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Research Note

Genetic variability, correlation, path coefficient and cluster analysis in Indian mustard (*Brassica juncea* L.)

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Abstract
Twenty-eight diverse mustard (*Brassica juncea* L.) genotypes were assessed for genetic variability, heritability, genetic advance, trait association and genetic diversity based on 14 yield and its attributing traits during the *Rabi* 2023 at the Post Graduate Research Farm, School of Agriculture, LPU, Punjab. The study revelled significant differences among the mustard genotypes both in their genotypic and phenotypic level. Significant differences were observed in traits such as number of siliquae per plant, number of siliquae on the main stem, and seed yield per plant. Moreover, it is noteworthy to emphasize that a pronounced degree of heritability was founded for all traits except for days to maturity. Significant genetic advancements expressed as a percentage mean was observed for all attributes examined, except for days 50% flowering and maturity. Seed yield per individual plant manifested a substantial and positive genotypic correlation with the number of secondary branches per plant, 1000-seed weight, biological yield per individual plant, and the harvest index. Path analysis further elucidated that biological yield per individual plant and the harvest index exerted the most substantial positive direct effects on seed yield per plant. Based on these findings, the study suggests that choosing mustard genotypes based on these particular traits could lead to a more effective strategy for increasing overall yield. Additionally, genotypes grouped into distinct clusters with higher average values for seed yield and other essential characteristics present an attractive opportunity for inclusion in hybridization efforts aimed at producing superior offspring.

Keywords: Brassica, Heritability, Correlation, Path analysis, Diversity

Brassica juncea, which is frequently referred to as rai, Raya, or laha in India, is a well-known plant species in the country is a member of the Cruciferae family. It is a naturally occurring amphidiploid that emerged from a cross between *Brassica campestris* and *Brassica nigra,* resulting in a chromosome count of $2n = 36$ (Nagaharu, 1935). This plant is believed to have originated in the Mediterranean region and has been cultivated for many centuries. While mustard is predominantly cultivated in temperate regions, it is also cultivated as a cold-weather crop in specific tropical and subtropical regions. Brown mustard prefers cool and moist growing conditions, typically with an average temperature range of 15–25 °C. Sandy soils are ideal for mustard growth, and the plant can reach a height of 60–70 cm or more (Pradhan, 2014). India is the second-largest country in terms of acreage dedicated to mustard cultivation, accounting for 19.81% of the global total, with Canada taking the first position. India contributes 10.37% of global production, placing it fourth in terms of production behind China, the European Union, and Canada (AICRP PC Report 2021, ICAR-DRMR). The average yield of brown mustard in India ranges from 1.2 to

1.5 tonnes per hectare, although this can vary depending on factors such as weather conditions, soil quality, and farming practises (Nautiyal *et al.,* 2012). Mustard seeds can be crushed to extract mustard oil, which is rich in unsaturated fatty acids and has a concentration of 38 to 46% in the seed oil (Smooker *et al*., 2011). Mustard oil serves multiple purposes, including being used as a hair oil, lubricant, and traditional remedy for ailments such as foot pain, rheumatism, and arthritis (Duke and Wain, 1981). Additionally, the leftover oilseed cake is used as fertiliser and livestock feed (Reed, 1976).

A comprehensive assessment of both genetic and non-genetic factors is necessary to understand the determinants of seed yield. Plant breeders must assess the genetic diversity present in the available germplasm for diverse qualities since it is essential to the success of crop improvement efforts. Heritability, genetic advance, genotypic coefficient of variation, and phenotypic coefficient of variation all serve as useful biometric indicators of genetic diversity (Manjunath *et al.,* 2017). Examining the traits that contribute to yield can assist breeders in identifying desirable genotypes for enhancing yield and other agronomic characteristics. By studying the genetic architecture of yield, breeders can select genotypes with high yield potential and desired combinations of traits (Khan and Dar, 2010). Despite being a complex crop, mustard is a favourable for scientific research in the areas of genetics and plant breeding due to its comparatively simple morphology. The components contributing to seed yield exhibit varying levels of correlation with both seed yield itself and with each other. Understanding the interrelationships among these components is crucial to identifying the most favourable combination within a single genotype.

Seed yield is influenced by multiple components, both directly and indirectly through other traits, posing challenges for breeders in their selection process. Path coefficient analysis, a methodology designed to dissect the correlation coefficient into direct and indirect effects of variables. This analytical tool has proven invaluable in understanding the factors influencing yield, empowering breeders to strategically identify superior accessions from gene banks (Wright, 1921). Genetic diversity is the base for survival of plants in nature and for crop improvement. Diversity in plant genetic resources provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits (high yield potential, large seed, etc.) and breeder-preferred traits (pest and disease resistance and photosensitivity, etc.).

The trial involving 28 mustard genotypes **Table 1**, sourced from the Directorate of Rapeseed Mustard Research, Bharatpur, Rajasthan, was conducted during the *Rabi* 2022 season at the Research Farm of the School of Agriculture, LPU, Punjab. The experiment was laid out in three replications in Randomized Block Design (RBD), with inter-row and intra-row spacing set at 45 cm and 20 cm, respectively and recommended cultural practices were followed for optimal growth and development of the plants.

Five plants were randomly selected from each entry across all replications for recording observations on plant height (PH) in centimetres, the number of primary branches per plant (NPBP), secondary branches per plant (NSBP), the length of the main raceme (LMR), the number of siliqua on the main raceme (NSMR), siliqua per plant (NSPP), siliqua length (LS), seeds per siliqua (NSPS), 1000 seed weight (TW) in grams, biological yield (BY) in grams, seed yield per plant (SYP), and harvest index (HI). Days to 50% flowering (DF) and maturity (DM) were recorded on plot basis.

Analysis of Variance was performed on the mean data, following the approach proposed by (Panse and Sukhatme, 1978). For assessing the genotypic and phenotypic coefficients of variation (GCV and PCV), techniques suggested by (Burton, 1952) were utilized. The broad-sense heritability was determined using the formula established by (Allard, 1960). To estimate the genetic advance, the method proposed by (Johnson *et al.,* 1955) was employed. The path analysis was conducted according to Dewey and Lu, 1959. Genetic diversity was assessed based on D^2 statistics as per Mahalanobis, 1936.

The ANOVA in this study identified significant difference among the genotypes for all studied characters, as detailed in **Table 2**. This implies the existence of genetic diversity within the utilized genotypes. Moreover, the mean performance of these genotypes exhibited a wide range, indicating significant variation among them. Similar finding were also observed by Kumar and Pandey, 2013, Shrivastava *et al.,* 2023 and Choudhary *et al.,* 2023.

PCV and GCV provide insights into the environmental impact on various characters. Small differences between PCV and GCV estimates illustrate the influence of environmental factors on trait expression. Low PCV and GCV values were observed for Days to 50% Flowering, length of siliqua, and length of the main raceme, and also for the number of seeds per siliqua. Similar findings were reported by (Gupta *et al.,* 2022) and (Shrivastava *et al.,* 2023), who emphasized the significance of considering these traits directly for selection.

Moderate values of both PCV and GCV were founded for traits such as primary branches per plant, number of secondary branches per plant, 1000 seeds weight, harvest index, and biological yield per plant **(Table 3)** indicating a substantial amount of genetic contribution in the expression of the traits and minimal environmental influence on these traits, suggesting the potential for

Table 1. List of diverse genotypes along with pedigree used in study

S. No.	Genotypes	Pedigree / Source (DRMR)
1.	BHAGIRATHI	SELECTION FROM PUSA JAI KISHAN
2.	NRCHB 101	BL-4 X PUSA BOLD
3.	VAIBHAV	DERIVED THOUGHT BIPARENTAL MATING INVOLVING VARUNA,KESHARI, CSU10 AND B1775, B1786, B1866
4.	TM 4	VARUNA X TM-1
5.	KRANTHI	SELECTION FROM VARUNA
6.	RH 30	SELECTION FROM P26/3-1
7.	DMH 1	DEVLOPED BY CMS
8.	RH 119	PUSA BOLD X RAJAT (PCR-7)
9.	KBS 3	PUSA KALYANI X YUKINA
10.	ROHINI	SELECTION FROM NATURAL POPULATION OF VARUNA
11.	URVASHI-18	VARUNA X KRANTI
12.	PUSA MUSTARD 24	PUSA BOLD X(LEB) X LES-29
13.	PUSA JAI KISHAN	SOMACLONE OF VARUNA
14.	GUJARATH MUSTARD-2	SELECTION FROM MATERIAL COLLECTED FROM VENDACHU, GUJRAT
15.	GUJARATH MUSTARD-1	MR 71-3-2 X TM-4
16.	PUSA BOLD	VARUNA X BIC1780
17.	SMR 9	-
18.	RH 749	DRMR
19.	BR 23	SELECTION FROM LOCAL GERMPLASM OF PURNEA, BHIHAR
20.	TL 17	-
21.	JD 6	PUSA BOLD X GLOSSY
22.	PUSA MUSTARD 28	SEJ8 X PUSA JAGANNATH
23.	RH 701	-
24.	PARWATHI MUSTARD	SELECTION FROM GAMMA IRRIGATED BT4
25.	PUSA MUSTARD 27	DIVYA/PUSABOLD/PR666EPS/PR704EPS2
26.	LAXMI RH-8812	-
27.	WR-2019	-
28.	RGN-48	RSM 204 X B 75

improvement in these genotypes. Similar conclusions were drawn by Kumar and Pandey, 2013. In case of days to maturity, low PCV and medium GCV values were observed, as reported by Tarkeshwar *et al.,* 2022.

Heritability and Genetic advance: As shown in **(Table 3)**, the estimated heritability ranged from 88.21% to 95.09% across various traits. In predicting the genetic gain and for effective selection for the improvement of concerned traits, considering heritability and genetic advance simultaneously has been found more helpful. However, it is not necessary that the character showing high heritability will also exhibit high genetic advance. High heritability was recorded for traits such as plant height, number of siliqua on the main raceme, number of siliqua per plant, length of siliqua, 1000 seed weight, biological yield per plant, harvest index, and seed yield per plant, indicating the presence of additive gene action. Similar findings were reported for the number of siliqua per plant by Tarkeshwar *et al.,* 2022 and plant height by Sur *et al.,* 2023. Moderate heritability was observed for traits like Days to 50% flowering, Number of primary branches, Number of secondary branches, Length of main raceme, and Number of seeds per siliquae. Choudhary *et al.,* 2023 also found similar results for Days to 50% flowering. On the other hand, low heritability was recorded for DM, consistent with the results reported by Patel *et al.,* 2021. The percentage of genetic advancement (GA) ranged from 10.60% (days to 50% flowering) to 41.87% (seed yield per plant). Traits like plant height, 1000 seed weight, biological yield, number of siliqua on the main raceme, number of siliqua per plant, seed yield per plant, and harvest index showed a high GA percentage, which aligns with the findings reported by Rahul *et al.,* 2022 and Shrivastava *et al.,* 2023 for seed yield per plant and biological yield per plant. Moderate GA percentages was observed for traits such as the number of primary branches per plant, number of secondary branches

Table 2. Analysis of variance for fourteen mustard character

S. No.	Characters	Source of variation			
		Replications	Genotypes	Error	CV%
	Degree of freedom	2	27	54	
1	Days to 50% flowering	2.08	25.53**	3.01	3.47
2	Days to maturity	11.98	24.50**	12.25	2.91
3	Plant height (cm)	23.56	1392.19**	50.19	4.88
4	Number of primary branches	0.09	0.69**	0.11	8.43
5	Number of secondary branches	0.06	2.11**	0.37	8.99
6	Length of main raceme	24.42	119.59**	22.00	7.50
7	Number of siliquae on main raceme	19.75	92.52**	8.04	8.80
8	Number of siliquae per plant	88.24	9652.79**	110.42	3.90
9	Length of siliquae	0.02	0.66**	0.04	2.90
10	Number of seeds per siliquae	0.23	6.28**	0.65	4.02
11	1000 seed weight	0.001	1.05**	0.01	2.19
12	Biological Yield	60.71	2095.98**	61.95	4.80
13	Harvest Index	8.38	72.84**	5.78	7.49
14	Seed yield per plant	14.74	378.05**	18.55	8.20

Table 3. Coefficient of variation, heritability, and genetic advance

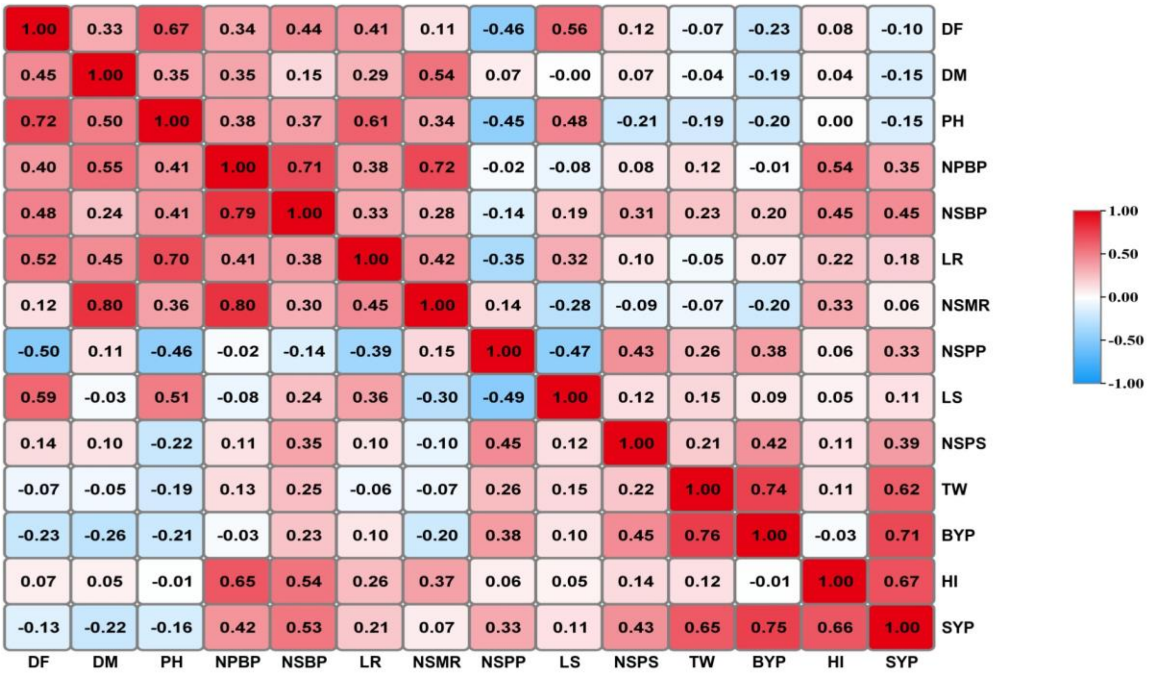
Characters	Range		General Mean	Co-Variance		Heritability Broad Sense (%)	Genetic Advance (5%)	GA% as mean
	Min.	Max.		GCV	PCV			
DF	45.33	54	50.02	5.48	5.83	88.21	5.30	10.60
DM	116.57	130.14	120.14	1.68	2.38	49.98	2.94	2.45
PH	102.40	168.4	145.13	14.57	14.84	96.40	42.78	29.48
NPBP	3.28	5.67	3.90	11.30	12.31	84.36	0.83	21.38
NSBP	5.31	8.39	6.77	11.24	12.38	82.41	1.42	21.02
LR	52.03	77.36	62.54	9.12	10.10	81.60	10.61	16.97
NSMR	24.80	55.87	32.24	16.46	17.22	91.31	10.45	32.40
NSPP	193.49	414.14	269.44	20.93	21.05	98.86	115.51	42.87
LS	5.77	7.71	6.75	6.73	6.94	94.18	0.91	13.46
NSPS	17.85	22.11	20.03	6.84	7.22	89.66	2.67	13.34
TW	3.36	5.36	4.21	13.98	14.03	99.19	1.21	28.67
BYP	120.28	213.81	163.98	15.88	16.12	97.04	52.84	32.22
HI	19.96	40.13	32.08	14.74	15.36	92.07	9.35	29.14
SYP	24.06	68.19	52.52	20.84	21.37	95.09	21.99	41.87

per plant, length of the main raceme, number of seeds per siliqua, and length of siliqua. Similar results were reported for the number of primary branches per plant by (Patel *et al.,* 2021) and (Rajendra *et al.,* 2022). Low GA percentages were observed for days to 50% flowering and days to maturity, consistent with the results reported by (Kumar *et al.,* 2013) and (Khan and Dar, 2010) for days to maturity. High or moderate heritability coupled with high or moderate genetic advance indicates preponderance of additive gene action in the inheritance of concerned traits and selection may be effective in this case, while high or moderate heritability coupled with low genetic advance or vice versa indicates preponderance of non- additive gene action.

Analysis of the: trait association: The genotypic and phenotypic correlation values were very similar, revelling that environmental factors don't affect these traits much. Seed yield per plant is linked positively with several variables, including primary branches per plant, secondary branches per plant, siliqua per plant, seeds per siliqua, 1000 seed weight, length of the main raceme, number of siliquae on the main raceme, biological yield per plant, and harvest index **(Fig. 1).** These characteristics exhibit positive relationships at both the phenotypic and genotypic level. Similar findings observed in a previous study conducted by (Shrivastava *et al.,* 2023). This suggests that these characters should be considered while selecting for improvement in seed yield per plant provided that the character should show high effect on yield, which is the real basis for selection.

On the other hand, traits negatively correlated in both phenotypic and genotypic correlations were observed for days to 50% flowering, days to maturity, and plant height. This is consistent with the findings of Sur *et al.*, 2023 and is not recommended for selection to improve seed yield.
The study of the path coefficient analysis revealed that seven traits, namely BY, HI, NSPP, TSW, PH, NPBP, and DF have direct positive effects on seed yield. Thus, these traits are considered valuable for selection to improve yield **(Table 4)**. Similar findings were reported for BY and HI by Choubey *et al.,* 2022 and Shekhawat *et al.,* 2014. It is proved that characters showing high positive effects on grain yield also exhibit high direct effects. In contrast, the traits showing a negative direct effect on seed yield were length of siliqua, days to maturity, number of secondary branches per plant, length of the main raceme, number of seeds per siliqua, and number of siliqua on the main raceme. Gupta *et al.,* 2022 and Chaubey *et al.,* 2022 also reported similar results for days to maturity and length of siliqua.

Genetic diversity analysis: A significant range of variation was found among the 28 evaluated mustard genotypes. Cluster analysis showed maximum intra-cluster differences in cluster I, followed by cluster II, while clusters III & IV displayed zero intra-cluster distances. Inter-cluster diversity ranged from 19.53 to 30.80, with the highest distance between clusters II and IV, followed by III and IV, and the lowest between clusters I and II, then I and III. It was observed that number of siliqua per plant was the highest contributor towards divergence followed by Number of siliqua on main raceme, Length of siliqua, biological yield, harvest index, plant height, test weight, Days to 50 % flowering, Days to maturity, Number of primary branches, Number of seeds per siliqua, Number of secondary branches, length of main raceme and seed yield per plant. **(Fig. 2)**. Crosses between genotypes within the same cluster are unlikely to produce transgressive segregants. Hence, hybridization strategies should prioritize utilizing parents from different clusters with maximum divergence to achieve desirable transgressive segregants (Kumar and Pandey, 2013). When choosing parent plants for a hybridization program, it's crucial to consider factors such as the extent of genetic diversity,



DF	DM	PH	NPBP	NSBP	LR	NSMR	NSPP	LS	NSPS	TW	BYP	HI	SYP	
1.00	0.33	0.67	0.34	0.44	0.41	0.11	-0.46	0.56	0.12	-0.07	-0.23	0.08	-0.10	DF
0.45	1.00	0.35	0.35	0.15	0.29	0.54	0.07	-0.00	0.07	-0.04	-0.19	0.04	-0.15	DM
0.72	0.50	1.00	0.38	0.37	0.61	0.34	-0.45	0.48	-0.21	-0.19	-0.20	0.00	-0.15	PH
0.40	0.55	0.41	1.00	0.71	0.38	0.72	-0.02	-0.08	0.08	0.12	-0.01	0.54	0.35	NPBP
0.48	0.24	0.41	0.79	1.00	0.33	0.28	-0.14	0.19	0.31	0.23	0.20	0.45	0.45	NSBP
0.52	0.45	0.70	0.41	0.38	1.00	0.42	-0.35	0.32	0.10	-0.05	0.07	0.22	0.18	LR
0.12	0.80	0.36	0.80	0.30	0.45	1.00	0.14	-0.28	-0.09	-0.07	-0.20	0.33	0.06	NSMR
-0.50	0.11	-0.46	-0.02	-0.14	-0.39	0.15	1.00	-0.47	0.43	0.26	0.38	0.06	0.33	NSPP
0.59	-0.03	0.51	-0.08	0.24	0.36	-0.30	-0.49	1.00	0.12	0.15	0.09	0.05	0.11	LS
0.14	0.10	-0.22	0.11	0.35	0.10	-0.10	0.45	0.12	1.00	0.21	0.42	0.11	0.39	NSPS
-0.07	-0.05	-0.19	0.13	0.25	-0.06	-0.07	0.26	0.15	0.22	1.00	0.74	0.11	0.62	TW
-0.23	-0.26	-0.21	-0.03	0.23	0.10	-0.20	0.38	0.10	0.45	0.76	1.00	-0.03	0.71	BYP
0.07	0.05	-0.01	0.65	0.54	0.26	0.37	0.06	0.05	0.14	0.12	-0.01	1.00	0.67	HI
-0.13	-0.22	-0.16	0.42	0.53	0.21	0.07	0.33	0.11	0.43	0.65	0.75	0.66	1.00	SYP

Fig. 1. Phenotypic (rp) correlations (above the diagonal) and genotypic (rg) correlations (below the diagonal) among 14 different traits in mustard

DF-Days to 50% flowering; DM-Days to maturity; PH- Plant height (cm); NPBP- Number of primary branches/plant; NSBP-Number of secondary branches/plant; LMR- Length of main raceme; NSMR-Number of siliquae on main ra-ceme; NSPP-Number of siliquae per plant; LS-Length of siliquae; NSPS-Number of seeds per siliquae; 1000 seed weight; BYP-Biological Yield/plant; HI-Harvest Index; SYP-Seed yield per plant

Table 4. Phenotypic direct and indirect effect of different characters on seed yield per plant in mustard

Characters	DFF	DM	PH	NPBP	NSBP	LMR	NSMR	NSPP	LS	NSPS	TSW	BYP	HI	SYP
DF	**0.0358**	0.0116	0.0239	0.0121	0.0159	0.0148	0.0038	-0.0166	0.0201	0.0044	-0.0023	-0.0082	0.0028	-0.1048
DM	-0.0155	**-0.0478**	-0.0165	-0.0166	-0.0072	-0.0139	-0.0259	-0.0032	0.0001	-0.0035	0.0017	0.0093	-0.0019	-0.1481
PH	0.0141	0.0073	**0.0211**	0.0081	0.0078	0.0129	0.0072	-0.0095	0.0100	-0.0044	-0.0040	-0.0042	0.0001	-0.1482
NPBP	0.0005	0.0005	0.0005	**0.0014**	0.0010	0.0005	0.0010	0.0001	-0.0001	0.0001	0.0002	0.0001	0.0007	0.3508
NSBP	-0.0069	-0.0023	-0.0057	-0.0110	**-0.0155**	-0.0051	-0.0044	0.0021	-0.0030	-0.0047	-0.0035	-0.0031	-0.0069	0.4503
LMR	-0.0070	-0.0049	-0.0103	-0.0063	-0.0056	**-0.0168**	-0.0070	0.0058	-0.0054	-0.0016	0.0008	-0.0013	-0.0037	0.1812
NSMR	-0.0008	-0.0041	-0.0026	-0.0054	-0.0021	-0.0032	**-0.0076**	-0.0011	0.0021	0.0007	0.0005	0.0015	-0.0025	0.0581
NSPP	-0.0152	0.0022	-0.0149	-0.0007	-0.0045	-0.0114	0.0047	**0.0329**	-0.0154	0.0142	0.0085	0.0123	0.0020	0.3250
LS	-0.0010	0.0001	-0.0008	0.0001	-0.0003	-0.0005	0.0005	0.0008	**-0.0017**	-0.0002	-0.0003	-0.0002	-0.0001	0.1101
NSPS	-0.0003	-0.0002	0.0004	-0.0002	-0.0006	-0.0002	0.0002	-0.0009	-0.0003	**-0.0021**	-0.0004	-0.0009	-0.0002	0.3881
TW	-0.0016	-0.0009	-0.0047	0.0029	0.0055	-0.0012	-0.0017	0.0063	0.0038	0.0051	**0.0246**	0.0182	0.0026	0.6246
BYP	-0.1623	-0.1377	-0.1413	-0.0077	0.1428	0.0528	-0.1403	0.2659	0.0651	0.3012	0.5245	**0.7089**	-0.0201	0.7126
HI	0.0553	0.0281	0.0026	0.3740	0.3131	0.1526	0.2275	0.0425	0.0347	0.0790	0.0743	-0.0198	**0.6989**	0.6718

R SQUARE = 0.9900 RESIDUAL EFFECT = 0.1002

DF-Days to 50% flowering; DM-Days to maturity; PH- Plant height (cm); NPBP- Number of primary branches/plant; NSBP-Number of secondary branches/plant; LMR- Length of main raceme; NSMR-Number of siliquae on main ra-ceme; NSPP-Number of siliquae per plant; LS-Length of siliquae; NSPS-Number of seeds per siliquae; 1000 seed weight; BYP-Biological Yield/plant; HI-Harvest Index; SYP-Seed yield per plant

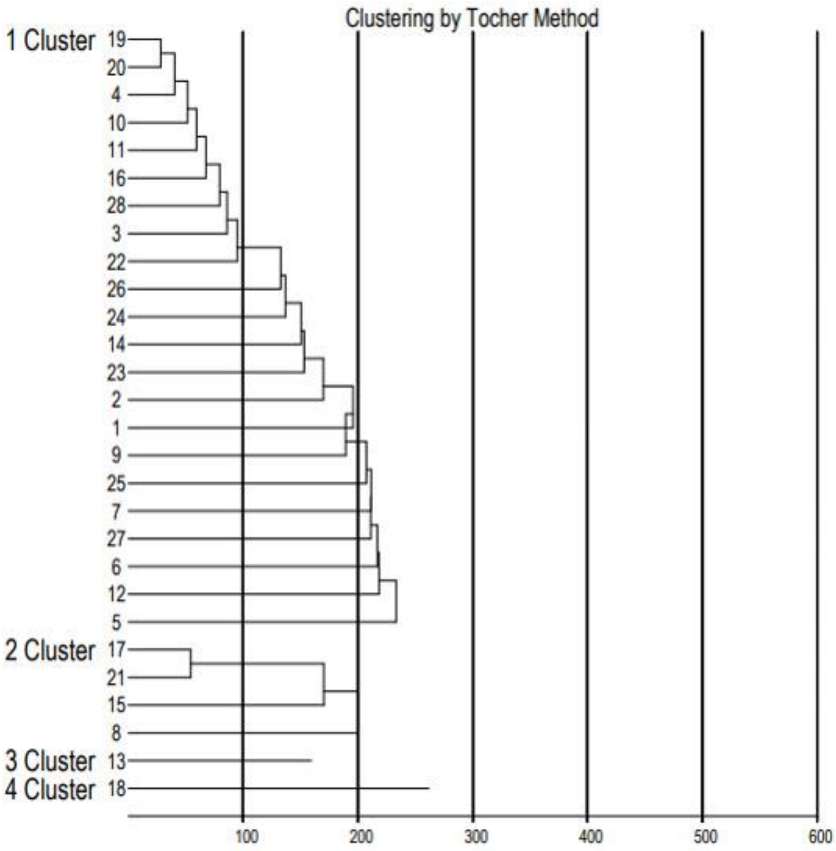


Fig. 2. Cluster diagram of 28 mustard genotypes based on 14 observable traits

Table 5. Average inter and intra-cluster distance values in mustard

Cluster	Cluster1	Cluster2	Cluster3	Cluster4
Cluster1	13.73	19.59	19.53	21.94
Cluster2	19.59	15.1	20.46	30.8
Cluster3	19.53	20.46	0.00	24.72
Cluster4	21.94	30.80	24.72	0.00

the individual traits contributions to overall divergence, and the cluster means for various trait performances. This strategic selection process is aimed at optimizing the potential for heterosis in the resulting hybrids. The variability between germplasms in clusters I & II, I & III, II & IV, IV & III, IV & I, and II & III is expected to result in high heterosis, as well as an accumulation of favorable genes in subsequent segregating generations **(Table 5)**.

In conclusion, the presence of favourable additive gene action, for traits like plant height, seed yield per plant, 1000 seed weight, and harvest index was indicated by the high heritability and significant genetic advance. The genotypic and phenotypic correlations further affirm the positive and direct effects of harvest index and number of seeds per siliqua on seed yield, emphasizing their importance for mustard breeding programs. Hence selection of these high-performing traits to increase mustard yield could yield desirable results. Genetic divergence analysis in mustard identified 28 genotypes grouped into four clusters, highlighting significant intra-cluster differences in clusters I and II. Genotypes from these clusters, exhibiting maximum diversity, are promising for future hybridization programs.

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