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STUDIES ON CLUSTER ANALYSIS OF FABA BEAN GENOTYPES (*VICIA FABA* L.) IN ANDHRA PRADESH, INDIA

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ABSTRACT

Present investigation on faba bean was conducted to assess genetic diversity among the genotypes of faba bean using Mahalanobis D^2 analysis. D^2 analysis grouped twenty genotypes into 5 distinct clusters. The highest inter cluster distance was recorded between cluster IV and cluster V. The lowest inter cluster distance was observed between clusters I and II. Cluster IV exhibited the maximum intra cluster distance. Among the clusters, cluster I was largest consisting of seven genotype followed by cluster III with six genotypes. Cluster IV consisting 5 genotypes while clusters II and V were solitary clusters. Cluster IV exhibited high cluster mean values for the majority of characters studied. Among the twenty-four traits studied, ascorbic acid content contributed maximum followed by non-reducing sugars, pod yield per plant, days to first harvest, protein content, pod yield, seed weight per pod, total sugars, germination percentage and pod yield per plot. This indicated that genotypes from distinct clusters were genetically more divergent and can be effectively used as parents in hybridization programs and selecting clusters that exhibited high cluster means can be effective in breeding programme.

Key words : Faba bean, Cluster analysis, Cluster distance, Mahalanobis D^2 technique, Tocher's method.

Introduction

Faba bean is a legume crop, botanically known as *Vicia faba* L. belongs to Fabaceae family (Harlan, 1969). This crop is known by many names, viz., Fava bean, Horse bean, Windsor bean, Tick beans (small types) and Bakela in Ethiopia, most of which refer to a particular subgroup rather than the whole species (Zohary and Hopf, 1973). In India, it is commonly called as Kala Matar and Bakala (Singh *et al.*, 2013). It is among the oldest crops in the world and worldwide it is the third most important grain legume after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) regarding area and production (Mihailovic *et al.*, 2005). Faba bean is grown in 66 countries with a global area of 0.27 M ha, production of 1.83 M tons and average productivity of 6.73 t/ha. Algeria ranked first regarding area and production with 0.032 M Ha and 0.287 M tons, respectively, followed by China with 0.187

M tons of production (FAOSTAT, 2023). Other important producers are Northern Europe, the Mediterranean region, Ethiopia, Central and East Asia and Latin America. Faba bean is considered as a minor, unutilized and underutilized crop in India due to its limited cultivation area and low productivity. It is cultivated in different states and considerable regions, particularly in the states of Uttar Pradesh, Bihar, Punjab, Haryana, Jammu & Kashmir, Rajasthan, Karnataka and Madhya Pradesh (Singh *et al.*, 2010).

Genetic diversity is important for selecting parents to recover transgressive segregants. For genetic diversity, germplasm is the reservoir which is quite often exploited to develop new improved genotypes. There is tremendous variability in this crop from nation to continent. So to have knowledge about inter cluster and intra cluster D^2 statistics is a sound statistical procedure in quantifying the degree

of divergence between different genotypes. Thus it helps in selection of parents which are diverse and suitable and assessment of genetic diversity in germplasm concerning yield and yield attributing characters is a prerequisite for a successful breeding programme.

Materials and Methods

Present investigation was conducted at College of Horticulture, Venkataramannadugem, Dr. Y.S.R. Horticultural University, West Godavari District, during *Rabi* season of 2024-25 to assess genetic divergence in 20 genotypes of faba bean. Statistical design followed was Randomized Block Design (RBD) with three replications. The plot size was 2.5 m length and 1 m width having 5 rows and row-to-row distance was 45 cm and plant-to-plant distances was maintained at 30 cm. Five randomly selected plants were used to record the data for twenty-four parameters. Recommended agronomic practices were followed to raise a healthy crop. Mahalanobis (1936) D^2 statistics was used to quantify the degree of divergence. Tocher's method was used to group genotypes in clusters following (Rao, 1952).

The following observations were recorded and presented hereunder.

Germination percentage : Germination percentage was calculated by dividing number of seeds germinated by number of seeds sown and then multiplying by 100 for each plot in each replication and mean value was taken as the germination percentage.

Plant height (cm) : Plant length was measured at final harvest from ground level to the tip of the main axis for 5 plants and the average was taken and expressed in centimeters.

Number of branches per plant : Total number of branches arising from main axis of each selected plant at final harvest was recorded for five plants and average was taken and expressed in number.

Number of nodes per plant : Number of nodes on each of the randomly selected five plants was counted, and the average value was calculated.

Days to 50% flowering : Days required from sowing to flowering in 50 percent of plants in a plot were counted to represent the days taken to 50 percent flowering for five plants and the average was taken.

Days to first harvest : Days taken to the first harvest were counted from the date of sowing to first harvest from randomly selected five plants for each plot and the average was calculated.

Number of pods per plant : Total number of pods

per plant was counted for five tagged plants at the time of each harvest and the average was taken.

Pod length (cm) : Length of the pod was measured in centimeters with the help of a scale from the point it was attached to the bunch up to the tip of the pod and the average length was computed. Ten pods from tagged plants of each plot were selected for this purpose at the time of harvesting and the average was taken.

Pod yield per plant (g) : Weight of fresh marketable pods from five plants at each harvesting of six pickings was recorded and the average pod yield per plant was worked out for each treatment and expressed in grams.

Pod yield per plot (kg) : Weight of pods from all plants in each plot at each harvesting of six pickings was recorded and cumulative yield was recorded.

Pod yield (t/ha) : As per the spacing of plants from row to row and plant to plant, total number of plants per hectare were calculated with pod yield per plant in each genotype and expressed in tonnes.

Number of seeds per pod : Total number of green seeds per pod, averaged over ten randomly selected pods in each plant was recorded and the average was expressed in number.

Seed yield per plant (g) : Five randomly selected plants from each entry were taken and their dried seeds were thrashed, winnowed and then weighed and averaged to record seed yield per plant in grams.

Seed weight per pod (g) : Weight of the green seeds was measured in grams with the help of a weighing balance from five pods and the average weight was computed. Five pods from tagged plants of each plot were selected for this purpose at the time of harvesting and the average was taken.

100 seed weight (g) : The weight of a hundred seeds selected randomly from each plant was expressed in grams.

Pod to seed ratio : Weight of pods and their seeds was measured from ten pods of tagged plants in each plot. Then weight of the pod was divided by weight of the seed to get the pod to seed ratio.

Crop duration : Number of days was counted from the date of sowing to the date of maturity of plant, i.e., when 90-95% of pods turn black.

Ascorbic acid content (mg/100 g) : Ascorbic acid content of faba bean pod samples was determined by 2,6-dicholophenol indophnol titration method as detailed by Ranganna (1986).

TSS ($^{\circ}$ Brix) : Total soluble solids (TSS) of pods

were recorded with the help of a digital refractometer (Atago, Pal-a, Made in Japan) by placing a drop of sample in the form of juice at room temperature. Average total soluble solids were calculated and expressed in degrees Brix.

Protein content (mg/100 g) : Protein content of the seeds under investigation was determined following the method of Lowry *et al.* (1951).

Titrateable acidity (%) : Titrateable acidity was analyzed by titrating a known aliquot of the sample against standard 0.1 N NaOH using phenolphthalein as an indicator and was expressed as per cent citric acid.

$$\text{Total acidity (\%)} = \frac{\text{Titration value} \times \text{Normality of alkali (0.1)} \times \text{Volume made up} \times \text{Equivalent weight of acid}}{\text{Weight or Volume of sample taken for estimation} \times 1000} \times 100$$

Reducing sugars (%) : Percentage of reducing sugars in the faba bean was determined by Lane and the Eyon method (1965).

Non reducing sugars (%) : Percentage of non reducing sugars in faba bean pulp was calculated by subtracting percentage of reducing sugars from the percentage of total sugars.

Total sugars (%) : Percentage of total sugars in faba bean was determined by Lane and Eyon method (1965).

Genetic divergence analysis

Data collected on different characters were analyzed using Mahalanobis's D^2 analysis (1936) to determine the genetic divergence among the genotypes.

D^2 value between the i^{th} genotypes for 'p' characters was calculated as

$$D_{ij}^2 = \sum_{t=1}^p (Y_i^t - Y_j^t)^2$$

Where,

Y_{it} is the uncorrelated mean value of the i^{th} genotype for 't' characters

Y_{jt} is the uncorrelated mean value of the j^{th} genotype for 't' characters

D_{ij}^2 is D^2 between the i^{th} and j^{th} genotypes.

For calculating the D^2 values, variance and covariance were calculated. Genotypes were grouped into different clusters. The population was arranged in order of their relative distances from each other. For including a particular population in clusters, a level of D^2 was fixed by taking maximum D^2 values between any two populations in the first row of the table, where D^2

values were arranged in increasing order of magnitude.

Results and Discussion

Grouping of genotypes into different clusters (D^2 analysis)

Twenty genotypes were grouped into five distinct clusters based on D^2 values as detailed in Table 1 and Fig. 1. Cluster I was the largest, containing seven genotypes (VRFB-II, VRFB-XI, VRFB-III, VRFB-V, Sambhat-75, VRFB-I and Basabeer). Clusters II and V each included a single genotype (Marawi and VRFB-VI, respectively). Cluster III comprised six genotypes (Sudan VI, VRFB-X, VRFB-VII, VRFB-VIII, Vikranth and VRFB-IX). The genotypes VRFB-IV, HFB-01, Hashbenge, HFB-02 and Hudeiba 93 were assigned to cluster IV.

The distribution pattern of different faba bean genotypes from different eco-geographical regions into 5 different clusters with different divergence values revealed that the geographical origin was responsible for this genetic diversity. The main source responsible for this genetic diversity other than geographical origin were natural selection, exchange of breeding material, genetic drift and environmental variation. These results are in

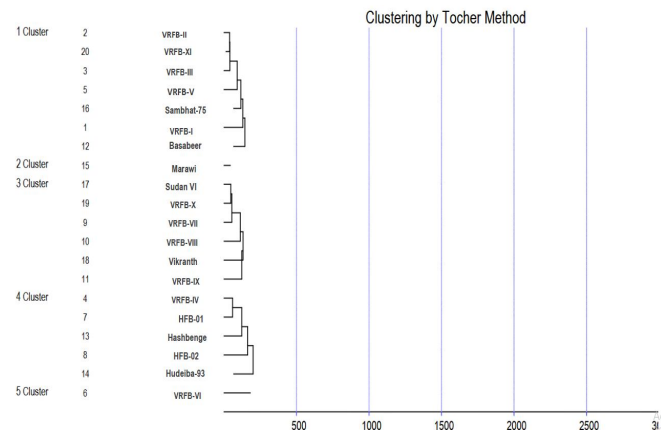


Fig. 1 : Dendrogram of clustering pattern of 20 genotypes of faba bean.

Table 1 : Clustering pattern of faba bean genotypes.

Cluster	Number of genotypes	Genotypes
I	7	VRFB-II, VRFB-XI, VRFB-III, VRFB-V, Sambhat-75, VRFB-I, Basabeer.
II	1	Marawi
III	6	Sudan VI, VRFB-X, VRFB-VII, VRFB-VIII, Vikranth, VRFB-IX
IV	5	VRFB-IV, HFB-01, Hashbenge, HFB-02, Hudeiba 93
V	1	VRFB-VI

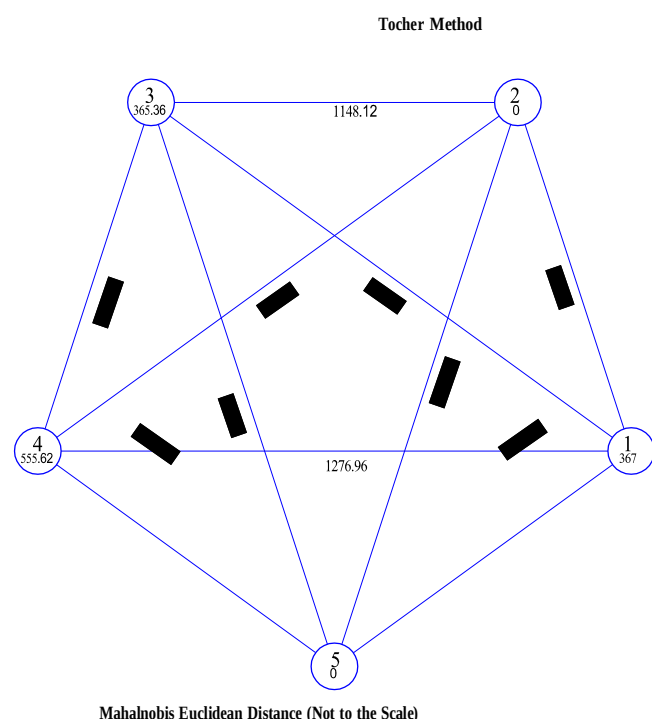


Fig. 2 : Diagram illustrating the clustering pattern in faba bean genotypes.

accordance with Susant and Vijay (2081) in dolichos bean.

Average intra and inter cluster distances

Highest inter cluster distance was recorded between cluster IV and cluster V (3313.76), indicating maximum genetic divergence and suggesting their potential use in hybridization programs to exploit heterotic advantage. In contrast, lowest inter cluster distance was observed between clusters I and II (559.19), highlighting their close genetic similarity. Since clusters II and V had only one genotype each, their intra cluster distance was 0.00, whereas cluster IV exhibited maximum intra cluster distance (555.62). (Table 2 and Fig. 2).

In the present investigation, the inter cluster distance was greater than the intra-cluster distance, indicating that genotypes within each cluster were relatively homogeneous, while heterogeneity existed between clusters. This suggested that genotypes from distinct clusters were genetically more divergent and can be effectively used as parents in hybridization programs. For breeding purposes, selecting genotypes from clusters with higher inter cluster distances, particularly those also exhibiting higher pod yield, was recommended to achieve desirable segregants in recombination breeding programs.

Cluster means of characters

Cluster mean values for all the characters in the clusters are presented in Table 3. For majority of the

Table 2 : Average intra and inter-cluster D^2 values for five clusters of faba bean genotypes.

Cluster number	I	II	III	IV	V
I	367.00	559.19	979.45	1276.96	1035.31
II		0.00	1148.12	747.71	1384.31
III			365.36	2057.99	939.91
IV				555.62	3313.76
V					0.00

characters studied Cluster IV presented high cluster mean values, including germination percentage (94.13), plant height (71.58 cm), number of branches per plant (4.95), number of nodes per plant (40.36), number of pods per plant (45.37), pod length (5.08 cm), pod yield per plant (113.79 g), pod yield per plot (1.57 kg), pod yield per hectare (8.43 t/ha), number of seeds per pod (3.77), seed yield per plant (49.76 g), seed weight per pod (2.91 g), 100 seed weight (29.76 g) crop duration (133.92), TSS (5.17%), reducing sugars (1.74%) and total sugars (2.08%). In Cluster II for pod to seed ratio (2.22) and titratable acidity (0.73%), whereas low cluster mean value for days to 50% flowering (40.20). In Cluster III high cluster mean values are exhibited by ascorbic acid content (18.35%), non-reducing sugars (0.41%) and protein content (25.83 mg/100 g). In cluster V, out of 24 characters, 19 characters exhibited low cluster mean values. It reported low cluster mean value for days to first harvest (61.00) whereas TSS ($^{\circ}$ Brix) reported low cluster mean value in both clusters II and V with 4.40 ($^{\circ}$ Brix).

Mean values of clusters can be effectively utilized depending on the specific objectives of the breeding program. For instance, if the breeder's goal was to improve earliness in the crop, priority should be given to selecting genotypes from clusters that show minimum mean values for trait days to 50% flowering and days to first harvest. For improving other parameters, selection should focus on clusters exhibiting maximum mean values for the respective traits. These results are in line with the work on fada bean by Singh *et al.* (2015)

Relative contribution of different traits towards divergence

Based on the relative contribution of characters towards diversity, ascorbic acid content contributed maximum followed by non reducing sugars, pod yield per plant, days to first harvest, protein content, pod yield, seed weight per pod, total sugars, germination percentage and pod yield per plot.

Knowing significance of genetic distance and relative

Table 3 : Mean values of clusters for twenty-four characters of faba bean genotypes.

S. no.	Character	Cluster number				
		I	II	III	IV	V
1	Germination (%)	85.57	90.96	88.31	94.13	80.45
2	Plant height (cm)	59.49	67.00	63.30	71.58	50.52
3	Number of branches per plant	3.44	4.07	3.88	4.95	2.30
4	Number of nodes per plant	28.52	33.33	31.73	40.36	25.40
5	Days to 50% flowering	48.96	40.20	46.90	46.88	46.00
6	Days to first harvest	64.35	71.80	68.72	74.19	61.00
7	Number of pods per plant	35.01	41.80	37.57	45.37	29.27
8	Pod length (cm)	4.78	4.96	5.02	5.08	4.34
9	Pod yield per plant (g)	76.33	94.91	85.68	113.79	63.04
10	Pod yield per plot (kg)	0.92	1.18	1.12	1.57	0.58
11	Pod yield (t/ha)	5.65	7.04	6.34	8.43	4.67
12	Number of seeds per pod	3.18	3.53	3.44	3.77	2.93
13	Seed yield per plant (g)	30.09	45.22	34.71	49.76	25.80
14	Seed weight per pod (g)	2.37	2.04	2.04	2.91	2.25
15	100 seed weight (g)	26.77	24.29	24.63	29.76	25.75
16	Pod to seed ratio	1.98	2.22	2.14	1.82	2.03
17	Crop duration	120.34	131.87	124.28	133.92	116.67
18	Ascorbic acid content (mg/100 g)	15.02	14.52	18.35	14.72	16.39
19	TSS (°Brix)	5.08	4.40	4.77	5.17	4.40
20	Protein content (mg/100 g)	22.84	19.93	25.83	24.54	19.46
21	Titrateable acidity (%)	0.70	0.73	0.72	0.71	0.64
22	Reducing sugars (%)	1.27	1.51	1.21	1.74	0.95
23	Non reducing sugars (%)	0.33	0.26	0.41	0.34	0.31
24	Total sugars (%)	1.60	1.77	1.62	2.08	1.26

contribution of individual traits to total divergence, the present study suggested that selecting parents for hybridization in faba bean improvement should not only focus on genotypes with high divergence but also prioritize those expressing traits with the greatest contributions to that divergence. When selecting parents based on D^2 values, it was particularly important to consider the relative influence of each trait on the observed divergence, as traits with higher contributions offer greater potential for selection and breeding progress. Similar findings were reported by Singh *et al.* (2021), Chaudhary *et al.* (2018), Sharifi and Aminpana (2014) and Chaubey *et al.* (2012) along with that Verma *et al.* (2015) in french bean.

Conclusion

Mahalanobis D^2 cluster analysis carried out on twenty genotypes of faba bean classified them into five distinct clusters, thereby revealing the presence of considerable genetic divergence among the genotypes. Highest inter cluster distance was recorded between cluster IV and cluster V along with that cluster VI reported maximum cluster mean values among the clusters.. Hence, selecting

genotypes from these clusters for breeding programme could be effective. Highest cluster mean values are reported for cluster VI.

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