



Multivariate Analysis of Genetic Diversity and Yield Attributing Traits in Indian Mustard [*Brassica juncea* (L.) Czern. and Coss.]

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ABSTRACT

Background: The present study aimed to evaluate genetic diversity and identify key traits contributing to yield improvement in 53 accessions of *Brassica juncea* using multivariate analyses.

Methods: Thirteen agro-morphological traits were assessed under an augmented randomized block design. Significant variation among genotypes was observed for most traits through ANOVA, highlighting the scope for genetic enhancement.

Result: Principal component analysis revealed that the first three principal components explained 75.5% of the total variance, primarily influenced by shoot length, plant height, siliquae traits and yield attributes. The PCA biplot showed clear trait associations and grouped similar genotypes such as Pusa Bold, RH 119 and 53-14-24D. Mahalanobis D² clustering organized the genotypes into seven distinct clusters, with maximum inter-cluster distance indicating high divergence between clusters VI and VII. The study provides useful insights for breeders to select diverse parents and prioritize traits for the development of high-yielding cultivars.

Key words: *Brassica juncea*, D² analysis, Genetic diversity, Principle component analysis.

INTRODUCTION

Brassica juncea (L.) Czern. and Coss, commonly referred to as Indian Mustard, is a vital oilseed crop from the Brassicaceae family. This amphidiploid species emerged as a result of interspecific hybridization between *Brassica rapa* (2n=2x=20, AA) and *Brassica nigra* (2n=2x=16, BB) with a chromosome number 2n=4x=36, AABB). Vavilov identified Afghanistan and adjacent regions in Central Asia as the primary origin of *B. juncea*, while secondary centres of genetic diversity were suggested to be Asia Minor, parts of China and eastern India (Zhang *et al.*, 2023). Rajasthan dominates Indian mustard cultivation, among other states accounting for approximately 45% of the nation's total output (Ola *et al.*, 2024). India's share in the global oilseed sector includes 7.4% of total oilseed production, 5.8% of edible oil output and 6.1% of oil meal (Jat *et al.*, 2019; Samal *et al.*, 2021). Oilseed crops are vital to global agriculture, playing a key role in the Indian economy, reflected in the significant outcomes of the Yellow Revolution (Kumar *et al.*, 2020; Saha *et al.*, 2024). However, the rising population has led to a gradual drop in the per capita oil availability due to environmental stresses and limited resources (Anushree *et al.*, 2025), making it vital to boost production to secure future needs (Ponniyath *et al.*, 2025).

Assessing genetic variation is the fundamental step for a successful breeding program (Lakra *et al.*, 2020; Singh *et al.*, 2021). Agro-morphological traits have been widely used to explore the extent of genetic variation and to understand the evolutionary linkages among different *Brassica juncea* accessions (Vinu *et al.*, 2013; Akhatar *et al.*, 2020; Saroj *et al.*, 2021). Multivariate analyses like Principal component analysis and D² analysis have been employed to assess the variability among the studied traits. By summarizing complex trait data, PCA provides insights into

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genetic diversity and population structure (Upadhyay *et al.*, 2022). Mahalanobis (1936) D² analysis has proven to be an effective method for evaluating genetic divergence among genotypes and allows for a comprehensive assessment of variability in quantitative traits (Rout *et al.*, 2019). Considering the present scenario, the study was undertaken to assess the genetic diversity and interrelationships of different traits among various genotypes of Indian mustard with the aim to identify useful traits that can help in selecting better genotypes for future yield improvement.

MATERIALS AND METHODS

53 accessions of *Brassica juncea* were procured from released sources (Table 1). The experiment was conducted in Rabi 2024-2025 at the Agriculture Research Farm, Department of Genetics and Plant Breeding, Lovely Professional University, Jalandhar, Punjab under an

Augmented randomized block design with the experimental layout consisting of paired rows, each 2 meters in length and spaced 45 cm apart with a total plot size of 176m². The experimental site is located at an altitude of 230 meters above mean sea level, at 31°14'31"N latitude and 75°41'47"E longitude. The region experiences a humid subtropical climate, marked by cool winters and extended periods of hot summers. Observations were recorded on five competitive randomly selected plants for 13 traits *viz.*, germination percentage, days to 50% flowering, plant height (cm), main shoot length (cm), primary branches per plant, secondary branches per plant, number of siliquae per plant, number seeds per siliqua, length of siliqua (cm), thousand seed weight (g), biological yield per plant (g), economic yield per plant (g) and harvest index (%). Cluster and principal component analysis (PCA) was performed on the recorded data for quantitative traits. Prior to cluster and PCA, mean of each parameter was standardized to avoid the effects of scaling differences. For all the pairs of

accessions, Euclidean distance co-efficient were calculated. The resulting Euclidean dissimilarity co-efficient matrices were utilized to estimate the association among the *B. juncea* germplasm through cluster analysis using the complete linkage method (Statistical version 7.0 and NTSYS-pc v 2.1).

RESULTS AND DISCUSSION

Principal component analysis (PCA)

PCA was first introduced by Karl Pearson (1901) to reduce data dimensionality and identify patterns in multivariate data. In the current study, the dataset was divided into 13 components (Table 2 and Fig 2a), corresponding to various parameters. Out of the 13 principal components, the first three PCs (PC1, PC2 and PC3) occupied most of the variability (75.5%). According to Kaiser's rule for PCA, PC's with eigenvalues ≥ 1 are considered significant and account for most of the variability. Similar results were reported by

Table 1: List of genotypes.

S. No	Name of the genotypes	S. No	Name of the genotypes	S. No	Name of the genotypes
1	RH-30	19	Vaibhav	37	Pusa vijay
2	RK GOLD	20	Basanti	38	Shivshi shivan
3	Parvathi mustard	21	BR-40	39	Jagannath
4	Pusa Bold	22	Rohini	40	JD-6
5	RH 119	23	RGN-73	41	BAVI-086
6	53-14-24D	24	RH-0749	42	45S46
7	Pusa jai kisan	25	BAUT 082	43	55-14-101
8	CS-60	26	PB-1-14-6	44	RN-593
9	RL-1359	27	Pusa Saag	45	JM-1
10	RGN-13	28	RH-8812	46	Pusa mustard-24
11	BR-23	29	PT-2025-10	47	KBS-3
12	NRCHB-101	30	RGN-73	48	Bhagirathi
13	DKMRIJ-31	31	Urvashi	49	Krishna
14	BAUM-019	32	Malika	50	SMR-9
15	Kranti	33	Pusa mustard 27	S. No	Check varieties
16	NRCDR	34	Laxmi	1	PBH-14
17	Durga Mani	35	Gujrat mustard	2	Gujrat Mustard -2
18	PUSA 00	36	RB-50	3	Maya

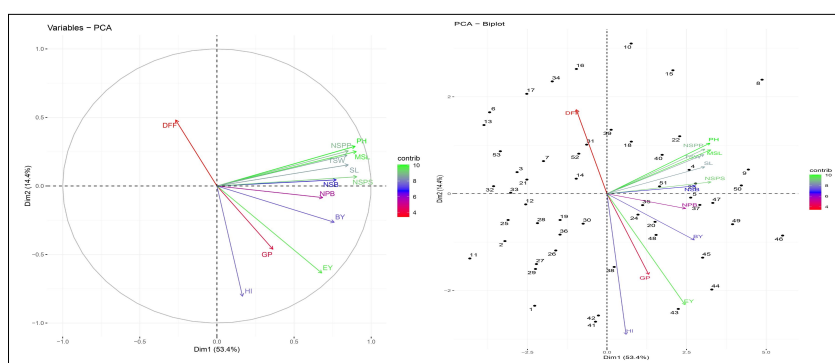


Fig 1: (a). Contribution of traits to PC (b). PCA biplot.

Table 2: Eigenvalues and coordinates of the eigenvectors of the 13 principal components in (*Brassica juncea*)

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Eigenvalue	6.946	1.867	1.001	0.821	0.694	0.529	0.399	0.281	0.184	0.152	0.098	0.027	0.001
Proportion	53.400	14.400	7.700	6.300	5.300	4.100	3.100	2.200	1.400	1.200	0.800	0.200	0.000
Cumulative	53.400	67.800	75.500	81.800	87.100	91.200	94.300	96.400	97.900	99.000	99.800	100.000	100.000
GP	0.138	0.338	0.433	-0.708	0.018	0.265	-0.054	-0.069	-0.269	-0.066	-0.149	0.029	-0.008
DFF	-0.102	-0.353	-0.625	-0.397	0.472	0.107	0.069	-0.022	-0.24	-0.07	-0.122	0.007	0.012
PH	0.34	-0.212	0.075	0.087	0.009	0.019	-0.32	0.344	-0.154	-0.195	-0.149	-0.721	0.037
MSL	0.343	-0.184	0.029	0.157	-0.041	-0.064	-0.31	0.267	-0.17	-0.212	-0.36	0.667	-0.043
NPB	0.26	0.062	-0.308	-0.32	-0.35	-0.573	-0.355	-0.322	0.002	-0.014	0.222	-0.024	0.004
NSB	0.294	-0.033	0.125	-0.147	0.097	-0.502	0.696	0.326	-0.011	-0.14	0.06	0.005	0.007
SL	0.323	-0.113	0.126	0.105	0.269	0.092	0.093	-0.61	0.381	-0.48	-0.13	-0.04	-0.004
NSPS	0.344	-0.049	-0.038	0.161	0.002	0.345	0.068	-0.044	-0.396	-0.118	0.736	0.115	-0.006
NSPP	0.323	-0.187	0.164	0.157	0.174	-0.104	0.081	-0.355	-0.299	0.707	-0.21	-0.046	0.001
TSW	0.32	-0.164	0.013	-0.275	0.169	0.188	-0.163	0.291	0.647	0.369	0.235	0.099	0.001
BY	0.289	0.194	-0.384	0.021	-0.401	0.311	0.281	0.009	0.072	0.07	-0.258	-0.086	-0.557
EY	0.258	0.465	-0.325	0.119	-0.002	0.133	0.09	0.048	0.038	0.055	-0.165	0.002	0.733
HI	0.063	0.589	-0.088	0.181	0.592	-0.21	-0.223	0.093	-0.027	0.013	0.077	-0.019	-0.385

Where, GP: Germination percentage, DFF: Days to 50% Flowering, PH: Plant height (cm), MSL: Main shoot length (cm), NPB: Number of primary branches, NSB: Number of secondary branches, SL: Silique length (cm), NSPS: Number of seeds per silique, NSPP: Number of silique per plant, TSW: Thousand seed weight (g), BY: Biological yield (g), HI: Harvest index, EY: Economic yield per plant.

Chakraborty *et al.* (2021), where the first three PCs accounted for the maximum variability indicating genetic consistency. PC1 had the highest eigenvalue of 6.946, contributing 53.4% of the total variance, having major contribution from the traits, main shoot length, plant height, number of siliquae per plant, siliquae length, test weight, number of seeds per siliqua, number of secondary branches, biological yield and number of primary branches. PC2 with an eigenvalue of 1.867, contributing 14.4% of the variance, for which harvest index, economic yield per plant, germination percentage and days to 50% flowering were

the major contributors. Similar traits were studied to contribute more to PC2 in the studies by Chakraborty *et al.* (2021) and Saikrishna *et al.* (2021). PC3 with an eigenvalue of 1.001, accounting for 7.7% of the variance, primarily represented by germination percentage, days to 50% flowering, biological yield, economic yield per plant and number of primary branches. PC4 to PC13 had eigenvalues less than 1 and accounted for the remaining 24.5% of the total variability. The scree plot in principal component analysis displays the percentage of total variance explained by each principal component (Fig 2c). Among the thirteen

Table 3: Distribution of 53 genotypes of Indian mustard into different clusters.

Cluster number	Number of genotypes in cluster	Name of genotypes in cluster
Cluster - I	17	JM-1, Krishna, BR-23, PBH-14, RH-8812, RGN-73, RH-749, Pusa Saag, Pusa Mustard-27, RNG-13, RNG-13, JD-6, Pusa Vijay, Gujrat mustard, PUSA 00, Laxmi, RH 119
Cluster- II	5	45S46, 55-14-101, KBS-3, Pusa Mustard-24, PT-2015-10
Cluster - III	16	Gujrat Mustard 02, Maya, DKMRIJ-31, Jagannath, Malika, NRCDR, PB-50, Urvashi, RH-30, Shivshi Shivan, BAUT-082, BAUM-019, Kranti, NRCHB-101, Pusa Jai Kisan, Durga Mani
Cluster - IV	12	Parvati Mustard, Vaibhav, Basanti, Pusa Bold, RK Gold, Bhagirathi, SMR-9, 53-14-24D, RL-1359, BR-40, Rohini, CS-60
Cluster - V	1	BAVI-086
Cluster - VI	1	PB-1-14-6
Cluster - VII	1	RN-393

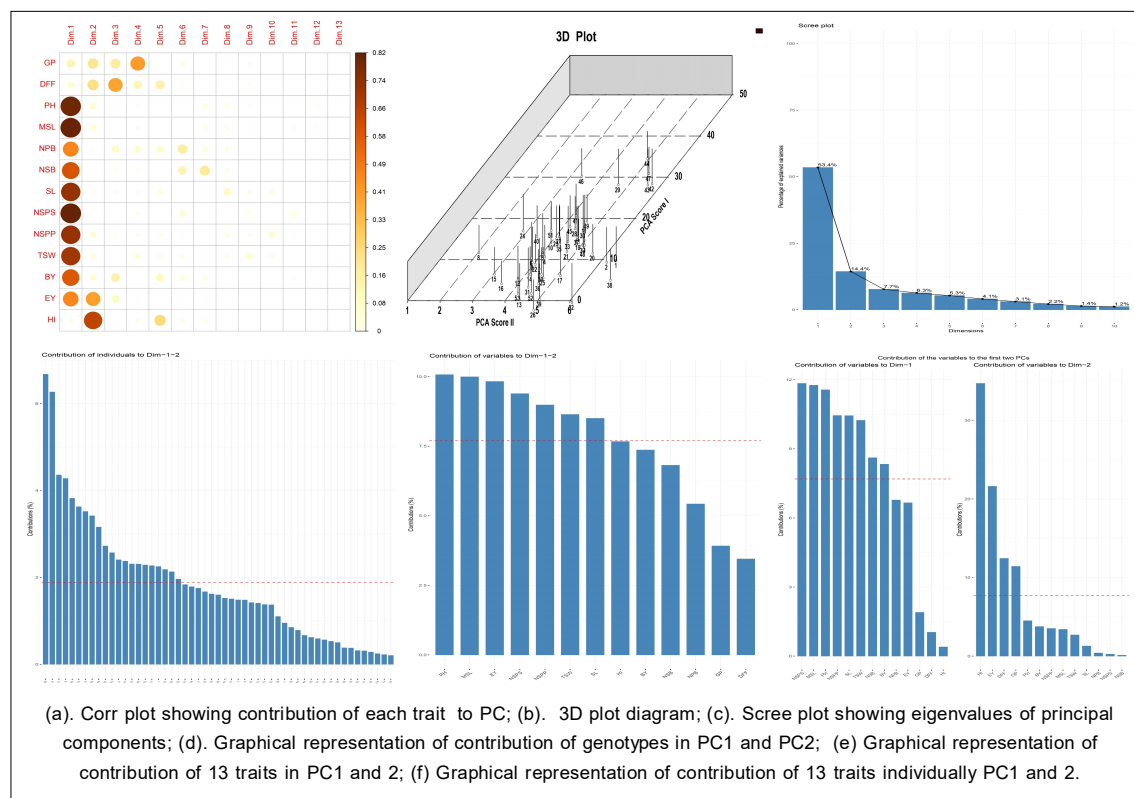


Fig 2: Graphical representation of principle component analysis (PCA).

PC's, PC1 had the highest contribution (53.4%) to the total variance (Fig 2e) followed by PC2 contributing 14.4% to the variance, PC3 accounting for 7.7% of the variance and increasing the cumulative variance to 75.5% (Fig 2c).

PCA-biplot analysis

The PCA biplot (Fig 1a and b) displayed the relationships among 13 traits and the distribution of 53 genotypes defined by PC1 and PC2 (Fig 2b,d, e and f). The traits, days to 50% flowering strongly influenced PC1 in the negative direction, while economic yield and biological yield contributed positively along PC1 and PC2 and were strongly correlated. Harvest index and germination percentage indicated negative strong correlation. The genotypes Pusa Bold, RH 119, 53-14-24D were closely located on the biplot indicating similar traits. The genotypes, 53-14-24D, Durga Mani, 55-14-101 were located close to the economic yield per plant indicating high yield.

Table 4: Contribution of various traits towards total genetic divergence.

Characters	Time ranked first	Contribution (%)
Germination percentage	1183	85.85%
Days to 50% flowering	4	0.29%
Plant height	7	0.51%
Main shoot length	13	0.94%
Number of primary branches	9	0.65%
Number of secondary branches	2	0.15%
Siliqua length	21	1.52%
Number of seeds per siliqua	15	1.09%
Number of siliquae per plant	26	1.89%
1000 seed weight	14	1.02%
Biological yield	34	2.47%
Harvest index	27	1.96%
Economic yield per plant	23	1.67%

D² analysis

D² analysis was carried out according to methods given by Mahalanobis (1936), which was later revised by Rao (1952). All the 53 genotypes were clustered into seven different clusters by using Tocher method (Table 3 and Fig 3). Maximum number of genotypes were present in Cluster-I, which contained 17 genotypes followed by Cluster-III (16), Cluster-IV (12). Least number of genotypes were present

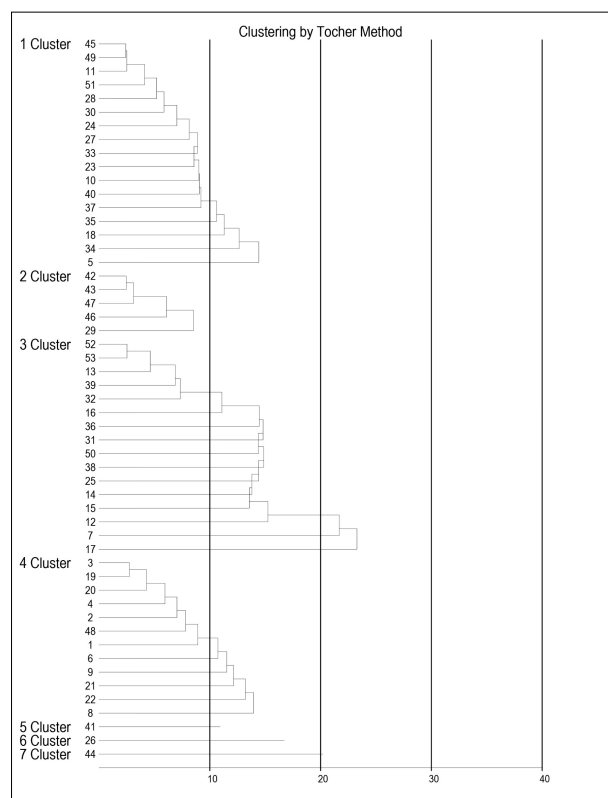


Fig 3: Clustering by tocher method.

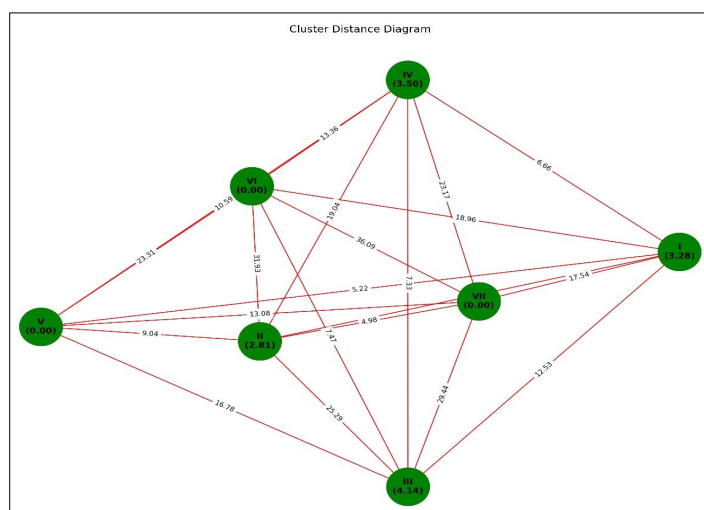


Fig 4: Intra and inter-cluster distance diagram.

Table 5: Cluster means for 13 traits in 53 genotypes of Indian mustard (*Brassica juncea*).

Cluster	GP	DFF	PH	MSL	NPB	NSB	SL	NSPS	NSPP	TSW	BY	HI	EY
I	21.25	68.16	183.61	177.91	6.09	5.25	4.33	223.66	8.38	3.74	266.04	18.21	47.62
II	31.6	64.47	174.8	179.98	6.4	6.27	4.64	211.64	8.41	3.84	272.81	18.72	45.95
III	11.74	67.59	177.14	176.85	5.83	5.68	4.44	205.6	8.35	3.6	259.41	17.86	45.65
IV	16.7	65.39	175.93	177.92	6.06	5.19	4.38	221.44	8.82	3.64	257.72	18.54	47.14
V	24.58	65	189.04	169.73	6.53	4.13	4.46	190.87	8.13	3.5	255.57	18.97	50.89
VI	6.37	65	182.51	179.4	6.13	6.73	4.67	208.4	7.73	3.64	253.39	18.72	47.11
VII	34.61	66.67	178.99	178.17	4.8	7.07	5.47	256.6	8.27	3.73	270.58	19.45	48.33

Where, GP: Germination percentage, DFF: Days to 50% Flowering, PH: Plant height (cm), MSL: Main shoot length (cm), NPB: Number of primary branches, NSB: Number of secondary branches, SL: Silique length (cm), NSPS: Number of seeds per silique, NSPP: Number of siliques per plant, TSW: Thousand seed weight (g), BY: Biological yield (g), HI: Harvest index, EY: Economic yield per plant.

in Cluster-II (5), Cluster-III (1), Cluster-V, Cluster-VI (1), Cluster-VII (1) and Cluster-IV (1). The clustering pattern revealed the presence of enough divergence to enable formation of individual clusters.

Contribution of various traits towards total genetic divergence

The contribution of various traits towards total genetic divergence (Table 4a) revealed that the trait germination percentage (85.85%) contributed the highest for divergence, followed by biological yield (2.47%) followed by harvest index (1.96%), economical yield (1.67%), number of siliques per plant (1.89%), silique length (1.52%), number of seeds per silique (1.09%), thousand seed weight (1.02%), main shoot length (0.94%), number of primary branches (0.65%), plant height (0.51%), days to 50% flowering (0.29%) and number of secondary branches (0.15%). Kumari and Kumari (2018) reported similar values for number of primary branches.

Cluster means

The range of mean values among different clusters was recorded for different characters in (Table 5). Cluster-I had the highest mean value for the trait days to 50% flowering (68.16). Cluster-II had the highest mean values for main shoot length (179.98), thousand seed weight (3.84) and biological yield (272.81). Cluster-III exhibited the lowest mean value for economic yield (45.65). Cluster-IV showed lowest mean for number of siliques per plant (8.82).

Cluster-V had the highest plant height (189.04), number of primary branches (6.53) and economic yield per plant (50.89). Cluster-VI had the highest mean value for silique length (4.67). Cluster VII exhibited the highest value for germination percentage (34.61), number of secondary branches (7.07), number of seeds per silique (256.6), harvest index (19.45). Similarly, Reddy *et al.* (2025) reported the highest cluster mean for plant height in Cluster VI, while Cluster I exhibited the highest mean value for the number of silique length. In contrast, Cluster II recorded the maximum mean for the number of seeds per silique. For 1000-seed weight, the highest mean was noted in Cluster VI. It's indicating that the genetic potential for that trait is higher in the genotypes of this cluster compared to others.

Inter and intra-cluster distances

The inter and intra-cluster distances revealed the extent of degree of genetic diversity among clusters (Table 6 and Fig 4). The maximum inter-cluster distance was found between clusters VI and VII (36.09) (Priyanka *et al.* 2021) followed by that between II and VI (31.93), III and IV (29.44), II and III (25.29) and IV and VII (23.17) Rout *et al.* 2019). whereas, minimum inter-cluster distance was found between clusters II and VII (4.98), followed by I and V (5.22), I and IV (6.66), III and IV (7.33) and III and VI (7.47). The intra-cluster distance observed were 3.28 (cluster I), 2.81 (cluster II), 4.14 (cluster III), 3.5 (cluster IV). The clusters V, VI and VII contained single

Table 6: Cluster distance in 53 genotypes of Indian mustard (*Brassica juncea*).

Cluster	I	II	III	IV	V	VI	VII
I	3.28	13.44	12.53	6.66	5.22	18.96	17.54
II		2.81	25.29	19.04	9.04	31.93	4.98
III			4.14	7.33	16.78	7.47	29.44
IV				3.5	10.59	13.36	23.17
V					0	23.31	13.08
VI						0	36.09
VII							0

genotypes and therefore, their intra-cluster distances were zero. The inter- and intra-cluster distances revealed considerable genetic diversity among the genotypes, with the highest inter-cluster distance observed between Clusters VI and VII (36.09), indicating their suitability as potential parents for hybridization to exploit heterosis. The highest intra-cluster distance was recorded in Cluster III (4.14), suggesting greater genetic variability within this cluster. Current findings are in line with Reddy *et al.* (2025).

CONCLUSION

The present investigation demonstrates considerable genetic diversity among 53 *Brassica juncea* accessions, with the first three principal components accounting for the majority of trait variation. Key traits influencing variability included main shoot length, plant height, siliquae per plant, silique length, test weight and yield-related characters. Genotypes Pusa Bold, RH 119 and 53-14-24D exhibited similar trait combinations, while 53-14-24D, Durga Mani and 55-14-101 were associated with higher economic yield. the trait germination percentage (85.85%) contributed the highest for divergence, followed by biological yield (2.47%) and minimum was from number of secondary branches (0.15%). The inter-cluster distances suggest ample scope for selecting genetically diverse parents. These findings can guide breeders in designing crossing programs to develop improved, high-yielding mustard varieties adapted to diverse conditions.

Conflict of interest

All authors declare that they have no conflict of interest.

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