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Integrating morphological and molecular diversity with heterosis and combining ability for seed yield improvement in mustard (*Brassica* spp.)

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ABSTRACT

The 23 genotypes of mustard were evaluated for morphological genetic diversity using Mahalanobis D^2 analysis reported five different clusters based of seed yield plant^{-1} and contributing traits. The maximum desirable inter-cluster distance observed between cluster I and IV (D^2 =15.23), while the selected genotypes were grouped into five different clusters using Intron Polymorphic (IP) markers were used to assess molecular diversity among the selected genotypes, which also confirmed diversity among the genotypes. On confirming the variability among genotypes, these genotypes were intermated in line × tester mating design with 15 testers and 8 lines to study heterosis and combining ability between them. The heterosis analysis coupled with gene action revealed that seed yield exhibited high σ^2_{SCA} effect. The crosses ACN-184 x DRMRMJB-35, CG-SARSON x LES-39, TAM 108-1 x RE-8, ACN-184 x RE-8 and CG-SARSON x DRMRIJ12-40 exhibited highly significant useful heterosis for seed yield plant^{-1} along with contributing characters. Additionally, the crosses CG-SARSON x RE-11 and NRCHB 101 x RE-11 showed superior performance over the check, possessing parents with significant variability that can be utilized in a hybridization program. The combining ability analysis indicated that among lines, CG-SARSON, ACN-184 and TAM 108-1, and among testers DRMRMJB-35, LES-39 and RE-11 were identified as good combiner parents. Among crosses, BHAWANI x DRMRMJB-35, ACN-184 x DRMRIJ-31, NRCHB 101 x LES-39, CG-SARSON x NPJ-112 and ACN-184 x RE-11 are recommended for further progression to the next generation, either through biparental mating, recurrent selection or diallel mating for further crop improvements.

Keywords: *D^2 Analysis, Molecular Diversity, Intron Polymorphic (IP) Marker, Heterosis, Combining Ability, Mustard, Line x Tester Mating Design*

1. Introduction

In India, among oilseeds, mustard is the second-largest crop after soybean, contributing around 20-22% to India's total oilseed production, making India the world's third-largest oilseed producer. In India, oilseeds occupy 14.1% of the cropped area, with rapeseed-mustard comprising 3% [1]. Mustard seeds with around 38-42% oil are crushed for affordable edible oil, renowned for its golden colour and aroma. Mustard cultivation in India focuses on oil extraction, facing challenges with declining cultivation area and stagnant production. Despite being the 7th largest global edible oil importer, India still imports 57% of its domestic requirements, necessitating the development of high-yielding mustard varieties [2]. In the Vidarbha region of Maharashtra, delayed planting after harvesting of paddy resulted in to powdery mildew infestation and poor yield, leading to a continuous decline in mustard cultivation [3].

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The improvement of crop plants is fundamentally dependent on the extent of genetic variability present in various quantitative traits [4]. Genetic diversity plays a crucial role in plant breeding, as hybrids derived from genetically diverse lines typically exhibit greater heterosis compared to those from closely related lines [5]. Genetic diversity can be assessed using morphological, biochemical, and molecular approaches [6]. Among these, the morphological approach associated with D^2 analysis of quantitative characters under study explains genetic diversity on the basis of morphological characters. However, the molecular approach will validate morphological diversity conclusions. Among the various molecular markers, Intron Polymorphic (IP) markers stand out due to their multi-allelic nature and their high resolution, scorability, and reproducibility. These characteristics make them an excellent tool for determining phylogenetic relationships among closely related taxa [7].

While combining ability analysis provides useful information for superior parents and crosses, especially through line x tester design developed by Kempthorne [8]. Heterosis studies are essential for enhancing mustard production, aiding in the identification of desirable crosses. This study aims to assess genetic diversity using morphological as well as molecular approaches along with combining ability and heterosis in

mustard genotypes, identifying superior combiners and hybrids for future breeding programs.

2. Materials and Methods

2.1. Plant Material

The 23 genotypes of Indian mustard collected form AICRP on Linseed and Mustard, College of Agriculture, Nagpur were planted in *rabi* 2020-21 in 3 replications using randomize block design for the evaluation of genetic diversity using Mahalanobis D^2 statistics based on morphological data [9]. To further dissect the genetic diversity among genotypes, belong to different cluster (I, IV &V) based on morphological data, 16 genotypes were selected for molecular diversity using Intron Polymorphic (IP) markers.

2.2. Experimental material and Field Evaluation

The research trial was conducted at AICRP on Linseed and Mustard, College of Agriculture, Nagpur (21°08'27.0"N, 79°04'24.6"E). In *rabi* 2021-22, 15 genotypes were used as testers, which were crossed with 8 genotypes as a line under study in line x tester mating design. In *rabi* 2022-23, all 23 parents and 120 crosses along with TAM 108-1 as check were planted in RBD in 3 replications to raise a healthy crop. The observations were recorded for days to 50% flowering, days to maturity on plot basis, whereas plant height, number of branches plant^{-1}, number of siliquae plant^{-1}, point to first siliqua, siliquae length, number of seeds siliqua^{-1}, siliqua density on main branch, 1000 seed weight and seed yield plant^{-1} were reported on five randomly selected plants.

2.3. Molecular marker analysis

The DNA were extracted from pooled fresh and young leaves (7days old seedling) of five plants per genotype by using CTAB method [10] and purified with phenol. The DNA extracted were quantified by using a spectrophotometer at UV absorbance ratio of A260/A280. The quality of concentration was examined by running on 0.8% agarose gel electrophoresis along with a lambda DNA ladder. Thirty-one (31) IP markers were used to study DNA polymorphism using DNA amplification in PCR (Appendix 1). PCR amplification was carried out in a 15 µL reaction volume containing 10X Taq buffer, 1.2µl $MgCl_2$, 2µl dNTPs, 0.75µl of each primer (Forward and Backward), 0.5 µl Taq DNA polymerase, and 2 µl of template DNA. The thermal profile consisted of 35 cycles, preceded by an initial denaturation at 95°C for 5 minutes. Each cycle involved denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was performed at 72°C for 10 minutes. The amplified PCR products were analyzed on a 2.5% agarose gel. Darwin software was used for dissimilarity coefficient calculation and dendrogram was constructed based on UPGMA.

2.4. Data Analysis

The analysis of variance was performed to test the significance of differences between the genotypes as per Panse and Sukhatme [11]. Mahalanobis D^2 statistics was used for the estimation of genetic divergence among 23 genotypes. D^2 values were clustered using Tocher's approach, as reported by Rao [12] and intra and inter-cluster distances were computed using the standard procedure given by Singh and Choudhary [13]. The molecular bands were scored based on presence (1) and absence (0) of the band, which were analysed using DARwin 6.0.21 to create dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) to cluster genotypes into different groups using Jaccard's similarity coefficient [14]. The combining ability analysis were carried out following the methodology with fixed effect model [8]. The analysis of heterobeltiosis and useful heterosis was calculated as per the formulae given by Fonseca and Patterson [15] and Meredith and Bridge [16], respectively.

3. Results and Discussion

3.1. Morphological Characterization

The genetic divergence was estimated by Mahalanobis D^2 statistics for evaluation of genetic diversity in plant breeding [12]. Twenty-three genotypes were clustered in five clusters using Mahalanobis D^2 statistics, represented in Table 1 and Figure 1. The cluster I was largest, comprising of 17 genotypes, followed by cluster IV comprising of 3 genotypes, cluster II, cluster III, cluster V comprising single genotypes in each cluster. The check TAM 108-1 grouped into cluster I along with 16 genotypes. This indicates that there are genotypes which were highly diverse from the check and hence offers good scope for improvement. Similarly, thirty-two germplasms of leafy mustard grouped into six clusters by Mahalanobis D^2 statistics [17]. 36 Indian mustard genotypes grouped into 11 clusters [18] whereas 56 genotypes clustered into 7 clusters representing the genetic diversity present in the available genotypes under study [19].

The intra cluster distance was ranged between 0.00 to 9.46 whereas cluster IV (9.46) reported highest intra-cluster distance followed by cluster I (6.93) and cluster II, III and V reported single genotypes in each having intra cluster distance 0.00. Cluster V comprises PC-6 (*Brassica carinata*) species reporting maximum inter-cluster distance between cluster V and cluster III (D^2 = 25.83) followed by cluster V and cluster II (D^2 = 22.84) and cluster V and Cluster I (D^2 = 20.97) showed the presence of available diversity between two species. However, PC-6 was subjected to the introduction of genes for powdery mildew resistance and terminal heat tolerance. Hence, PC-6 cannot be selected as a suitable cross combination. The following cluster combination followed between cluster III, IV and cluster II, IV which comprises NPJ-112 and CN-105364 respectively, which found non-significant performance for yield over check which reject the combination. The next desirable combination with high inter cluster distance between cluster I and cluster IV (D^2 = 15.23) which comprises following genotypes RE-8, DRMRIJ12-40, DRMRIJ-31, M-34, ACN-141, ACN-9, DRMRIJ12-48, ACN-184, TAM 108-1, NRCHB 101, IC-597880, TN-3, LES-39, DRMRMJB-35, BHAWANI, CG-SARSON, RLC-3 and RE-11, NC-37362, RE-44 respectively. The crosses between genotypes of these diverse clusters might be having more chances of getting desirable segregants [20]. However, all the possible cluster combinations reported high inter cluster value than mean $\overline{D}$ value ($\overline{D}$ = 5.94) which allow to select all the genotypes under crossing programme to study gene action associated with the characters. Similarly, maximum inter cluster distance between cluster II and V followed by cluster IV and V and cluster III and V which select the possible cross combinations coupled with high heterosis [21]. The findings were confirmed with study conducted by Vanukuri and Pandey reported the maximum inter cluster distance between cluster II and V which could be concluded that genotypes in these two clusters showed maximum degree of diversification [22].

The cluster mean performance for all characters revealed that cluster V reported maximum mean for plant height, number of

branches plant^{-1}, number of siliquae plant^{-1}, siliquae density on main branch and seed yield plant^{-1}, whereas point to first siliquae was found minimum which is desirable under selection. Whereas, cluster III reported high siliqua length and number of seeds siliqua^{-1} with early days to 50% flowering and days to maturity, which showed minimum *per se* performance for plant height, number of siliquae plant^{-1} and siliqua density on main branch. However, cluster II reported highest 1000 seed weight with early in days to 50% flowering but reported lowest mean for number of branches plant^{-1}, number of siliquae plant^{-1} and seed yield plant^{-1}. The cluster I reported maximum point to first siliqua whereas cluster IV reported the minimum mean values for siliqua length. Thus, it can be reported that parents may be selected for hybridization on the basis of seed yield plant^{-1}, number of siliquae plant^{-1}, plant height, point to first siliquae. Similar results were reported by [18], [23] & [24].

Table 1. Clustering of 23 mustard genotypes in different clusters

Cluster	Number of genotypes	Name of the genotypes
I	17	RE-8 (7), DRMRIJ12-40 (10), DRMRIJ-31 (11), M-34 (15), ACN-141 (16), ACN-9 (18), DRMRIJ12-48 (9), ACN-184 (17), TAM 108-1 (22), NRCHB 101 (21), IC-597880 (5), TN-3 (13), LES-39 (12), DRMRMJB-35 (8), BHAWANI (23), CG-SARSON (20), RLC-3 (6)
II	1	CN-105364 (4)
III	1	NPJ-112 (14)
IV	3	RE-11 (1), NC-37362 (3), RE-44 (2)
V	1	PC-6 (19)

**Numbers in parenthesis represents genotypes in the figure 1*

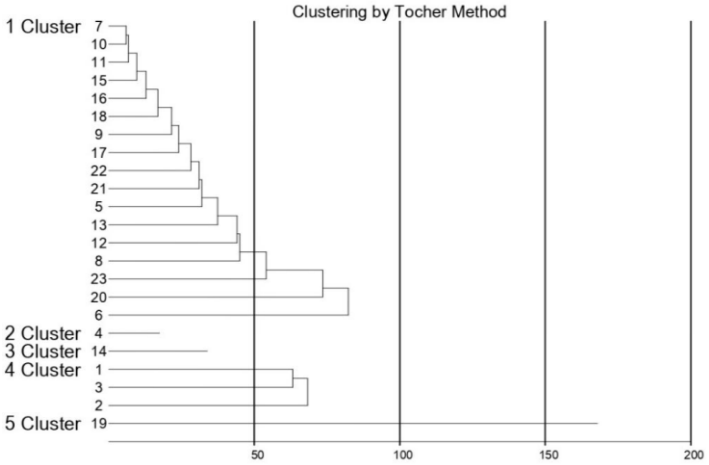

Figure 1. Dendrogram showing clustering by Tocher's method

Table 2. Average intra and inter cluster distance D^2 values in mustard

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	**6.93**	9.97	9.94	15.23	20.97
Cluster II		**0.00**	7.80	16.12	22.84
Cluster III			**0.00**	19.12	25.83
Cluster IV				**9.46**	14.06
Cluster V					**0.00**

$\bar{D}$ = 5.94 (Bold figures are average intra cluster distance)

3.2. Molecular Characterization

A total of 31 primer pairs were used to study genetic variation among the 16 selected genotypes based on the morphological performance. These markers collectively generated 93 scorable bands. Out of the 93 bands, 91 were determined to be polymorphic, yielding a high overall percentage of polymorphism reported 97.85%. The remaining two bands, originating from the IP15 and IP62 markers, were found to be monomorphic across all 16 genotypes and thus exhibited zero genetic variation. The high polymorphism rate confirms the effectiveness of the IP markers in discriminating among the tested genotypes [25]. The number of bands amplified by the primer varied between 1 to 5, with an average of 3 bands per primer.

The UPGMA dendrogram grouped the genotypes into five major clusters (Figure 2) *viz.,* Cluster I, II, III, IV and V using Jaccard's similarity coefficient. Cluster I comprises LES-39, Bhawani, NRCHB 101, ACN-9, RE-44, CG-SARSON, TAM108-1 which are found to be closely related. Cluster II comprises RLC 3, RE 8, DRMR-IJ-12-48, DRMR-IJ-12-40, RE-11, moderately diverse subgroup, showing some differentiation from Cluster I.

Cluster III consist of DRMR MJB-35, NC 37362, however cluster IV consist of IC 597880 which is genetically divergent genotype grouped, highlighting moderate differentiation. Cluster V represent PC 6 (*Brassica carinata*) formed an entirely independent branch, confirming its unique allelic constitution and maximum divergence from different species. The basic study conducted by [7] highlighted the importance of Intron Polymorphic (IP) markers among *Brassica spp.*, whereas the *Brassica carinata* found to be significantly grouped into different cluster compared to *Brassica juncea*. However, similar studies conducted by [26] using SSR marker grouped 36 genotypes into different clusters reporting the available genetic diversity among the genotypes.

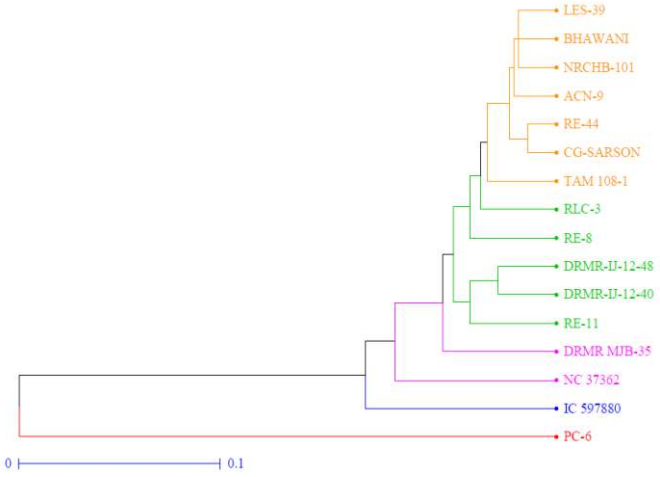

Figure 2. UPGMA dendrogram of 16 Indian mustard genotypes using IP markers

3.3. Analysis of heterosis

The analysis of variance for heterosis were presented in Table 3. For all characters, whereas mean square due to parents and crosses were found to be highly significant for all the characters which revealed the choice of exploiting heterosis for all the characters. However, mean square due to the interaction effect of parents *vs.* crosses was found to be non-significant for all characters except seed yield plant^{-1}. Similar results were reported by [27], [28] and [29].

For days to 50% flowering, NRCHB 101 x RLC-3 (-22.70%) reported the highest negative significant heterobeltiosis. However, NRCHB 101 x NPJ-112 (-18.88%) reported highest negative significant useful heterosis over check TAM 108-1. For days to maturity, ACN-184 x M-34 (-9.59%) reported highest

negative significant heterobeltiosis and ACN-9 x RE-8 & CG-SARSON x DRMRIJ-31 (-5.25%) reported highest negative useful heterosis over check. The cross NRCHB 101 x RE-11 (25.97%) reported highest positive heterobeltiosis for plant height, and positive heterosis over check with highest significance reported in the cross PC-6 x M-34 (48.75%). Highest positive significant heterobeltiosis for number of branches plant^{-1} was recorded by the cross CG-SARSON x RE-11 (53.06%). However, positive significant useful heterosis over check for the number of branches plant^{-1} with maximum useful heterosis reported by ACN-184 x DRMRMJB-35 (88.10%). For trait point to first siliqua, negative significant heterobeltiosis is desirable whereas thirty crosses found negative significant heterobeltiosis, whereas ACN-141 x DRMRMJB-35 (-40.06%) had highest negative heterobeltiosis and negative useful heterosis with the cross ACN-141 x DRMRMJB-35 (-32.97%). For siliqua length, the cross ACN-184 x NC-37362 (31.93%) had highest positive heterobeltiosis. The maximum positive significant heterobeltiosis for number of seeds siliqua^{-1} was recorded in the cross CG-SARSON x DRMRIJ12-40 (31.09%), whereas CG-SARSON x DRMRIJ12-40 (31.09%) reported the highest positive significant useful heterosis. The cross ACN-184 x IC-597880 (106.09%) had highest positive significant heterobeltiosis for the number of siliquae plant^{-1} and the cross CG-SARSON x DRMRIJ-31 (69.53%) reported highest useful heterosis along. For trait siliqua density on main branch, positive heterobeltiosis reported in ACN-9 x DRMRMJB-35 (9.71%) and positive significant useful heterosis over check with the highest value for the cross PC-6 x DRMRIJ12-40 (20.57%). Out of 120 crosses, none of the cross showed positive significant heterobeltiosis and useful heterosis over check TAM 108-1 for 1000 seed weight. For seed yield plant^{-1}, positive and significant heterobeltiosis

Table 3. Analysis of variance for heterosis for seed yield and yield contributing characters in mustard

Sources of variation	d. f.	Mean squares										
		Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches plant^{-1}	Point to first siliqua (cm)	Siliqua length (cm)	Number of seeds siliqua^{-1}	Number of siliquae plant^{-1}	Siliquae density on main branch	1000 seed weight (g)	Seed yield plant^{-1} (g)
Replications	2	12.09	40.86	438.28	0.98	350.28	0.01	2.87	5492.04	0.01	0.24	0.28
Genotypes	142	29159.45**	11210.48**	124193.64**	159.17**	65708.14**	61.62**	466.11**	1533144.55**	0.58**	67.66**	8439.88**
Parents	22	3041.30**	1500.55**	20600.85**	14.76**	11264.47**	11.51**	46.64**	161392.01**	0.12**	8.05**	283.06**
Crosses	119	96667.86**	546520.78**	1051156.38**	444.22**	265854.08**	877.89**	8370.36**	2202694.11**	19.13**	1086.31**	3420.79**
Parents *vs* Crosses	1	-70549.71	-536810.85	-947563.59	-299.81	-211410.40	-827.78	-7950.90	-830941.57	-18.67	-1026.69	4736.03**
Error	284	611.24	2526.48	28429.80	70.67	22148.87	31.87	225.29	338608.23	0.32	25.03	530.21

**, **=Significant at 5% and 1% level respectively.*

Table 4. Analysis of variance for combining ability for yield and its attributing characters

Sources of variation	d. f.	Mean squares										
		Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches plant^{-1}	Point to first siliqua (cm)	Siliqua length (cm)	Number of seeds siliqua^{-1}	Number of siliquae plant^{-1}	Siliquae density on main branch	1000 seed weight (g)	Seed yield plant^{-1} (g)
Females (f)	7	3409.28**	1078.21**	8694.11**	8.82**	2966.18**	1.39**	5.67**	106488.73**	0.008**	2.82**	315.36**
Males (m)	14	25.07**	30.89**	770.42**	0.72**	451.71**	0.61**	3.96**	6570.17**	0.017**	0.78**	26.10**
Females × Males (f × m)	98	19.39**	16.40**	322.80**	0.74**	276.07**	0.28**	3.11**	5422.23**	0.002**	0.29**	45.79**
Error	238	2.11	9.22	75.88	0.28	68.75	0.10	0.66	1052.70	0.001	0.09	2.09
GCA vs SCA		0.99	0.99	0.97	0.93	0.93	0.88	0.76	0.95	0.93	0.93	0.88

**, **=Significant at 5% and 1% level respectively.*

with highest positive heterobeltiosis in ACN-184 x DRMRMJB-35 (291.19%). Whereas, positive significant heterosis over check was reported by ACN-184 x DRMRMJB-35 (395.92%).

The cross ACN-184 x DRMRMJB-35 reported with superior useful heterosis along with high mean performance for seed yield plant^{-1} along with the characters plant height, number of branches plant^{-1}, number of siliquae plant^{-1}, number of seeds siliqua^{-1} and siliqua density on main branch followed by the cross CG-SARSON x LES-39 for seed yield plant^{-1}, days to 50% flowering, plant height, number of seeds siliqua^{-1} and number of siliquae plant^{-1}. Similarly, for the cross TAM 108-1 x RE-8 for seed yield plant^{-1}, plant height, number of branches plant^{-1}, number of siliquae plant^{-1} and siliqua density on main branch.

3.4. Analysis of combining ability

The mean performances of parents and crosses is presented in Figure 3 using violine graph. The shape and width of graph represent the variability present among parents and F_1 crosses. A wider region represents a higher concentration of the data points representing uniform performance. However, the narrow region represents the higher variability and less number of genotypes (parents and crosses). The central tendency of traits is visually represented between parents and crosses. The wider violins show wide range of performance within population, while narrower violin shows more consistent expression for the trait.

The analysis of variance for combining ability has been presented in Table 4. The mean square due to lines, testers and line × tester was significant for all the characters under study. For all the characters studied, the predictability ratio was found to be more than 0.50 which reveals that both GCA effects of parents and SCA effect of crosses for their exploitation to recover transgressive segregates. The present findings were in accordance with the results reported earlier by [1] and [2] who also found a predictability ratio close to unity.

The genetic components of variance and dominance are presented in table 5. Among all the characters under study, days to 50% flowering, days to maturity, plant height, number of siliquae plant^{-1} and 1000 seed weight reported high σ^2_{GCA} compared to σ^2_{SCA}, while number of branches plant^{-1}, point to first siliqua, the siliqua length, number of seeds siliqua^{-1}, siliquae density on main branch and seed yield plant^{-1} shows high σ^2_{SCA} compared to σ^2_{GCA}. The ratio of $\sigma^2_{GCA}/\sigma^2_{SCA}$ shows less than 1 for all characters except days to 50% flowering, days to maturity, plant height and number of siliquae plant^{-1}. The mean degree of dominance $(\sigma^2_{SCA}/\sigma^2_{GCA})^{0.5}$

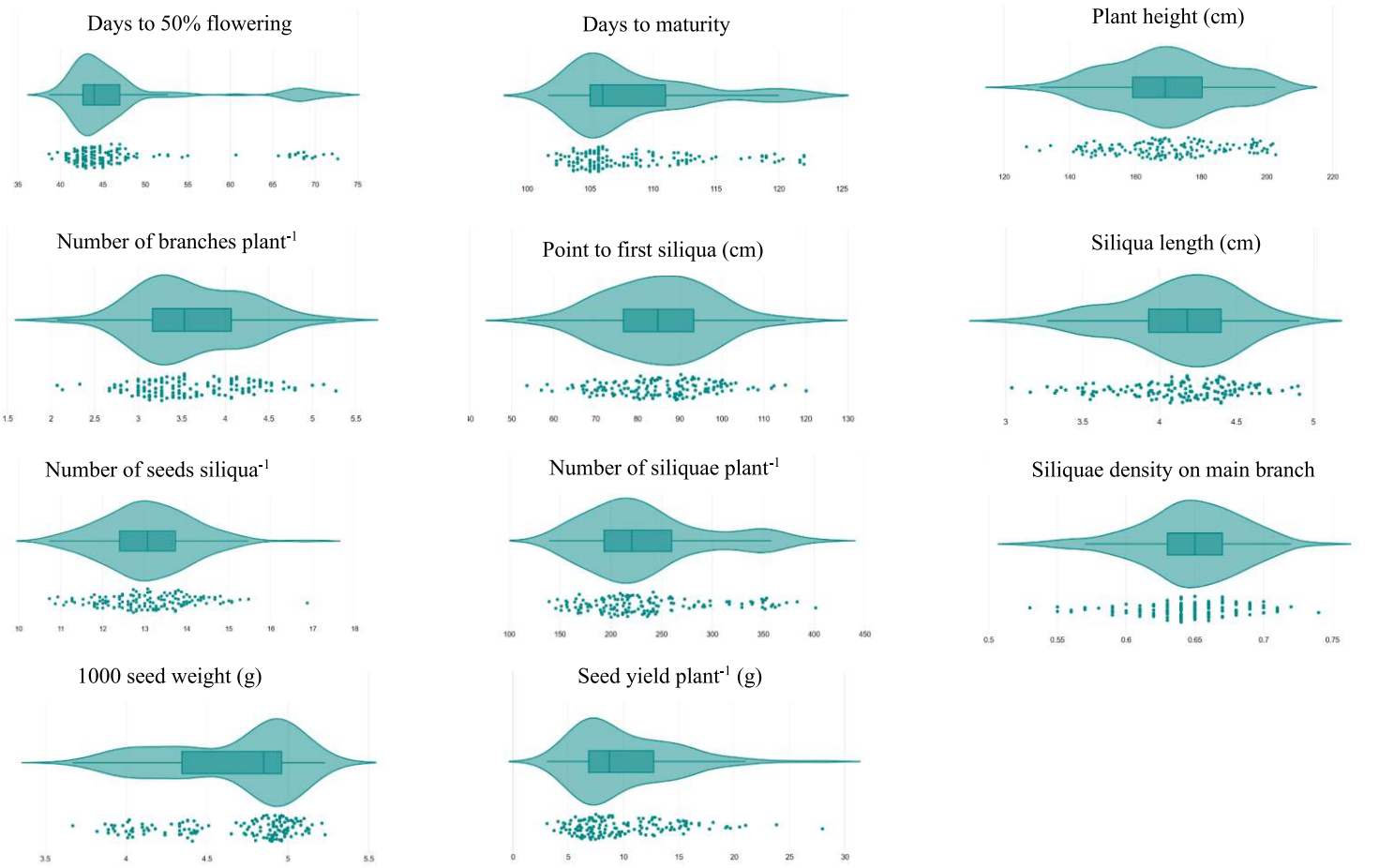

Figure 3. Violine graph representing mean performance of parents and crosses for seed yield plant-1 and other contributing characters in Indian mustard (Brassica spp.)

Table 5. Assessment of genetic variance components and level of dominance in relation to seed yield and its attributing characters

Sources of variation	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches plant^{-1}	Point to first siliqua (cm)	Siliqua length (cm)	Number of seeds siliqua^{-1}	Number of siliquae plant^{-1}	Siliquae density on main branch	1000 seed weight (g)	Seed yield plant^{-1} (g)
σ^2_{gca}	49.211	15.598	127.81	0.117	41.533	0.021	0.049	1,481.37	0.001	0.031	3.621
σ^2_{sca}	5.761	2.393	82.485	0.154	69.107	0.058	0.817	1,456.51	0.000	-0.013	14.57
$\sigma^2_{gca}/\sigma^2_{sca}$	8.54	6.52	1.55	0.76	0.60	0.36	0.06	1.02	0.00	-2.38	0.25
$(\sigma^2_{sca}/\sigma^2_{gca})^{0.5}$	0.34	0.39	0.80	1.15	1.29	1.66	4.08	0.99	0.00	0.00	2.01
σ^2_A	98.422	31.197	255.62	0.234	83.065	0.042	0.099	2,962.74	0.001	0.061	7.243
σ^2_D	5.761	2.393	82.485	0.154	69.107	0.058	0.817	1,456.51	0.000	-0.013	14.57
σ^2_A/σ^2_D	17.08	13.04	3.10	1.52	1.20	0.72	0.12	2.03	0.00	-4.69	0.50
$\sigma^2_A/\sigma^2_A+\sigma^2_D$	0.94	0.93	0.76	0.60	0.55	0.42	0.11	0.67	1.00	1.27	0.33
Gene action	Additive	Additive	Additive	Additive	Additive	Dominance	Dominance	Additive	Additive	Additive	Dominance

Table 6. Parents selected on the basis of GCA effect for seed yield plant^{-1} and its attributing characters

Selected parents	GCA effect significant for other characters
Lines	
CG-SARSON	Point to first siliqua, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Siliqua length, Siliqua density on main branch, Seed yield plant^{-1}
ACN-184	Plant height, Point to first siliqua, Number of siliquae plant^{-1}, Siliqua density on main branch, 1000 seed wright, Seed yield plant^{-1}
TAM 108-1	Point to first siliqua, Siliqua length, 1000 seed weight, Seed yield plant^{-1}
Testers	
DRMRMJB-35	Days to 50% flowering, Siliqua length, Number of seeds siliqua^{-1}, Seed yield plant^{-1}
LES-39	Number of siliquae palnt^{-1}, Number of seed siliquae^{-1}, 1000 seed weight, Seed yield plant^{-1}
RE-11	Days to 50% flowering, Plant height, Point to first siliqua, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Seed yield plant^{-1}

reported more than 1 for number of branches plant^{-1}, point to first siliquae, siliqua length, number of seed siliqua^{-1} and seed yield plant^{-1} shows preponderance of over dominance. However, the additive σ^2_A and dominance σ^2_D the component helps to clarify the gene action involved more efficiently. The ratio of σ^2_A/σ^2_D reported more than 1 for all characters indicating additive gene action except siliqua length, number of seed siliqua^{-1} and seed yield plant^{-1} governs dominance gene action. The predictability ratio $(\sigma^2_A/\sigma^2_A+\sigma^2_D)$ consistently reported more than 0.05 for all characters under study. The study conducted by [30], [31], [32] and [33] reported similar results for seed yield and yield contributing characters, confirming the findings of the study.

The estimate of GCA effect among lines and testers observed wide variation in level of significance for various characters. Among lines, CG-SARSON had highly significant positive GCA effect for point to first siliqua, number of branches plant^{-1}, number of siliquae plant^{-1}, siliqua length, siliquae density on main branch and seed yield plant^{-1}. Also, the line ACN-184 had high positive significant GCA effect for plant height, point to first siliqua, number of siliquae plant^{-1}, siliquae density on main branch, 1000 seed weight and seed yield plant^{-1}. Likely, the line TAM 108-1 exhibited high positive significant GCA effect for point to first siliquae, siliquae length, 1000 seed weight and seed yield plant^{-1}. Therefore, line CG-SARSON, ACN-184 and TAM 108-1 were recorded as the best general combiner for yield and yield contributing characters. (Table 6)

From the testers, DRMRMJB-35, LES-39 and RE-11 found to be the best general combiner for yield and yield contributing characters presented in Table 6. The tester DRMRMJB-35 reported highly significant positive GCA effect for days to 50% flowering, siliquae length, number of seeds siliquae^{-1} and seed yield plant^{-1}. The tester LES-39 also reported highly significant positive GCA effect for the number of siliquae plant^{-1}, number of seed siliquae^{-1}, 1000 seed weight and seed yield plant^{-1}. Similarly, RE-11 exhibited positive significant GCA effect for days to 50% flowering, plant height, point to first siliqua, number of branches plant^{-1}, number of siliquae plant^{-1} and seed yield plant^{-1}.

On considering useful heterosis for all 120 crosses with D^2 analysis presented in Table 7, the cross CG-SARSON x RE-11 reported superior for useful heterosis for seed yield plant^{-1}, plant height, number of branches plant^{-1}, number of siliquae plant^{-1} and siliqua density on main branch, whereas parents associated with the cross belongs to diverse cluster under study. However, the cross NRCHB 101 x RE-11 reported parents with high genetic variability along

Table 7. Crosses selected for heterosis breeding on the basis of mean performance, useful heterosis, SCA effect of crosses for yield and other character

Sr. No.	Crosses	Mean	Useful heterosis	SCA	GCA		SCA Significant for another trait	Heterosis superior over best check	Significant superior performance over best check
					P1	P2			
1	CG-SARSON x RE-11	23.82	322.34**	8.92**	2.93**	1.29**	Days to maturity, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Siliqua length	Plant height, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Siliqua density on main branch	Number of branches plant^{-1}, Number of siliquae plant^{-1}
2	NRCHB 101 x RE-11	15.55	175.71**	3.97**	-0.40**	1.29**	Point to first siliqua, 1000 seed weight, Days to 50% flowering	Plant height, Number of branches plant^{-1}, Point to first siliqua	Days to 50% flowering, Number of branches plant^{-1}
3	ACN-184 x DRMRMJB-35	27.97	395.92**	12.57**	2.72**	2.00**	Plant height, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Number of seeds siliqua^{-1}	Plant height, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Number of seeds siliqua^{-1}, siliqua density on main branch	Days to 50% flowering, Number of branches plant^{-1} Number of siliquae plant^{-1}, Number of seeds siliqua^{-1}
4	CG-SARSON X LES-39	21.03	272.87**	5.66**	2.93**	1.76**	Number of siliquae plant^{-1}	Days to 50% flowering. Plant height, Number of seeds siliqua^{-1}, Number of siliquae plant^{-1}	Days to 50% flowering, Number of siliquae plant^{-1}, Number of seeds siliqua^{-1}
5	TAM 108-1 x RE-8	20.48	263.12**	7.91**	1.88**	0.01	Number of siliquae plant^{-1}, Siliqua length	Plant height, Number of branches plant^{-1}, Number of siliquae plant^{-1}, Siliqua density on main branch	Days to 50% flowering, Number of branches plant^{-1}, Number of siliquae plant^{-1}
6	ACN-184 X RE-8	19.65	248.40**	6.24**	2.72**	0.01	Days to maturity, Plant height, Number of branches plant^{-1}, Number of siliquae plant^{-1}	Days to 50% flowering, Days to maturity, Plant height, Number of branches plant^{-1}, Siliqua density on main branch	Days to 50% flowering, Number of branches plant^{-1}
7	CG-SARSON X DRMRIJ12-40	19.35	243.09**	6.33**	2.93**	-0.59**	Days to 50% flowering, Plant height, Point to first siliqua, Siliqua length, Number of seeds siliqua^{-1}, 1000 seed weight	Days to 50% flowering, Plant height, Number of branches plant^{-1}, Number of seeds siliqua^{-1}, Siliqua density on main branch	Days to 50% flowering, Number of branches plant^{-1}, Number of seeds siliqua^{-1}

(, **=Significant at 5% and 1% level respectively.)*

with high useful heterosis for seed yield plant^{-1}, plant height, number of branches plant^{-1} and point to first siliqua. The results were supported by [4], PRai 1118 x Prakash and RLM 185 x RLM 514 to exhibit high SCA effect for seed yield and oil yield along with mid parent heterosis and heterobeltiosis with parents from diverse clusters. The results were confirmed with the study conducted by [34] whereas the cross TM-4 x Vardan belongs to cluster II and I, respectively among all heterotic crosses with higher heterosis for all characters studied.

Whereas, on considering useful heterosis along with *per se* performances and significant positive SCA effect of 120 crosses were presented in Table 7. The cross ACN-184 x DRMRMJB-35 reported with superior useful heterosis for seed yield plant^{-1} along with the characters plant height, number of branches plant^{-1}, number of siliquae plant^{-1}, number of seeds siliqua^{-1} and siliqua density on main branch followed by the cross CG-SARSON x LES-39 for seed yield plant^{-1}, days to 50% flowering, plant height, number of seeds siliqua^{-1} and number of siliquae plant^{-1}. The cross TAM 108-1 x RE-8 followed for the character seed yield plant^{-1}, plant height, number of branches plant^{-1}, number of siliquae plant^{-1} and siliqua density on main branch and the cross ACN-184 x RE-8 reported superior performance over check for seed yield plant^{-1} with traits days to 50% flowering, days to maturity, plant height, number of branches plant^{-1} and siliqua density on main branch. However, the cross CG-SARSON x DRMRIJ12-40 also reported with superior performance over best check for the characters seed yield plant^{-1}, days to 50% flowering, plant height, number of branches plant^{-1}, number of seeds siliqua^{-1} and siliqua density on main branch. Hence, the crosses ACN-184 x DRMRMJB-35, CG-SARSON x LES-39, TAM 108-1 x RE-8, ACN-184 x RE-8 and CG-SARSON x DRMRIJ12-40 were utilized in the development of hybrids in heterosis breeding program after converting female lines into male sterile lines. Similarly, the cross RH-749 x Pusa Mustard-31 had positive significant useful heterosis over check for seed yield along with other traits which can be utilized after evaluation in various yield trials for development of hybrids after conversion of the female line into CMS background [35].

Among 120 crosses, BHAWANI x DRMRMJB-35, ACN-184 x DRMRIJ-31, NRCHB 101 x LES-39, CG-SARSON x NPJ-112 and ACN-184 x RE-11 reported high negative significant SCA effect for seed yield and its attributing characters along with significant performance for yield plant^{-1} and some yield contributing characters presented in Table 8. The presence of negative SCA effect for several yield components in the above crosses indicates the predominant role of additive gene action for yield components, which can be attributed to

Table 8. Potential crosses identified on the basis of mean performance, GCA effect of parents and SCA effect of crosses for yield and other traits

Crosses	Characters	Mean	SCA effect	GCA Effect	
				P_1	P_2
BHAWANI x DRMRMJB-35	Seed yield plant^{-1}	8.41	-4.59**	0.32**	2.00**
	Number of branches plant^{-1}	2.80	-0.59*	-0.19**	-0.05
	Days to 50% flowering	43.00	-1.48	-3.48**	0.68**
	Days to maturity	104.67	-0.12	2.84**	-0.44
	Number of seeds siliqua^{-1}	13.07	-0.19	-0.35**	0.43**
ACN-184 x DRMRIJ-31	Seed yield plant^{-1}	8.63	-4.55**	2.72**	-0.22**
	Number of branches plant^{-1}	2.73	-0.85**	-0.04	-0.01
	Number of siliquae plant^{-1}	193.00	-61.21**	12.77**	5.34
	Plant height	167.53	-5.16	5.43**	-2.21
	Days to maturity	108.67	-0.21	0.38	0.44

NRCHB 101 x LES-39	Seed yield plant^{-1}	7.85	-4.20**	-0.40**	1.76**
	Number of seeds siliqua^{-1}	14.40	-0.31	0.19	0.93**
	Number of siliquae plant^{-1}	190.00	-34.20*	-25.48**	12.85*
	Siliqua length	4.21	-0.16	-0.07	0.01
	1000 seed weight	4.41	-0.33*	-0.04	0.13*
	Plant height	164.53	-1.03	-3.77**	-0.13
	Siliqua density	0.64	-0.01	0.02	-0.02
	Days to maturity	103.33	-2.02	-2.11**	-0.61
CG-SARSON x NPJ-112	Seed yield plant^{-1}	9.69	-3.57**	2.93**	-0.35**
	Plant height	145.07	-11.18*	-5.05**	-8.17**
	Number of seeds siliqua^{-1}	12.07	-1.56	0.16	0.30
	Point to first siliqua	69.80	-13.93*	1.20	-2.89
	Days to maturity	104.00	-0.31	-2.49**	-1.27*
	Number of siliquae plant^{-1}	239.00	-18.60**	14.74**	5.90
	Siliqua length	4.18	-0.20	-0.09*	0.29**
ACN-184 x RE-11	Seed yield plant^{-1}	11.37	-3.32**	2.72**	1.29**
	Plant height	172.27	-9.99**	5.43**	7.36**
	Number of branches plant^{-1}	3.33	-0.65**	-0.04	0.40**
	Point to first siliqua	90.07	-5.26*	3.61**	6.29**

**, **=Significant at 5% and 1% level respectively.*

Appendix 1. List of 31 forward and Reverse sequence of Intron Polymorphic (IP) primers

Primer Symbol	Loci Name	Forward Sequence	Reverse Sequence
IP 12	At1g01290	5' - CGAAAAGGGCTTTCAGCAGTA - 3'	5' - TGTCAACACTAAAATCCTCAGGGTT - 3'
IP 15	At1g36380	5' - GGAATCTCCTTCGTTCCTTGAC - 3'	5' - CTCCCACGAGATTCAGGTTT - 3'
IP 18	At1g02410	5' - ATGGAGGTACTGTTCAACGCAA - 3'	5' - AGGAGTCGCTGCTCCTCA - 3'
IP 21	At1g79040	5'-TCATCGGTGACGTTGAAACC -3'	5'-ACAAGAAGAGCTCCTCCGGC -3'
IP 22	At3g63420	5'-AAGCACATGATCCTTGCGGAGC	5'-CTTCAAACCACCGGTCCCATCC
IP 26	At3g21865	5'-GTGATTTGTATCTCATGGAAAGAGT -3'	5'-ATTGACTGCGAGGTGAACAC -3'
IP 27	At3g55005	5'-GATCGACAGTCACGTATGCTG-3'	5'-TCAGCTATCGTAGGTCAAG-3'
IP 29	At3g09925	5'- ATATGGATTGGTTCGGCTGC -3'	5'- GGGAAGTAGACAGGCCAGTTGTA -3'
IP 30	At3g13120	5'- TCCAGAAACTCTCGATGAACCC -3'	5'- GAGGCACCCAGTATGATCTAAGC -3'
IP 33	At3g24800	5'-TTCTCTGCTGCGTTTGCCT -3'	5'-GGAAAGTGAACGTAAGGGTCTCTAC-3'
IP 40	At2g46390	5'- AGATGATCTACCGAAAGTGGAGT -3'	5'- CACTTGTTTGATGAGACATTCTTCT -3'
IP 41	At2g46390	5'- AGATGATCTACCGAAAGTGGAGT -3'	5'- CACTTGTTTGATGAGACATTCTTCT -3'
IP 43	At2g31490	5'- CGGAATCTTCGGCATCGC -3'	5'- TGTGGTCTGCCTGCATCTTC -3'
IP 45	At2g35790	5'- CGCCGTCTCTCTTCCTCC -3'	5'- GATGCCCACGTTCTCTGG -3'
IP 47	At2g19560	5'- ACGCACTCAACGTCTTCCA -3'	5'- ATGCTTCGACTAGATTCCCAAC -3'
IP 50	At2g28880	5'-GATGAGTGGACGTGGGAAGAA -3'	5'- AAATCAGAGTTTCTCCCGGATG-3'
IP 51	At2g30200	5'- AATGCGCTCACTGCTTCAC -3'	5'- AACACAAATGTCCAGAAGATCATAC -3'
IP 61	At1g69390	5' - GCGAGCTTASTWWCTCCTTATCA - 3'	5' - CATCTTGAGCCGCTGCTT - 3'
IP 62	At1g69980	5'- TCATCATCGTTTCTCGCCA -3'	5'- GATAACCCGTTTCACTGCAAC -3'
IP 64	At4g30310	5'-AGGAACTGG GTC TGT TRG CTG GA -3'	5'- TAA GKG CAG MTA YAA ATG GAG AAC TTG-3'
IP 66	At4g35000	5'-GGC TAA ACA TCC CAA AAT CAC A -3'	5'- CAC TGC TAC CAC ACC AGC AAG -3'
IP 71	At4g21150	5'-GCTACACGTCTCGCTCTATGC -3'	5'- TCGACTGGTACGAAGACATCC -3'
IP 72	At4g24440	5'-GAG CCA AGT GAA GAC CAA GGT GTC-3'	5'-CAC AGA ACC TGT AAG TGT GCA GGT G-3'
IP 75	At4g15520	5'-CTGCGTTTCTTCTCGGTAATGAG-3'	5'-GTAACATTCAAAGAGGCAGTGCC-3'
IP 80	At4g18400	5'-AAACGTCTACTCCTCCGGCTACA-3'	5'-TCTAACCTCGTCCTTTCTCTTTTGG-3'
IP 87	At5g37630	5' -CCGGAGATTGCTCTTTACTGG-3'	5' -CATCGCAGTAGCAGCATCAG-3'
IP 89	At5g41560	5' - CCTCACAATTTCAGTCAACATCGT - 3'	5' - GAGGTGGAAGAGTACGGTTGTG - 3'
IP 93	At5g19150	5' - TCCACCCTGTTCTTGAGGAATC - 3'	5' - ACAGCAAGCGGATAGCTGT -3'
IP 96	At5g53920	5' - TGCACCTTACCTCTCAGTTCGTA - 3'	5' - CTCGGCGATTTCAACAGGCT - 3'
IP 97	At5g63520	5' -GGAACATCGTCTGCAATCG-3'	5' -GTAGCCAATGGTCAACAGTATAGC-3'
IP 100	At5g08280	5' - CGAACTGCTATCATCAGAATTGG - 3'	5' - CCGTTTATCAAGGCCTCGTC - 3'

The next generation. Biparental mating may be used in selected progeny and further selection of segregant generation or recurrent selection or diallel mating may also be used for improvement of yield and yield components. The study conducted by [2] reported that the cross ACN 9 x PC-6, TAM 108-1 x Chhattisgarh Sarson and Bio-902 x Chhattisgarh Sarson exhibited a highly significant SCA effect along with high mean performance which suggested to forward for next generation. NRCHB-101 × Pusa Mustard-31 and Pusa Mustard-31 × Kranti reported significant negative SCA effects for seed yield [36] and most of its contributing characters and also possessed significant mean for most of yield contributing characters, suggesting the suitability of biparental mating in selected progeny and further selection in segregating generation in mustard.

4. Conclusion

All 23 genotypes were grouped into 5 clusters supported by 5 clusters of IP markers confirming the diversity among the genotypes were crossed using line x tester mating design to study gene action among the genotypes. Among the lines CG-SARSON, ACN-184 and TAM 108-1, and among testers DRMRMJB-35, LES-39 and RE-11 were identified as good combiners. The crosses ACN-184 x DRMRMJB-35, CG-SARSON x LES-39, TAM 108-1 x RE-8, ACN-184 x RE-8 and CG-SARSON x DRMRIJ12-40, along with CG-SARSON x RE-11 and NRCHB 101 x RE-11, show potential for utilization in heterosis breeding programs after converting female lines into male sterile lines. Conversely, the crosses BHAWANI x DRMRMJB-35, ACN-184 x DRMRIJ-31, NRCHB 101 x LES-39, CG-SARSON x NPJ-112 and ACN-184 x RE-11 is recommended for further breeding programs, which could involve biparental mating, diallel mating or recurrent selection in subsequent generations.

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Conflict of Interest
The authors declare that there is no conflict of interest.

REFERENCES

1. Sapkal A. D., Kamdi, S. R., Rani A. Jadhav and Meshram M. P. (2023). Genetic analysis of yield and its components in F_1 and F_2 population of Indian mustard [*Brassica juncea* (L.) Czern and Coss]. *AATCC Review*, 28-39. https://doi.org/10.58321/AATCCReview.2023.11.01.05

2. Chaudhari, Vasundara, Kamdi, S. R., Jyoti Shinde, Sapana Baviskar, Meshram M. P. and Diksha Tajne. (2022a). Combining ability studies in different species of mustard. *J. Pharm. Innov.*, 11(12): 3324-3328.

3. Ingole, H. P., Kamdi, S. R., Shanti R. Patil, Bhure, S. S., Aditi S. Deshmukh and Thikare, D. S. (2021). Stability analysis in Indian mustard. *J. Soils and Crops*, 31(2): 360-364.

4. Gupta, V. P., Sekhon, M. S. and Satija, D. R. (1991). Studies on genetic diversity, heterosis and combining ability in Indian mustard [*Brassica juncea* (L.) (Czern and Coss)]. *Indian J. Genet.*, 51(4): 448-453.

5. Naznin, S., Kawochar, M. A., Sultana, S., Zeba, N. and Bhuiyan, S. R. (2015). Genetic divergence in *Brassica rapa* (L.) *Bangladesh J. Agric. Res.*, 40(3): 421- 433.

6. Mohammadi, Seyed Abolghasem, and Prasanna, B. M. (2003). Analysis of genetic diversity in crop plants—salient statistical tools and considerations. *Crop sci.,* 43 (4): 1235-1248.

7. Chaudhary, R., Yashpal, Pardeshi P., Nanjundan J., Yadav S., Vasudev S., Yadava D.K. and Saini N. (2022). Relationship and genetic diversity analysis of *Brassica juncea* and U tringle species using intron polymorphic markers. *Indian J. Genet.*, 82(2): 177-185.

8. Kempthorne, O. (1957). An Introduction to General Statistics. John Wiley and Sons. Inc. New York. Chapman and Hall Ltd. London. p. 468-470.

9. Mahalanobis, P. C. (1936). Study on the generalized distance in statistics. Proceedings of the National Institute of Sciences of India, Calcutta, 2: 49-55.

10. Doyle, J. J. and Doyle, J. L. (1990). Isolation of Plant DNA from fresh tissue. Focus, 12, 13-15.

11. Panse, V. G. and Sukatme, P. V. (1954). Statistical methods for agricultural workers. ICAR. Publication, New Delhi, 2: 63-66.

12. Rao, C. R. (1952). Advanced statistical methods in biometrical research, Ist Edn. John Wiley and Sons, New York.

13. Singh, R. K. and Chaudhary, B. D. (1985). Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India.

14. Perrier, X., Flori, A., Bonnet, F. (2003). Data analysis methods. Genetic diversity of cultivated tropical plants. Eds. In Hamon, P, Seguin, M, and. Glaszmann, J.C., Enfield, Science publisher, Montpellier, pp.4376.

15. Fonseca, S., and Patterson, F. L. (1968). Yield component heritabilities and interrelationships in winter wheat (*Triticum aestivum* L.). *Crop sci.*, 8(5), 614-617.

16. Meredith Jr, W. R., and Bridge, R. R. (1972). Heterosis and gene action in cotton (*Gossypium hirsutum* L.). *Crop Sci.*, 12(3), 304-310.

17. Bhandari, D. and Singh, A. (2021). Genetic divergence in leafy mustard (*Brassica juncea var. rugosa*) germplasm grown under Tarai condition of Uttarakhand. *Indian J. Agric. Res.*, A-5795: 1-5.

18. Margam, B. K., Chakraborty, N. R. and Sadhu, A. (2022). Assessment of genetic diversity based on agro-morphological traits in Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] germplasm. *EJPB*, 13(3): 1019-1023.

19. Sur, B., Rout, S., Singla, S., Mandal, R., Nath, S., Maying, B., Sadhu, S., Chakraborty, M., Hijam, L., Debnath, M. K. and Roy, S. K. (2023). Evaluation of mustard genotypes [*Brassica juncea* (L.) Czern and Coss] for quantitative traits and character association of seed yield and yield components at sub-Himalayan region of West Bengal (India). *Plant Sci. Today*, 10(1): 166-178.

20. Arunachalam, V., Bandyopadhyay, A., Nigam, S. N. and Gibbons R. W. (1984). Heterosis in relation to genetic divergence and specific combining ability in groundnut (*Arachis hypogaea* L.). *Euphytica,* 33: 33-39.

21. Yadav, D., Singh, L., Jafri, S. K. F., Mishra, U. K., and Singh, V. (2022). Study of genetic divergence in different genotypes of Indian mustard [*Brassica juncea* (L.) Czern and Coss]. *J. Pharm. Innov.*, 11(5): 375-379.

22. Vanukuri, A. R. and Pandey, M. K. (2022). Genetic variability and genetic diversity studies in Indian mustard [*Brassica juncea* (L.)]. *J. Pharm. Innov.*, 11(7): 1357-1360.

23. Lien, H.T.P., Avtar, R., Kumari, N., Kumar, N., Singh, V.K., Singh, M. and Bishnoi, M. (2021). Diversity assessment among exotic and indigenous lines of Indian mustard for exploitation in breeding programme. *EJPB*, 12(3): 1048-1053.

24. Tudu, V. K., Kumar, A. and Rani, V. (2018). Assessment of genetic divergence in Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] based on yield attributing traits. *JPP*, 7: 2093-2096.

25. Hromadová, Zuzana, Zdenka Gálová, Lucia Mikolášová, Želmíra Balážová, Martin Vivodík, and Milan Chňapek. (2023). Efficiency of RAPD and SCoT Markers in the Genetic Diversity Assessment of the Common Bean. Plants 12, no. 15: 2763. https://doi.org/10.3390/plants12152763

26. Saini, Nupur and Archana Rai. (2020). Molecular analysis of diversity presents in Brassica juncea genotypes with the help of SSR markers. *IJCMAS*, 9(7): 2987-2993.

27. Singh, M., Choudhary, R. R., Avtar, R., Punia, R., Kumar, P., Kumari, N. and Khedwal, R. S. (2022). Heterosis for yield and its component traits in Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. *J. Pharm. Innov.*, 11(1): 1842-1851.

28. Chaudhari, Vasundara, Kamdi, S. R., Barde, S. R. and Jyoti Shinde. (2022b). Heterobeltiosis and useful heterosis studies in different species of mustard (*Brassica* sp.). *J. Soils and Crops*, 33(1): 162-167.

29. Sapkal, A., Kamdi, S. R., Nair, B., Deotale, R. D., Raut, P. D., Bhure, S. S. and Parbat, J. M. (2021). Heterosis for seed yield and yield contributing characters in Indian Mustard [*Brassica juncea* (L.)]. *J. Soils and Crops*, 31(1): 152-157.

30. Rout, S., Roy, S. K., Mandal, R., Singla, S., Rahimi, M., Sur, B., Ghimiray, T. S. (2025). Genetic analysis and heterosis breeding of seed yield and yield attributing traits in Indian mustard (*Brassica juncea* (L.) Czern & Coss.). *Sci. Rep.*, 15(1), 2911.

31. Singh, M., Singh, R., Yadav, K. & Chaurasiya, J. P. (2022). Genetic analysis for seed yield and its contributing traits in Indian mustard [(*Brassica juncea* (L.) Czern & Coss)]. *J. Oilseeds Res.,* 37, 66–67.

32. Patel, A., Prajapati, K. P., Patel, P. J., Shah, S. K., and Patel, P. S. (2015). Genetic study for seed yield and seed quality traits in Indian mustard [*Brassica juncea* L. Czern & Coss]. *EJPB*, 6, 672–679.

33. Synrem, G., Rangare, N., Choudhari, A., Kumar, S., Myrthong, I. (2015). Combining ability analysis for seed yield and component traits in Indian mustard [*Brassica juncea* (L.) Czern & Coss]. *EJPB*, 6, 445–453.

34. Monalisa P., Singh, N. B., Singh, N. G. and Laishram, J. M. (2005). Genetic divergence and combining ability in relation to heterosis in Indian mustard [*Brassica juncea* (L.) Czern. and Coss.] for seed yield, its attributes and oil yield. *Indian J. Genet.*, 65(4): 302-304.

35. Raut, P. D., Kamdi, S. R., Beena Nair, Deotale, R. D., Sapkal, A. D., Bhure, S. S., Parbat, J. M. and Dadas, N. D. (2021). Heterosis for seed yield and yield contributing characters in Indian Mustard [*Brassica juncea* (L.) Czern and Coss]. *J. Soils and Crops*, 31(1): 105-109.

36. Kamdi S. R., Raut, P. D., Beena Nair, Sapkal, A. D., Diksha Tajane and Bhure, S. S. (2023). Combining ability analysis for yield and it's contributing characters in Indian mustard. *J. Oilseeds Res.,* 40: 153-154.