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Diversity Analysis for Phenotypic and Qualitative Traits of Broad Bean (*Vicia faba* L.): An Underutilized Vegetable Crop of India by Multivariate Analysis

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ABSTRACT

Background: Broad bean (*Vicia faba* L.) is an important protein rich underutilized vegetable crop cultivated for its tender green pods and dry seeds. The present study was carried out to assess the genetic divergence among the broad bean genotypes collected for developing high-yielding cultivars.

Methods: The experimental material consisted of 20 broad bean genotypes, raised in randomized block design with three replications at Horticultural Research Station, Nilgiris, Tamil Nadu, India during *Kharif* 2023. Data concerning 12 quantitative traits and 7 qualitative traits underwent examination using Mahalanobis D^2 statistic, principal component analysis and stepwise multiple regression analysis.

Result: The cluster analysis categorized the genotypes into nine clusters, with the highest number of genotypes in Cluster II (4) and the lowest in Clusters VII, VIII and IX (1 each). Cluster IV displayed the highest intra-cluster distance (D^2=3358.2), signifying substantial variation among the genotypes within this cluster. The largest inter-cluster distance (D^2=316.9) was observed between Clusters I and VII, indicating significant divergence from other clusters, suggesting that genotypes within these clusters could be valuable as diverse parents for hybridization and selection. The PCA combined with stepwise multiple regression analysis highlighted the primary variations and traits influencing pod yield.

Key words: Mahalanobis D^2, PCA, Regression analysis.

INTRODUCTION

The broad bean (*Vicia faba* L.; Family: Fabaceae) is an important underutilized winter sown legume crop and serves as a valuable source of protein (20-30%) for both human and animal consumption (Qahtan *et al*., 2021). The cultivation of broad beans traces its roots to the Stone Age, marking it as one of the earliest crops to be domesticated (Duc *et al*., 2010). It is known by several other names, bakla bean (Hindi name), faba bean, horse bean, winter bean, English bean, field bean and Windsor bean (Singh *et al*., 2017), whereas in Tamil Nadu it is recognized as "double beans". They are well-suited to a wide range of climatic conditions and contribute to the overall sustainability of cropping systems. Both green and dry cotyledons of broad bean are consumed as vegetable and pulse crop respectively in the hilly regions of India (Gasim and Link, 2007). In the Nilgiris District of Tamil Nadu, broad beans are used in culinary preparations during important occasions and festival times.

Out of over 50 countries that produce broad beans, approximately 90% of the production is centred in the regions of Asia, European Union and Africa (FAO, 2020). In India, despite its agricultural potential for producing high yields and protein, it is regarded as an underutilized crop and cultivation in India is confined to limited acreage in Bihar, Madhya Pradesh and some part of Uttar Pradesh (Singh *et al*., 2012). Lack of a good cultivar along with proper package of practises may be hindering the expansion of this crop (Raja *et al*., 2023). By adopting improved, nutritionally rich and stable genotypes in cultivation, it is possible to transform the status of this crop from being underutilized to a potentially high-value crop.Therefore, there is a need to evaluate and identify the genotype that consistently delivers both high yield and quality.

Harnessing the genetic diversity within the gene pool is of utmost significance for developing high-yielding broad

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bean cultivars. Multivariate methods such as D^2 analysis, principal component analysis and multiple regression analysiscan serve as valuable tools for assessing genetic diversity. Hence, the current research was planned to evaluate various broad bean genotypes based on specific traits identified through multivariate techniques and effective selection criteria to identify superior genotypes among them, which can be used as potential parents in future breeding programs.

MATERIALS AND METHODS

The experimental material consisted of twenty broad bean genotypes collected across different geographical locations of The Nilgiris district of Tamil Nadu ranging from 918 to 2192 m above MSL were collected. The crop was raised in randomized block design (RBD) with three replications at the experimental farm (Woodhouse farmat an elevation of 2535 MSL; latitudes of 11.4°N and 76.4°E) of Horticultural Research Station, TNAU, Udhagamandalam, Nilgiris, Tamil Nadu, India during *Kharif* 2023. The experimental plot consisted of three rows each separated by 4 m. The spacing between rows and individual plants was maintained at 45 cm and 15 cm, respectively.

The observations were recorded on five randomly selected plants in each replications for 12 quantitative and 7 qualitative traits *viz*., plant height (cm), number of branches per plant, days to 50% flowering, days to maturity, pod length (cm), pod width (cm), number of pods per cluster, number of pods per plant, number of seeds per pod, 100 seed weight (g), seed yield per plant (g), pod yield per plant (g), moisture (%), dry matter (%), ascorbic acid (mg/100 g), protein content (%), total phenol (%), carbohydrate (%) and total soluble solids (°Brix) to study the genetic divergence. Multivariate analysis using Mahalanobis D^2 statistic (Mahalanobis, 1936) based on minimum genetic distance using Tocher2 s method as described by Rao (1952) was performed using TNAUSTAT statistical software. The software R version 3.4.3 was utilized to conduct Principal Component Analysis by Kaiser, 1958 and Jeffers, 1967.Stepwise multiple regression analysis described by Lush (1940) was carried out using SPSS software 2.0.

RESULTS AND DISCUSSION

The overall mean for various traits across the broad bean genotypes indicated a considerable range of diversity among the genotypes for each trait (Table 1). The plant height varied from 72.67 to 113.67 cm, days to 50% flowering with 52.71 to 63.97days, days to maturity varied from 147.00 to 163.50 days. The average number of pods per plant was 17.25. The mean values of 100 seed weight and seed yield per plant are 109.78 g and 64.73 g respectively. The average pod yield per plant is 160gram, protein is 21.53%. The maximum coefficient of variation (10.87) is observed for number of pods per plant. These results highlight that there was substantial variability within the material, making it suitable for further investigations into various traits. Additionally, the coefficient of variation was found to be below 10 percent for all the characters assessed, indicating that the experiment was carried out with precision. Abbas and Rodany (2009); Ammar *et al.* (2015); Arab *et al.* (2018); Boghara *et al.* (2016); Dahiya *et al.*

Table 1: Variations in morphological and qualitative traits of broad bean genotypes.

Characters	Mean	Range		S.Em. (±)	CD at 5%	CV (%)
		Minimum	Maximum			
Plant height (cm)	95.27	72.67	113.67	2.40	3.57	7.11
Number of branches per plant	2.60	1.90	3.70	0.20	10.89	0.59
Days to 50% flowering	58.24	52.71	63.97	0.49	1.19	1.46
Days to maturity	156.25	147.00	163.50	0.78	0.71	2.31
Pod length (cm)	11.07	9.48	12.85	0.03	0.41	0.10
Pod width (cm)	1.58	1.49	1.76	0.01	0.48	0.02
Number of pods per cluster	2.43	1.65	3.35	0.18	10.22	0.52
Number of pods per plant	17.25	11.17	29.00	0.64	5.24	1.89
Number of seeds per pod	2.58	1.88	3.25	0.10	5.50	0.30
100 seed weight (g)	109.78	79.50	139.25	0.91	1.18	2.70
Seed yield per plant (g)	64.73	45.00	81.50	0.56	1.23	1.67
Pod yield per plant (g)	160.00	51.67	273.67	3.67	3.25	10.87
Moisture (%)	12.93	11.25	14.50	0.20	2.16	0.58
Dry matter (%)	87.08	85.50	88.75	0.20	0.32	0.58
Ascorbic acid (mg/100 g)	16.42	13.41	19.45	0.26	2.27	0.78
Protein content (%)	21.53	15.64	26.13	0.34	2.22	1.00
Total phenol (%)	0.16	0.12	0.20	0.01	7.83	0.03
Carbohydrate (%)	48.60	43.90	52.77	0.43	1.25	1.27
Total soluble solids (°Brix)	7.76	6.93	8.26	0.13	2.38	0.39

(2021) and Ertiro *et al.* (2023) have also reported similar observations for analysis of variance in broad beans.

Genetic divergence analysis

The data collected on different morphological and qualitative characters of broad bean genotypes underwent an analysis for genetic diversity based on Mahalanobis D^2 statistic and principal component analysis. The significant values indicated substantial variability within the studied genotypes, resulting in genetic diversity. The percent contribution of all the 19 characters towards genetic divergence is presented Fig 1 based on the number of times that character appeared in the first rank. The maximum contribution towards diversity was shown by Total soluble solid (44.74%) followed by carbohydrate (41.05%), moisture (4.21%), phenol (2.63%), pod length (2.11%), ascorbic acid (2.11%), 100 seed weight (1.05%), dry matter (1.05%), protein (0.53%) and number of seeds per pod (0.53%) whereas the contribution of other traits was zero per cent. Jadhav *et al.* (2023) also reported similar contribution of the traits towards divergence.

Using Tocher's method, 20 genotypes were categorized into nine clusters based on the relative magnitude of their D^2 estimates. Table 2 illustrates the distribution of various genotypes within each cluster. Among the nine clusters, cluster II was the largest cluster with 4 genotypes followed by the clusters I, III and IV each with three genotypes. Cluster V and VI comprised of 2 genotypes, whereas the clusters VII, VIII and XI are monogenotypic consisting of only one genotype. The diversity within the genotypes is apparent due to the cluster arrangement, indicating significant variability. The findings broadly align with the reports by Rebaa *et al.* (2017) and Dewangan *et al.* (2022).

Table 2: Mahalanobis D^2 clustering pattern of broad bean genotypes.

Cluster number	No. of genotypes	Name of genotypes
1	3	Vf 7, Vf 20, Vf 6
2	4	Vf 3, Vf 16, Vf 18, Vf 13
3	3	Vf 2, Vf 8, Vf 1
4	3	Vf 12, Vf 15, Vf 5
5	2	Vf 4, Vf 9
6	2	Vf 10, Vf 14
7	1	Vf 17
8	1	Vf 11
9	1	Vf 19

The intra and inter-cluster D^2 values among nine clusters were calculated and presented in Table 3. The maximum intra-cluster distance was recorded in cluster IV (3358.2) followed by cluster II (D^2=2899.7), cluster III (D^2=2359.3) and cluster VI (D^2=2298.6), whereas the least intra-cluster distance was observed in cluster I (D^2=2258.9) and cluster V (D^2=1805.8). Cluster VII, VIII and IX had no intra-cluster distance(D^2=0.0) as they contained only one genotype each. Genotypes clustered together are expected to display minimal variation among the measured characteristics. The largest inter-cluster D^2 values were found between cluster I and VII (316.9) and cluster I and III (315.9), indicating a broader genetic diversity among the genotypes in these clusters. The smallest inter-cluster distance (62.7) was observed between cluster VI and cluster XI, as well as between cluster VII and Cluster VIII (64.6), indicating a strong similarity or close relationship among the genotypes in these clusters.

The findings revealed that inter-cluster distances surpassed intra-cluster distances, signifying substantial genetic variations between clusters compared to the limited variations within clusters. The genetic diversity was notably higher in clusters III, V, VII and VIII compared to the remaining clusters, as they displayed the greatest genetic distance. The genotypes within these clusters might be

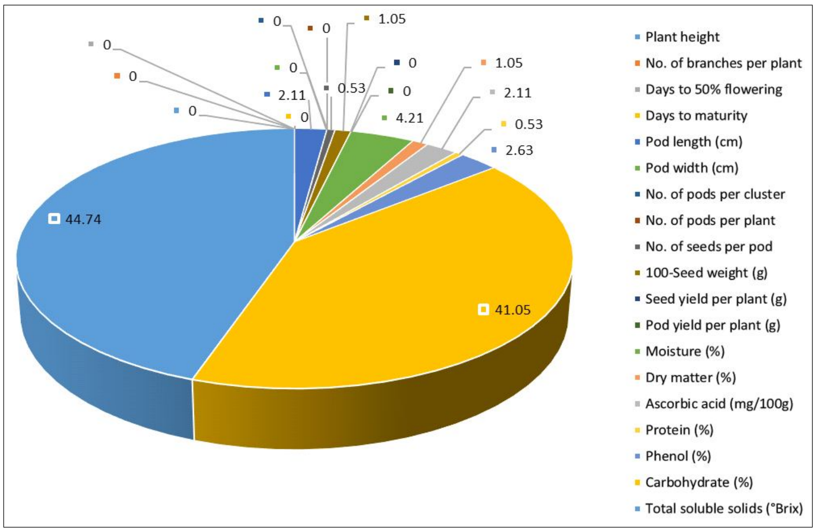

Fig 1: Per cent contribution of genetic diversity in broad bean genotypes.

valuable for future breeding programs, with the potential to produce improved offspring in subsequent generations. The results in similar lines were also reported by Singh *et al.* (2015) and Singh *et al.* (2021). The nearest and farthest clusters from each cluster can also be interpreted based on D^2 values in Table 3.

The cluster means of all the 19 phenotypic and qualitative characters were presented in Table 4. This cluster means value indicates the average performance of all genotypes present in a particular cluster for different traits. The data indicated a wide range of mean values between the clusters.The highest cluster mean value for number of pods per cluster (3.12), number of pods per plant (27.50), seed yield per plant (78.67), pod yield per plant (251.39), protein (24.47) and carbohydrate (52.18) were recorded in cluster III, phenol content (0.19) in cluster IV, number of branches (3.35) in cluster V, for total soluble solids (8.17) in cluster VI, pod length (12.85)in cluster VII, number of seeds per pod (3.25) and ascorbic acid (18.36); similarly for pod width (1.76), 100-seed weight (134.00) and dry matter (88.55) in cluster XI.

The cluster I had the lowest mean values for plant height (74.72). The lowest mean values for days to 50% flowering (54.34) was observed in cluster VIII, days to maturity (150.00) in cluster XI, moisture content (11.25) in cluster XI. Comparative study of cluster mean values suggested that clusters III, V and XI had highest cluster means for pod yield and its contributing traits, therefore, these clusters may be considered superior for selecting promising genotypes for utilizing in future studies. These results largely align with the discoveries made by Dubey *et al.* (2022) and Dewangan *et al.* (2022).

The top-performing genotypes selected from various clusters, considering multiple characteristics is given in Table 5. The promising broad bean genotypes identified from both the divergence and cluster mean analysis were Vf 2, Vf 8, Vf 1 from cluster III; Vf 9 and Vf 4 from cluster V; Vf 10 and Vf 14 form cluster VI and Vf 19 from cluster XI based on various pod yield and its component traits. These genotypes hold potential for future broad bean breeding programs, enabling the selection, hybridization and recovery of transgressive segregants with the highest yield potential.

Principal component analysis (PCA)

Principal component analysis (PCA) is a commonly employed statistical method for assessing genetic diversity within plant genotypes and identifying the key variables that contribute to this variation (Price *et al*., 2006). The variability within the genotypes was distributed across 18 principal components (PCs). Out of these 18 PCs, four had eigenvalues exceeding 1, collectively representing 78.23 per cent of the total variation for yield and its contributing traits among the 20 genotypes. This indicates that the traits identified within these components notably influenced the genotypes. It was in accordance with the findings of Tiwari and Singh (2019) and Dewangan *et al.* (2022).

Table 3: Average intra (diagonal) and inter cluster (above diagonal) distance (D^2) values of clusters in broad bean genotypes.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9
Cluster 1	2258.9 (47.5)	14244.4 (119.3)	99792.7 (315.9)	4748.5 (68.9)	55267.2 (235.1)	37992.2 (194.9)	100457.1 (316.9)	92352.7 (303.9)	43782.1 (209.2)
Cluster 2		2899.7 (53.8)	44855.5 (211.8)	7155.9 (84.6)	17418.8 (132.0)	8531.5 (92.4)	47290.5 (217.5)	38902.4 (197.2)	12655.7 (112.5)
Cluster 3			2359.3 (48.6)	77008.6 (277.5)	9632.0 (98.1)	18240.8 (135.1)	5177.0 (72.0)	4628.4 (68.0)	14144.7 (118.9)
Cluster 4				3358.2 (58.0)	39247.7 (198.1)	24245.8 (155.7)	79514.5 (282.0)	69567.3 (263.8)	29792.6 (172.6)
Cluster 5					1805.8 (42.5)	4185.4 (64.7)	11520.6 (107.3)	7848.7 (88.6)	5595.8 (74.8)
Cluster 6						2298.6 (47.9)	19626.8 (140.1)	13352.8 (115.6)	3930.2 (62.7)
Cluster 7							0 (0.0)	4176.6 (64.6)	19779.9 (140.6)
Cluster 8								0 (0.0)	14911.4 (122.1)
Cluster 9									0 (0.0)

In a scree plot graph, eigenvalues associated with a factor were plotted in descending order against the number of principal components to illustrate the proportion of variance attributed to these components. The scree plot revealed that, apart from the first four principal components, the rest of the PCs exhibited a negligible amount of variation (Fig 2). The scree plot clearly showed that PC1 held the most significant level of variation, suggesting that choosing genotypes based on traits from PC1 would be advantageous.

The first principal component (PC 1) accounted for approximately 50.29 per cent of the total variation and possessed an eigen value of 9.556. Notably, traits such as plant height (0.697), number of branches per plant (0.745), pod width (0.776), number of pods per cluster (0.690), number of pods per plant (0.842), number of seeds per pod (0.729), 100 seeds weight (0.845), seed yield per plant (0.919), pod yield per plant (0.937), dry matter content (0.711), carbohydrate (0.881) and total soluble solids (0.684) made the most significant contributions to the divergence observed in PC 1. The second principal component (PC 2) possessed an eigen value of 2.884 and explained approximately 15.18 per cent of the total variation. Key contributors to PC 2 included traits like days to 50% flowering (0.720), days to maturity (0.696), dry matter (0.608) and protein content (0.685). The third principal component (PC 3), with an eigen value of 1.370, contributed about 7.21 per cent of the total variation. The major contributor to PC 3 was pod length (0.638). Meanwhile, the fourth principal component (PC 4) contributed roughly 5.54 per cent of the total variation and had an eigen value of 1.053 with pod width (0.413) contributing to the total variation. Similar findings were reported in the studies conducted by Zayed *et al.* (2022) and Dewangan *et al.* (2022).

The first two principal components were very important and accounted for approximately 65.47% of the total available variability; hence, they were plotted graphically to demonstrate the relationship among the broad bean genotypes. For easy visualization, both genotypes and variables were combined into a single bi-plot graph, which centered on two primary principal components, namely PC1 and PC2 (Fig 3). The length of each trait2 s vector within the graph illustrates its contribution to overall divergence. The trait pod yield per plant displayed the longest vector length, indicating its substantial contribution to overall divergence, which was followed by seed yield per plant, dry matter, carbohydrate, protein and number of pods per plant.These observations concur with those of Malek *et al.* (2021).

The angle formed between the trait vectors signifies the direction of association between the traits. It reflected that pod yield per plant, seed yield per plant, pod width and number of pods per plant mostly contributed towards variability among genotypes *viz.*, Vf 1 and Vf 2. On the other hand, traits such as number of branches per plant and number of pods per cluster contributed significantly to the variability among genotypes Vf 8, Vf 10 and Vf 19. The

Table 4: Cluster mean values for various traits among 20 broad bean genotypes.

	PH	NBP	DF	DM	PL	PW	NPC	NPP	NSP	SWT	SYP	PYP	MC	DYM	ASC	PRO	PHE	CAR	TSS
Cluster 1	74.72	2.10	61.44	163.70	9.97	1.53	2.05	12.56	2.04	95.33	45.75	78.33	14.00	85.96	16.08	19.97	0.17	45.14	7.17
Cluster 2	94.54	2.25	59.51	159.69	10.57	1.55	2.21	15.46	2.44	106.81	63.00	133.33	12.81	86.99	15.55	20.53	0.17	48.36	7.85
Cluster 3	103.22	3.17	55.61	152.54	12.00	1.67	3.12	27.50	2.83	129.83	78.67	251.39	11.75	88.25	18.24	24.47	0.14	52.18	8.15
Cluster 4	92.56	2.40	57.91	157.50	10.44	1.54	2.33	12.78	2.38	95.67	55.58	127.50	13.33	86.61	15.98	21.78	0.19	46.85	7.47
Cluster 5	93.00	3.35	58.82	155.46	11.15	1.59	1.83	17.75	2.75	114.38	75.63	201.58	13.25	86.58	14.88	21.36	0.14	50.81	7.66
Cluster 6	96.25	2.55	55.48	156.23	11.56	1.55	2.75	15.92	2.88	110.88	70.88	166.75	13.00	86.94	17.41	21.28	0.14	49.28	8.17
Cluster 7	113.67	2.30	60.24	156.36	12.85	1.60	2.15	22.84	3.25	107.00	63.00	162.00	13.25	86.54	18.36	20.63	0.16	45.39	7.67
Cluster 8	113.67	2.60	54.34	151.72	12.33	1.61	3.00	16.50	3.00	114.25	76.00	192.17	13.00	86.73	15.04	19.70	0.13	49.65	8.02
Cluster 9	102.84	3.30	58.67	150.00	11.22	1.76	2.85	18.00	2.50	134.00	70.50	204.17	11.25	88.55	17.27	24.14	0.12	50.83	8.08

PH= Plant height, NBP= No. of branches per plant, DF= Days to 50% flowering, DM= Days to maturity, PL= Pod length (cm), PW= Pod width (cm), NPC= No. of pods per cluster, NPP= No. of pods per plant, NSP= No. of seeds per pod, SWT= 100-Seed weight (g), SYP= Seed yield per plant (g), PYP= Pod yield per plant (g), MC= Moisture (%), DYM= Dry matter (%), ASC= Ascorbic acid (mg/100 g), PRO= Protein (%), PHE= Phenol (%), CAR= Carbohydrate (%), TSS= Total soluble solids (°Brix).

genotypes Vf 1, Vf 2, Vf 8, Vf 7, Vf 20, Vf 6, Vf 14, Vf 11, Vf 11 and Vf 19 were positioned farthest from the origin in the biplot, signifying that they accounted for more trait variability associated with their respective principal components compared to other genotypes. These outcomes are consistent with the findings of Tiwari and Singh (2019).

Table 5: Superior and diverse broad bean genotypes selected from different clusters for various phenotypic and qualitative traits.

Earliness	No. of branches per plant	No. of pods per plant	Seed yield per plant	Pod yield per plant	Protein	Ascorbic acid
Vf 14 (VI)	Vf 4 (V)	Vf 1 (III)	Vf 1 (III)	Vf 2 (III)	Vf 8 (III)	Vf 10 (VI)
Vf 11 (VIII)	Vf 1 (III)	Vf 8 (III)	Vf 2 (III)	Vf 8 (III)	Vf 19 (XI)	Vf 8 (III)
Vf 2 (III)	Vf 8 (III)	Vf 2 (III)	Vf 8 (III)	Vf 1 (III)	Vf 16 (II)	Vf 2 (III)
Vf 15 (IV)	Vf 19 (XI)	Vf 17 (VII)	Vf 11 (VIII)	Vf 9 (V)	Vf 10 (VI)	Vf 17 (VII)
Vf 1 (III)	Vf 9 (V)	Vf 19 (XI)	Vf 4 (V)	Vf 19 (XI)	Vf 2 (III)	Vf 19 (XI)

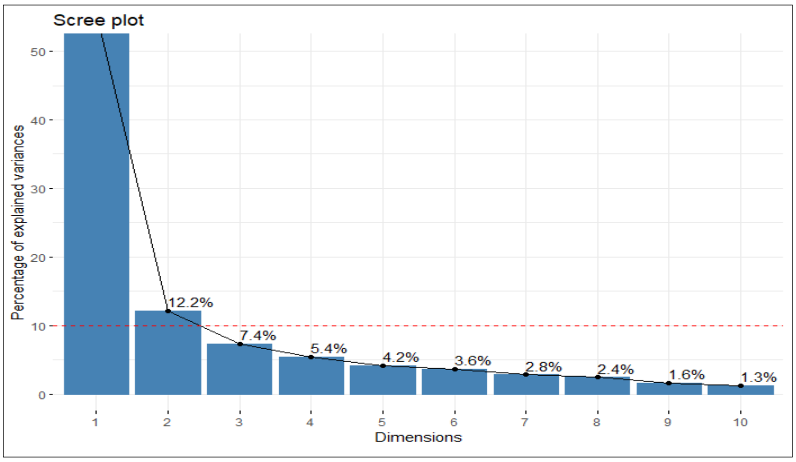

Fig 2: Scree plot showing contribution of various principal components towards divergence.

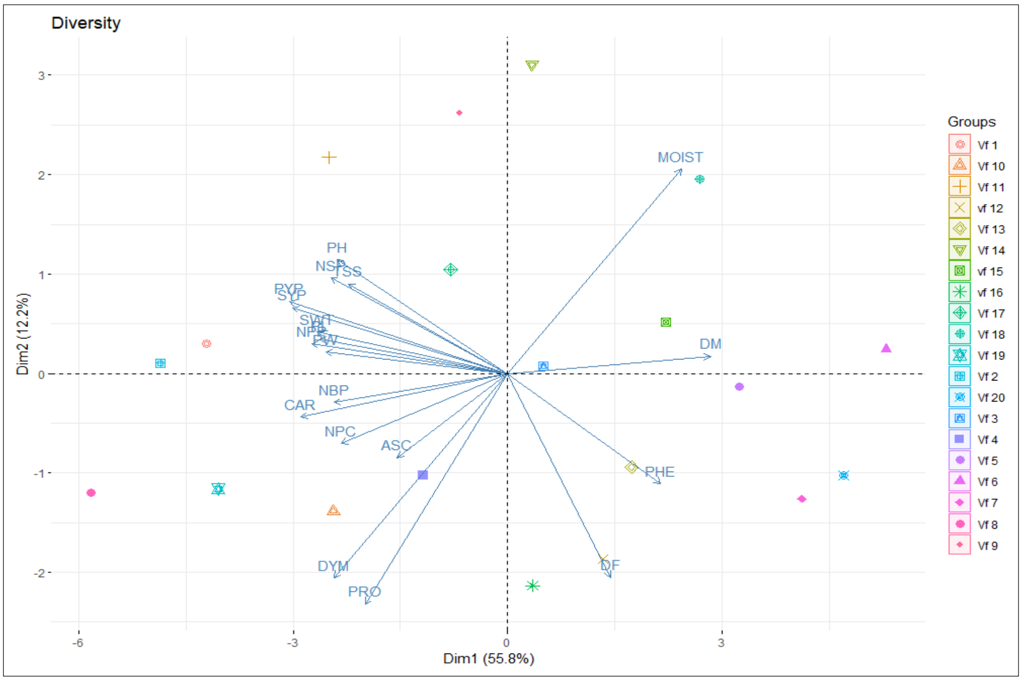

Fig 3: Biplot illustrating the relationship between PC1 and PC2.

Table 6: Summary of stepwise multiple regression analysis for pod yield and its component traits.

Steps	Entered variables	Bi	R^2 value	Adjusted R^2	SE	t value
1	Seed yield per plant	0.896	0.802	0.791	26.074	6.036**
2	No. of pods per plant	0.933	0.870	0.855	21.752	2.977**

**Significant at 1% level of significance, R^2= Coefficient of determination, R= Multiple correlation coefficient, SE= Standard error.

Stepwise multiple regression analysis

The pod yield per plant was regarded as the dependent variable, while the remaining traits served as independent variables. Initially, the model included the seed yield per plant, elucidating 79.1% of the total observed variations, followed by the number of pods per plant. The cumulative variations explained by the combination of the number of pods per plant with the seed yield per plant amount to 85.5% (Table 6). Thus, the stepwise multiple regression analysis identified the crucial economic traits on pod yield of broad beans. These findings could be valuable in effectively selecting promising broad bean genotypes in subsequent generations which agree with those of Janghel *et al.* (2020) and Dewangan *et al.* (2022).

CONCLUSION

The broad bean genotypes Vf 2, Vf 8, Vf 1, Vf 9, Vf 4 and Vf 19 could be utilized in hybridization programmes to enhance the heterotic response for increased yields. The findings from this study offer essential guidance for the selection of diverse broad bean genotypes and will serve as basis for research in hybridization programmes as well as contributing to germplasm conservation.

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Conflict of interest

All authors declared that there is no conflict of interest.

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