



Identification of Superior Faba bean (*Vicia faba* L.) Accessions for Key Morphological and Yield-related Traits

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ABSTRACT

Background: Faba bean (*Vicia faba* L.) is a multipurpose legume whose genetic improvement relies on exploiting existing morphological variation. Rigorous phenotypic evaluation provides insights into heritability, trait associations and divergence patterns, which are crucial for identifying superior genotypes and its selections in breeding programs.

Methods: Forty-five genotypes, including three checks, were evaluated across two Rabi seasons in a randomized block design for 20 agro-morphological traits spanning phenology, plant architecture, pod/seed attributes and yield. Best Linear Unbiased Estimates (BLUEs) were computed across replications and seasons. Broad-sense heritability, Genotypic variation, genetic divergence, D^2 statistics, trait associations and the Multi-trait Genotype-Ideotype Distance Index (MGIDI) were applied to identify superior accessions.

Result: Analysis of variance showed significant genotypic variation for most of the traits, with high heritability. However, leaflet width and seeds per pod had moderate heritability. Mahalanobis D^2 divided genotypes into four clusters. Cluster IV displayed strong yield traits and significant differences from Cluster I, indicating potential for hybrid vigour. Correlation analysis revealed positive relationships, such as maturity with pods per plant and pod length, along with some trade-offs. MGIDI found nine promising accessions that combine earliness, good pod and seed traits and appealing structure. Overall, these results confirm a wide range of phenotypic diversity and provide a clear set of accession for improving faba bean.

Key words: Best linear unbiased estimates, Cluster analysis, Faba bean, Heritability, Multi-trait genotype-ideotype distance index (MGIDI), Phenotypic selection.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an annual legume belonging to the family Fabaceae. It is widely known by several names such as broad bean, horse bean, windsor bean, tick bean and bakela (Hawtin and Hebblethwaite, 1983). Global production of faba bean continues to rise, with production of approximately 6.77 million tonnes of dry beans and 1.81 million tonnes of green beans in 2022, cultivated over ~6.63 million and 0.64 million acres, respectively (FAOSTAT, 2024). Recent market forecasts further emphasize the growing demand. IMARC Group (2025) reported that global faba bean production reached 9.3 million tonnes in 2024 and it is projected to expand to 12.9 million tonnes by 2033, growing at a compound annual growth rate of 3.5%.

Although it is grown in limited areas in India and considered a minor crop, it holds promise due to its nutritional value, adaptability and potential role in crop diversification (Singh *et al.*, 2012a; Singh *et al.*, 2013). Faba bean is highly valued as a source of plant-based protein, with seeds containing 22-35% protein, 58% carbohydrates and 25% dietary fibre (USDA, 2021; Martineau-Côté *et al.*, 2022). In addition to its nutritional role, the crop provides several bioactive compounds, including flavonoids, phenolics and L-DOPA (a precursor of dopamine), which have potential medicinal applications in the treatment of Parkinson's disease (Vered *et al.*, 1997). The crop also serves as an important source of food and feed in developing countries, while in developed

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nations it is mainly utilized as animal feed (Gasim and Link, 2007; Singh and Bhatt, 2012a).

Globally, more than 30,000 germplasm of faba bean are conserved in gene banks (Singh and Bhatt, 2012b). This genetic variability presents significant opportunities for crop improvement, but effective utilization requires a systematic characterization and evaluation. Morphological characterization remains the first and most practical step in germplasm evaluation, providing information on the extent of variation for traits of agronomic and breeding importance (Salem, 2009; Hiywotu *et al.*, 2022). Grouping accessions and identifying important genotypes for trait-specific breeding are made easier by multivariate techniques like principal component analysis and cluster analysis Zayed *et al.* (2022) for instance, used PCA to identify the characteristics that contributed to significant phenotypic divergence in faba bean accession and to direct parent selection.

MATERIALS AND METHODS

Plant material and field conditions

A total of 42 diverse faba bean accessions, along with three checks (HFB 1, HFB 2, Vikrant), were obtained from ICAR-NBPGR, New Delhi and evaluated for morphological traits. The trial was conducted in an RBD at the Horticultural Research Farm, SHUATS, Prayagraj, during rabi 2022-23 and 2023-24. Each accession was grown in six-row plots (1.5×2.0 m²), with five tagged plants per replication for data recording.

Data collection

In the present study, a set of 20 phenotypic traits was evaluated for faba bean to understand their breeding importance. All the traits have been characterized according to the DUS standard. These traits included: days of germination (DG), days to first flowering (DFF), days to 50% flowering (DF), number of branches per plant (NBP), number of nodes on the main branch (NNMB), number of flowers per plant (NFP, number), plant height (PH), leaflet length (LL), leaflet width (LW), number of pods per plant (NPP), pod length (PL), pod width (PW), number of seeds per pod (NSP), biological yield (BY), economical yield (EY), harvest index (HI), days to first pod formation (DPF), seed index (SI), days to maturity (DM) and yield per hectare (YPH).

Statistical analysis

For each trait recorded, data were analyzed for the Analysis of Variance (ANOVA) along with the significance levels of * $p < 0.05$ (significant), ** $p < 0.01$ (highly significant) and *** $p < 0.001$ (extremely significant) and Broad sense heritability using the agricolae package of R. Each replication in the two seasons comprised data collected from five plants per replication and the Best Linear Unbiased Estimates (BLUEs) values were computed using the lme4 package in R (Bates, 2010) for each genotype across the three replications. This approach minimized the environmental variation and provided unbiased estimates of each accession.

Based on the BLUE values estimated, summary statistics have been calculated using the agricolae package in R to verify the central tendency and trait performance across the accessions. Furthermore, the distribution and trends of each trait among the accessions were visualized using histograms overlaid with frequency curves, generated with the ggplot2 package in R (Wickham, 2011).

D² clustering analysis

A D² analysis was performed to group the accessions into various clusters based on their phenotypic BLUE values for all traits, enabling us to identify accessions with identical or distinct phenotypic characteristics. This clustering enables us to understand the nature of variation within and between accessions, which is essential in the breeding program for selecting parents. Phenotypic divergence of accessions was measured using a generalized distance (D²) proposed by Mahalanobis (1936), a multivariate distance that considers correlations among traits and accurately differentiates among accessions. Furthermore, intra and inter-cluster distances were determined to assess the relationships of clusters and the extent of dissimilarity among them to identify highly dissimilar accessions to hybridize and succeed in improving faba bean traits.

Correlation analysis

Correlation analysis determines the level of association between the traits under study, thereby establishing key conclusions. The 20 morphological traits were compared in pairs to produce the estimated pairwise correlations using the corr_plot function of the metan package in R (Olivoto and Lúcio, 2021). The analysis was performed to determine the strength and relationship of association between traits, to identify interdependent traits that may serve as indirect selection criteria during the breeding process. The significance of the correlations was also tested, with correlations referred to as significant (* $p < 0.05$), highly significant (** $p < 0.01$) and extremely significant (***) $p < 0.001$).

Selection of the best accessions

The Multi-Trait Genotype-Ideotype Distance Index (MGIDI) was calculated according to the procedure outlined by (Olivoto *et al.*, 2022). The pre-determined desired ideotype trait performance used the BLUE values of each accession. Factor analysis was conducted to reduce multicollinearity and generalize trait variation and the genotype scores were projected in the reduced factorial space. The MGIDI of each accession was computed as the euclidean distance between the genotype and the ideotype, with lower values denoting a closer resemblance to the ideotype. All calculations were performed in R version 4.1.2 (R Core Team, 2020), utilizing the metan package (Olivoto and Lúcio, 2021) and visualized with ggplot2 (Wickham, 2011).

RESULTS AND DISCUSSION

Analysis of variance and heritability

Analysis of variance revealed significant differences among accessions for most of the traits (DG, DFF, D50F, NBP, NNMB, NFP, PH, LL, NPP, PL, PW, BY, EY, HI, DPF, SI, DM, YPH). This confirms substantial genetic variation. LW and NSP showed moderate variation ($P < 0.05$). Replication effects were non-significant (Table 1), indicating that differences were mostly due to genotypes.

Reproductive traits (DFF, D50F, NFP, NPP) and morphological traits (PH, LL, PL, PW, HI) showed strong effects from genotypes, making them useful for selection. Broad-sense heritability was high ($>60\%$) for most of the traits. LW had a moderate value of 38.1% , while NSP was also moderate; PW had the highest value at 98.2% . No traits fell in the low heritability range.

The frequency distribution (Fig 1) showed that most accessions yielded 32-36 g/plant, with only four exceeding 37 g/plant. Seventeen accessions flowered at 60-62 days, with four flowering earlier. Nine accessions produced the highest number of flowers (25-28) and seven matured early (~ 110 days), while most matured around 120 days.

D² clustering and cluster trait variation

D² cluster analysis grouped the 45 accessions into four clusters based on 20 traits (Fig 2). Cluster I included 13 accessions, such as IVT HB18-10, IVT HB19-10 and the check Vikrant. Cluster II had 11 accessions, including IVT HB19-12, AVT I HB16-15 and the check HFB-2 (Table 2). Both Cluster I and Cluster II showed lower average values for yield traits. Cluster III had 9 accessions, while Cluster IV

contained 11 accessions. Clusters III and IV recorded higher averages, with Cluster IV surpassing the others in biomass yield, grain yield, flowering, maturity and harvest index. This highlights its potential for high yield (Fig 3).

The variation within clusters was highest in Cluster IV (7.68) and Cluster II (7.66) and lowest in Cluster III (4.23). The largest differences between clusters happened between Cluster I and Cluster IV (7.68) and between Cluster I and Cluster III (6.91). The smallest difference was between Cluster II and Cluster I (4.3) (Fig 4). These results show clear differences in traits, with Cluster III being the smallest and Cluster IV exhibiting the most tremendous internal variation.

Correlation

Correlation analysis of morphological characteristics in faba bean demonstrated that morphological characteristics were differently associated with flowering, pod and yield characteristics, as illustrated in (Fig 5). The days to first flowering had a strong positive and significant correlation with days to maturity ($r = 0.45$), but a negative and non-significant correlation with the pod weight ($r = -0.45$). Days to fifty percent flowering is positively correlated and highly significant with the biological yield ($r = 0.99$) and the economic yield ($r = 0.66$). There were positive and significant relationships between days to maturity and the number of pods per plant ($r = 0.87$), pod length ($r = 0.91$) and number of Seeds per pod ($r = 0.59$).

Pod length is negatively correlated with pod width ($r = -0.02$) and highly significantly correlated positively with the number of seeds per pod ($r = 0.63$) and seed index ($r = 0.86$). Pod width is negatively correlated with the number

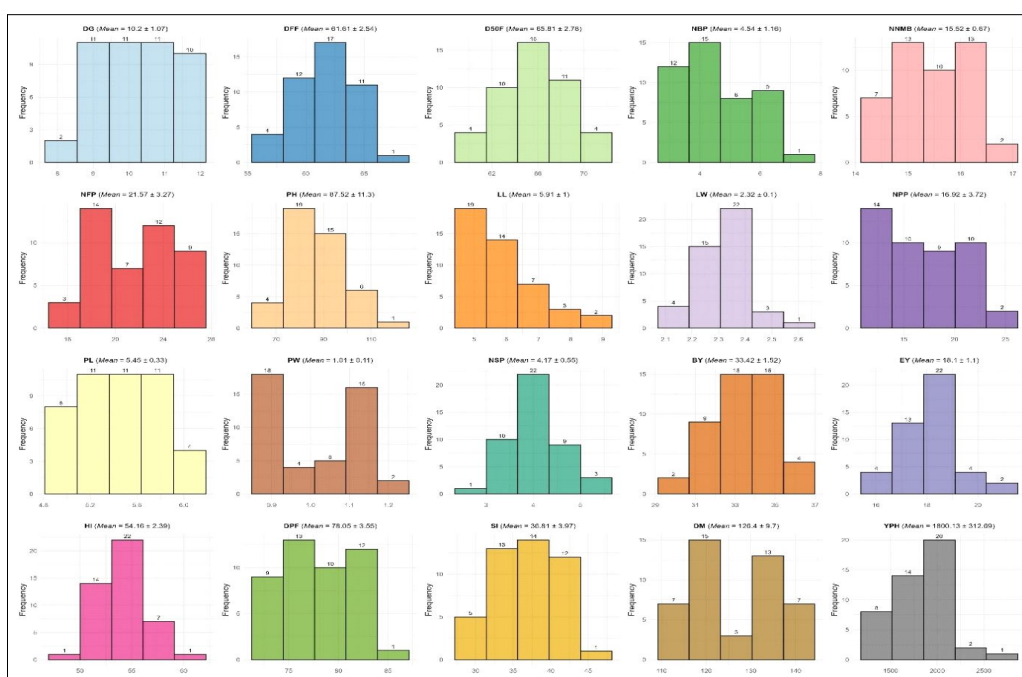


Fig 1: Frequency histogram for the plant morphological traits for the faba bean accessions.

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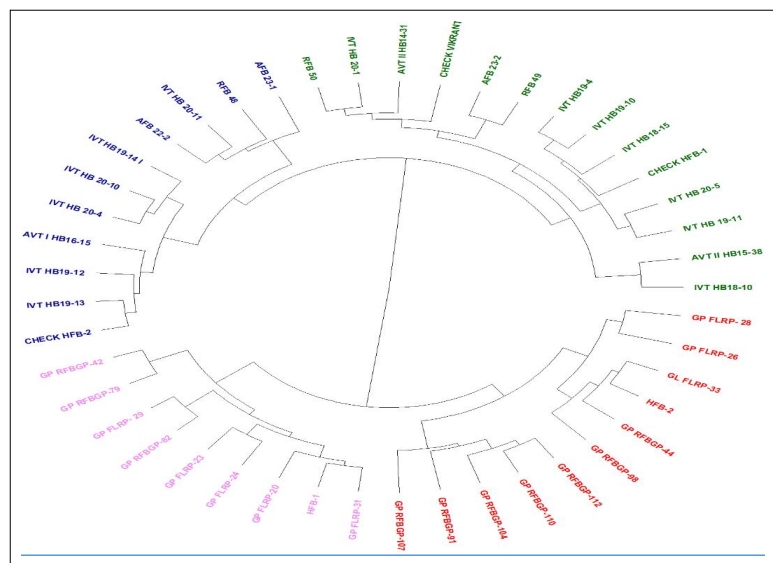
Table 1: Anova table.

Trait	Effect	Df	Sum_Sq	Mean_Sq	F_value	Pr	Significance
DG	Genotypes	44	152.448	3.464727	9.41037	6.24E-19	***
	Rep	2	6.81E-29	3.41E-29	9.25E-29	1	ns
	Residuals	88	32.4	0.368182			
	Heritability (H2)						H2 = 0.894
DFF	Genotypes	44	854.184	19.41327	9.2553	1.07E-18	***
	Rep	2	0.417333	0.208667	0.099482	0.905408	ns
	Residuals	88	184.5827	2.09753			
	Heritability (H2)						H2 = 0.892
D50F	Genotypes	44	1021.309	23.21156	2.669485	4.58E-05	***
	Rep	2	4.647259	2.32363	0.267233	0.766114	ns
	Residuals	88	765.1727	8.695145			
	Heritability (H2)						H2 = 0.625
NBP	Genotypes	44	178.8148	4.063973	4.375765	1.99E-09	***
	Rep	2	2.27037	1.135185	1.222278	0.29951	ns
	Residuals	88	81.72963	0.928746			
	Heritability (H2)						H2 = 0.771
NNMB	Genotypes	44	60.11733	1.366303	2.649586	5.18E-05	***
	Rep	2	0.001333	0.000667	0.001293	0.998708	ns
	Residuals	88	45.37867	0.515667			
	Heritability (H2)						H2 = 0.623
NFP	Genotypes	44	1411.248	32.07382	5.072918	5.04E-11	***
	Rep	2	8.948148	4.474074	0.707637	0.49559	ns
	Residuals	88	556.3852	6.322559			
	Heritability (H2)						H2 = 0.803
PH	Genotypes	44	16860.36	383.1901	5.996431	5.61E-13	***
	Rep	2	136.5333	68.26667	1.068285	0.34801	ns
	Residuals	88	5623.467	63.90303			
	Heritability (H2)						H2 = 0.833
LL	Genotypes	44	132.762	3.017319	14.53136	1.89E-25	***
	Rep	2	0.534527	0.267264	1.287137	0.281206	ns
	Residuals	88	18.27249	0.207642			
	Heritability (H2)						H2 = 0.931
LW	Genotypes	44	1.211784	0.027541	1.615515	0.028703	*
	Rep	2	0.073336	0.036668	2.150928	0.122456	ns
	Residuals	88	1.500184	0.017048			
	Heritability (H2)						H2 = 0.381
NPP	Genotypes	44	1821.782	41.40413	17.11187	4.65E-28	***
	Rep	2	3.833926	1.916963	0.79226	0.456023	ns
	Residuals	88	212.9261	2.419614			
	Heritability (H2)						H2 = 0.942
PL	Genotypes	44	14.21232	0.323007	8.431736	2.14E-17	***
	Rep	2	0.102117	0.051059	1.332824	0.269002	ns
	Residuals	88	3.37115	0.038309			
	Heritability (H2)						H2 = 0.881
PW	Genotypes	44	Genotypes	44	1.688733	0.03838	***
	Rep	2	Replication	2	0.000138	6.89E-05	ns
	Residuals	88	Residuals	88	0.028329	0.000322	
	Heritability (H2)					H2 = 0.982	

Table 1: Continue..

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NSP	Genotypes	44	39.91481	0.907155	1.641959	0.024724	*
	Rep	2	0.048148	0.024074	0.043574	0.957382	ns
	Residuals	88	48.61852	0.552483			
	Heritability (H2)						H2 = 0.391
BY	Genotypes	44	304.3643	6.917369	3.358993	6.70E-07	***
	Rep	2	1.287077	0.643539	0.312495	0.732428	ns
	Residuals	88	181.2235	2.059358			
	Heritability (H2)						H2 = 0.702
EY	Genotypes	44	160.8493	3.655665	2.959081	7.60E-06	***
	Rep	2	0.051033	0.025516	0.020654	0.979562	ns
	Residuals	88	108.7157	1.235406			
	Heritability (H2)						H2 = 0.662
HI	Genotypes	44	751.6362	17.08264	2.762944	2.56E-05	***
	Rep	2	3.60189	1.800945	0.291285	0.748021	ns
	Residuals	88	544.0836	6.182768			
	Heritability (H2)						H2 = 0.638
DPF	Genotypes	44	1666.179	37.86771	3.627603	1.37E-07	***
	Rep	2	1.702938	0.851469	0.081568	0.92174	ns
	Residuals	88	918.6119	10.43877			
	Heritability (H2)						H2 = 0.724
SI	Genotypes	44	2080.469	47.28338	12.78157	1.85E-23	***
	Rep	2	15.5517	7.775851	2.101956	0.128312	ns
	Residuals	88	325.5419	3.69934			
	Heritability (H2)						H2 = 0.922
DM	Genotypes	44	12423.41	282.3503	78.03743	1.14E-54	***
	Rep	2	9.937037	4.968519	1.373225	0.258662	ns
	Residuals	88	318.3963	3.61814			
	Heritability (H2)						H2 = 0.981
YPH	Genotypes	44	12906611	293332.1	23.64886	2.03E-33	***
	Rep	2	0.014815	0.007407	5.97E-07	0.999999	ns
	Residuals	88	1091521	12403.65			
	Heritability (H2)						H2 = 0.958

**Fig 2:** D² clustering of the faba bean accessions based on phenotypic traits.

of pods per plant ($r=-0.04$). The number of seeds per pod is highly significant and positively correlated with the seed index ($r = 0.86$), as well as showing a positive correlation

with both biological yield ($r = 0.29$) and economic yield ($r = 0.15$). Biological yield is positively correlated with yield per hectare ($r = 0.07$). Biological yield is positively

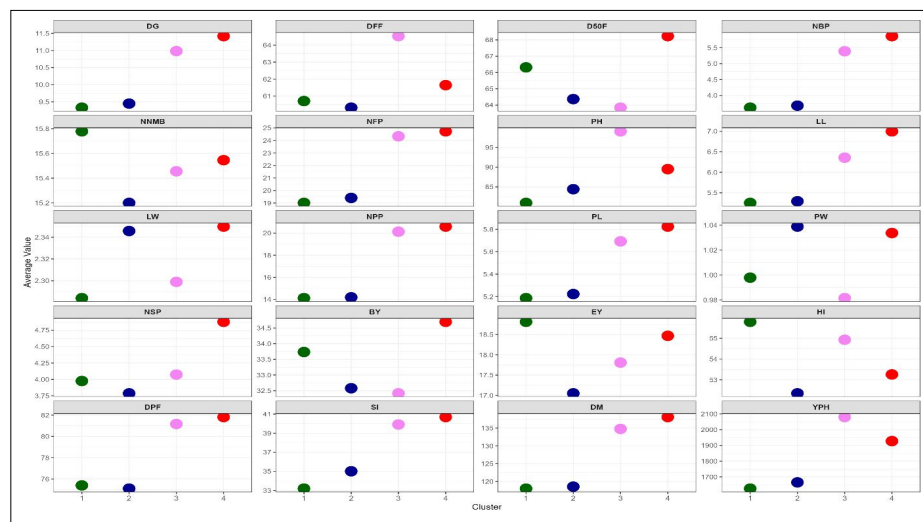


Fig 3: Cluster wise variation of the traits in each cluster.

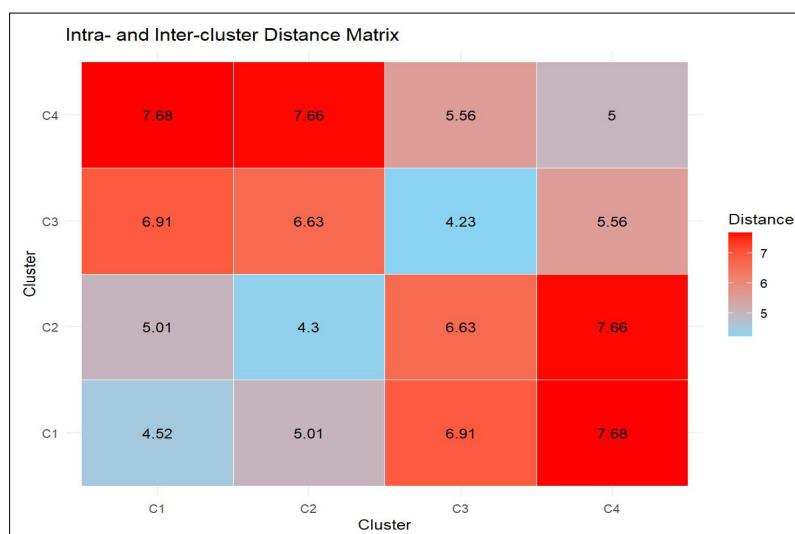


Fig 4: Intra and inter cluster distance matrix.

Table 2: List of genotypes under D2 clusters.

Cluster	Genotypes	Number of genotypes
1	IVT HB18-10, IVT HB19-10, IVT HB18-15, IVT HB19-11, IVT HB19-4, AVT II HB14-31, AVT II HB15-38, CHECK HFB-1, CHECK VIKRANT, IVT HB20-1, IVT HB20-5, RFB 49, RFB 50, AFB 23-2	13
2	IVT HB19-12, IVT HB19-13, IVT HB19-14 I, AVT I HB16-15, CHECK HFB-2, IVT HB20-11, IVT HB20-10, IVT HB20-4, RFB 48, AFB 22-2, AFB 23-1	11
3	HFB-1, GP FLRP-20, GP FLRP-23, GP FLRP-24, GP FLRP-29, GP FLRP-31, GP RFBGP-42, GP RFBGP-79, GP RFBGP-82	9
4	HFB-2, GP FLRP-26, GP FLRP-28, GL FLRP-33, GP RFBGP-44, GP RFBGP-91, GP RFBGP-98, GP RFBGP-104, GP RFBGP-107, GP RFBGP-110, GP RFBGP-112	11

Clustering signifies the phenotypic trait variation

The clustering by Mahalanobis D² revealed distinct groups of accessions, reflecting strong phenotypic influence that can be strategically exploited in breeding. Accessions from clusters separated by large inter-cluster distances are particularly valuable for crossing because they maximize the potential for heterosis and recovery of transgressive segregants. At the same time, clusters with higher intra-cluster variability provide opportunities for effective within-

cluster selection. Comparable findings were reported by Dewangan *et al.* (2022), who observed clear cluster differentiation among faba bean lines, with distant clusters offering the best parental combinations for yield improvement. Similarly, Upadhyay and Pandey (2025) demonstrated that clusters with superior mean performance for seed yield and related traits are prime candidates for hybridization, thereby broadening the genetic base and enhancing productivity.

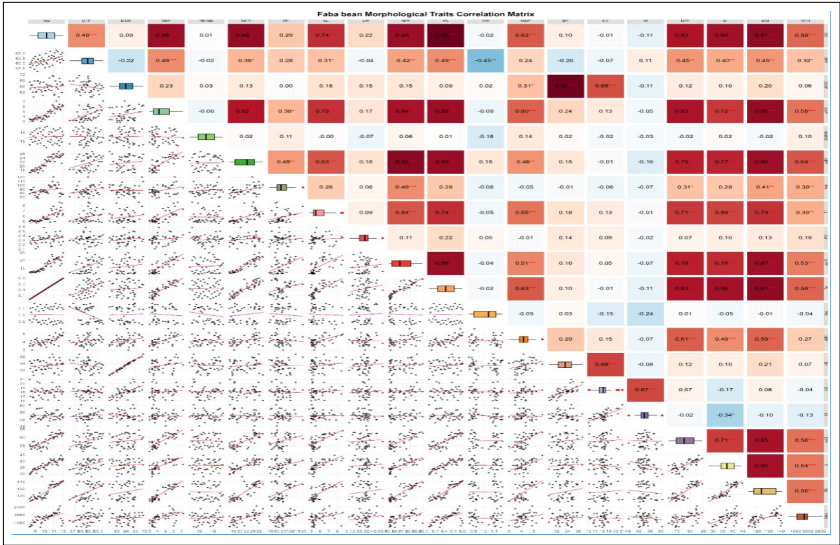


Fig 6: MGIDI analysis for the selection of superior faba bean accessions.

Table 4: Factor analysis (FA)-Factor loadings after rotation.

Trait	Factor	Xo	Xs	SD	SD (%)	Sense	Goal
DG	FA1	10.2	10.9	0.673	6.60	Decrease	0
DFF	FA1	61.6	64.0	2.37	3.85	Decrease	0
NBP	FA1	4.54	5.26	0.722	15.9	Increase	100
NFP	FA1	21.6	23.7	2.17	10.1	Increase	100
LL	FA1	5.91	6.12	0.214	3.62	Increase	100
NPP	FA1	16.9	19.0	2.07	12.2	Increase	100
PL	FA1	5.45	5.66	0.207	3.79	Increase	100
NSP	FA1	4.17	4.15	-0.022	-0.533	Increase	0
DPF	FA1	78.1	80.4	2.32	2.97	Decrease	0
SI	FA1	36.8	39.0	2.21	5.99	Increase	100
DM	FA1	126.0	133.0	6.49	5.13	Decrease	0
YPH	FA1	1800	2036	236	13.1	Increase	100
D50F	FA2	65.8	64.5	-1.31	-1.99	Decrease	100
BY	FA2	33.4	32.7	-0.679	-2.03	Decrease	100
EY	FA2	18.1	17.9	-0.155	-0.857	Increase	0
HI	FA3	54.2	54.8	0.658	1.21	Increase	100
NNMB	FA4	15.5	15.2	-0.271	-1.75	Increase	0
PW	FA4	1.01	0.976	-0.037	-3.65	Increase	0
PH	FA5	87.5	96.8	9.24	10.6	Increase	100
LW	FA5	2.32	2.36	0.037	1.59	Increase	100

Correlation across the morphological traits

The patterns of correlation between flowering, maturity, pod characteristics and yield highlight the intricate relationship between faba bean productivity. In line with Mishra *et al.* (2023), who found that harvest index and biological yield had a significant direct impact on seed yield, positive correlations between reproductive timing and yield components imply that enhancing one can improve others. However, the existence of both positive and negative correlations highlights the importance of careful selection in balancing trade-offs. Hiywotu *et al.* (2023) reported similar results, showing that traits related to pods and seeds had a

significant impact on grain yield through direct and indirect effects, indicating their usefulness as selection criteria.

Superior faba bean accessions selection

Using the MGIDI framework enabled simultaneous, balanced selection across phenology, yield and architecture while controlling for multicollinearity via factor analysis; by ranking genotypes on their distance to an agronomic ideotype, MGIDI prioritizes accessions that deliver multi-trait gains without over-weighting redundant, correlated traits (Olivoto and Lúcio, 2021; Olivoto *et al.*, 2022). The analysis captured by the leading principal components together with high trait communalities

Table 5: Selection differential (SD) for MGIDI analysis.

Trait	FA1	FA2	FA3	FA4	FA5	Communality	Uniqueness
DG	-0.97	0.01	0.02	-0.03	0.10	0.95	0.05
DFF	-0.52	0.38	-0.38	0.31	0.07	0.66	0.34
D50F	-0.12	-0.97	0.04	0.04	0.02	0.96	0.04
NBP	0.92	0.12	0.09	0.01	-0.13	0.88	0.12
NNMB	-0.03	0.11	-0.18	-0.76	-0.05	0.63	0.37
NFP	0.88	0.04	-0.12	0.07	-0.31	0.89	0.11
PH	0.29	-0.07	-0.01	-0.26	-0.73	0.69	0.31
LL	0.80	0.11	0.07	0.01	0.04	0.66	0.34
LW	0.09	0.18	0.02	0.35	-0.52	0.43	0.57
NPP	0.88	0.05	0.00	-0.07	-0.25	0.85	0.15
PL	0.97	-0.01	-0.02	0.02	-0.10	0.95	0.05
PW	-0.03	0.09	-0.50	0.64	0.00	0.66	0.34
NSP	0.70	0.27	-0.03	-0.14	0.43	0.76	0.24
BY	-0.12	-0.97	0.01	0.02	0.03	0.95	0.05
EY	0.00	0.73	0.66	0.05	0.00	0.97	0.03
HI	-0.11	0.02	0.91	0.08	0.02	0.85	0.15
DPF	-0.90	-0.02	-0.06	-0.03	0.03	0.82	0.18
SI	0.87	-0.03	-0.24	-0.07	-0.08	0.82	0.18
DM	-0.94	-0.09	0.00	0.00	0.14	0.92	0.08
YPH	0.58	-0.01	-0.09	-0.13	-0.46	0.57	0.43

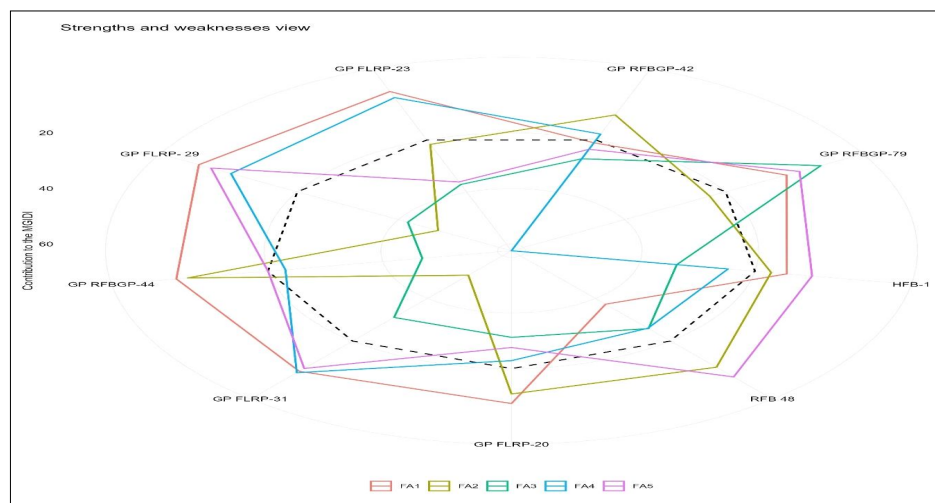


Fig 7: Contribution of traits to MGIDI across five factors in superior genotypes selection.

supports the reliability of the multivariate signal underlying the MGIDI ranking; comparable patterns-where the first PCs explain most agronomic variation and clearly separate superior, often earlier and higher-yielding faba bean genotypes-are reported in recent faba bean multivariate studies (Soliman *et al.*, 2024). In this context, positive selection differentials for pod-and seed-related traits alongside negative differentials for flowering and maturity are consistent with ideotype-based improvement that jointly targets sink capacity and earliness, as advocated in faba bean PCA-guided selection and in cross-crop MGIDI applications (Olivoto and Lúcio, 2021; Olivoto *et al.*, 2022).

CONCLUSION

This study revealed substantial genetic variability among 45 faba bean genotypes for key morphological and yield-related traits, with high heritability supporting the effectiveness of direct phenotypic selection. Cluster and divergence analyses identified Cluster IV as a valuable source of superior genotypes. They highlighted inter-cluster combinations, particularly between Clusters I and IV, as promising for heterosis and broadening the genetic base. Correlation analysis confirmed pods per plant, pod length and seed index as reliable indirect selection traits, while weaker associations emphasized the importance of managing trade-offs in breeding. Multivariate approaches, including PCA and MGIDI, effectively integrated trait information and prioritized ideotype-proximal genotypes such as GP RFBGP-42, GP RFBGP-79 and HFB-1 RFB-48 for advancement. Collectively, these findings provide a clear framework for selecting parents and advancing promising lines.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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