Black Sea Journal of Agriculture
Open Access Journal
e-ISSN: 2618 - 6578

BS Publishers

Research Article
Volume 2 - Issue 4: 203-211 / October 2019

GENETIC DIVERGENCE AND CLUSTERING OF ETHIOPIAN FENUGREEK (*Trigonella foenum-graecum* L.) GENOTYPES IN SOUTH WOLLO ZONE OF AMHARA REGION, ETHIOPIA

Yimam ALI[1]*, Arega GASHAW[1], Alemu ABATE[2]

[1]*Amhara Agricultural Research Institute, Sirinka Agricultural Research Center, Ethiopia*

[2]*Bahir Dar University College of Agriculture and Environmental Science, Ethiopia*

Received: *August 16, 2019;* ***Accepted:*** *September 18, 2019;* ***Published:*** *October 01, 2019*

Abstract

Fenugreek is multipurpose plant originated in the Mediterranean region. Lack of information on phenotypic characteristic and association of traits are main problems in fenugreek production. Field experiment was conducted at Jamma district of South Wollo, Amhara National Regional State, in 2018/19 main rainy season to estimate genetic distance among Ethiopian fenugreek genotypes. Sixty two nationally collected fenugreek genotypes along with standard and local checks were evaluated in simple lattice design. Analysis of variance showed the presence of significant ($p<0.05$) difference among genotypes for most of the traits examined, indicating the presence of genetic variability. Seed yield ranged from 651 kg ha-1 to 2148 kg ha-1. A total of 30 and 35 genotypes had yield advantage up to 85 % and 98% than local and standard checks respectively. Genetic distances of fenugreek genotypes measured by Euclidean distance ranged from 9.6 to 73.07. Genotypes were grouped in to 6 major and 2 standalone clusters based on their genetic pedigree rather than geographical location, suggesting the presence of genetic variability and higher chance of developing varieties through direct selection or crossing of genotypes using distantly related genotypes to produce heterotic hybrids. Developing different breeding program in addition to yield and intensive direct selection of local collection of genotypes is used to improve the productivity of fenugreek.

Keywords: Clustering, Euclidean distance, Fenugreek, Simple lattice

***Corresponding author:** Amhara Agricultural Research Institute, Sirinka Agricultural Research Center, Ethiopia
E mail: yimamali121102@gmail.com (Y.ALI)
Yimam ALI https://orcid.org/0000-0002-4646-4615
Arega GASHAW https://orcid.org/0000-0001-7854-7805
Alemu ABATE https://orcid.org/0000-0000-0000-0000

Cite as: Ali Y, Gashaw A, Abate A. 2019. Genetic divergence and clustering of Ethiopian fenugreek (*trigonella foenum-graecum* L.) genotypes in South Wollo zone of Amhara region, Ethiopia. BSJ Agri, 2(4): 203-211.

1. Introduction

Fenugreek is multipurpose plant and originated from the Mediterranean region (Petropoulos, 2002). India is the leading producer and Ethiopia, Egypt, Algeria and Morocco are major producer in Africa. Cluster analysis is to group "like" observations together when the underlying structure is unknown. Genetic diversity plays an important role in plant breeding because hybrids

between lines of diverse origin generally display a greater heterosis than those between closely related strains (Million et al., 2012). This is carried out through a variety of methods, all of which use some measure of distance between data points as a basis for creating groups. Typically this distance is the standard Euclidian distance (ED). Essentially, data points with the smallest distances between them are grouped together. Then the data with the next smallest distances are added to each group, etc. until all observations end up together in one large group.

Divergence analysis is a technique used to categorize genotypes that are similar to one group and others into different groups (Tariyal et al., 2017). D-square statistics (D2) developed by Mahalanobis (1936) has been used to classify the divergent genotypes into different groups. Generalized genetic distance by using multiple measurements that are subjected to multivariate statistical analysis can provide generalized distance as indicated by D2 statistics. The value of D2 statistics (Mahalanobis, 1936) has been demonstrated effective in choosing the parental stocks for cross breeding of fenugreek (Wojo et al., 2015). The diverse genotypes characterized by maximum inter cluster distance will differ in phenotypic performance and therefore, selection of divergent parents should be based on these cluster distances to obtain favorable hybrids and transgressive segregants in fenugreek (Jadhav *et al*., 2018).

Genotypes within the same group or cluster are relatively homogeneous and those grouped in different clusters are relatively heterogeneous. 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 are expected from crosses that involve parents from the clusters characterized by significant distances. Therefore this research is conducted to asses' genetic distance among fenugreek genotypes in Ethiopia.

2. Material and Method

2.1. Description of the Experimental Site

The experiment was conducted at Jamma research site of Sirinka Agricultural Research Center (SARC) at Jamma District during the main growing season of 2018. Jamma is located at 10°27'N and 39°16'E on an altitude of 2622 meters above sea level, South Wollo, Amhara National Regional State, Ethiopia. Based on the last ten years (2008-2017) meteorological data obtained from Ethiopian Meteorological Agency, Kombolcha station, Jamma receives an average annual rainfall of 1047 mm and minimum and maximum temperature of 9.2 °C and 26.2 °C, respectively. Jamma is 120 km and 320 km away from Dessie and Addis Ababa, respectively. The dominant soil type in the District is Vertisol. Passport data of genotypesis presented in Table 1

Table 1. Passport data of genotypes

S.no	Accession Number	Region	Zone	S.no	Accession Number	Region	Zone
1	53003	Oromiya	N/Shewa	33	201627	NA	
2	53008	Amhara	S/ Gondar	34	201632	NA	
3	53009	Amhara	S/Gondar	35	202121	NA	
4	53014	Amhara	S/ Wollo	36	202122	NA	
5	53016	Oromiya	W/ Harerge	37	202124	NA	
6	53021	Amhara	E/Gojam	38	202125	NA	
7	53023	Oromiya	N/ Shewa	39	202126	NA	
8	53026	Amhara	E/Gojam	40	202127	NA	
9	53027	Amhara	E/Gojam	41	202129	NA	
10	53028	Amhara	E/Gojam	42	202132	NA	
11	53035	Amhara	E/Gojam	43	202133	NA	
12	53037	Amhara	E/Gojam	44	207361	Amhara	S/ Gondar
13	53039	Amhara	E/Gojam	45	207362	Amhara	N/ Gondar
14	53040	Amhara	E/Gojam	46	207363	Amhara	N/Gondar
15	53041	Amhara	E/Gojam	47	207364	Amhara	N/ Gondar
16	53042	Amhara	E/Gojam	48	207365	Amhara	N/ Gondar
17	53045	Amhara	E/Gojam	49	207390	Amhara	N/Gondar
18	53055	Amhara	E/Gojam	50	207391	Amhara	S/ Gondar
19	53056	Amhara	E/Gojam	51	207394	Amhara	S/ Gondar
20	53057	Amhara	E/Gojam	52	208680	Oromiya	E/ Harerge
21	53058	Amhara	E/Gojam	53	210864	NA	
22	53059	Amhara	E/ Gojam	54	212549	Amhara	N/ Shewa
23	53080	Amhara	E/ Gojam	55	212552	Amhara	N/ Shewa
24	53085	Oromiya	Bale	56	212777	Amhara	E/ Gojam
25	53086	Oromiya	N/Shewa	57	213115	Amhara	S/ Wollo
26	53094	SNNP	S/Omo	58	213116	Amhara	S/ Wollo
27	53097	Amhara	E/ Gojam	59	214942	Amhara	N/ Shewa
28	53098	Amhara	E/ Gojam	60	215056	Oromiya	Borena
29	53099	Amhara	E/ Gojam	61	216898	Oromiya	Arssi
30	53106	Amhara	N/ Shewa	62	216899	Oromiya	Arssi
31	53108	Amhara	N/Gondar	63	Jamma		
32	201577	NA		64	Local		

NA = not identified and SNNP=South Nation Nationalist People

2.2. Experimental Materials, Design and Procedure

Sixty two fenugreek genotypes collected from Debre-Zeit Agricultural Research Center (DZARC) along with local and standard checks were evaluated at Jamma testing site of SARC. The experiment was laid out using simple lattice design (8x8) on plot size of 1.6 m2, with an inter-row of 20 cm and intra-row spacing of 5 cm. The genotypes were collected from different parts of the country.

Clean fenugreek seeds were sowed of 20 and 5 cm between rows and plants, respectively, as per the national recommendation. Each genotype was planted on a gross plot size of 1.6 m2 (0.8 m width x 2 m length). The distance between plots and blocks were maintained at 0.5 m and 1 m, respectively. Being fenugreek is leguminous crop, fertilizer were not applied at all. Weeding and thinning were practiced at the appropriate time. Data were recorded from the central two rows with net plot size of 0.8 m2 (0.4 m x 2 m).

2.3. Data Collection

2.3.1. Data collected on plot basis

Data from plot basis were recorded from the central two rows, leaving a guard row from either side of the plot. The following data were recorded from plot basis.

Days to 50 % flowering: Days to 50% flowering was recorded as the number of days from planting to the time when 50 % of the plants in the plots produced flower.

Days to 90 % Maturity: It was recorded as number of days from planting to the time when 90 % of the plants in the plot reach physiological maturity.

Pod Filling Period: Number of days from flowering or exertion of pods to the time when 50% of the pod forms seeds.

Biomass yield (Above ground): It was taken as the total above-ground biomass weight of the plants from the central two rows. Total above-ground biomass was harvested and sun-dried and weighed using spring balance

Seed Yield: It was taken from the central two rows. Entire plants were harvested, threshed and winnowed. Clean seed was measured using electronic sensitive balance.

Thousand Seeds Weight: Thousand seeds were counted and weighed using electronic sensitive balance for each replication.

Harvesting Index: It was calculated as the ratio of seed yield to biomass yield in percent

2.3.2. Data collected on plant basis

The data on plant basis was recorded from five randomly taken but representative plants from the central two rows.

Plant Height: plant height was measured from the main stem, measured from the ground level to the tip of the plant using measurement tape at 90% physiological maturity.

Pod length: pod length was measured from the tip to petiole of the pod at 90% physiological maturity.

Number of Branches Plant^{-1}: The total number of branches arising from the main stem was counted at 90% physiological maturity.

Number of Pods Plant^{-1}: The total number of pods per plant was counted at physiological maturity.

Number of Seeds Pod^{-1}: The total number of seeds per pod was counted at physiological maturity.

2.4. Data analysis

Multivariate analysis (clustering and PCA) was carried out to group genotypes in to homogeneous sets based on the measured variations. Genetic distance of genotypes PCA is calculated by using SAS and Dendrogram is constructed by JMP. Genetic divergence analysis was computed based on multivariate analysis using Mahalanobis D^2 statistic (Mahalanobis, 1936) by using SAS software. Genetic distance of 64 fenugreek genotypes was estimated using Euclidean distance (ED) calculated from the quantitative traits after standardization (subtracting the mean value and dividing it by the standard deviation) as established by Sneath and Sokal, (1973) as follows:

$$EDjk = \sqrt{\sum_{i=1}^{n}\left(Xij - Xik\right)}^{\frac{2}{2}}$$

Where, EDjk = distance between genotypes j and k; xij and xik= agro-morphology traits mean values of the i^{th} trait for genotypes j and k, respectively; and n= number of traits used to calculate the distance. Dendrogram of genotypes was conducted using Unweighted Pair-group Method with Arithmetic means (UPGMA) method based on Euclidean distance (ED) matrices. The calculated value was tested against the tabulated X^2 value for p*(g-1) degree of freedom at 5% or 1% probability level.

Where; p = number of characters studied and g-1 = degree of freedom of genotypes.

3. Results and Discussion

3.1. Estimation of Euclidean Distance and Clustering of Genotypes

Genetic distance of the 64 fenugreek genotypes were estimated using Euclidean distance (ED) and the mean ED of each genotype with other 63 genotypes is presented in Table 2. Pair of genotypes distribution in different ranges of Euclidean distance is presented in Figure 1. The ED of pair of genotypes ranged from 9.6 between genotypes 53008 and 53009 and 73.07 between genotypes 53016 and 201577. The overall mean ED was 20.78 with 5.43 and 26.15% standard deviation and coefficient of variation, respectively.

Among 2016 pairs of genotypes, 2 and 118 pairs had ED of <9.92 (<mean-2SD) and >37.07 (>mean+3SD), respectively. Other 466, and 62 pair of genotypes had ED ranged from 9.92 (>mean2SD) to 15.35 (<mean-1SD) and 31.64 (>mean + 2SD) to 37.07 (mean + 3SD), respectively (Figure 2). The rest 120 and 1248 pairs of genotypes ranged from 26.2 (>mean + 1SD) to 31.64

(<mean+ 2SD) to 31.64 (and 15.35 (<mean-1SD) to 26.21 (>mean +1SD). These wide range of ED result indicated that fenugreek genotypes had wide range of genetic distances. This will allow breeders to select genotypes for character(s) of interest in addition to yield.

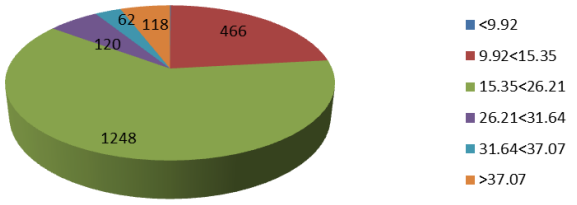

Figure 1. Pair of genotypes distribution in different ranges of Euclidean distance

The number of genotypes with their list in each cluster is given in Table 3. The high number of clusters indicated that the presence of wide genetic variability among the tested fenugreek genotypes. Distribution of the genotypes revealed that the maximum genotypes grouped in Cluster V (31) shared 48% of the genotypes, followed by Cluster VII and VI each comprised 10 and 13 genotypes, respectively. On the other hand, Cluster III and VIII each contained three genotypes in which they shared 9.4% of the total genotypes. Other one Cluster; Cluster II comprised 2 genotypes, which constituted only 3% of the total genotypes. The two Standalone clusters; cluster I and IV both contributed 3% of genotypes from the total distribution.

Table 2. Mean Euclidean distance of 64 fenugreek genotypes tested

SN	Gen	Min	Max	Mean	SD	CV%	SN	Gen	Min	Max	Mean	SD	CV%
1	53003	12.84	62.92	19.84	7.42	37.42	33	201627	15.12	43.97	25.12	6.19	24.65
2	53008	9.59	65.56	22.10	8.64	39.09	34	201632	11.31	46.76	18.49	6.21	33.58
3	53009	9.59	58.58	22.52	8.34	37.04	35	202121	9.99	40.23	15.92	4.91	30.86
4	53014	12.16	55.52	19.41	5.76	29.66	36	202122	10.65	47.37	16.46	5.95	36.17
5	53016	31.21	73.07	51.51	8.08	15.69	37	202124	11.59	54.08	22.39	7.86	35.08
6	53021	10.50	64.42	20.94	8.31	39.71	38	202125	10.59	46.68	17.76	6.12	34.45
7	53023	17.92	65.93	28.65	8.28	28.89	39	202126	10.06	45.24	15.54	5.78	37.20
8	53026	12.46	42.11	19.67	5.42	27.54	40	202127	11.75	48.06	18.19	6.06	33.31
9	53027	11.62	45.92	18.63	5.27	28.30	41	202129	10.07	55.32	20.83	8.31	39.88
10	53028	12.44	45.00	22.13	6.28	28.39	42	202132	14.26	56.97	21.96	5.97	27.20
11	53035	10.80	52.92	17.57	6.22	35.38	43	202133	11.03	51.59	18.63	6.29	33.74
12	53037	14.82	58.81	27.37	8.04	29.39	44	207361	9.99	47.16	15.82	5.72	36.13
13	53039	11.32	53.46	19.46	6.91	35.49	45	207362	12.44	48.17	19.00	6.62	34.85
14	53040	11.31	58.50	22.69	8.05	35.49	46	207363	12.44	58.63	22.02	7.49	34.02
15	53041	9.73	58.13	18.43	7.51	40.76	47	207364	9.73	52.60	18.23	6.77	37.10
16	53042	12.53	50.61	21.51	7.22	33.59	48	207365	10.37	54.60	17.59	6.42	36.49
17	53045	10.75	50.37	17.70	6.03	34.05	49	207390	13.33	48.43	22.14	6.53	29.48
18	53055	12.57	46.12	19.03	5.55	29.16	50	207391	14.38	46.07	23.51	5.37	22.83
19	53056	11.42	43.90	18.79	5.73	30.49	51	207394	24.38	73.07	40.51	7.02	17.33
20	53057	10.71	48.37	17.59	6.49	36.87	52	208680	10.99	60.88	20.01	7.50	37.49
21	53058	10.50	62.24	19.01	7.60	39.96	53	210864	15.87	66.84	25.17	7.84	31.16
22	53059	12.77	46.05	21.55	6.66	30.89	54	212549	11.37	55.38	17.75	6.61	37.25
23	53080	11.03	50.37	17.97	6.18	34.41	55	212552	11.16	59.24	18.55	7.49	40.37
24	53085	10.28	51.60	17.40	6.53	37.53	56	212777	11.25	48.93	17.71	6.03	34.05
25	53086	10.71	47.19	19.57	6.79	34.69	57	213115	10.04	50.24	20.76	7.07	34.05
26	53094	15.43	61.05	25.26	7.89	31.24	58	213116	11.61	46.56	20.13	6.46	32.09
27	53097	12.77	45.97	20.98	6.89	32.82	59	214942	10.37	51.95	17.04	6.43	37.77
28	53098	11.67	47.79	18.71	6.46	34.50	60	215056	12.31	55.10	23.84	8.37	35.11
29	53099	11.76	58.12	18.18	6.88	37.83	61	216898	11.32	45.53	17.21	5.92	34.40
30	53106	11.99	45.73	19.62	6.39	32.58	62	216899	10.04	55.18	19.29	6.84	35.48
31	53108	10.06	51.19	15.52	6.25	40.28	63	Jamma	10.83	45.46	17.82	5.82	32.64
32	201577	14.27	42.67	21.57	5.97	27.66	64	Local	13.09	51.14	23.49	7.83	33.35

Min =minimum, Max= maximum, SD= standard deviation, CV= coefficient of variation

3.2. Cluster Mean Analysis

Mean values of the seven characters of different clusters has been presented in Table 4. Significant differences in cluster mean values were marked for all the characters (Kakani et al., 2015). The sixty four fenugreek genotypes were grouped into eight clusters (Cluster I- Cluster VIII). Clustering was done by where different members within a cluster were assumed to be more closely related in terms of the trait under consideration with each other than those members in different clusters. Similarly, members in clusters with non-significant distance were assumed to have more close relationship with each other

than they were with those in significantly distant clusters (Norbert and Habtamu, 2017).

Cluster I is a solitary cluster contains only 1 genotype from Oromiya region. The genotype was characterized by less number of seed pod-1, and had better number of pod plant-1, biomass yield plant-1, seed yield plot-1 and harvesting index. Cluster II contained 2 genotypes from Amhara and Oromiya regions. This cluster was better in plant height and number of pod plant-1, intermediate in biomass yield, seed yield and harvesting index and lowest in number of seed plant-1. Cluster III was better in harvesting index and thousand seed weight and lower biomass yield plot-1, number of pod plant-1, and seed yield plot-1. The cluster comprised of 2 genotypes from Amhara and 1 genotype from Oromiya regions.

Cluster IV was characterized by intermediate in most traits except better in biomass yield plot-1 and lowest in plant height and harvesting index. This cluster also standalone (solitary) cluster, only 1 genotype from Amhara region was grouped.

Table 3. Distribution of genotypes in to 8 clusters based on Euclidean distance of 64 fenugreek genotypes tested

Cl	N.G	Code of Genotypes	Region
I	1	53003	O
II	2	53023 and 212549	O and NA
III	3	53016, 53027 and 207394	2 A and 1 O
IV	1	201577	NA
V	31	53014, 53028, 53035, 53039, 53041, 53042, 53056, 53057, 53085, 53086, 53094, 53097, 53099, 53108, 201627, 202125, 202126, 202127, 202129, 207361, 207362, 207363, 207365, 210864, 212777, 213115, 213116, 214942, 215056, 216898 and Local	19A, 4O +6 NA+1SNNP
VI	10	53055, 53098, 53106, 202122, 202124, 202132, 207364, 207391, 216899 and Jamma	5A + 1O and 3NA
VII	13	53008, 53009, 53021, 53026, 53040,53045, 53058, 53059, 53080, 201632, 202121, 208680, 212552,	11A + 2NA
VIII	3	53037, 207390 and 216899	2A + 1O

N.G = number of Genotype, A – Amhara, O – Oromiya, SNNP –South Nation National People and NA – not identified

Cluster V was the largest cluster sharing 48% of the genotypes. In this cluster 19 genotypes from Amhara, 4 genotypes from Oromiya, 1 genotype from SNNP and 6 genotypes were unidentified their location were grouped. This cluster showed lower performance in most traits. Cluster VI was characterized by intermediate in most of the traits but lower in biomass yield polt-1 and seed yield plot-1. This cluster contained 5 genotypes from Amhara, 1 genotype from Oromiya regions and 3 genotypes with unidentified location. Cluster VII contained a total of 13 genotypes, 11 genotypes from Amhara and 2 genotypes with unidentified location and characterized by better in thousand seed weight and seed yield plot-1. Cluster VIII contained 3 genotypes collected 2 genotypes from the Amhara and 1 from Oromiya regions. This cluster was best in plant height and number of seed plant-1, while lowest in thousand seed weight.

Table 4. Cluster means of 7 characters of the 64 fenugreek genotypes tested

Cluster	PH	BM	NP	NS	SY	HI	TSW
CL-I	27.7	7.00	11.3	7.6	2.148	0.30	20.2
CL-II	32.3	5.47	13.3	7.6	1.159	0.21	20.5
CL-III	29.7	2.96	6.6	8.4	0.973	0.33	20.6
CL-IV	27.3	10.63	7.0	8.4	1.168	0.12	19.8
CL-V	26.2	5.84	6.5	8.8	1.095	0.19	19.7
CL-VI	29.7	5.29	9.0	8.7	1.012	0.20	19.8
CL-VII	28.7	6.53	7.6	8.1	1.645	0.26	21.5
CL-VIII	31.4	6.96	9.3	10.1	1.450	0.21	18.3
Mean	29.1	6.3	8.8	8.4	1.3	0.23	20.0
Stdv	2.1	2.2	2.4	0.8	0.4	0.1	0.9
CV	7.1	34.2	27.5	9.2	30.1	29.0	4.7

PH=plant height, NB= number of branch per plant, BM=biomass, NP=number of pod/plant, SY=seed yield, HI=harvesting index, TSW=thousand seed weight, Stdv= standard deviation and CV=coefficient of variation.

3.3. Intra and Inter Cluster Genetic Distance

The mean genetic distances of genotypes within cluster and each cluster members with other 7 clusters were calculated to understand which cluster was containing diverse genotypes and which cluster members were distant from other groups of genotypes. The two genotypes constructed solitary Clusters; I and IV and the three major clusters; cluster II, III and VIII had higher mean genetic distance than the overall mean. But, the mean intra cluster distance of the three clusters (V, VI

and VII) was lower than overall mean. This indicated that the three clusters have small divergence among themselves; and more closely related fenugreek genotypes were grouped in this cluster than genotypes in other clusters. This showed that the members of genotypes in these clusters were more diverse among them, and were more distant to other genotypes in other clusters, while the genotypes that constructed cluster II were distant from these genotypes. This suggested that crossing of the members of these clusters among them and with other genotypes in other clusters might produce heterotic progenies (Mathur, 1992). Singh et al. (1987) stated that crosses involving parents belonging to the most divergent clusters would be expected to noticeable maximum heterosis and wide variability in genetic architecture. Cluster II had maximum intra cluster distance (3.93) followed by cluster III and VIII (6.12). This indicates genotypes in these clusters were more diverse, while cluster VI had the smallest intra cluster distance (1.45) followed by cluster VII (3.19). The smallest intra cluster distance indicates the more homogeneous genotypes were grouped in these clusters (Table 5).

Table 5. Intra cluster (bold diagonal) and Inter cluster (off diagonal) Pair wise generalized squared distance (D^2) among 8 clusters constructed from 64 fenugreek genotypes tested.

C	I	II	III	IV	V	VI	VII	VIII
I	8.32	23.11***	53.61***	58.43***	24.16***	23.31***	23.52***	15*
II		6.93	45.16***	73.76***	39.02***	20.99**	27.81***	20.81**
III			6.12	58.17***	14.55*	16.77**	16.23*	31.59***
IV				8.32	27.32***	33.93***	23.79***	36.49***
V					1.45	15.21*	8.05	17.91**
VI						3.71	7.057	13.46*
VII							3.19	13.68*
VIII								6.12

C, *, **, ***= stands for Cluster, significant at 5, 1 and 0.1%, respectively, X^2 = 12.6, 16.8 and 22.5 at 5, 1 and 0.1% respectively.

Cluster V which contained 31 genotypes had lowest mean genetic distances 1.45 intra cluster distance and cluster VI contained 10 genotypes with 3.71 intra cluster genetic distances within genotypes. These cluster contained local and standard checks, respectively. This indicated at least 23 fenugreek genotypes had a genetic distance different from the checks; and therefore, they could be good sources of parents for crossing for the improvement of fenugreek genotypes.

In general, all of the twenty-eight possible pairs of clusters, differences between 6 pairs were significant ($P<0.05$), 4 pairs were highly significant ($P<0.01$) and 16 pairs were very highly significant ($P<0.001$), while the rest 2 pairs of cluster were not significantly different from each other. The maximum inter distance was found between cluster I and IV (D2=58.43). These two clusters are standalone clusters. The next maximum distance was between cluster III and IV (58.17). The three genotypes constructed cluster III are 2 from Amhara region; South Gonder, East Gojam and 1 from West Hararghe of the Oromiya region. The minimum inter cluster distance was found between cluster VI and VII (D2=7.06). These clusters contained 10 and 13 genotypes from different regions, respectively. The second minimum inter cluster distance was between cluster V and VII (8.05). Therefore, crossing between clusters I and IV would produce maximum segregation at F2. Therefore, hybridization between genotypes from cluster I and cluster IV could produce better segregants in segregating populations for the studied characters (Jyoti et al., 2018); and crossing between genotypes from cluster VI and VII can produce minimum segregants.

In the present study, genotypes from different locations were grouped together. This indicated that clustering was based on genetic divergence rather than geographical diversity. Genetic pedigree and selection forces under different environments could cause greater diversity rather than geographical diversity. The findings were in agreement with findings of Tariyal et al. (2017), Kole and Goswami (2015), and Jain et al. (2006) who worked similar investigation in different genotypes from different sources.

3.4. Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to find out the characters, which accounted more to the total variation (Wojo et al., 2015). The principal component analysis revealed that the first eight principal components from thirteen components with as explained by the eigen values of 22.3, 6.01, 5.03, 4.55, 2.72, 2.22, 2.03 and 1.01, respectively, accounting about 95% of the total variations recorded among fenugreek genotypes for twelve quantitative traits examined (Table 6).

Table 6. Eigen vectors and values of the eight principal components for 12 traits of fenugreek genotypes.

Eigenvectors	PCA1	PCA 2	PCA 3	PCA 4	PCA 5	PCA 6	PCA 7	PCA 8
DF	0.13	0.16	-0.16	-0.14	0.23	0.18	-0.30	0.15
DM	-0.07	0.21	-0.13	0.07	0.05	0.14	0.20	0.80
PFP	-0.08	0.24	0.24	0.27	-0.09	-0.06	-0.05	0.00
PH	0.07	0.52	-0.52	-0.24	0.27	-0.20	0.23	-0.25
NB	0.08	-0.07	0.08	0.19	0.13	0.44	-0.40	-0.38
BM	0.56	0.35	0.62	-0.38	0.02	0.05	0.08	-0.13
NP	0.12	0.11	-0.36	-0.27	0.19	0.61	0.54	-0.05
NS	0.03	0.11	-0.02	-0.37	-0.23	-0.06	-0.45	0.24
PL	0.16	-0.42	0.36	-0.02	-0.10	-0.22	0.35	-0.08
SY	0.97	0.04	-0.16	0.16	0.04	0.09	-0.02	-0.04
HI	0.38	-0.07	-0.63	0.63	-0.09	0.08	-0.02	0.07
TSW	0.15	0.14	0.18	0.36	0.86	0.03	0.11	0.13
eigen values	22.30	6.01	5.03	4.55	2.72	2.22	2.03	1.10
Percent	46%	12%	10%	9%	6%	5%	4%	2%
Com. %	46	58	69	78	84	89	93	95

DF= date of flowering, DM= date of maturity, PEP=pod filling period PH=plant height, NB= number of branch per plant, BM=biomass, NP=number of pod/plant, PL=pod length, SY=seed yield, HI=harvesting index, TSW=thousand seed weight and PCA= principal component analysis.

The relative magnitudes of eigen vectors for the first principal component having share of about 46% indicated that seed yield plot-1, biomass yield plot-1 and harvesting indexes were the first and the most important contributing traits for the total variation.

This indicates these traits had higher contribution for clustering of genotypes. Similar result was reported by Millon et al. (2012), indicating greater influence by seed yield plot-1 and biomass yield plot-1 for PCA1. The second principal component contributed about 12% of the total variation. Plant height, pod length and biomass yield plant-1 were the most predominant traits for this principal component and these traits also had high contribution for grouping of fenugreek genotypes.

The third principal component contributed 10% of total variation on the traits of plant height, biomass yield plot-1, pod length and harvesting index. Zandi et al. (2011) also reported higher effect of plant height for PCA2, pod length and harvesting index for PCA3. The fourth principal component contributed 9% of the total variation with the traits harvesting index, thousand seed weight, number of seed plant-1 and biomass yield plot-1 had high share for this variation. The fifth principal component contributed 6% of the total variation with traits days to flowering, plant height, number of seed plant-1 and thousand seed weight contribute more exertion for it. The sixth, seventh and eighth principal component contribute 5%, 4% and 2%, respectively from the total variability. Traits number of branch plant-1 and number of pod `plant-1 for principal component sixth, number of seed pod-1, number of branch plant-1 and number of pod plant-1 for principal component seventh and number of branch plant-1 and days to maturity for eighth principal component had highest contribution. It could be concluded that maximum variation was present from the first principal component (Jadhav et al., 2018). Therefore, selection of fenugreek genotypes should be targeted based on traits that contribute maximum magnitude for first principal component.

4. Conclusions

Based on Euclidean distance matrices, 64 fenugreek genotypes were grouped into 6 major and 2 standalone clusters. A cluster contained genotypes from different geographical locations. This indicated that genotypes were grouped based on their genetic divergence rather than geographical location. From the two standalone clusters one is from Oromiya collection and eight genotypes from Oromiya region were distributed in to six different clusters. This indicates genotypes from Oromiya region are more diverse than the other locations. In addition, genotypes grouped in different clusters were characterized by one or more desirable traits. The study results showed that genotypes were diverse with significant variations among them for agromorphological traits. This suggested that higher chance of selecting genotypes for traits of interest to develop as variety directly or crossing of the genotypes among them to produce heterotic progenies. PCA showed that seed yield plot-1, biomass yield plot-1, harvesting index, plant height and pod length had highest contribution for clustering of fenugreek genotypes.

As recommendation, wide genetic diversity indicates possibility of genetically diverse breeding program and can creates genetic diversity. This implies these genotypes can be used as good sources of crossing material and can be directly developed as variety not only for yield, but also other traits of interest. Therefore Ethiopian biodiversity institute maintain these high yielder genotypes. To increase the income generated to

the farmers and foreign earning of the country from fenugreek, researchers focus to intensive use of local collection fenugreek genotypes to maximize low fenugreek yield on farmer's field.

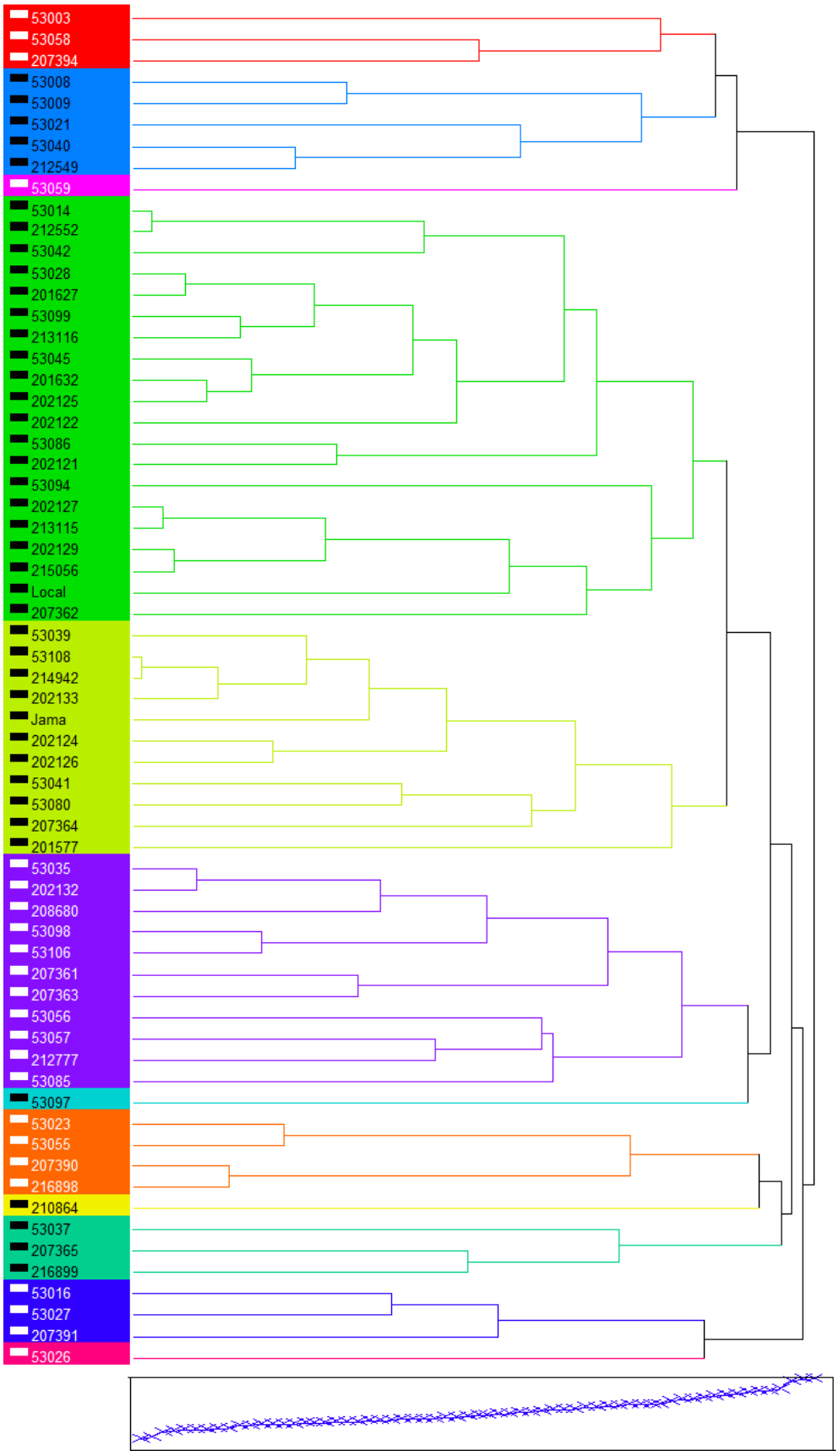

Figure 2. Dendrogram of 64 fenugreek genotypes constructed on the basis of agronomic and qualitative traits using hierarchical clustering Method of average

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors acknowledge Amhara Agricultural Research Institute (AARI) and Sirinka Agricultural Research Center for financial support.

References

Asati J, Desai P, Patel RK. 2018. Genetic diversity analysis for seed yield and its attributes in Fenugreek (*Trigonella foenum-graecum* L.). Int J Curr Microbiol App Sci, ISSN: 2319-7706, 7: 12.

Dewar N, Matthew H. 2017. Genetic diversity for morpho-physiological traits and associations between the geographic origins of the germplasm. Global J Plant Breed Genet, 4(2): 337-346.

Fikreselassie M, Zeleke H, Alemayehu N. 2012. Genetic variability of Ethiopian fenugreek (*Trigonella foenum-graecum* L.) landraces. J Plant Breed Crop Sci, 4(3): 39-48.

Jadhav S, Dileepkumar AM, Mahantesha BN, Tatagar MH, Sumangala K. 2018. Estimation of genetic diversity in fenugreek (*Trigonella foenum graceum* L.) genotypes for productivity traits. Inter Chem Stud, 6(5): 2701-2703.

Jain U, Singh D, Balai O, Shiva K. 2011. Genetic divergence in fenugreek (*Trigonella foenum-graecum* L.) germplasm. J Spices Arom Crops, 15(1): 52-57.

Jain UK, Singh D, Balai OP, Shiva KN. 2006. Genetic divergence in fenugreek (*Trigonella foenum-graecum* L.) germplasm. J Spices Arom Crops, 15(1): 59-62.

Kakani RK, Singh G, Saxena SN. 2015. Genetic divergence in fenugreek (*Trigonella foenum-graecum* L.) Inter J Seed Spices, 5(1): 37-40.

Kole PC, Goswami T. 2015. Genetic divergence in fenugreek grown under sub-humid subtropical Red lateritic belt of eastern India. Inter J Bio Env Agri Sci, 1(3): 97-102.

Mahalanobis PC. 1936. Studies on the generalized distance in statistics. In: Proceedings of Institute of Science, India; 2: 49-55.

Mathur VL. 1992. Genetic divergence in fenugreek (*Trigonella foenum-graecum* L.). Indian J Genet Plant Breed, 52: 432-434.

Petropoulos GA. 2002. Fenugreek: The genus Trigonella. Taylor & Francis, London and New York, p. 255.

Singh SK, Singh RS, Maurya DM, Verma OP. 1987. Genetic divergence among lowland rice cultivars. Indian J Genet Plant Breed, 47(1): 11-14.

Sneath PHA, Sokal RR. 1973. Numerical taxonomy: the principles and practice of numerical classification. San Francisco: Freeman, 573 p.

Tariyal, YS., Bisht, SS., Pant, SC. and RS Chauhan. 2017. Study of genetic divergence in fenugreek (*Trigonella foenum-graecum* L.). J Pharmac Phytochem, 6(5): 1551-1552.

Wojo AA, Alamerew S, Nebiyu A, Menamo T. 2015. Genotype and phenotype variability studies in fenugreek (*Trigonella foenum-graecum* L.). J Spices Arom, 25(2): 159-168.

Zandi P, Shirani– Rad AH, Daneshian J, Bazrkar– Khatibani L. 2011. Agronomic and morphologic analysis of Fenugreek (*Trigonella foenum-graecum* L.) under nitrogen fertilizer and plant density via factor analysis. African J Agri Res, 6(5): 1134-1140.