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Cluster Analyses based on Yield and Yield Components in Fenugreek (*Trigonella Foenum-Graecum* L.) Accessions

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Abstract- In Ethiopia, there are insufficient information on genetic diversity in the Ethiopian fenugreek germplasms and need to study associations among seed yield and yield related traits. The overall objective of this investigation was cluster analysis using divergence and ranging the traits which were the most contributors to variation by principal component analysis. It was undertaken on 36 geographically diverse Ethiopian germplasms of fenugreek. The germplasms were collected from different regions of the country. These germplasms were tested in a 6x6 simple lattice at South Nation and Nationality Peoples Region (NNPR), Gesha Woreda, Kefa Zone, Deka, Ethiopia in 2013 main cropping season. Distances square (D^2) analysis showed that 36 fenugreek germplasms grouped into six clusters. The distances were significant difference which helps for maximum genetic segregation and genetic recombination for crosses as well as obtaining heterotic response in breeding program for improving yield and yield related traits. Clustering pattern of genotypes was not related to geographical differentiation. Principal component analysis showed that the first four principal components explained about 72.88% of the total variation. Generally, this study revealed that there was a good scope of concurrent improvement in yield by exploiting the Ethiopian germplasms of fenugreek.

Keywords: variability, divergence, principal component analysis.

I. Introduction

Fenugreek belongs to the Trigonella genus, which is closely related to the Medicago and Melilotus genera (Small, 1989). It is best known for presence of the distinctive, pungent aromatic compounds in the seed that impart flavor, color and aroma to foods, making it a highly desirable supplement for use in culinary applications (Max, 1992). As a spice, it constitutes one of the many ingredients that make up curry powders (Srinivasan, 2006). In countries such as India, fenugreek leaves are consumed as leafy vegetables in the diet (Sharma, 1986), while in Ethiopia and Egypt, the plant is used as a supplement in maize and wheat flour for bread-making (Al-Habori and Raman, 2002).

Clustering using D^2 (genetic distance) matrix is useful for analyzing the divergence of the population to identify genotypic variability. The D^2 statistic measures the forces of differentiation at intra- and inter-cluster levels and determines the relative contribution of each component trait to the total divergent (Sharma *et al.*, 1990). Clusters separated by the largest D^2 (genetic distance) show the maximum divergence, while the genotypes in the same clusters or groups are less divergent (Chaudhary and Singh, 1975).

Several measurements of distances have been proposed over the past decades to suit various objectives (Amsal, 2001). Generalized genetic distance by using multiple measurements that are subjected to multivariate statistical analysis can provide such measure based on generalized distance as indicated by D^2 statistics (Mahalanobis, 1936; Radhakrishna Rao, 1952). A number of workers observed that D^2 statistics was a powerful tool in describing divergence among different lines based on multiple characters; fenugreek (Banerjee and Kole, 2004; Jain *et al.*, 2006) and wheat(Deshmukh *et al.*, 1999; Debebe *et al.*, 2000; Amsal, 2001; Mustefa, 2006).

In Ethiopia, selection of high yielding and promising genotypes and pure line development has thoroughly being carried out at Sinana and DebreZeit Agricultural Research Centers. As a result, one variety (Chella) was officially released by DZARC in 2004 (DZARC, 2004). The present investigation was aimed to study the extent of genetic divergence and cluster the 36 genotypes into different homogeneous groups which were collected from different region of Ethiopia.

II. Materials and Methods

a) Study Area

The experiment was conducted in South Nation and Nationality Peoples Region (NNPR), Gesha Woreda, Kefa Zone, Deka, Ethiopa in 2013 main cropping season. It was done at Ethiopian Evangelical church Mekane Yesus development farm site with the permission and approvals obtained from Terfasa Meko. The experiment site is about 585km southwest of Addis Ababa and lies at 6.24^{0}8.13' N and 35.48^0 36.78' E. The site is situated at an altitude of 2200 m.a.s.l. The mean annual rainfall is 2200mm, while the mean annual minimum and maximum temperatures of the area are

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10.1 and 27.5^0_C, respectively (Ayele, 2004).The area is characterized by Nitisols.

b) Experimental materials and design

Thirty six fenugreek accessions samples random were selected. The accessions were collected and maintained by the Ethiopia Institute of Biodiversity Conservation (IBC) from different region of the country (Table 1) and were obtained kindly from IBC. The experiment was laid out in a 6x6 simple lattice design with 2x1m plot size. The plot consists of four rows with 20x10cm spacing. Weeding and other cultural practices were done based on the recommendations adopted for the respective sites (Fikreselassie *et al.*, 2012).

Table 1 : List of fenugreek germplasms with collection regions and passport data in IBC

SN	Accession No.	Regional areas					
		regions	Zones	districts	longitude	latitude	altitude
1	53008	Amhara	South-Gonder	Tach-gayint	08-07-00N	40-12-00E	2480
2	53009	Amhara	South-gonder	Este	08-26-00N	32-15-00E	2330
3	53012	Amhara	South-wollo	Wereilu	06-19-00N	27-45-00E	2240
4	53013	Amhara	South-wollo	Wereilu	-	-	NA
5	53014	Amhara	South-wollo	Legambo	12-02-00N	41-32-00E	2300
6	53026	Amhara	East-gojam	Enarg-enawg	-	-	2115
7	53055	Amhara	East-gojam	Shebel-benta	-	-	2045
8	53059	Amhara	East- Gojam	Shebel-benta	-	-	NA
9	239065	Amhara	Bahrdar	Bahir dar	16-05-00N	26-08-00E	2000
10	239066	Amhara	West-Gojam	Merawi	10-06-00N	28-12-00E	2050
11	239068	Amhara	West-Gojam	Around-Bahirdar	11-39-18N	37-13-41E	1930
12	215261	Amhara	North-Wello	Guba-lafto	11-48-00N	39-33-00E	1910
13	215731	Amhara	South-Wello	Werebabu	08-44-00N	41-16-00E	2050
14	53060	Amhara	East-Gojam	Shebel-beret	-	-	1845
15	53063	Amhara	Agawui	Danigla	09-23-00N	32-14-00E	NA
16	53056	Amhara	East-Gojam	Shebel-bere	-	-	1950
17	53057	Amhara	East-Gojam	Shebel-bere	-	-	2115
18	53066	Benshangul	Metekel	Dangur	-	-	2000
19	53006	Oromia	Arsi	Gedeb	-	-	NA
20	53016	Oromia	East- Hararge	Tullo	09-07-00N	41-01-00E	2400
21	51012	Oromia	Harerghe	Deder	09-28-00N	28-12-00E	2410
22	53020	Oromia	Bale	Goro	09-32-00N	42-16-00E	2560
23	53022	Oromia	East-Wollega	Amuru-garte	-	-	NA
24	237984	Oromia	Balle	Gasserana	-	-	2330
25	230536	Oromia	East- Hararge	Gursum	09-24-00N	42-1700E	2200
26	237982	Oromia	West-Shewa	Decho	-	-	2110
27	53072	Oromia	Arsi	Chole	08-22-00N	39-53-00E	2520
28	53074	Oromia	Arsi	Chole	08-40-00N	39-50-00E	2660
29	53064	Oromia	Bale	Agrfa	07-17-00N	39-50-00E	2450
30	53030	Oromia	Harerghe	Girawa	-	-	2420
31	53032	SNNP	Kaffa	Telo	-	-	2010
32	Local	SNNP	Kaffa	Gesha	-	-	NA
33	238247	Tigray	Mehaklegnaw	Laelay-maychew	14-05-00N	39-06-00E	1990
34	234033	Tigray	Mehaklegnaw	Naederadet	14-03-00N	38-44-00E	2120
35	234034	Tigray	Mehaklegnaw	Laelay-maychew	14-04-00N	38-43-00E	2120
36	53065	Tigray	Adwa	Adwa	-	-	NA

Source: Ethiopian Institute of Biodiversity Conservation, (2012). NA: information is not available

c) Data collected and analysis

Ten quantitative traits were recorded on five randomly selected plants from the two middle rows of each plot: included flowering days, maturity days, Plant height at maturity, number of primary branches, number of secondary branches, Pods number per plant, seeds number per pod, thousand seeds weight, Biological yield per plot and seed yield per plot. Cluster analysis was performed by canonical roots method using procedures of SAS version 9.2 (Institute Inc, 2008) based on divergence value. Principal component analyses were also performed by using correlation matrix by employing procedure print comp corr of SAS version 9.2 (Institute Inc, 2008).

III. Result and Discussion

a) Cluster Analysis

Divergence analysis is a technique used to categorize genotypes that are similar into one group and others into different groups. D-square statistics (D^2) developed by Mahalanobis (Mahalanobis, 1936), has been used to classify the divergent genotypes into different groups. The D^2 values based on the pooled mean of genotypes resulted in classifying the 36 fenugreek accessions in to six groups which were four clusters and two standalone based on ten traits (Table 2). Previous studied also showed that 36 fenugreek genotypes grouped into six clusters (Jain *et al.*, 2011). These groups were not depended the geographical locations which were collected. For instance, cluster I contained 13 germplasms: which were six from Amhara region (53012, 53026, 215731, 239066, 53063 and 53075) one Benishangul (53016), five Oromia (53016 51012 53074 53064 and 53030) and one SNNP (53032). This might indicate less environmental effect on the phenotypes expression. The distribution pattern of genotypes in different clusters indicated that the cluster was based genetic divergence rather than geographical diversity i.e. genetic drift and selection forces under diverse environments could cause greater diversity than geographical diversity (Jain *et al.*, 2011). It might be due to diversity of their pedigree along with natural and direction pressure for certain agronomic traits (Jain *et al.*, 2011). Similarly, the distribution pattern of Fenugreek genotypes in different clusters indicated that genetic divergence not related to geographical differentiation (Kole and Saha, 2009; Jain *et al.*, 2011).

The F- test for the six groups indicated that there was statistically accepted difference between all clusters (Table 3). The extent of diversity present between germplasms determines the extent of improvement gained through selection and hybridization. Cluster I was included 13 accessions (36.11%) which contained relatively plant height, seeds number per pod and thousand seeds weight when compared to other clusters. Cluster II was contained 10 accessions (27.77%). This cluster had high number of primary and secondary branches per plant and low in thousand seeds weight. Cluster III was contained four accessions (11.11%). This cluster was characterized by high number of pod per plant, seeds per pod, biological yields and seed yield per plot. It also had early maturity character. Seven accessions were included in Cluster IV. Cluster IV characterized by early flowering and low number of primary and secondary branches per plant, number of pod per plant, number of seeds per pod, thousand seeds weight and number of seed yield per plot. Each group V and VI was containing only one accession.

The maximum distance was found between group five and six (Table 3). The second most divergent clusters were group three and six (D^2= 290.87). The third most divergent clusters were group four and five (D^2=254.22), the forth most divergent clusters were between group one and six (D^2=217.42), followed by cluster four and three (D^2=145.67), cluster two and five (D^2=129.47), cluster two and six (D^2=126.78), cluster two and three (D^2=63.67).

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 germplasms from cluster five and six will give rise to maximum genetic segregation. On the other hand, crossing between cluster II and VI, there may be a chance to recombine genes which are early maturity with high seed yields per plant. Among the six clusters formed, cluster one showed the maximum intra-cluster D^2 value of 8.04 followed by cluster two with 5.89. Since cluster six contains a single accession, the intra-cluster D^2 value is zero.

Table 2 : Distribution of genotypes in to six clusters based on D^2 analysis for 36 Fenugreek genotypes tested at Deka, 2013

group	accessions	Proportion (%)	Mean									
			DF	DM	PH	NPB	NSB	NPP	SNP	TSW	BY	SYP
Cluster I	53012, 53026, 215731, 239066, 53063, 53057, 53066, 53016, 51012, 53074, 53064, 53030 & 53032	36.11	47.92	145.81	49.81	4.42	5.31	19.74	10.68	14.90	776.23	207.46
Cluster II	Local ,234034, 234033, 230536, 237984, 53072, 53020, 53006, 53060 & 239065	27.77	47.6	141.5	49.30	4.65	5.45	19.52	9.53	13.85	744.60	193.20
Cluster III	53055, 239068, 237982 & 53065	11.11	46.25	134.5	48.37	4.5	5.25	19.94	10.62	14.65	789.80	222.50
Cluster IV	53022, 238247, 53056, 215261, 53014, 53009 & 53008	19.44	45.64	140.71	48.85	4.14	4.86	19.18	9.28	14.21	772.27	190.42
V	53013	2.77	48.00	128.50	56.00	5.00	4.50	21.5	11.00	14.40	723.80	186.50
VI	53059	2.77	45.50	141.00	45.5	4.00	5.00	19.75	11.40	15.40	807.80	219.00

DF=days to flowering, DM=days to maturity, PH=Plant height at maturity, NPB=Number of primary branches/plant, NSB=Number of secondary branches/plant, NSP= number of seeds per pod, NPP=Number of pods/plant, TSW=Thousand seeds weight(g), BY=Biological yield per plot(g), SY=seed yield per plant (g)

Table 3 : Pair-wise generalized squared distance (D^2) among 6 clusters constructed from 36 *T. foenum-graecum* landraces

Group	Cluster 1	Cluster 2	Cluster 3	Cluster 4	5	6
Cluster 1	8.04*	14.16*	22.17**	45.33**	58.65**	217.42**
Cluster 2		5.63*	63.67**	23.56**	129.47**	126.78**
Cluster 3			3.89*	145.67**	23.19*	290.87**
Cluster 4				3.28*	254.22**	77.40**
5					2.19*	489.27**
6						0.00

,, significant at 5 and 1%, respectively.*

b) Principal Component Analysis

Principal component analysis (PCA) is one of the multivariate statistical techniques which are a powerful tool for investigating and summarizing underlying trends in complex data structures (Legendre and Legendre, 2012). Principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation (Sharma *et al.*, 1990).

The principal component analysis (Table 4) revealed that four principal components PC1 to PC4 with Eigen values 3.66, 1.82, 1.36, and 1.16, respectively. The first two principal components PC1 and PC2 with value of 33.3% and 16.55% respectively, contributed more to the total variation. According to Chahal and Gosal (Chahal and Gosal, 2002), characters with largest absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero. Therefore, in the present study, differentiation of the genotypes into different cluster was because of a cumulative effect of a number of characters rather than

to the small contribution of each character (± 0.01-0.54). Characters having relatively higher value in the first principal component (PC1) like day to maturity, seeds number, a thousand seeds weight, biomass yield, pods number per plant, number of primary and secondary branches per plant, number of seeds per pod and seed yield had more contribution to the total diversity and they were the ones that most differentiated the clusters. Likewise, 16.55% of the total variability among the tested germplasms for the second principal component originated from variation in day to flowering, day to maturity, number of primary branches, number of secondary branches and number of seeds per pod. Similarly, the third principal component, which accounted 12.4% of the total variation were obtained from day to flowering, plant height at maturity, number of primary and secondary branches, and biomass yield per plot . Further, the fourth principal component which explained 10.5% of total variations were chiefly obtained from variations of plant height at maturity, number of primary branches, a thousand seeds weight and harvest index.

Table 4 : Eigen vectors and Eigen values of the first four principal components (PCs) for ten Characters of 36 Fenugreek accessions tested at Daka (2013)

Traits	Eigen vectors			
	PC1	PC2	PC3	PC4
Days to 50% flowering	0.52	0.45	0.43	-0.15
Days to maturity	0.22	0.51	0.2	0.01
Plant height(Dekkers and Hospital)	0.01	-0.17	0.49	0.51
No of 1^0 branches/plant	0.39	-0.43	0.38	-0.06
No of 2^0 branches/plant	0.30	-0.31	0.35	-0.27

IV. Conclusion and Recommendation

Genetically, distant parents could be used in hybridization program to get better yielding recombinants. Thus, based on the relative squared distance values (D^2) between any two genotypes, the 36 fenugreek germplasms were grouped into six distinct clusters. Clusters I and II contained large number of germplasms (13 and 10 germplasms respectively) followed by Cluster IV (7 germplasms) and Cluster III (4 germplasms) whereas Cluster V and cluster VI had the least number of genotypes (1 germplasms each).The maximum distance was found between cluster five and six (D2=489.27) and the maximum intra-cluster D^2 value in cluster one (8.04).

Cluster analyses indicated that the geographic and genetic diversity might not necessarily to be in a group i.e. germplasms collected from the same geographic collection region fell in different cluster groups whereas those collected from different geographic region tended to be grouped in the same cluster. However, the analysis suggested that there was considerable diversity among the germplasms. There is a very good scope to bring about improvement through hybridization and selection by crossing germplasms from different clusters.

The principal component analysis revealed that four principal components (PCs) having Eigen values between 1.16 and 3.66, extracted a cumulative of about 72.8% of the total variation noted among the germplasms. It was also noted that differentiation of the germplasms into different cluster was because of a cumulative effect of a number of characters rather than the small contribution of each character.

In conclusion, the present investigation indicated that there is wide range of genetic variability and diversity in Ethiopian fenugreek germplasms though the present investigation was conducted on only a part of it. There is large scope of simultaneous improvement in seed yield through selection. Hybridization among germplasms from different clusters identified in this study could lead to considerable genetic improvement by following appropriate selection strategies in the segregating generations.

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