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Diversity and Principal component analysis in Fenugreek (*Trigonella foenum-graecum* L.) genotypes

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Abstract

A study comprising of 71 genotypes of fenugreek was laid out in ABD. Enough variability was observed among the genotypes under study as revealed by ANOVA. Genetic divergence analysis grouped the genotypes into three clusters based on 10 phenotypic traits. Cluster III (31) contains maximum number of genotypes followed by cluster I (26) and cluster II (14). Maximum inter cluster distance was obtained between cluster I and III. This revealed the presence of wider genetic diversity between two clusters and crossing between the members will results in high heterosis. PCA revealed that five main components contributed a cumulative variance of 76.51%, out of which PC 1 contributed (28.1%), followed by PC 2 (15.11%). A contribution of 11.77%, 10.92% and 10.61% was made from PC 3, PC 4 and PC 5, respectively. The bi-plot of PC-I and PC-II showed presence of a considerable variability through dispersion pattern of studied genotypes.

Keywords: Fenugreek, genetic diversity, cluster analysis and PCA

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Introduction

India is renowned as the "Land of Spices." The genus Trigonella belongs to the family Fabaceae and sub-family Papilionaceae [1], encompassing two economically significant species: *Trigonella foenum-graecum*, commonly known as "fenugreek" or "methi," and *Trigonella corniculata*, known as "kasthuri methi." Fenugreek is cultivated as fodder and spice to enhance the flavor and nutritional content of the food. Believed to originate from Iran and North India [2], fenugreek is extensively grown in India, especially in Rajasthan (>80%), Madhya Pradesh, Gujarat, Tamil Nadu, Andhra Pradesh, and Uttar Pradesh. Fenugreek seeds and leaves are utilized in the preparation of Ayurvedic medicines due to their mucilaginous, demulcent, diuretic, carminative, astringent, and aphrodisiac properties. The low productivity of fenugreek in India can be primarily attributed to the lack of suitable high-yielding varieties for different agro-ecoclimatic regions and inadequate crop management practices. In any crop breeding program, germplasm plays a crucial role as it provides the necessary variability for various traits. Proper screening and evaluation of germplasm lines help assess their potential as suitable parents for use in varietal development programs and enhancing fenugreek productivity. Selection and hybridization approaches are commonly employed to achieve quantitative improvements and desired enhancements. In conventional plant breeding, morphological markers are pivotal tools for studying genetic variability [3]. These markers offer a straightforward and cost-effective means to assess variability, providing valuable preliminary insights [4]. Biometrical techniques such as correlation analysis, path coefficient analysis, principal component analysis, and D^2 analysis are commonly employed to estimate genetic variability across crops. Cluster analysis is another method used to group genotypes based on shared characteristics, aiding in the identification of patterns and the understanding of genetic diversity. To assess the existing genetic diversity among the genotypes D^2 technique of Mahalanobis (1936) is intensively and widely used in crop improvement programmes. For knowing the source of genes for particular trait within the available germplasm the evaluation of genetic diversity present within the germplasm is also very important. Principal Component Analysis (PCA) is a robust statistical method used to analyse complex datasets and unveil underlying patterns in multivariate data [5]. By reducing the dimensionality of data while retaining essential variation, PCA allows for the visualization of relationships among variables and samples [6]. PCA achieves this by transforming correlated variables into a smaller set of uncorrelated variables called principal components. These components are ordered by the amount of variance they explain, with the first few components capturing the majority of the variability present in the original data. By examining the loadings of variables on these principal components, researchers can discern which traits are most influential in distinguishing genotypes from one another. Therefore, the present study was carried out to estimate the nature and magnitude of genetic diversity present

fenugreek genotypes and to enhance genotype selection processes and ultimately advance breeding programs by focusing on traits that significantly impact fenugreek performance and quality.

Materials and Methods

The experiment consisted of a total of 71 fenugreek genotypes, which included four reference checks RMt-143, RMt-305, AFg-2 and AFg-3 (**Table 1**). These genotypes were received from different geographical regions and were evaluated in augmented design with 5 blocks during *Rabi*-2022-23 at ARS, Mandor, Agriculture University, Jodhpur. Each genotype was accommodated in single row plot of 3.0 m length with a spacing of 50 cm between row and 10 cm between plants. A total of 10 phenotypic traits were observed including SY- seed yield (kg/ha), BY- Biological yield (kg/ha), BP- Number of branches/plant, DF- Days to 50% flowering, DM- Days to maturity, PH- plant height (cm), PD- pods/plant, SP-seeds per pod, PL-pod length (cm), TW- Test weight (1000 seed weight in g). The observation on phenotypic traits were recorded from five randomly selected plants for each genotype except for DF and DM which was recorded on a plot basis. Multivariate analysis of D^2 was done for all fifteen characters by using Mahalanobis Statistics (1936) [7] and different clusters were formed by following the Ward's method as described by Rao (1952) [8]. PCA analysis was performed using R STUDIO software (2023.03.1+446).

Table 1 List of genotypes used in the present study and their source

Sn.	Genotypes Names	Source	Sn.	Genotypes Names	Source
1	EC-510632	NBPGR Jodhpur	37	UM-9	SKNAU Jobner
2	EC-510630	NBPGR Jodhpur	38	UM-10	SKNAU Jobner
3	EC-510565	NBPGR Jodhpur	39	UM-11	SKNAU Jobner
4	EC-510609	NBPGR Jodhpur	40	UM-12	SKNAU Jobner
5	IC-373433	NBPGR Jodhpur	41	UM-13	SKNAU Jobner
6	EC-510616	NBPGR Jodhpur	42	UM-14	SKNAU Jobner
7	EC-510617	NBPGR Jodhpur	43	UM-15	SKNAU Jobner
8	EC-510577	NBPGR Jodhpur	44	UM-17	SKNAU Jobner
9	EC-510608	NBPGR Jodhpur	45	UM-18	SKNAU Jobner
10	EC-510712	NBPGR Jodhpur	46	UM-19	SKNAU Jobner
11	EC-510623	NBPGR Jodhpur	47	UM-20	SKNAU Jobner
12	IC-333183	NBPGR Jodhpur	48	UM-21	SKNAU Jobner
13	EC-510588B	NBPGR Jodhpur	49	UM-22	SKNAU Jobner
14	EC-510740	NBPGR Jodhpur	50	UM-23	SKNAU Jobner
15	EC-510615	NBPGR Jodhpur	51	UM-24	SKNAU Jobner
16	EC-510737	NBPGR Jodhpur	52	UM-25	SKNAU Jobner
17	EC-510715	NBPGR Jodhpur	53	UM-26	SKNAU Jobner
18	EC-510590	NBPGR Jodhpur	54	UM-27	SKNAU Jobner
19	EC-510709	NBPGR Jodhpur	55	UM-28	SKNAU Jobner
20	EC-510711	NBPGR Jodhpur	56	UM-29	SKNAU Jobner
21	EC-510580	NBPGR Jodhpur	57	UM-30	SKNAU Jobner
22	EC-510713	NBPGR Jodhpur	58	AM-71	NRCSS Ajmer
23	EC-510727	NBPGR Jodhpur	59	AM-108	NRCSS Ajmer
24	EC-510583A	NBPGR Jodhpur	60	AM-280	NRCSS Ajmer
25	EC-510710	NBPGR Jodhpur	61	AM-281	NRCSS Ajmer
26	EC-510576	NBPGR Jodhpur	62	AM-282	NRCSS Ajmer
27	EC-510604	NBPGR Jodhpur	63	AM-284	NRCSS Ajmer
28	IC-333214	NBPGR Jodhpur	64	AM-291	NRCSS Ajmer
29	UM-1	SKNAU Jobner	65	AM-293	NRCSS Ajmer
30	UM-2	SKNAU Jobner	66	AM-310	NRCSS Ajmer
31	UM-3	SKNAU Jobner	67	AM-323	NRCSS Ajmer
32	UM-4	SKNAU Jobner	68	RMt-143 (Check-1)	SKNAU Jobner
33	UM-5	SKNAU Jobner	69	RMt-305 (Check-2)	SKNAU Jobner
34	UM-6	SKNAU Jobner	70	AFg-2 (Check-3)	NRCSS Ajmer
35	UM-7	SKNAU Jobner	71	AFg-3 (Check-4)	NRCSS Ajmer
36	UM-8	SKNAU Jobner			

Results and Discussion

The significant treatment mean square revealed presence of adequate amount of variability among the genotype for all the characters studied [9-11]. Although the analysis of variance revealed presence of variability among the genotypes, but to know the extent of genetic diversity present among the genotypes, cluster analysis was performed. On basis of observed distance among genotypes, 71 genotypes were grouped into 3 clusters. Cluster III contains maximum number of genotypes i.e. 31 followed by 26 in cluster I and 14 in cluster II (**Table 2**).

Table 2 Clustering pattern of fenugreek genotypes

Cluster	No. of genotypes	Name of genotypes
1	26	AM-280, AM-281, AM-282, AM-284, AM-291, AM-293, AM-310, AM-323, IC-333214, IC-373433, EC-510565, EC-510580, EC-510588B, EC-510590, EC-510604, EC-510608, EC-510609, EC-510615, EC-510616, EC-510617, EC-510623, EC-510630, EC-510710, EC-510727, EC-510737, RMt-305
2	14	UM-13, UM-14, UM-20, UM-22, UM-23, UM-24, UM-26, UM-27, UM-28, UM-29, UM-30, AM-71, AM-108, EC-510632
3	31	UM-1, UM-2, UM-3, UM-4, UM-5, UM-6, UM-7, UM-8, UM-9, UM-10, UM-11, UM-12, UM-15, UM-17, UM-18, UM-19, UM-21, UM-25, EC-510576, EC-510577, EC-510583, EC-510709, EC-510711, EC-510712, EC-510713, EC-510715, EC-510740, IC-333183, RMt-143, AFg-2, AFg-3

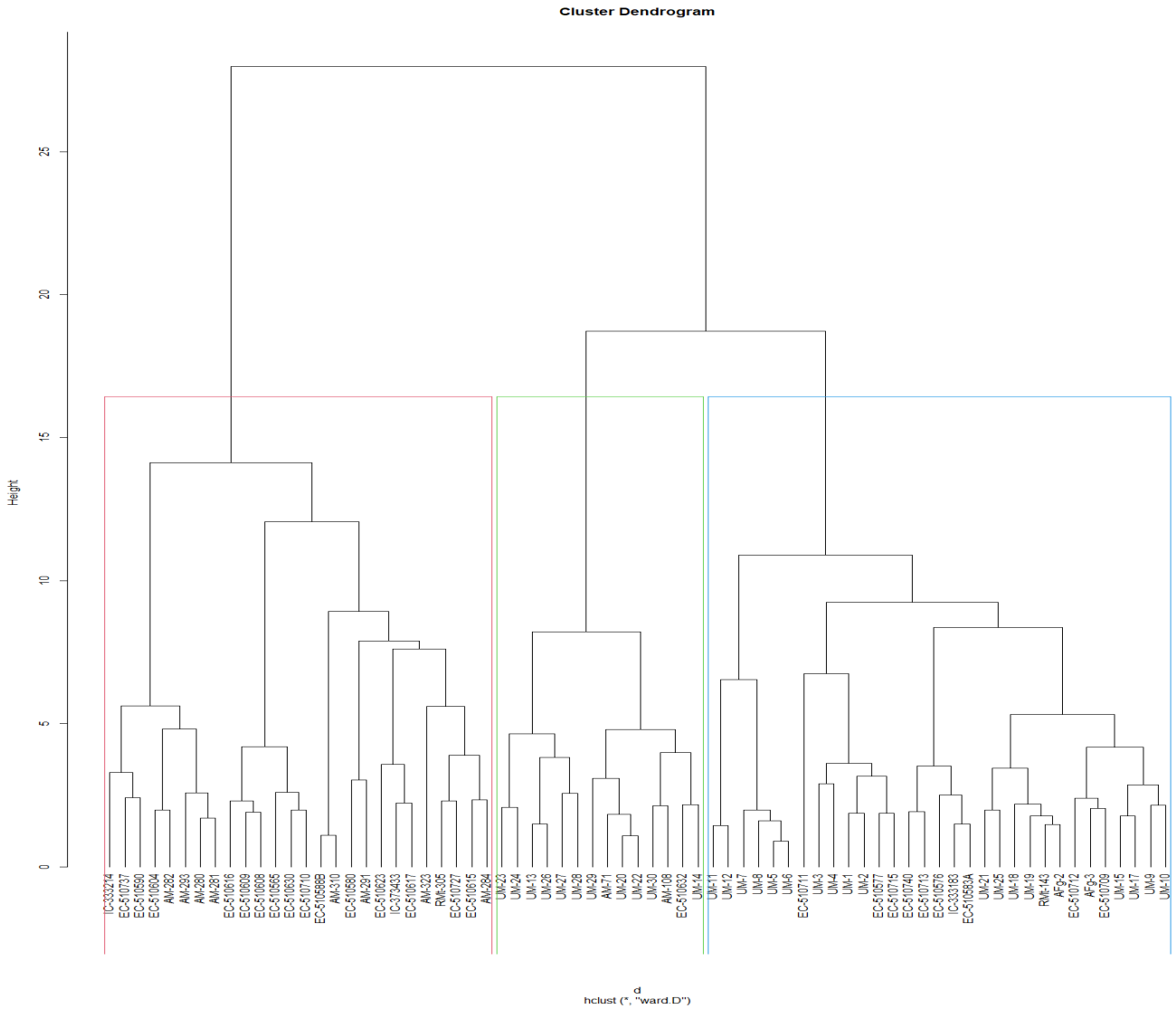

Figure 1 Dendrogram showing the clustering pattern of different fenugreek genotypes

Taking into consideration (**Table 3**) average inter cluster values, maximum was obtained between cluster I and III. This result revealed the presence of wider genetic diversity between these two clusters and crossing between the members will helps in exploiting high heterosis. While at intra cluster level, maximum values were recorded for cluster III (D^2= 4.22) followed by cluster II (D^2=3.57) and cluster I (D^2=3.52). This result revealed that Cluster III showed maximum variability present within the cluster because it included exotic collections from NBPGR, Jodhpur, germplasms from SKNAU, Jobner and three checks out of Four (**RMt-143**, **AFg-2**, **AFg-3).** In the dendrogram, genotypes within the same cluster exhibit less variation compared to those in different clusters, as shown in **Figure 1**. Similar findings were previously reported in Sorghum [12]. This similarity in their genetic makeup present despite of being originated at different location or environments. so clustering pattern revealed that genotypes from same origin may present in the same cluster (AM-280 and AM-281) *i.e.* cluster I or may be in the different clusters (AM-280 and AM-108) *i.e.* cluster I and cluster II (Table 2) Similar findings were previously reported in groundnut [4, 13] and onion [14].

Table 3 Average of intra (diagonal) and inter-cluster genetic distance in 71 genotypes of Fenugreek

Clusters	1	2	3
1	3.520446		
2	4.289724	3.57431	
3	5.511005	4.746513	4.22438

Clusters means for different morphological traits studied revealed presence of wide range differences among clusters with respect to these traits as shown in **Table 4**. The average cluster means for different characters demonstrated that cluster I has highest mean for seed yield (1434.75), biological yield (4025.64) and plant height (67.28) with minimum test weight (12.53). Minimum seed yield (328.20), minimum biological yield (1203.59) and minimum plant height (54.89) along with maximum test weight (13.73) has showed by cluster III.

Table 4 Cluster means of 10 different morphological traits of 71 genotypes

Cluster	SY	BY	BP	DF	DM	PH	PD	SP	PL	TW
1	1434.75	4025.64	6.85	48	119	67.28	39	17	8.90	12.53
2	1266.72	3023.19	7.47	47	128	60.96	56	16	10.57	13.36
3	328.20	1203.59	9.56	47	133	54.89	53	14	8.47	13.73

PCA was calculated for a dataset comprising 71 fenugreek genotypes, including 10 phenotypic traits. Components having an eigen value more than one were selected and the primary components contributing most significantly to the overall variation were identified through a scree plot (**Figure 2**).

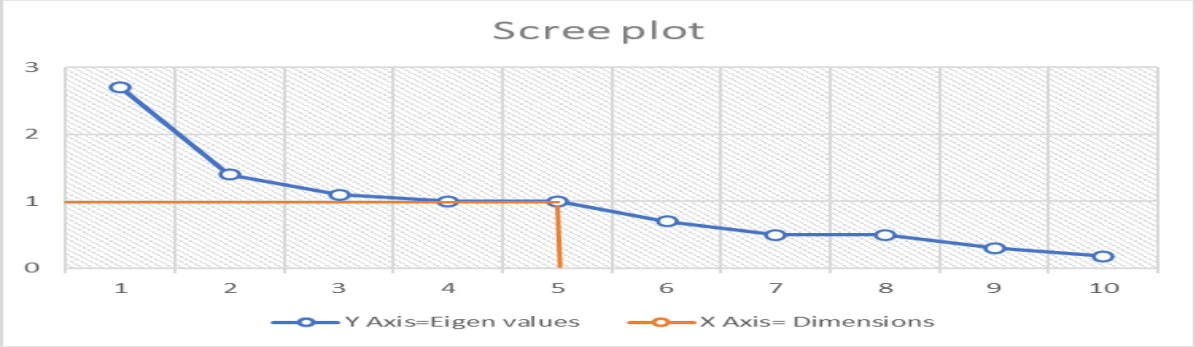

Figure 2 Scree plot depicted eigen values for respective principal components

These five main components contributed a cumulative variance of 76.51%, out of which PC 1 contributed (28.1%), followed by PC 2 (15.11%). A contribution of 11.77%, 10.92% and 10.61% was made from PC 3, PC 4 and PC 5, respectively (**Table 5** and **Table 6**).

Table 5 Variance attributable to principal components

Variances	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.67	1.22	1.08	1.04	1.02
Eigen values	2.7	1.4	1.1	1.0	1.0
Proportion of variance (%)	28.1	15.11	11.77	10.92	10.61
Cumulative proportion (%)	28.1	43.21	54.98	65.90	76.51

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

Traits	PC1	PC2	PC3	PC4	PC5
SY	-0.437	-0.214	0.066	-0.366	0.076
BY	-0.515	-0.048	0.016	-0.342	0.068
BP	0.317	0.126	0.373	-0.466	0.211
DF	-0.117	-0.269	-0.531	0.073	0.552
DM	0.304	-0.111	-0.661	-0.154	-0.191
PH	-0.389	-0.269	-0.003	-0.068	-0.118
PD	0.301	-0.481	0.014	-0.220	-0.055
SP	-0.308	0.334	-0.151	0.319	-0.338
PL	0.018	-0.455	0.076	-0.037	-0.643
TW	0.011	-0.478	0.329	0.589	0.244

The bi-plot of PC-I and PC-II showed presence of a considerable variability through dispersion pattern of studied genotypes. Similar results were previously reported in oat, dolichos bean [15], oat [16] and fenugreek [17]. The biplot visualization revealed that the diverse genotypes located farther from the point of origin (**Figure 3**). The traits with the longest vectors in the direction of PC1 were the most influential *i.e.* PH, SY and BY, while PC2 was favourably impacted by PD, BP, DM, DF. PH and SY are closely aligned, suggesting that they were positively correlated. DM and BM were found closer to each other, indicating presence of a positive correlation. The opposite directions of BY and PD suggest a negative correlation between these traits. Traits like DM and DF pointed towards the negative side of PC2, indicated that higher values of these traits were associated with lower PC2 scores. PC1 can be interpreted as a dimension of overall crop vigor or productivity. Breeding programs that aim to maximize "Plant height", "Seed yield" and "Biological yield" should focus on moving genotypes towards the positive end of PC1. PC2 can be interpreted as a dimension distinguishing between early vs. late maturity traits and yield structure. Breeding programmes with the objective of traits early flowering or a higher number of pods per plant can use the insights from PC2 to select appropriate genotypes [15-17].

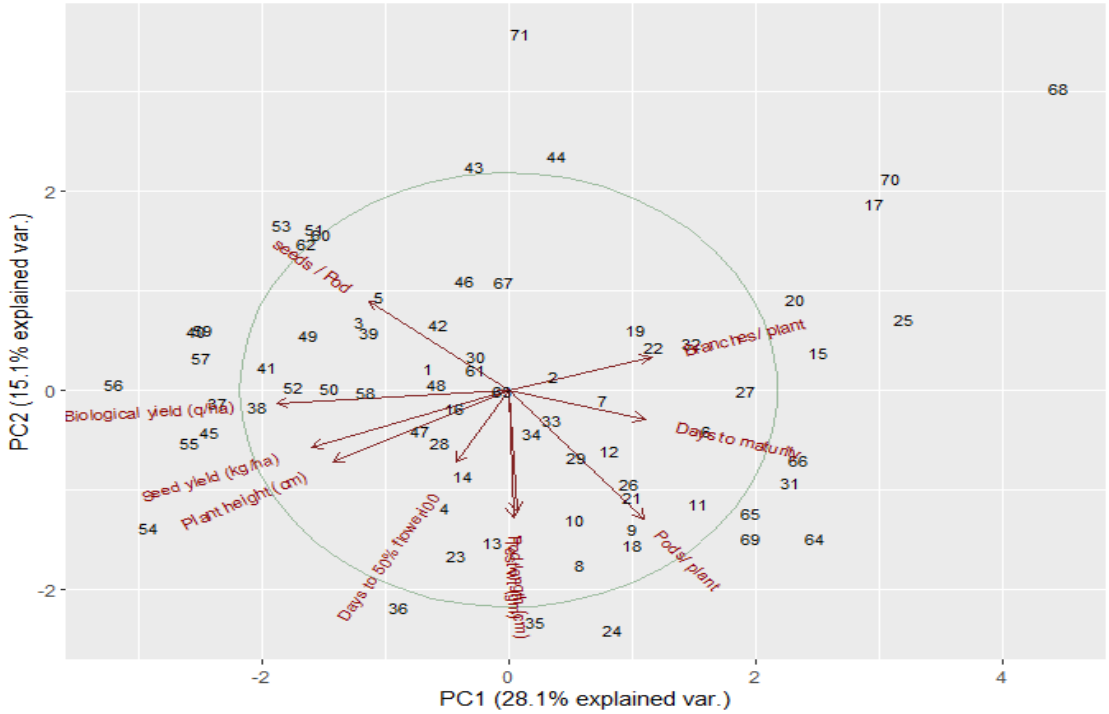

Figure 3 Biplot showing relationships between traits and genotypes in a multivariate dataset

Conclusion

The study revealed presence of substantial variability among fenugreek genotypes for various traits, confirmed by both analysis of variance and cluster analysis. Genotypes were grouped into three clusters, with cluster III showing the highest intra-cluster variability, indicating diverse genetic backgrounds within each group. While principal component

analysis highlighted significant contributors to trait variation, with PC1 primarily influenced by traits like plant height, seed yield, and biological yield, while PC2 reflected traits associated with maturity and yield structure. This analysis provides valuable insights for breeding programs aiming to enhance specific agronomic traits in fenugreek genotypes, guiding selection strategies for improved crop performance and adaptation.

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