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Cluster and principal component analysis in fenugreek (*Trigonella foenum-graecum* L.) genotypes based on yield and yield related traits

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Abstract
The present study was conducted to identify the nature and magnitude of genetic divergence among forty eight genotypes of fenugreek based on phenotypical traits using the multivariate analysis. Based on cluster analysis, the genotypes were best fitted into five clusters. The maximum and minimum genotypes were grouped in cluster I (21) and cluster V (1) respectively. The inter cluster distance was maximum between clusters II and V (D2=8.115) followed by IV and V (D2=7.856) revealing that the genotypes of these clusters were highly diverse from others and can be used as divergent parents for hybridization and selection. Thus, for getting high heterosis for recovering transgressive segregants, genotypes from cluster II and V can be used as distant parents in any breeding programme for successful fenugreek improvement. Whereas, the maximum intra-cluster distance was shown by cluster IV (D2=4.679) indicating maximum difference among the genotypes within. Among the nine characters studied for genetic divergence, seeds per pod contributed the maximum accounting for 22.78% of total divergence, followed by pods per plant (20.39%) and plant height (16.13%). The result of PCA revealed that all the three principal components (PC-I, PC-II and PC-III) contributed 66.62% of the total variability. The results of present study could be exploited in the future genetic improvement programme of fenugreek genotypes.

Keywords: Cluster analysis, fenugreek, genetic diversity, multivariate, PCA

Introduction
India is well known as "Land of Spices". The genus *Trigonella* is one of the largest genera of the tribe *Trifoliate* in the family *Fabaceae* and sub-family *Papilionaceae* (Balodi and Rao, 1991) [1] which includes two economically important species *viz., Trigonella foenum-graecum* or the commonly called "fenugreek/methi" and *Trigonella corniculata* or the "kasthuri methi." Fenugreek is a seed spice diploid species with chromosome number of 2n=16 grown for fodder or spices and condiments to improve the flavor and nutritive value of food. Fenugreek is believed to originate from Iran and North India (Smith, 1982). It is extensively cultivated in India, particularly in the states of Rajasthan (>80%), Madhya Pradesh, Gujarat, Tamil Nadu, Andra Pradesh, Uttar Pradesh. Fenugreek is an annual 30 to 90 cm tall herb with light green pinnately trifoliate leaves. The flowers are papillonaceaus with white or yellow in colour and produces slander, beaked pods of approximately 10-15 cm long and each pod contains 10-20 small hard yellowish brown seed possessing smooth and oblong, about 3 mm long, each grooved across one corner, giving them a hooked appearance. Fenugreek seeds are also used in preparation of ayurvedic medicines due to its mucilaginous, demulcent diuretic, carminative, astringent and aphrodisiac properties. The low productivity of fenugreek in India is mainly due to non-availability of suitable high yielding varieties for various agro climatic region and poor crop husbandry. The productivity of any crop is mainly attributed to the contribution of the yield components. In any crop breeding programme, germplasm serve as the most valuable reservoir in providing variability for various traits. Proper screening and evaluation of germplasm lines would provide an estimate about their potential value as suitable genotype for utilization in varietal development programme. Selection and hybridization approaches are easily followed in bringing about the quantitative improvement in order to bring about desired improvement.
Assessment of genetic divergence in fenugreek genotype is important for long term crop improvement programme.

In the process of genetic improvement of any crop, genetic diversity among germplasm plays a major role, since it opens the way to determine the most divergent parents based on the contribution of different qualitative and quantitative traits, for further utilization in any breeding programme. Therefore, the exploration of genetic diversity in the available germplasm is a pre-requisite in a breeding programme for effective selection of the superior genotypes. The D2 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) [17]. Clusters separated by the largest D2 (genetic distance) show the maximum divergence, while the genotypes in the same clusters or groups are less divergent (Chaudhary and Singh, 1975) [3]. A plant breeder has to identify the source of favorable genes to incorporate them into the breeding populations and select for a combination of desirable traits that might result in the isolation of productive genotypes and cultivars. Thus, present study is undertaken to understand the magnitude of genetic divergence for identifying more diverse parents for fenugreek genetic improvement.

Materials and Methods

The present investigation consisting of forty eight genotypes of fenugreek was carried out at Agronomy Farm, S.K.N. College of Agriculture, Jobner, Jaipur (20º 6' N, 75º 25' E and 420 m above mean sea level) during *rabi* 2016-17 to evaluate the amount of genetic diversity and relatedness among the genotypes. The Randomized Block Design was used to raise these genotypes in three replications. In each replication the seeds were sown in a single row plot of 3 m length in which row to row and plant to plant spacing was 30 cm and 10 cm, respectively. All the recommended agro-practices were followed to ensure a healthy crop growth and development. Observation were recorded on five randomly selected plants situated under the same field condition for nine morphological quantitative traits *viz.*, days to 50 per cent flowering, days to maturity, plant height (cm), branches per plant, pods per plant, pod length (cm), seeds per pod, test weight (g) and seed yield per plant (g). The magnitude of genetic diversity among forty eight fenugreek genotypes was determined by using D2 Mahalanobis genetic distance statistics (Mahalanobis, 1936) [11]. Hierarchical clustering using Tocher's method, as described by Rao (1952) [14] was followed for the grouping of genotypes into distinct clusters. The principal component analysis was done using R software (Venables and Ripley, 2002) [23].

Results and Discussion

The data obtained from the observations recorded on nine morphological quantitative traits were subjected to the statistical scrutiny. It was evident from the analysis of variance that mean sum of squares due to 48 genotypes were highly significant for all the traits (Table 1), giving the clear picture of presence of wide spectrum of variability among the genotypes. This suggested that response to selection may be accepted in the breeding programme for seed yield or any of its supporting characters. These results are in agreement with the findings of Singh and Kaur, 2007 [20]; Yadav et al., 2013 [26]; Kole *et al.*, 2014 [7]; Sharma *et al.*, 2015 and Singh and Naula, 2017 [18]. Although the analysis of variance revealed sufficient variability among the genotypes, but the extent of genetic diversity present among the genotypes could not be explained, therefore, cluster analysis was performed to quantify the genetic divergence between any two genotypes or group of genotypes.

Table 1: Mean sum of squares for different characters in fenugreek (ANOVA)

S. No.	Source of variation (with d.f.)	Replications (2)	Genotypes (47)	Error (94)
1	Days to 50% flowering	2.80	8.92**	1.86
2	Days to maturity	7.92	19.08**	2.87
3	Plant height (cm)	2.18	75.80**	8.10
4	Branches per plant	0.19	0.42**	0.16
5	Pods per plant	11.23	120.55**	13.05
6	Pod length (cm)	0.01	0.59**	0.08
7	Seeds per pod	0.06	2.46**	0.23
8	Test weight (g)	1.17	1.79**	0.48
9	Seed yield per plant (g)	0.87	3.74**	0.61

** represents significant at 1% level of significance

Based on the relative magnitude of their Mahalanobis D2 values using Tocher's method, all the 48 genotypes of fenugreek under study were grouped into five clusters. The clustering patterns of Fenugreek genotypes into five clusters are presented in Table 2. Maximum number of genotypes (21) was grouped in cluster I namely: UM-55, UM-66, UM-8, RMt-1, UM-4, UM-52, UM-61, UM-13, UM-10, UM-37, UM-57, RMt-305, UM-36, UM-304, UM-302, UM-53, UM-301, UM-41, UM-50, UM-44, UM-163 Whereas, cluster III contained 15 genotypes; UM-62, UM-65, UM-63, UM-48, UM-49, UM-54, UM-47, UM-118, UM-124, UM-26, UM-29, UM-58, UM-46, UM-45, UM-24, cluster II comprises of 8 genotypes namely: RMt-143, UM-40, UM-51, UM-27, UM-64, UM-55, UM-38, UM-28 followed by cluster IV comprises UM-112, UM-60, RMt-361 and Cluster V comprises UM-100. Clustering pattern of genotypes was not related to geographical differentiation. The assignment of genotypes to different clusters was reported by several research groups in different legume crops (Banerjee and Kole, 2004; Parihar *et al.*, 2013) [7, 13].

Table 2: Clustering pattern of 48 genotypes on the basis of their mahalanobis genetic divergence

Cluster	No. of genotypes	Name of genotypes
1	21	UM-55, UM-66, UM-8, RMt-1, UM- 4, UM-52, UM-61, UM-13, UM-10, UM-37, UM-57, RMt-305, UM-36, UM-304, UM-302, UM-53, UM- 301, UM-41, UM-50, UM-44, UM-163
2	8	RMt-143, UM-40, UM-51, UM-27, UM-64, UM-55, UM-38, UM-28
3	15	UM-62, UM-65, UM-63, UM-48, UM-49, UM-54, UM-47, UM-118, UM-124, UM-26, UM-29, UM-58, UM-46, UM-45, UM-24
4	3	UM-112, UM-60, RMt-361
5	1	UM-100

The average intra and inter-cluster D2 values with their corresponding intra and inter-cluster distance are presented in Table 3. The inter-cluster distances were greater than intra-cluster distances, which indicated the presence of considerable amount of genetic diversity among the genotypes studied. The greater the magnitude of inter and intra cluster distance the higher the variability among the cluster and within the cluster and *vice versa*. The results are in concurrence with the findings of Kumar *et al.* (2006) [8]; Singh and Mishra (2008) [19]; Sen and De (2017) [15] and Kumar *et al.* (2021) [9] in different legumes. Since cluster V contains a single accession, the intra-cluster D2 value is zero. Whereas, maximum value of intra-cluster distance was observed in cluster IV (D2=4.679) revealing the existence of maximum differences among the genotypes falling in this cluster, followed by divergence for cluster I (D2=3.310), cluster III (D2=3.058) and cluster II (D2=2.567). Hence, selection within these clusters may be exercised based on the highest area of desirable traits. In any breeding programme where the nature of crosses is to be evaluated, choice of diverse parents is of paramount importance as they produce superior off-springs due to maximum genetic segregation and genetic recombination than the closely related ones.

The inter-cluster distance (D2) being the main criterion for selection of genotypes was also worked-out as crossing of genotypes within the same cluster would not produce superior off-springs. A range of 3.529 to 8.115 was observed when inter-cluster D2 values were used to study the diversity among the clusters. The minimum value of inter-cluster distance (D2=3.529) was found between cluster II and III indicating close relationship and similarity for most traits among the genotypes included in these clusters. Whereas, cluster II and V showed maximum value of inter-cluster distance (D2=8.115), followed by cluster IV and V (D2=7.856) and cluster III and V (D2=7.497) indicating that the genotypes included in these clusters are not so closely related showing good amount of diversity. Hence, these genetically diverse genotypes can be used as promising parents for hybridization. These results are corroborated with the findings Jain *et al.*, 2006 [4]; Kakani *et al.*, 2015 [5]; Wojo *et al.*, 2015 [24] as they also gave similar conclusion.

Table 3: Average of intra- and inter-cluster genetic distance

Clusters	1	2	3	4	5
1	3.310				
2	4.472	2.567			
3	3.672	3.529	3.0578		
4	6.007	5.256	4.9192	4.679	
5	7.242	8.115	7.4971	7.856	0.000

Diversity among the genotypes was also estimated based on the considerable amount of variation in cluster means for different character. Different clusters exhibited distinct mean values for almost all the nine characters which reflect the genetic differences between the clusters (Table 4). Highest mean value of days to 50% flowering (56.00), days to maturity (111.00), branches per plant (5.33) and test weight (12.13) was observed in cluster IV whereas highest mean value of plant height (65.81), pods per plant (44.58) and seed yield per plant (7.98) was observed in cluster III and highest mean value of pod length (10.67) and seeds per pod (16.61) was observed in cluster I (Table 4). Comparative assessment of cluster means showed that for improving specific characters, the genotypes should be selected from the cluster having high mean value for that particular character. This comparison indicates that clusters III and IV had better cluster means for most of the characters; therefore, these clusters might be considered better for selecting genotypes as divergent parents. The similar results are exhibited with the findings of Kole and Mishra (2002) [6]; Yadav *et al.* (2018) [25] and Meena *et al.* (2021) [12].

Table 4: Cluster wise mean values of 9 morphological traits

Cluster	DF	DM	PH	BPP	PPP	PL	SPP	TW	SYPP
1	47.99	103.14	56.70	5.21	31.87	10.67	16.61	10.60	5.61
2	46.97	102.08	64.70	4.74	38.87	10.57	16.57	10.65	6.89
3	47.06	101.36	65.81	5.20	44.58	10.08	15.44	11.62	7.98
4	56.00	111.00	48.60	5.33	41.93	10.40	15.53	12.13	7.62
5	48.46	103.07	62.95	4.87	33.33	10.04	15.67	10.86	5.94

Where, DF= days to 50% flowering, DM= days to maturity, PH = plant height, BPP= branches per plant, PPP= pods per plant, PL= pod length, SPP= seeds per pod, TW= test weight and SYPP= seed yield per plant

The contribution of various characters to divergence in fenugreek was recorded and found that seeds per pod had contributed highest (22.78 %) followed by pods per plant (20.39 %) and plant height (16.13 %). In contrast, number of branches per plant (1.86 %) contributed least (Table 5). These findings are in conformity with Magalingam *et al.* (2013) [10] in dolichos bean. Dendrogram obtained from hierarchical clustering based on mahalanobis genetic distance depicted the clear relationship and exact position of genotype in clusters (Fig 1). This study on genetic diversity assessment identified diverse genotypes that could be used as parental clones in hybridization and breeding programme in fenugreek.

Table 5: Contribution percentage of nine characters towards genetic divergence in fenugreek

S. No.	Traits	Contribution (%)
1	DF	4.78
2	DM	7.71
3	PH	16.13
4	BPP	1.86
5	PPP	20.39
6	PL	14.80
7	SPP	22.78
8	TW	4.25
9	SYPP	7.26

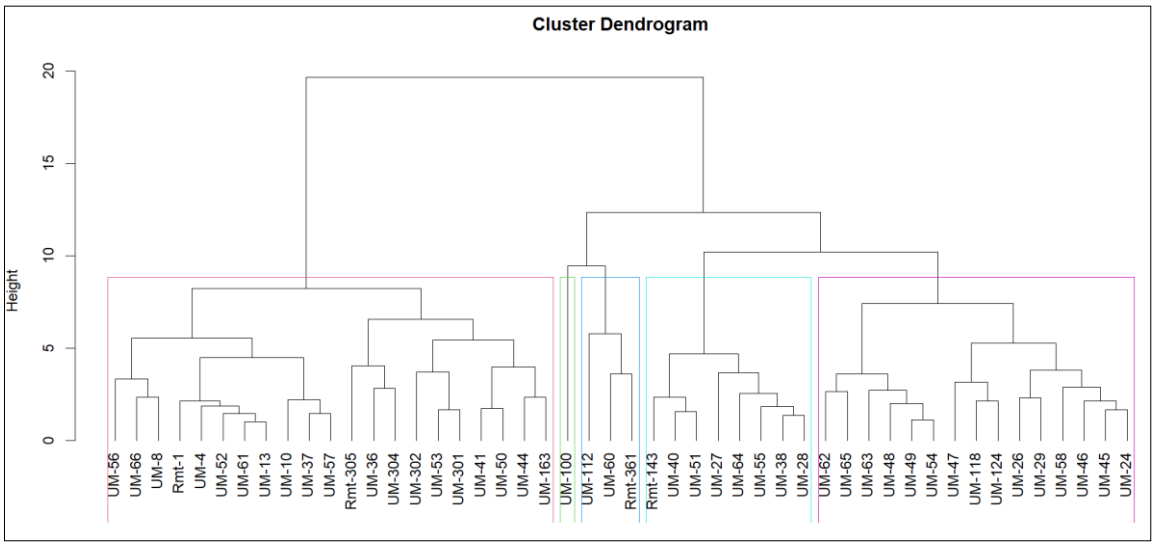

Fig 3: Dendrogram showing relationship among fenugreek genotypes using hierarchical clustering based on mahalanobis genetic distance

The principal component analysis (PCA) is one of a series of techniques for collecting high-dimensional information and using the dependence between the variables in a more tractable form without any loss of information. It represents the major contributor to the total
difference in each differentiation axis and helps in identification and ranking of genotypes and important economic traits contributing in genetic diversity for developing superior hybrid in fenugreek. Eigen values with more than one have high explanatory power of original variables. Based on the analysis the first three principal components having Eigen values greater than one contributed 66.62 per cent of the total variability in 48 fenugreek genotypes (Table 6). Proportion of variance for the first 3 components was 28.96, 24.12 and 13.53 per cent respectively. The variability on the first PC (28.96%) was accounted for by high positive loadings for PPP, SYPP, PH, TW and PL, whereas, PC-II had high positive loadings for BPP, DF, TW, DM, PPP and SYPP. PC-III showed positive contribution with all traits except PH and BPP. Screen plot explain the percentage of variation associated with each principal component obtained by drawing a graph between Eigen value and principal components (Fig 2). The bi-plot of PC-I and PC-II showed a considerable variability presenting a dispersion pattern of delineated genotypes. The scatter plot generated through PCA illustrated the diverse genotypes located farther from the point of origin (Fig 3). It was also noted that differentiation of the genotypes into different cluster was because of a cumulative effect of a number of characters rather than the small contribution of each character. These diverse genotypes may be employed as parents in hybridization in future breeding program. Similar findings were reported by Singh *et al.* (2021) [21] in dolichos bean.

Table 6: Factor loadings for yield contributing traits in fenugreek (*Trigonella foenum-graecum* L.)

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
DF	-0.682	0.483	0.227	-0.208	0.207	0.394	-0.227	0.327	-0.058
DM	-0.702	0.406	0.189	0.279	0.069	0.287	0.346	-0.122	0.065
PH	0.702	-0.242	-0.351	-0.052	0.382	0.256	0.209	0.253	-0.014
BPP	-0.05	0.535	-0.081	0.674	-0.433	0.182	0.093	0.135	-0.028
PPP	0.733	0.403	0.200	-0.314	-0.303	0.090	-0.120	0.085	0.196
PL	0.022	-0.559	0.724	0.080	-0.111	-0.217	0.242	0.189	0.014
SPP	-0.159	-0.755	0.328	0.183	0.008	0.455	-0.003	-0.056	0.023
TW	0.258	0.468	0.342	0.478	0.586	-0.075	-0.037	-0.091	0.091
SYPP	0.721	0.401	0.459	-0.180	-0.038	0.124	0.005	-0.120	-0.207
Eigen values	2.607	2.171	1.218	0.979	0.835	0.468	0.348	0.274	0.100
Proportion of variance	28.961	24.124	13.533	10.882	9.279	5.198	3.866	3.045	1.110
Cumulative proportion (%)	28.961	53.085	66.619	77.502	86.781	91.978	95.845	98.890	100

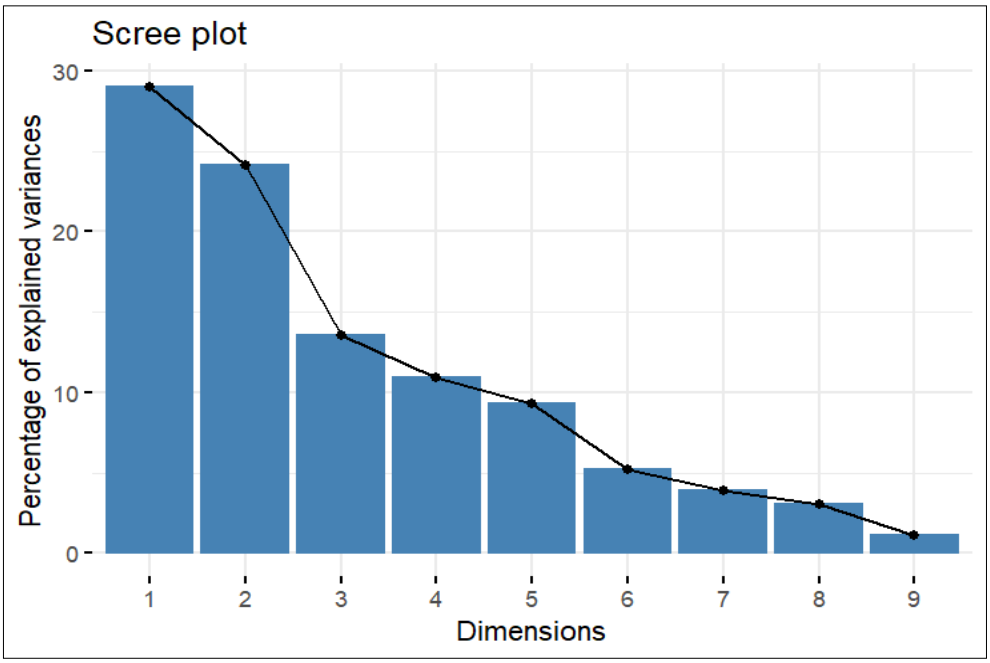

Fig 2: Scree plot showing variance and along with principal components

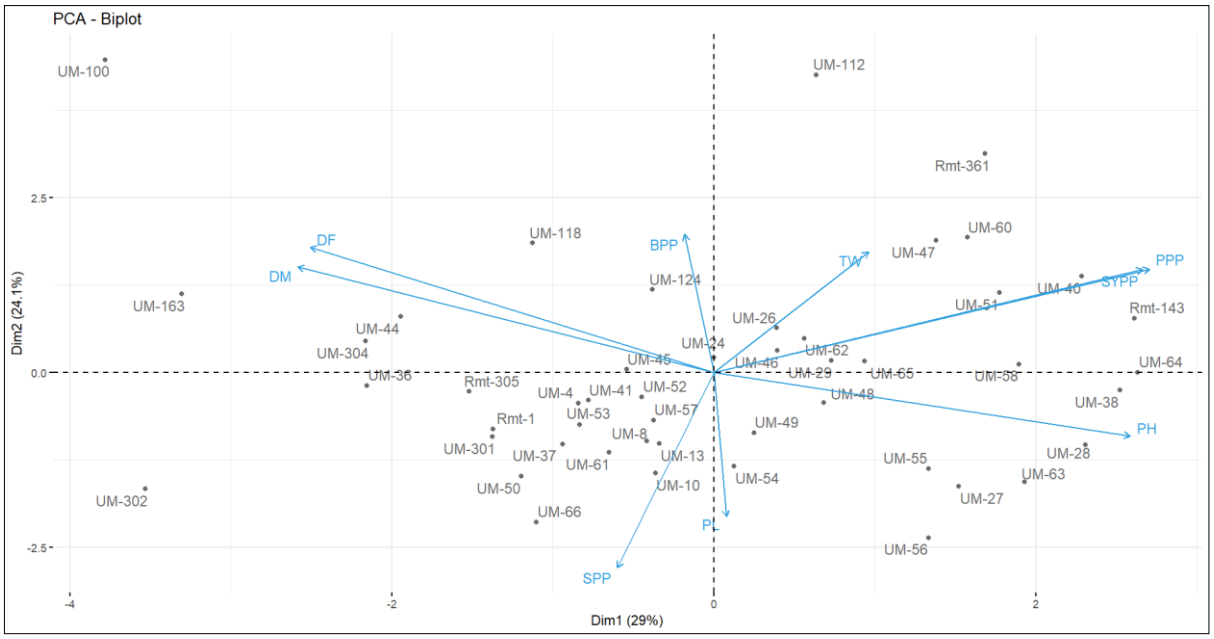

Fig 3: Scattered plot of fenugreek genotypes using first two principal components

Conclusion

Based on analysis of variance, cluster analysis and principle components analysis it can be concluded that the fenugreek genotypes in the present study can be successfully used for planning future breeding programmes. The information on genotypes and distinct traits contributing for more variation facilitates breeders to develop newer high yielding cultivars. Therefore, from the present study genotypes from farthest clusters based on their high inter-cluster distance and high cluster mean values can be hybridized as the potential parents to produce superior off-springs in the segregating generations and to improve fenugreek productivity. This study would certainly provide guidelines in the selection of parents, effective selection of promising fenugreek genotypes and also have paramount importance in formulating plant model in selection of segregating generations.

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