fertilizer, the increase of shoot and root dry matter of maize was remarkable.

Fenugreek has a high proportion of protein as well as amino acid, 4-hydroxyisoleucine, which has high potential for insulin stimulating activity. The fenugreek seed is quite rich in protein content in comparison with cereal grain and other legume seeds (Petropoulos 1973; Awadala *et al.* 1980; Ullah 1982). The increasing protein deficiency all over the world, justifies every effort made for the genetic improvement of fenugreek in this direction. Petropoulos (1973) reported genetic variability for protein content among a collection of 123 hybrid lines of fenugreek ranging from 20.4 to 39.3% and Duke (1986) found an average of 32.2%. Hidvergi *et al.* (1984) reported a protein content of 26.4%. Feysal (2006) reported seed protein content, from 36 Ethiopian fenugreek landraces which ranged from 24.94 to 37.54%.

Knowledge of the extent and pattern of variability particularly of genetic variability present in a population of a given crop is indisputably essential for further improvement. Therefore, in order to best exploit the available genetic wealth, unraveling the information on the extent and nature of genetic diversity of the population would help in formulating efficient scheme of selection based on the traits of importance. However, only little of such vital information on fenugreek landraces is present under Ethiopian conditions. Therefore, the objective of this study was to assess the extent and pattern of genetic diversity for morpho-physiological and nitrogen fixation traits.

MATERIALS AND METHODS

Experimental location and layout

The field experiment was conducted at Ambo and Adadi during 2007 main cropping season. Ambo has an altitude of 2300 m.a.s.l. and average annual rainfall of 1000 mm, while Adadi has an altitude of 2050 m.a.s.l. and average annual rainfall of 900 mm. The soil at Ambo is characterized as a vertisol with a pH of 6.1 while that of Adadi is light vertisol with a pH of 7.5.

Fenugreek accessions (143 samples) along with one commercial variety '*Challa*' were considered in this study. Treatments

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were arranged in a 12 × 12 simple lattice design and seeding was done in a plot of four rows with 2 m length and spacing of 10 cm between plants and 25 cm between rows. The layout and randomization were as per the standard procedure set by Cochran and Cox (1957).

Collection of data

The following traits were collected on whole plot or on a plant basis as:

Days to maturity (DM): Recorded as the number of days from the date of sowing to the date at which 90% of the pods reached the stage of physiological maturity.

Grain filling period (GFP): This was recorded as the number of days from days to flowering to days to maturity.

Seed protein content (CP): Seed samples from each randomly selected 50 plots were oven-dried to constant moisture, ground to pass through a 2-mm size mesh sieve for determination of nitrogen content at Holetta Research Center using the Kjeldahl technique. Grain protein contents were estimated by multiplying percentage of N in the dried seed samples by the standard conversion factor of 6.25 (AOAC 1970). The results obtained from these fifty sample plots were used for calibration and validation of Near Infrared Reflectance Spectroscopy (NIRS), which is also available at Holetta Research Center. Thus the remaining experimental material was analyzed by taking 3 g of intact seeds of each plot and scanned by a monochromator model 6500 (NIR Systems, Maryland, USA).

Number of nodes per plant (NPPL): This was determined as an average number of nodes from the ten sample plants.

Number of podding nodes per plant (PNPPL): This was determined as an average number of nodes that bear pod from the ten sample plants.

Number of primary branches per plant (PBR): The total number of primary branches which gave rise to other seed-bearing branches of higher order or born seeds themselves was determined as an average number of productive primary branches from the ten sample plants. Counting was done at the time when flowering was completely over and pods were still green but old enough to judge that they would give seeds.

Number of secondary branches per plant (SBR): The average number of secondary or any other higher order productive branches of the same plants used to determine the number of primary branches.

Number of nodules/plant (NL): The average number of nodules obtained from the five sample plants. The results were scored using a 0-4 scale, where 0 = no nodules, 1 = 1 to 10 nodules/plant; 2 = 11 to 20 nodules/plant; 3 = 21 to 30 nodules/plant and 4 = > 30 nodules/plant.

Fresh weight of nodules/plant (FWNL): The average weight in gram per plant of the fresh nodules obtained from the plants used to determine number of nodules per plant.

Number of effective nodules/plant (ENL): The number of effective nodules was visually identified at the time of flowering from the same plants used to determine number of nodules per plant and fresh weight of nodules per plant. The effective nodules were differentiated from ineffective ones by their color whereby the deep red- or brown-colored nodules, which reflect high leg-haemoglobin content, were designated as effective, while all others were designated as non-effective nodules (Carter 1995).

Data analysis

The data were subjected to the analyses of variance (ANOVA) and combined analysis of variance over locations for simple lattice design was performed using the SAS software (SAS 1996).

For the sake of covariance to estimate the expected mean square the analyses of variances in this investigation were conducted using RCBD as per the standard procedure set by Cochran and Cox (1957) and Gomez and Gomez (1984).

The total variability for the traits was quantified using pooled analyses of variance over locations using the following model:

$$P_{itjk} = \mu + l_t + r_{i(t)} + b_{j(i)(t)} + g_k + (gl)_{kt} + e_{itjk}$$

where P_{itjk} = the phenotypic value in the i^{th} replication, t^{th} location, j^{th} incomplete block within replication i and location t and from the k^{th} accession, μ = the grand mean, l_t = the effect of location t, $r_{i(t)}$ = the effect of replicate i within location t, $b_{j(i)(t)}$ = the effect of the incomplete block j within replication i and location t, g_k = the effect of the k^{th} accession, $(gl)_{kt}$ = the effect of interaction of the k^{th} accession and the t^{th} location and e_{itjk} = the residual.

The coefficients of variations at phenotypic and genotypic levels were estimated using the formula adopted by Johnson *et al.* (1955). Broad-sense heritability (h^2) for the traits was quantified using pooled analyses of variance over locations using the following model:

$$h^2 = [\ \frac{(\text{Varg})}{\text{Varg} + \text{Varge}/l + \text{Vare}/rl}\ ] \times 100$$

where Varg = genotypic variance, Varge = genotype by environment variance, Vargr = genotypic by replication variance, Vare = environmental variance, l = number of locations and r = number of replications

Genetic advance in absolute unit (GA) and percent of the mean (GAM), assuming selection of the superior 5% of the genotypes, was estimated in accordance with the methods illustrated by Johnson *et al*. (1955).

Genetic diversity between clusters was computed based on multivariate analysis using Mahalanobis D^2 statistic (Mahalanobis 1936). The important traits in each principal component that significantly contributed to the variation observed were identified as suggested by Johonson and Wichern (1988).

Squared distance (D^2) for each pair of genotype combinations was computed using the following formula adopted by Singh and Chaudhary (1999). Based on the squared distances (D^2), clustering of genotypes was done using Tocher's method as described by Singh and Chaudhary (1999).

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance for the traits studied under both locations is given in **Table 1**. It revealed from the results that mean squares due to locations were highly significant for all traits, except for PBR and SBR that were significant and non-significant, respectively. Mean squares due to genotypes were highly significant for all the traits studied, except for DM, GFP, and SBR which were significant, but non significant for FWNL revealed the presence of variability for these traits in fenugreek germplasm accessions investigated. All, except DM, PBR and SBR that exhibited non-significant effects, mean squares due to the interactions between locations and genotypes were highly significant. The result confirmed that the Ethiopian fenugreek landraces evaluated in this study showed significant phenotypic variability in terms of phenology, yield component and protein content. These results are similar with the findings of other scholars like Banya (1973), Cornish *et al.* (1983), Provorov *et al.* (1996), Feysal (2006), McCormick (2009) and Fikreselassie *et al.* (2012), whose findings are highlighted in **Table 2**.

In general, the accessions showed shorter days to maturity and grain filling periods thus may be suitable to low rainfall regions whereas the late types can be adapted to the highland areas with dependable rainfall. Thus, the variability that has been exhibited by these accessions can offer great opportunity for the development of suitable varieties for the various agro-ecological zones of Ethiopia as well as other continents of the world.

From the results, the broad spectrum of variability observed among these collections of fenugreek for different characters generally indicates possibilities for genetic improvement of the crop through selection and cross breeding.

Estimation of genotypic and phenotypic variations

High genotypic coefficient of variation (68.95%) was observed for the trait SBR followed by ENL (43.72%) and NL

Table 1 Analysis of variance for 10 traits of *T. foenum-graecum* landraces tested in 2007 over two locations (Adadi and Ambo).

Traits	MSL(1) [a]	MSR(1)	MSB(22)	MSG(143)	MSLG(143)	MSE	CV (%)
[b]DM	325.755**	4.168 [ns]	19.144*	13.762*	12.958 [ns]	10.566	2.51
GFP	4.344**	0.185*	0.130**	0.063*	0.066**	0.044	2.40
NPPL	17.488**	11.630**	0.592[ns]	0.672**	0.762**	0.454	12.34
PNPPL	45.699**	4.624**	0.623*	0.569**	0.685**	0.352	18.65
PBR	0.212*	3.331**	0.084[ns]	0.080**	0.065[ns]	0.054	13.06
SBR	0.015[ns]	3.129**	0.240[ns]	0.300*	0.287[ns]	0.232	66.61
NL	36.544**	4.958**	1.039*	1.192**	1.350**	0.666	23.37
ENL	11.301**	0.322[ns]	0.314*	0.412**	0.453**	0.198	28.74
FWNL	0.111**	0.067**	0.007[ns]	0.006[ns]	0.008**	0.006	20.87
CP%	26.518**	74.570**	1.206 [ns]	3.141**	3.845**	1.128	3.46

*, ** Significant at 0.05 and 0.01 probability level respectively and [ns] non significant
MSL = Mean Square due to location, MSR = Mean Square due to replication, MSB = Mean Square due to block, MSG = Mean Square due to genotypes, MSLG = Mean Square due to the interaction between location and genotypes, MSE = Mean Square due to error, CV% = Coefficient of variation in percentage.
[a] Figures in parenthesis indicate degrees of freedom.
[b] DM = Days to 90% maturity, GFP = Grain filling period, NPPL = Number of nodes per plant, PNPPL = Number of podding nodes per plant, PBR = Number of primary branches per plant, SBR = Number of secondary branches per plant, NL = Number of nodules per plant, ENL = Number of effective nodules per plant, FWNL = Fresh weight of nodules in g per plant, CP = Crude protein of the seed

Table 2 Assessing variability of different traits among *Trigonella foenum-graecum* L. from different work done

Reference	Investigated trait (s)	Result	Remarks
Cornish *et al.* 1983	Monohydroxysapogenin yield	Significant genetic segregation in cross indicating genetic distances	Shows viability
Provorov *et al.* 1996	Fresh wt, seed yield, nodulation ability resistance to root rot, duration to inter-stage and growing periods, leafiness and biochemical composition	Significant difference existed among fenugreeks except for the traits leafiness and biochemical composition	Shows viability
Feysal 2006	Days to flowering and maturity, plant height, number of primary and secondary branches, pod length, number of pods and seeds per plant, seed yield, biomass yield, thousand seed weight, protein content of the seed	Significant for all traits except Number of secondary and biomass yield in 36 fenugreek accessions	Shows viability
McCormick 2009	Cotyledon size, leaf size, plant growth habit, nodulation, plant height, seed color, biomass, seed yield per plant, harvest index and yield components	Significant difference in 204 different fenugreek collections across the world	Shows viability
Fikreselassie *et al.* 2012	Days to flowering, plot uniformity, thousand seed weight, number of pods per plant, plant height, number of seeds per plant, number of seeds per pod, seed color, seed shape, seed yield, biomass yield, harvest index.	Significant for all traits except harvest index	Shows viability

Table 3 Estimates of minimum, mean and maximum value, variance and coefficient of variation at phenotypic (σ^2p), genotypic (σ^2g) level, heritability in broad sense ($h^2\%$), genetic advance in absolute (GA) and percent of mean (GAM) for 10 traits of *T. foenum-graecum*.

Traits	Min	Mean	Max	σ^2p	σ^2g	GCV%	PCV%	$h^2\%$	GA	GAM
[b]DM	123.80	129.71	135.38	18.318	4.564	1.65	3.30	48.63	2.14	1.65
GFP	69.73	75.64	81.58	24.800	6.220	3.30	6.58	2.92	0.15	0.20
NPPL	16.95	29.90	44. 88	114.490	28.623	17.89	35.79	30.81	3.40	11.37
PNPPL	3. 86	10.31	19.48	34.106	8.547	28.36	56.64	14.74	0.89	8.62
PBR	1.47	3.16	4.76	1.346	0.339	18.44	36.71	35.97	0.43	13.68
SBR	0.00	0.62	2.02	0.960	0.183	68.95	158.06	17.38	0.17	28.22
NL	3.39	12.69	27.17	73.616	18.376	33.78	67.61	7.07	0.63	4.93
ENL	0.39	2.53	6. 82	4.928	1.224	43.72	87.75	4.32	0.10	3.89
FWNL	0.08	0.13	0.24	0.004	0.001	24.92	46.15	8.10	0.01	4.16
CP%	28.70	30.65	33.66	3.460	0.858	3.02	6.07	12.94	0.25	0.81

Min, Mn and Max stands for minimum, mean and maximum value values and [b]DM = Days to 90% maturity, GFP = Grain filling period, NPPL = Number of nodes per plant, PNPPL = Number of podding nodes per plant, PBR = Number of primary branches per plant, SBR = Number of secondary branches per plant, NL = Number of nodules per plant, ENL = Number of effective nodules per plant, FWNL = Fresh weight of nodules in g per plant, CP = Crude protein of the seed in percentage

(33.78%), PNPPLt (28.36%), FWNL (24.92%), PBR (18.44%) and NPPL (17.89%). Nevertheless, low for the temporal data such as number of DM (1.65%) and GFP (3.30%) as well as CP content of the seed (3.02%) (**Table 3**).

Likewise, phenotypic coefficient of variation was high for SBR (158.06%) followed by ENL (87.75%) and NL (67.61%), PNPPL (56.64%) and FWNL (46.15%). In general, the environmental variance was greater than the genetic variance for all the traits.

The estimated values of phenotypic variances were in the range of 0.004 for FWNL to 114.49 for NPPL (**Table 3**). The lowest and highest genotypic variances were found for FWNL (0.001) and NPPL (28.62) per plant, respectively.

The results depicted in **Table 3** showed that estimates of heritability in broad sense were moderate for DM (48.63%), PBR (35.97%) and NPPL (30.81%). However, low values of heritability were estimated for GFP (2.92%), ENL and NL (4.32 and 7.07%, respectively), FWNL (8.10%), CP content of the seed (12.94%), PNPPL (14.74%) and SBR (17.38%), indicating limited possibility of improvement for those characters through selection.

In earlier studies, high heritability estimates for days to maturity, number of primary branches and protein content (Feysal 2006) were reported (**Table 4**). The probable cause of discrepancy between the present investigations with that of the previous are might be due to the masking effect of environments over the genetic components since the experiment conducted over two locations.

Table 4 Comparison the broad sense heritability of multi-location *vis-à-vis* single location experiments.

Authors	Days to maturity	Number of primary branches	Crude protein content of the seed	Remarks
Feysal 2006	95.04	64.34	56.29	Executed at one environment
Current finding	48.63	35.97	12.94	Executed at two environments

Estimation of expected genetic advance

Genetic gains that expected from selecting the top 5% of the genotypes, as a percent of the mean, varied from 0.20%

Table 5 The eigen values and vectors of the correlation matrix for 10 traits of 144 *T. foenum-greacum* landraces.

Parameter	[a]PRIN1	PRIN2	PRIN3	PRIN4	PRIN5
Eigen value	3.27	1.84	1.53	1.05	0.91
% variance	29.69	16.70	13.95	9.52	8.24
Cumulative	29.69	46.39	60.34	69.86	78.10
Character					
[b]DM	0.315	0.169	0.520	-0.247	-0.205
GFP	0.262	0.169	0.628	-0.049	-0.075
NPPL	0.472	0.014	-0.121	0.190	0.207
PNPPL	0.476	0.032	-0.130	0.155	0.215
PBR	0.374	0.007	-0.311	0.005	0.003
SBR	0.414	0.091	-0.120	0.259	-0.177
NL	-0.080	0.585	-0.250	0.093	-0.147
ENL	-0.115	0.558	-0.041	-0.018	0.253
FWNL	-0.095	0.518	-0.032	0.010	-0.173
CP	0.133	0.083	-0.136	-0.760	0.503

[a]PRIN1, PRIN2, PRIN3, PRIN4 and PRIN5 = Principal component 1, 2, 3, 4 and 5 respectively, [b]DM = Days to 90% maturity, GFP = Grain filling period, NPPL = Number of nodes per plant, PNPPL = Number of podding nodes per plant, PBR = Number of primary branches per plant, SBR = Number of secondary branches per plant, NL = Number of nodules per plant, ENL = Number of effective nodules per plant, FWNL = Fresh weight of nodules in g per plant, CP = Crude protein of the seed

for GFP to 28.22% for SBR, indicating an increase and/or improving with the magnitude of 0.20% to 28.22% can be made by selection based on these traits under similar conditions to this study. The low values of expected genetic advance for the traits like DM in spite of moderate (48%) heritability is due to low variability for the trait indicated by the low GCV and PCV values. This indicates the importance of genetic variability in improvement through selection. This result is also in conformation with that of Feysal (2006).

As observed in the present investigation, the low expected genetic advance for PBR and SBR, CP content and all nitrogen-fixing traits (NK, ENL and FWNL) were due to low variability existed for the traits.

Principal component analysis

In order to assess the pattern of variations as worked by Elfalleh *et al*. (2009) and Zandi *et al.* (2011), principal component analysis (PCA) was done by considering all the ten variables simultaneously. Five of the ten principal components (PCs) accounted for more than 78% of the total variation in the Ethiopian fenugreek landraces (**Table 5**).

The first PC accounted for 29.69% of the total variation and seven of the ten traits considered in this study exerted positive effects on this component, while the rest traits exerted negative effects of different magnitudes. Among those traits having positive and greater influence includes, number of podding nods, total nods and secondary branches per plant. Conversely, N-fixing traits (NL, ENL and FWNL) had all negative weights on this component. The second component accounted for an additional 16.70% of the total variation. All of the traits exerted positive but different magnitude and the N-fixing traits (NL, ENL and FWNL) were among the traits which have positive and maximum impacts on the second component. The third PC accounted for about 13.95% of the total variation and except temporal traits (DM and GFP), all traits exerted negatively in this component. CP content of the seed exerted high but negative influence on the fourth component and inversely, it exerted high and positive effect on the fifth component.

Clustering of genotypes and divergence analysis

Genetic diversity plays an important role in plant breeding since hybrids between lines of diverse origin generally display a greater heterosis than those between closely related lines (Welsh 1990).

The average linkage technique of clustering produced a more understandable portrayal of the 144 fenugreek accessions by grouping them into five clusters, whereby different members within a cluster being assumed to be more closely related in terms of the ten trait under consideration with each other than those members in different clusters (Gemechu *et al.* 2005a; 2005b; Nigussie and Becker 2002). Similarly, members in clusters with non-significant distance were assumed to have more close relationship with each other than they are with those in significantly distant clusters. **Table 6** indicates the range, mean, standard deviation and coefficient of variation in some morphological and N-fixing traits of the five clusters and **Fig. 1** shows trends of each cluster on each of the ten traits. The detail account of the characteristics of each cluster is presented hereunder.

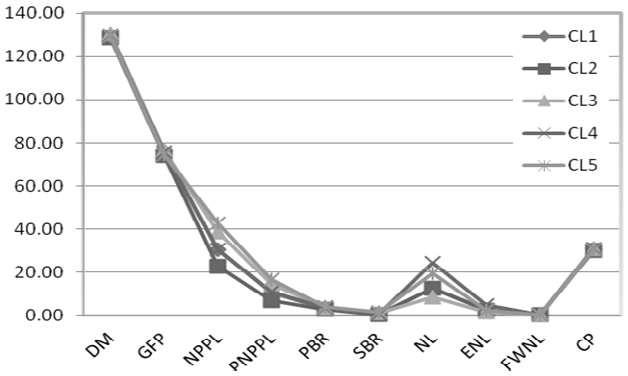

Fig. 1 Trend of the five clusters of the 144 *Trigonella foenum-graecum* L. germplasms over the ten traits. Y-axis scale based upon the units mentioned under the material and methods section for each parameters.

Cluster I: consisted of 94 landraces collected from the entire regions of the country and required longer period for grain filling and bears larger NPPL indicating accessions takes longer time for maturity after flowering which might be suited for the areas with prolonged rainfall.

Cluster II: consisted of 29 landraces, which exhibited early in DM and GFP. The major contributing factors that cause differentiation of this cluster from the rests of the clusters were nitrogen fixing traits (**Table 5**) which also confirmed from the standard deviations of the traits in the cluster as compared with the other traits (**Table 6**).

Cluster III: consisted of 14 landraces which required longer phonological traits but they exhibited lower in nitrogen fixing traits. The major contributing traits that cause differentiation of this cluster from the rests of the clusters were such phonological traits as days to maturity and grain filling period.

Cluster IV: had 5 landraces which superior in all nitrogen fixing traits and protein content of the seed. Among the other traits, CP is the most negatively contributing traits that create variability of this cluster from the rest.

Cluster V: consisted of 2 landraces, which were relatively superior in most morpho-physiological traits. Among the other traits, as of cluster IV, CP and ENL are the most contributing traits that create variability of this cluster from the rest (**Table 6**).

In general, the differences between the clusters were mainly attributed to the variation in DM. Other traits such as, NPPL, the N-fixing traits (NL, ENL and FWNL) and CP content of the seed have contributed equally well for cluster constellations. These traits were also the major contributors to the PC one and two.

From the estimated distance analysis, out of ten possible pairs of clusters, differences between six pairs were highly significant ($P \leq 0.01$) while those between the rest (four pairs) of clusters were non-significant (**Table 7**).

The maximum distance was found between cluster two and five ($D^2 = 64.07$). Cluster two constitutes twenty nine while cluster five constitutes two accessions. The second most divergent clusters were cluster two and three ($D^2 = 37.17$). Cluster three constitutes fourteen accessions and the third most divergent clusters were cluster three and four ($D^2 = 31.54$). Cluster four constitutes five accessions. The forth

Table 6 Mean and range of genetic divergence in morphological and nitrogen traits of the five clusters of *T. foenum-graecum*.

Cluster	Traits	DM	GFP	NPPL	PNPPL	PBR	SBR	NL	ENL	FWNL	CP
I	Min	124.58	70	24.95	6.93	1.9	0.03	5.82	0.51	0.08	28.83
	Mn	129.85	76.01	30.59	10.61	3.22	0.65	12.58	2.59	0.13	30.73
	Max	135.38	81.58	38.14	16.79	4.54	2.02	20.23	4.85	0.21	33.66
	SD	2.15	2.52	3.07	2.06	0.51	0.46	3.46	0.94	0.03	0.93
	CV%	1.65	3.32	10.02	19.38	15.74	70.19	27.52	36.2	21.76	3.03
II	Min	123.8	69.73	16.95	3.86	1.47	0	3.39	0.39	0.08	28.7
	Mn	128.76	74.14	22.57	6.8	2.64	0.21	12.62	2.41	0.14	30.24
	Max	132.88	79.25	26.01	10.52	3.43	0.81	18.91	6.17	0.22	32.32
	SD	2.08	2.18	2.32	1.43	0.47	0.2	4.02	1.2	0.04	0.91
	CV%	1.62	2.93	10.28	21.03	17.82	94.28	31.87	49.79	26.62	3.01
III	Min	126.76	72.51	35.69	12.41	2.95	0.35	6.35	0.75	0.09	29.57
	Mn	130.73	76.37	38.51	14.56	3.66	1.07	8.51	1.61	0.12	30.84
	Max	133.92	81.21	44.88	17.33	4.76	1.73	12.02	2.79	0.17	32.36
	SD	1.82	2.2	2.56	1.64	0.49	0.48	1.87	0.66	0.03	0.76
	CV%	1.39	2.88	6.65	11.24	13.36	44.7	22.01	40.71	22.65	2.48
IV	Min	128.02	73.92	27.33	8.42	2.61	0.49	21.96	3.15	0.15	29.79
	Mn	129.71	75.768	30.292	10.804	3.39	0.738	24.164	4.636	0.184	31.024
	Max	132.94	78.04	31.93	14.28	4.38	1.24	27.17	6.82	0.24	32.64
	SD	2.07	1.85	1.76	2.37	0.66	0.3	2.05	1.59	0.04	1.03
	CV%	1.59	2.45	5.8	21.98	19.54	40.67	8.47	34.32	20.55	3.33
V	Min	129.49	74.01	41.83	13.43	3.67	1.6	17.86	1.28	0.15	30.22
	Mn	129.56	74.45	42.74	16.455	4.01	1.685	19.36	2.365	0.175	30.54
	Max	129.63	74.89	43.65	19.48	4.35	1.77	20.86	3.45	0.2	30.86
	SD	0.1	0.62	1.29	4.28	0.48	0.12	2.12	1.53	0.04	0.45
	CV%	0.08	0.84	3.01	26	11.99	7.13	10.96	64.88	20.2	2.98

Min, Mn and Max stands for minimum, mean and maximum value, SD = standard deviation, DM = Days to 90% maturity, GFP = grain filling period, NPPL = Number of nods per plant, PNPPL = Number of podding nods per plant, PBR = Number of primary branch per plant, SBR = Number of secondary branch per plant, NL = Number of nodule per plant, ENL = Number of effective nodules per plant, FWNL = Fresh weight of nodules in g per plant, CP = Crude protein of the seed

Table 7 Pair-wise generalized squared distance (D^2) among five clusters constructed from 144 *T. foenum-graecum* landraces.

Cluster	C_1	C_2	C_3	C_4	C_5
C_1	0.89	10.00^{ns}	10.29^{ns}	12.88^{ns}	28.38**
C_2		1.46	37.17**	24.49**	64.07**
C_3			3.02	31.54**	15.12^{ns}
C_4				2.17	27.59**
C_5					5.23

most divergent clusters were between cluster one and five ($D^2 = 28.38$), cluster one was constituted from ninety-four accessions collected from different part of the country.

Genotypes grouped into the same cluster presumably diverge little from one another as the aggregate characters are measured. Generally, maximum genetic segregation and genetic recombination is expected from crosses that involve parents from the clusters characterized by significant distances. In the present investigation, therefore, crossing of accessions from cluster two and five will give rise to maximum genetic segregation.

Among the five clusters formed, cluster five showed the maximum intra-cluster D^2 value of 5.23 followed by cluster three and four with 3.02 and 2.17, respectively. The result revealed that even though cluster one contains the largest number of accessions (65.28% of the total accessions), it had the shortest intra-cluster distance. This indicates that the accessions grouped in this cluster are more similar as compared with the rest of the accessions in the rest of the clusters. In a similar fashion, there were only two accessions for cluster five but they were more divergent as compared with the accessions present in the rest of the clusters.

It is worthy to note that in calculating cluster mean, the superiority of a particular accession with respect to a given character could get diluted by other accessions that are grouped in the same cluster but are inferior or intermediate for the character in question. Hence apart from selecting genotypes from the clusters which have higher inter-cluster distance for hybridization one can also think of selecting parents based on the extent of divergence with respect to a character of interest.

CONCLUSION

From the results obtained in our work, it can be concluded that there is the presence of variability for most of the traits in Ethiopian fenugreek germplasm accessions and, generally, indicates possibilities for genetic improvement of the crop through selection and cross breeding. The result also reveals from genetic gains estimate an increase and/or improving with the magnitude of 0.20% to 28.22% can be made by selection based on these traits under similar conditions to this study.

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