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RESEARCH ARTICLE

An integrative analysis approach for combining ability, heterosis and SSR-based genetic diversity in Indian mustard (*Brassica juncea* (L.) Czern. & Coss.) for seed yield and quality traits

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Abstract

This study integrates a line × tester analysis with SSR marker-based diversity assessment in Indian mustard (*Brassica juncea* (L.) Czern. & Coss.) to identify promising parent combinations for breeding. Ten elite mustard lines and four testers were crossed (line × tester) to produce 40 hybrids, which along with parents were evaluated in a randomized block design with 3 replicates for 15 agro-morphological and quality traits. Analysis of variance (ANOVA) revealed significant genetic variability in both general combining ability (GCA) and specific combining ability (SCA) effects for most traits, indicating the involvement of both additive and non-additive gene actions. Notably, lines such as PR-2019-9 and PR-2019-2 showed high positive GCA for seed yield and key yield components, while certain crosses like PR-2019-2 × Albeli and PR-2017-11 × RB-101 exhibited high SCA for yield. Significant heterosis for seed yields were observed, with better-parent heterosis reaching approximately 68 % and standard heterosis up to 50 %, the highest standard heterosis being recorded in the cross PR-2019-2 × Albeli. Molecular characterisation with 20 polymorphic SSR markers yielded a high polymorphism information content (PIC) up to 0.79. Pairwise Jaccard similarity ranged from 0.45 to 0.85, with the most genetically distant genotypes being PRD-14-13 and PR-2019-9. The UPGMA clustering grouped the 14 genotypes into 3 major clusters, broadly separating the parental lines and identifying distinctly divergent genotypes. Interestingly, line PR-2019-2 and tester Albeli fell into different clusters, reflecting their divergent genetic backgrounds. These findings demonstrate how integrating phenotypic combining ability analysis with molecular diversity assessment can guide the strategic selection of parent lines for developing high-yield mustard hybrids.

Keywords: *Brassica juncea*; genetic divergence; heterosis; Indian mustard; line × tester analysis; SSR markers

Introduction

Indian mustard (*Brassica juncea* (L.) Czern. & Coss.) is a major oilseed crop in South Asia, especially in India, where it plays a key role in edible oil production and supports rural livelihoods. In India, rapeseed-mustard is cultivated on approximately 6 to 7 million hectares, producing 9 to 11 million tons annually. This accounts for about 25 % of the country's total oilseed output (1). Despite its importance, the national average yield remains low at around 1.2 to 1.3 tons per hectare, mainly due to a narrow genetic base and susceptibility to both biotic and abiotic stresses(2). The demand for mustard oil is steadily rising. To meet the projected edible oil demand of 34 million tons by 2025, about 14 million tons must come from domestic mustard production (3). This calls for significant genetic improvement in yield. The impact of climate change has further intensified this challenge, as Indian mustard is highly sensitive to temperature and sowing time. A delay of just one month in sowing can result in yield losses exceeding 40 % (4). Therefore, developing high-yielding varieties and hybrids is a crucial breeding objective.

Combining ability and heterosis in mustard breeding is a proven strategy to boost crop yield potential. India's first commercial hybrid, DMH-1, delivered about 25–30 % more seed yield than the best check varieties in multi-location trials (5). Recent work continues to show large heterotic gains for seed-yield up to 74 % over the better parent was recorded in a 48-crosses of line × tester set (6). To harness this potential, breeders must identify parental lines that combine well. For that the line × tester designs remain the method of choice because they quantify general combining ability (GCA) which reflects additive gene effects of a parent (average performance in hybrids) and specific combining ability (SCA) reflects non-additive (dominance and epistatic) gene interactions in particular cross combinations (7–9). Many Indian studies report both additive and dominant/epistatic gene action for yield traits, some find non-additive gene action control predominantly (10). Generally, a predominance of additive effects (high GCA) suggests traits can be improved via selection, whereas dominant gene action (high SCA, heterosis) implies hybrid development would be effective. In practice, this means selecting parents with high GCA to ensure good transmission of traits whereas targeted crosses with high SCA and heterosis helps to create superior hybrids.

The important step in hybrid breeding is to measure how genetically different the parent lines are from each other. When 2 parents come from wider gene pools, their progeny is more likely to combine complementary alleles and show strong heterosis (11). Pedigree information and observable traits provide only indirect measures of genetic distance and may be influenced by environmental effects, whereas DNA markers offer a more objective assessment of genetic divergence. Among them, simple-sequence repeats (SSRs) are especially useful because they are plentiful in *Brassica* genome, multi-allelic, co-dominant and highly polymorphic (11). Using 20 SSR loci, (12) clustered 48 mustard lines into 3 groups and recorded polymorphic-information-content (PIC) values as high as 0.69. A larger survey of 87 released cultivars with 200 SSRs produced an average PIC of 0.39 and resolved two clear sub-populations (13). Such data helps breeders choose the most genetically distant parents. Links between marker distance and hybrid performance are encouraging but not absolute. In Indian mustard, found a positive relationship of molecular and phenotypic divergence with hybrid performance (14). These findings suggest that molecular-diversity metrics are a useful guide, but they should be combined with GCA/SCA tests to select parents that both combine well and widen the crop's genetic base. Therefore, incorporating molecular diversity data can guide the choice of parental lines in hybrid breeding programs to ensure a broad genetic base and maximise heterotic gains.

The present study was designed to integrate phenotypic and molecular analyses to support hybrid breeding in Indian mustard. We conducted a line × tester mating analysis using 14 diverse genotypes to estimate combining ability and heterosis for seed yield and its related traits. Simultaneously, genetic diversity among the parental genotypes was assessed using SSR marker analysis and cluster-based grouping. The objective of this study is to identify parental lines and testers with high GCA, cross combinations with high SCA and heterosis for yield components. Also to characterised the genetic relationships among these parental genotypes using SSR markers and evaluate the extent to which molecular diversity complements combining ability and heterosis effects. By integrating phenotypic and molecular approaches, this study aims to provide a more comprehensive strategy for selecting parents and cross combinations in Indian mustard breeding programs, ultimately contributing to the development of high-yielding hybrids and varieties.

Materials and Methods

Plant material (Genotypes and pedigrees)

Fourteen Indian mustard genotypes (10 lines and 4 testers) were used in this study as the breeding material. These parental genotypes were selected based on their agronomic performance, genetic background and adaptation to the Tarai agro-climatic region. These lines represented diverse pedigree backgrounds and advanced breeding material including lines developed at G.B. Pant University of Agriculture & Technology (Pantnagar, India) and a few released varieties, representing a broad genetic base. The list of genotypes and their pedigrees is given in Table 1. The female parent lines (denoted L1–L10) comprise selections from diverse cross backgrounds. The male testers (T1–T4) were chosen for their higher general combining ability. The PR-20 (a bold-seeded selection from Varuna), Albeli (an out-cross derivative), Giriraj (HB-9908 × HB-9916) and RB-101 (RH-30 × MCN-8). Two check varieties – Kranti (national check) and RH-749 (recent high-yielding variety).

Table 1. List of mustard genotypes and their pedigree

Line/ Testers	Indian mustard lines	Pedigree
L1	PR-2016-8	PR-05-24 × Maya
L2	PR-2016-4	PR-05-24 × Varuna
L3	PR-2015-5	PBR-375-2 × RW-8559
L4	PR-2017-8	(PRK-28 × Kranti) × PAB-9511
L5	PR-2019-9	Urvashi × PRB-2006-5
L6	PR-2019-2	Krishna × NRCHB-101
L7	PR-2017-11	MCN-15-25 × Kranti
L8	PRL-2017-7	EC-552579 × Vaibhav
L9	RGN-73	-- Not available --
L10	PRD-14-13	Varuna × PAB-9511
T1	PR-20	Bold seeded selection From Kranti
T2	Albeli	-- Not available --
T3	Giriraj	HB-9908 × HB-9916
T4	RB-101	RH-30×MCN-8

Hybridization and field experiment

A line × tester mating design was employed during the 2020–2021 rabi season to generate hybrid seeds. Each of the 10 female lines was crossed with each of the 4 male testers to produce 40 F_1 hybrids. Crosses were made by hand-emasculation and pollination in isolation. The resulting F_1 progeny along with the 14 parents, were evaluated during the 2021–2022 rabi season at the Norman E. Borlaug Crop Research Centre, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India (29° N, 79° E; 243.8 m above mean sea level). The region falls under a sub-humid, sub-tropical climate. The soil is predominantly Mollisols, with loam to sandy clay loam texture. The trial was laid out in a randomized block design (RBD) with 3 replications. Each entry (parent or F_1 hybrid) was grown in a single 3 m row plot, with inter-row spacing of 30 cm and plant spacing of ~10 cm (thinned after emergence). Standard agronomic practices for mustard were followed and border rows were planted to minimise edge effects.

Traits recorded

A total of 15 characters (12 agronomic and 3 seed quality traits) were recorded on five random plants per plot for each entry. The morphological traits included: 1. Days to 50 % flowering (DF), 2. Days to maturity (DM), 3. Plant height (PH), 4. Length of main raceme (LMR), 5. Number of siliquae on main raceme (SMR), 6. Number of primary branches per plant (PB), 7. Number of secondary branches per plant (SB), 8. Siliqua length (SL), 9. Number of seeds per siliqua (SS), 10. Siliqua density (SD) (siliquae on main raceme divided by raceme length) and 11. 1000-seed weight (TW). In addition, three seed quality traits were measured on harvested seed samples: 12. Oil content (OC), 13. Protein content (PC) and 14. Glucosinolate content (GC). Standard procedures were used for quantifying OC, PC and GC via Near-Infrared Reflectance (which was calibrated with wet chemistry). 15. Seed yield per plant (SY) was computed from the total seed weight (g) from randomly selected 5 plants and their average was taken to calculate the seed yield per plant.

Molecular characterisation (SSR marker analysis)

Genomic DNA was extracted from young leaves of each genotype using the CTAB (cetyltrimethylammonium bromide) method of

previous researchers, with minor modifications (15). The quality and concentration of DNA were checked and samples were diluted to approximately 100 ng/µL for polymerase chain reaction (PCR) analysis. A set of 20 SSR markers known to be polymorphic in *Brassica* was used for molecular characterisation (primer details are listed in Table 2). These included both genomic SSRs and EST-SSR primers such as At5g41940, BRMS-033, Ni2-D10 and sORA43-selected based on earlier reports (13). Although the 20 SSR markers used were polymorphic and informative, the marker density represents a moderate coverage of the genome. Therefore, the diversity patterns observed should be considered indicative rather than exhaustive of genome-wide variation. The PCR amplification was performed in 15 µL reactions containing template DNA, 0.2 µM of each primer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 1× PCR buffer and 1 U Taq DNA polymerase. The thermal cycling conditions were as follows: initial denaturation at 94 °C for 3 min; followed by 35 cycles of 94 °C for 45 sec, an appropriate annealing temperature (50–60 °C depending on the primers) for 45 sec and 72 °C for 1 min; with a final extension at 72 °C for 7 min. The PCR products were resolved by electrophoresis on 2.0 % agarose gels or 3.5 % agarose (for higher resolution of close alleles) and stained with ethidium bromide. Gel images were visualised under UV light and recorded. Fig. 1 and 2 show examples of SSR banding patterns for all 14 genotypes (gel images of loci NA10-C01c and Ni2-A07), confirming clear polymorphic profiles. For each SSR locus, the presence [1] or absence [0] of allelic bands was scored for all genotypes, generating a binary matrix. The polymorphism information content (PIC) was calculated for each marker using the formula $PIC = 1 - \Sigma (p_i^2)$, where p_i is the frequency of the i th allele among the genotypes. The mean PIC across all loci was also determined to assess the overall informativeness of the marker set.

Combining ability analysis

The line × tester experimental data were first subjected to analysis of variance (ANOVA) to confirm significant differences among entries (parents and hybrids). Thereafter, combining ability analysis was performed following standard line × tester methodology (fixed effects model) as described earlier (8). The GCA effects were estimated for each parent (lines and testers) and SCA effects for each hybrid cross, for all 15 traits. Mean square expectations from ANOVA tested the significance of GCA and SCA variances. Desirable GCA effects were considered positive for yield-enhancing traits (e.g. seed yield, branches, siliqua number) and negative for traits where a lower value is favorable (e.g. days to flowering/maturity, plant height for lodging developing lodging resistant lines). Similarly, SCA effects of crosses were noted and t-tests were used to identify significantly positive or negative combining ability effects.

Table 2. List of primers used in this study

Sl. No	Locus	Forward sequence (5'-3')	Reverse sequence (5'-3')
1.	At5g41940	TGGCCGTTCCTACTTGGAGT	CGACACTGTCTCCGCAGGT
2.	O110B11	AAAATGTGAGGCTGTTTGGG	TTTCGCAGCAGTAAACATGG
3.	BN6A3	GCTACCCACTCATGTCCTCTG	CCAAGCTTATCGAATCTCAGCTA
4.	Ni2D10	GATGCCCCAAATCTGTTACG	CAATTCGTGAAAAATAGCCG
5.	BrgMS339	CTACCTGAAGATGACCCAGACG	GCATACAAACCTCGTCCTAAGC
6.	BN25A	CACGTGGTATGTTGGTATTGGG	TGATTCTCCTCCGACGCATGC
7.	At5g41560	CCTCACAATTTCAGTCAACATCGT	GAGGTGGAAGAGTACGGTTGTG
8.	MB5	AACATCTTTTTGCGTGATAT	AATAGCATTGAAGCCTTAC
9.	BRMS-033	GCGGAAACGAACACTCCTCCCATGT	CCTCCTTGTGCTTTCCCTGGAGACG
10.	Ni3C05	TTTCGTGCTTTGGTGTGAAG	TCCCCAAATCGAACCATAAG
11.	BrgMS329	TCATCATCATAGCTTTCGCTTC	AAAACCTCCTCCTCCTCCTC
12.	sORA43	GCGCGTGTGGGATCAGAA	CTTCTCCACCGTCGATCG
13.	BRMS-006	TGGTGGCTTGAGATTAGTTC	ACTCGAAGCCTAATGAAAAG
14.	NA10-C01c	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC
15.	Ni2B03	ACTTCTTGCCCTCCTCACC	AAATACTCACTGCAATACCCAGG
16.	NI02-D08a	TTTAGGGAAAGCGAATCTGG	ACAACAACCATGTCTTCCG
17.	BrgMS1237	ATCAAAAGATGCAGGGAGAGAG	GTCCTCAATGGATTACACATGC
18.	BrGMS70	TACAATGAAGATGTGATCCCGA	CGTGCGTGAGCTTATCAATACA
19.	SSR Ni2-A07	GGAACCCAACAAGTGAGTCC	AGAGCTTGAGACACATAACACC
20.	BRMS-017	GGAAAGGGAAGCTTCATATC	CTGGAAAGCATACACTTTGG

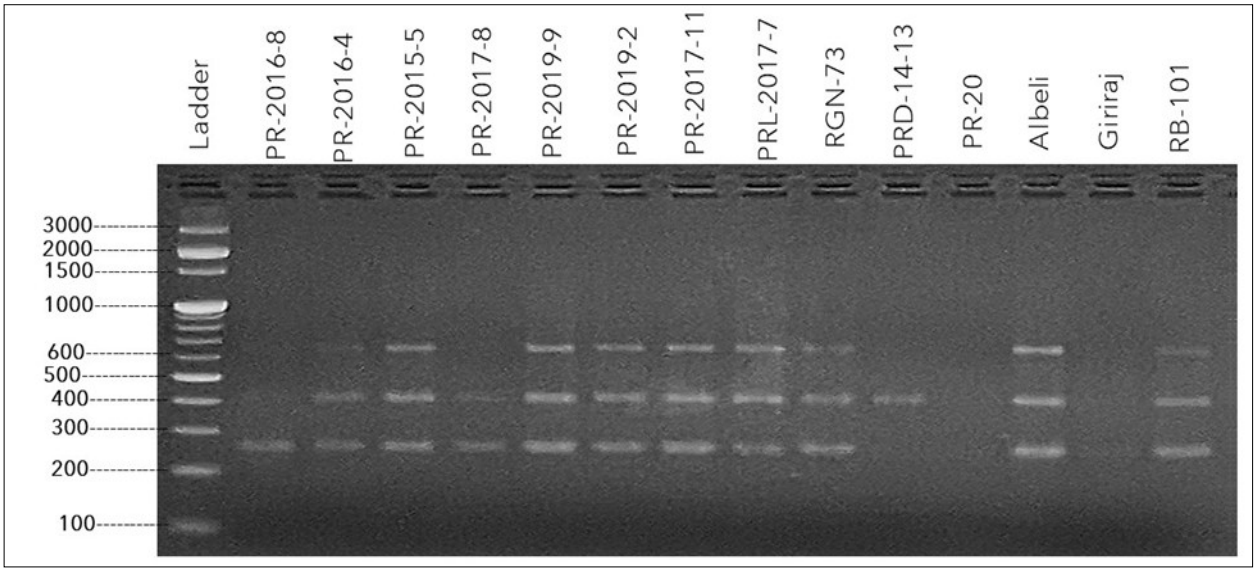

Fig. 1. NA10-C01c SSR loci amplicons pattern among 14 genotypes under study.

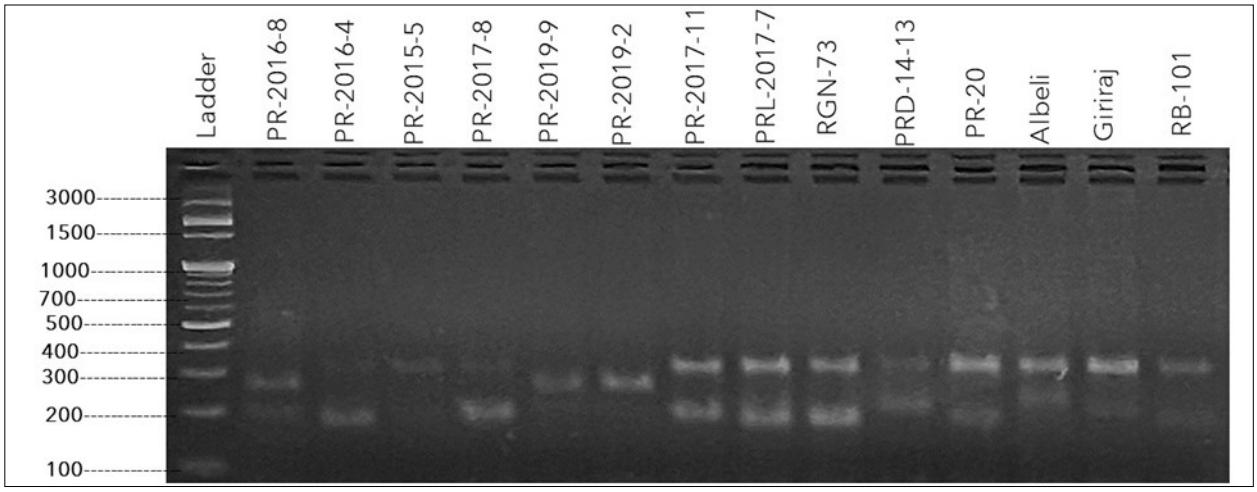

Fig. 2. SSR Ni2-A07 loci amplicons pattern among 14 genotypes under study.

Heterosis estimation

For each hybrid, three types of heterosis were calculated for key yield traits:

(1) Mid-parent heterosis = [(F_1 – average of parents)/average of parents] × 100

(2) Better-parent heterosis = [(F_1 – superior parent)/superior parent] × 100

(3) Standard heterosis = [(F_1 – check variety)/check variety] × 100, using RH-749 as check.

Heterosis values were tested for significance with appropriate t-tests. The magnitude and direction of heterosis for each cross and trait were analysed to identify crosses with significant desirable heterosis.

Molecular data analysis

The SSR marker data were scored as a binary matrix (presence = 1, absence = 0) and used to estimate pairwise genetic similarity among genotypes using Jaccard's similarity coefficient:

$$J_{ij} = \frac{a}{a + b + c}$$

where a represents the number of shared bands between genotypes i and j, b denotes bands present in i but absent in j and c denotes bands present in j but absent in i. Genetic distance was calculated as:

$$D = 1 - J_{ij}$$

A Jaccard similarity matrix (14 × 14) was generated to describe genetic relatedness among all genotype pairs. Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on the Jaccard distance matrix. The dendrogram was constructed using DARwin version 6.0 to classify genotypes according to SSR-based genetic diversity.

To further validate clustering patterns, Principal Coordinate Analysis (PCoA) was conducted on the same similarity matrix using DARwin. The first two principal coordinates were used to visualise genetic relationships among genotypes in a two dimensional space, enabling independent confirmation of clusters and identification of potential outliers. Cluster delineation was determined using an empirical similarity threshold from the UPGMA dendrogram and was supported by the grouping patterns observed in the PCoA.

Statistical analysis

Analysis of variance (ANOVA) and combining ability analyses were performed using SPAR version 2.0. Estimation of heterosis and statistical parameters was carried out using INDOSTAT. Significance was assessed at $p < 0.05$ and $p < 0.01$, wherever applicable.

Results and Discussion

Genetic variability

The analysis of variance revealed considerable genetic variability among the set of mustard parents and their hybrids. The treatment means squares (genotypes) were highly significant ($p < 0.01$) for all 15 traits, confirming the presence of substantial phenotypic variation. This included significant differences among the parents, among the F_1 hybrids and between parents vs hybrids, for key yield components (Table 3). The significance of "parents vs crosses" comparison for most traits signalled overall heterosis effects. The combining ability ANOVA (Table 4) further revealed that both GCA (due to lines and testers) and SCA (due to line × tester interaction) variances were significant for all traits.

Importantly, for most yield-contributing traits (branches per plant, siliqua number, seeds per siliqua, seed yield), the SCA variance exceeded GCA variance (GCA/SCA ratio<1, pointing to a preponderance of non-additive gene action). This was evidenced by the high heterosis observed and aligns with the expectation that yield is a complex trait often influenced by dominance interactions (16). The significant variability for all traits and the predominance of SCA over GCA for most yield components are consistent with earlier line × tester studies, where yield and its associated traits frequently showed stronger non-additive control, making heterosis breeding advantageous (17–19). Classical quantitative genetic theory also supports that complex traits such as yield are often governed by dominance and epistatic interactions, whereas traits like maturity and plant height may show relatively greater additive contribution and respond well to selection (9, 20, 21).

The substantial phenotypic variability observed among the traits was supported by genetic parameter estimates (Table 5). High PCV and GCV were recorded for secondary branches and seed yield per plant, with seed yield showing PCV of 26.26 % and GCV of 21.15 %. Secondary branches exhibited high heritability (>80 %) along with high genetic advance (GA) as percentage of mean (>40 %), indicating strong heritable control. Seed yield per plant showed moderate

Table 3. ANOVA for Line × Tester mating design including parents and crosses for all 15 traits

Source of Variation	Mean sum of squares															
	df	DF	DM	PH	LMR	SMR	PB	SB	SL	SS	SD	TW	PC	GC	OC	SY
Replication	2	0.93	4.91	6.12	0.86	2.15	0.10	4.69	0.01	0.24	0.007	0.02	2.33	1.78	0.12	3.98
Treatments	53	19.52 **	16.04 **	382.34 **	108.91 **	48.02 **	1.49**	15.23 **	0.26 **	4.62 **	0.01	0.38 **	2.35 **	187.31 **	4.43 **	7.5 **
Parent	13	20.72**	10.95**	564.54**	114.19**	32.81**	1.51**	7.19**	0.25**	4.880**	0.02*	0.49**	2.35**	203.66**	6.14**	7.02**
Crosses	39	17.13**	16.85**	240.45**	100.45**	44.36**	1.50**	17.87**	0.26**	4.58**	0.01**	0.35**	1.82**	149.24**	3.34**	7.63**
Parent v/s Crosses	1	97.10**	50.48**	3547.42**	370.07**	388.75**	0.61**	16.88**	0.33**	2.71	0.01	0.52**	23.27**	1459.67**	24.94**	8.88**
Error	106	1.53	1.75	8.61	4.42	0.93	0.05	1.18	0.02	1.33	0.01	0.03	0.59	4.38	0.83	1.21

** =Significance at 1 % probability level; * = Significance at 5 % probability level.

Table 4. ANOVA for combining ability for 15 traits

Source of Variation	Mean sum of squares															
	df	DF	DM	PH	LMR	SMR	PB	SB	SL	SS	SD	TW	PC	GC	OC	SY
Line	9	243	24.04 *	431.24 *	143.88	37.62	2.78 *	41.64 **	0.43	11.01 **	0.024 *	0.826 **	1.8	208.68	3.23	10.4
Tester	3	4.89	59.96 **	438.05	58.81	62.57	1.43	10.38	0.31	4.47	0.01	0.52	4.25	478.99 **	5.06	16.91 *
Line × Tester	27	16.16 **	9.66 **	154.89 **	90.59 **	44.58 **	1.08 **	10.79 **	0.19 **	2.45 *	0.008 **	0.178 **	1.55**	92.78 **	3.18**	5.67 **
Error	106	1.53	1.75	8.61	4.42	0.93	0.05	1.18	0.02	1.33	0.01	0.03	0.59	4.38	0.83	1.21

** =Significance at 1 % probability level; * = Significance at 5 % probability level.

Table 5. Estimates of genetic variability, heritability and genetic advance for all traits

Characters	PCV (%)	GCV (%)	ECV (%)	H^2_{bs} (%)	GA	GA as % of Mean
DF	4.99	4.48	2.2	0.81	4.57	8.27
DM	1.85	1.6	0.93	0.75	3.85	2.85
PH	6.05	5.9	1.35	0.95	23.3	11.84
LMR	10.26	9.45	4	0.85	11.11	17.93
NSMR	10.29	10.01	2.38	0.95	8.12	20.06
PB	12.32	11.81	3.49	0.92	1.36	23.34
SB	24.49	22.22	10.31	0.82	4.2	41.51
SL	6.99	6.44	2.72	0.85	0.55	12.22
NSPS	11.89	8.55	8.26	0.52	1.58	12.66
SD	11.25	9.37	6.22	0.69	0.11	16.09
TW	11.31	10.19	4.9	0.81	0.68	18.91
PC	4.3	3.05	3.03	0.5	1.12	4.46
GL	8.24	7.96	2.15	0.93	15.41	15.82
OC	3.56	2.81	2.19	0.62	1.91	4.57
YPP	26.26	21.15	15.58	0.65	2.4	35.07

heritability (65 %) coupled with high genetic advance (35.07 % of mean), suggesting the involvement of both additive and non-additive gene effects (17). These results indicate that while selection can be effective for improving yield-related traits, hybrid breeding approaches may also offer advantages for enhancing seed yield.

General combining ability (GCA) of carents

The GCA effects for both lines and testers for all evaluated traits in this study are presented as a heatmap in Fig. 3 to facilitate comprehensive interpretation of results. Positive GCA effects generally indicate that a parent transmits favourable alleles for increasing the value of a trait, whereas negative effects are typically considered unfavourable for yield traits, except in cases where trait reduction is beneficial such as for days to flowering to achieve earlier maturity and plant height.

Among the lines, PR-2019-9 (L5) exhibited the highest and highly significant positive GCA effect for seed yield per plant (SY = +1.29, $p < 0.01$), along with significant positive effects for siliquae on the main raceme (SMR = +3.13) and seeds per siliqua (SS = +1.91). This highlights PR-2019-9 as a strong general combiner for yield improvement. The PR-2019-2 (L6) also demonstrated a significant positive GCA for seed yield (SY = +1.25), oil content (OC = +0.70) and seeds per siliqua (SS = +1.10), making it a strong candidate for breeding programs targeting enhanced yield and oil content. The PR-2016-8 (L1) stood out for its significantly negative GCA for plant height (PH = –12.01), a desirable attribute for developing semi-dwarf and lodging-resistant varieties that can be grown at higher densities and may provide improved harvest index without compromising yield. In contrast, RGN-73 (L9) displayed a significantly negative GCA for seed yield (SY = –1.67, $p < 0.01$), suggesting a tendency to transmit alleles associated with reduced yield. Notably, RGN-73 had a high positive GCA for days to flowering (DF = +2.81), indicating delayed flowering, which is generally undesirable when breeding for earliness.

Among the testers, PR-20 (T1) showed the highest positive GCA for seed yield (SY = +1.12, $p < 0.01$), along with a positive effect for siliquae on the main raceme (SMR = +0.52). RB-101 (T4) exhibited a significant negative GCA for plant height (PH = -3.40) and days to maturity (DM = -1.41), indicating its usefulness for developing shorter and earlier genotypes.

In summary, lines such as PR-2019-9 and PR-2019-2, along with the tester PR-20, emerged as superior general combiners for seed yield and key agronomic traits. These parents represent useful

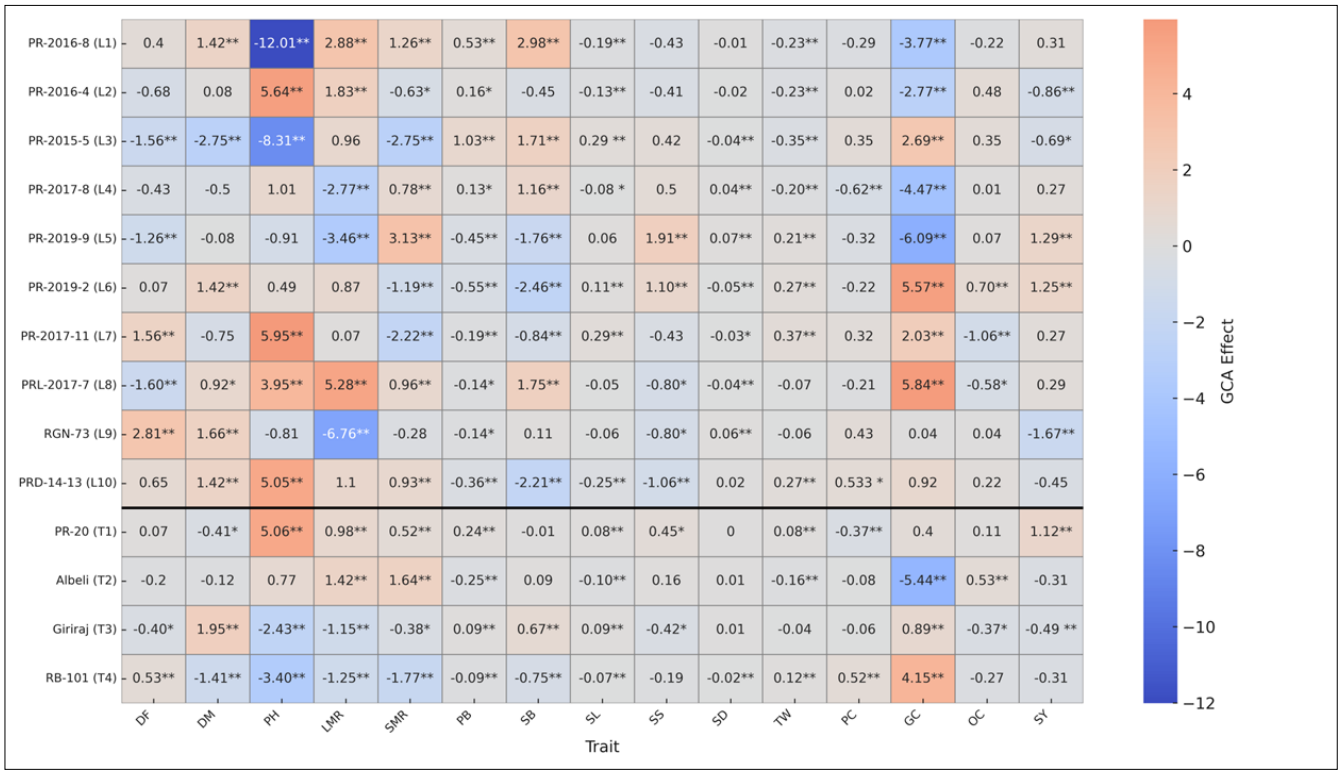

Fig. 3. Heatmap of general combining ability effects of parents for all 15 traits

genetic resources for hybrid breeding programs targeting yield improvement. Lines with significant negative GCA for flowering time (such as PR-2015-5 and PRL-2017-7) and for plant height (such as PR-2016-8 and Giriraj) can also be strategically utilised to develop early maturing and semi-dwarf varieties. However, as non-additive effects were also prominent it is important to examine SCA effects and heterosis.

Specific combining ability (SCA) and heterosis of F_1's

While GCA identifies good parents, SCA pinpoints parental combinations that produce better progeny, which are due to dominance/epistatic gene effects. In this study, several crosses displayed significant positive SCA effects for seed yield per plant and other traits which are presented as a heatmap in Fig. 4. The top SCA cross for seed yield was PR-2019-2 × Albeli, which had the highest SCA effect i.e., SCA = +2.59 among the 40 hybrids. Interestingly, this cross involves PR-2019-2 (a line with highest GCA for yield) and Albeli (tester with modest GCA), suggesting an interaction where Albeli's specific genetic background complements PR-2019-2. Another notably high SCA was observed in cross of PR-2017-11 × RB-101 which have low GCA's. In general, crosses involving the top GCA parents tended to feature among the best SCA performers, a pattern seen in case of PR-2016-8 × Giriraj (high SCA for plant height and branches) and PR-2016-4 × PR-20 (high SCA for raceme length and glucosinolate content). This indicates some "good × good" parent combinations yielded best hybrids, aligning with the conventional expectation that good combiners can produce superior crosses if their gene complementation is favorable.

However, we also observed instances where a parent of only average GCA, when paired with a good combiner, resulted in high SCA i.e. "good × average" combinations giving heterotic crosses. For example, RGN-73 × Albeli (neither parent was top in GCA) showed one of the highest SCA for the number of siliquae on the main raceme and that translated into high heterosis for that trait. This suggests the presence of complementary epistasis, certain allele combinations from these genetically distant parents unlocked new potential in F_1. Such crosses would particularly benefit from heterosis breeding, as their performance cannot be predicted by GCA alone. Indeed, crosses with good × average or even good × poor combiners exhibiting high SCA underline the value of maintaining broad genetic diversity among parents. This is consistent with theoretical expectations that more divergent parents can create novel positive interactions in hybrids.

The heterosis analysis for yield (Table 6) was consistent with the SCA results. We found substantial mid-parent and better parent heterosis in many hybrids for yield and its components. The maximum better-parent heterosis for seed yield was +68.74 %, observed in the cross PRL-2017-7 × PR-20. Standard heterosis over the best check (RH-749) was also pronounced, reaching a maximum in PR-2019-2 × Albeli. The crosses showing the highest heterosis largely correspond to those with high SCA effects, indicating the contribution of non-additive gene action in yield expression. The PRL -2017-7 × PR-20 and PR-2019-2 × Albeli emerged as promising combinations for seed yield improvement. These results indicate that specific parental combinations can generate substantial hybrid vigor. The close association between high SCA effects and high heterosis for seed yield in the present study supports the role of non-additive gene action in hybrid performance (19, 20). However, not all crosses between good general combiners ("good × good") produced the highest heterosis. This indicates that GCA alone may not fully predict hybrid performance, as heterosis also depends on the interaction and complementation of alleles between parental genomes (20).

From a practical breeding perspective, we can highlight several superior hybrids that could be advanced for further testing or used in breeding programs: 1. PR-2019-2 × Albeli – top for yield per se and standard heterosis; 2. PRL-2017-7 × PR-20 – top for heterobeltiosis in yield and also early maturing; 3. PR-2016-8 × Giriraj – good for branch number; 4. PR-2019-9 × PR-20 – very high seeds/siliqua and yield; and 5. PR-2017-11 × Albeli – notable for 1000-seed weight and oil content improvement. Some could be potential

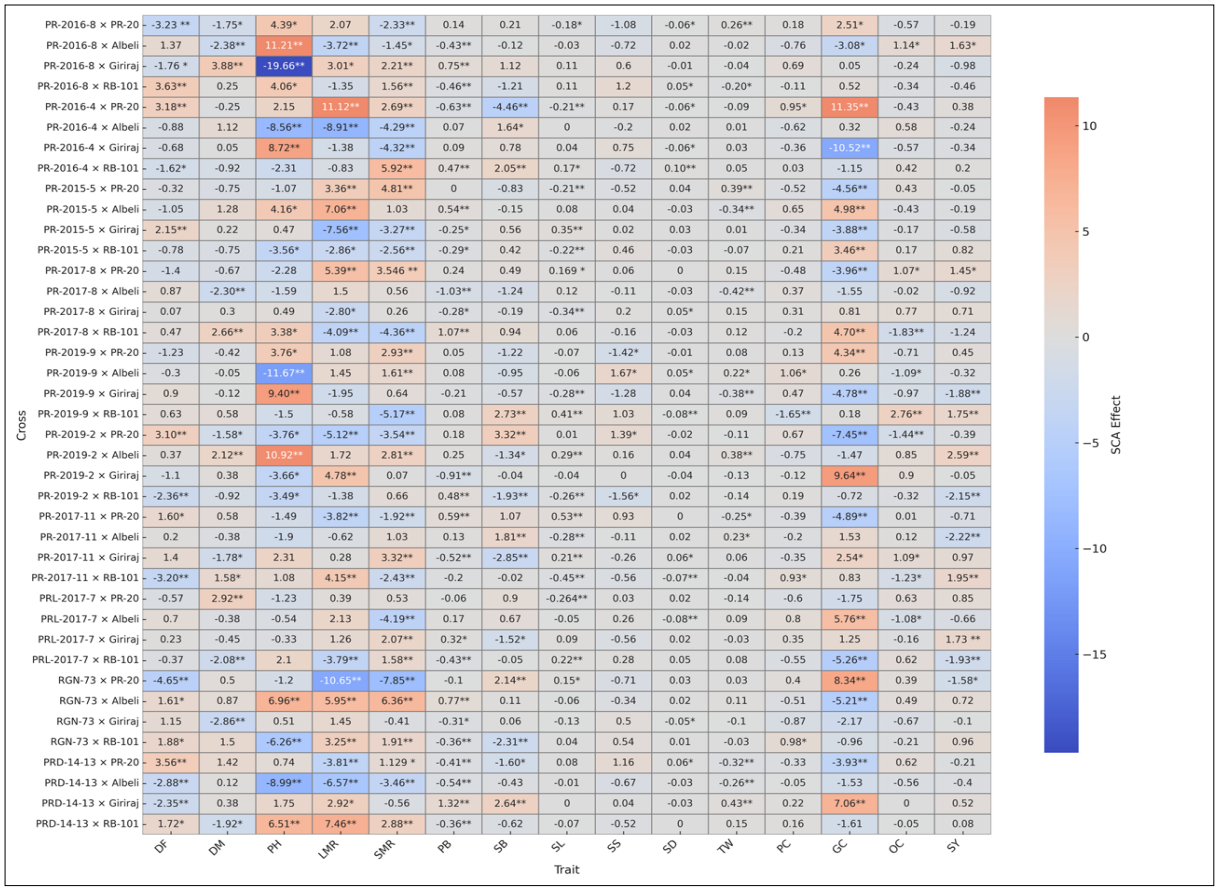

Fig. 4. Heatmap of SCA effects of all 40 crosses for all 15 traits.

Table 6. Estimates of all three heterosis (%) for seed yield per plant

Sl. No.	Crosses	Mid parent	Better parent	Standard
1	PR-2016-8 × PR-20	50.14 **	50.08 **	51.49 **
2	PR-2016-8 × Albeli	60.96 **	57.15 **	58.64 **
3	PR-2016-8 × Giriraj	-8.97	-20.46	7.4
4	PR-2016-8 × RB-101	-12.32	-30.71 **	20.46
5	PR-2016-4 × PR-20	40.72 **	39.22 *	40.41 *
6	PR-2016-4 × Albeli	5.48	4.13	2.78
7	PR-2016-4 × Giriraj	-16.36	-27.61 *	-2.25
8	PR-2016-4 × RB-101	-18.54	-36.15 **	11.01
9	PR-2015-5 × PR-20	31.41 *	28.35	35.76 *
10	PR-2015-5 × Albeli	5.89	1.09	6.92
11	PR-2015-5 × Giriraj	-19.93	-28.61 *	-3.6
12	PR-2015-5 × RB-101	-10.2	-27.78 **	25.56
13	PR-2017-8 × PR-20	68.68 **	59.11 **	81.01 **
14	PR-2017-8 × Albeli	5.87	-2.31	11.13
15	PR-2017-8 × Giriraj	10.65	1.94	37.65 *
16	PR-2017-8 × RB-101	-26.75 **	-39.41 **	5.33 s
17	PR-2019-9 × PR-20	65.01 **	52.52 **	81.26 **
18	PR-2019-9 × Albeli	31.19 *	18.68	41.05 *
19	PR-2019-9 × Giriraj	-14.13	-19.28	9
20	PR-2019-9 × RB-101	22.31 *	2.96	79.00 **
21	PR-2019-2 × PR-20	20.6	-4.59	65.26 **
22	PR-2019-2 × Albeli	43.78 **	11.81	93.66 **
23	PR-2019-2 × Giriraj	-7.96	-18.1	41.85 *
24	PR-2019-2 × RB-101	-38.58 **	-38.69 **	6.58
25	PR-2017-11 × PR-20	12.23	-6.4	41.32 *
26	PR-2017-11 × Albeli	-29.25 *	-42.09 **	-12.57
27	PR-2017-11 × Giriraj	-0.39	-5.65	42.44 *
28	PR-2017-11 × RB-101	1.03	-5.61	64.09 **
29	PRL-2017-7 × PR-20	70.47 **	68.74 **	70.18 **
30	PRL-2017-7 × Albeli	19.24	17.65	16.24
31	PRL-2017-7 × Giriraj	34.03 **	16.05	56.70 **
32	PRL-2017-7 × RB-101	-31.51 **	-46.29 **	-6.63
33	RGN-73 × PR-20	-7.8	-11.08	-10.32
34	RGN-73 × Albeli	11.22	9.77	5.57
35	RGN-73 × Giriraj	-23.69	-35.38 **	-12.74
36	RGN-73 × RB-101	-17.76	-36.72 **	10
37	PRD-14-13 × PR-20	32.70 *	29.37	37.37 *
38	PRD-14-13 × Albeli	6.31	1.3	7.56
39	PRD-14-13 × Giriraj	0.46	-10.27	21.16
40	PRD-14-13 × RB-101	-16.82	-33.01 **	16.46

candidates for developing commercial hybrid cultivars if a viable hybrid seed production system (e.g. cytoplasmic male sterility) is in place for mustard. The superior crosses identified in this study warrant further multi-environment evaluation to assess their yield stability and commercial potential. For instance, hybrids such as PR-2019-2 × Albeli demonstrated substantial yield advantages under the present experimental conditions. However, its performance should be validated across multiple environments to confirm the stability and consistency of this heterotic response.

It is also important to examine GCA, SCA and heterotic profiles simultaneously. In several cases, one parent exhibited high GCA while the other showed moderate effects, suggesting that favourable hybrid performance may arise from the interaction of additive and non-additive gene effects. Crosses between 2 high GCA parents ("good × good") generally produced consistent F_1 performance; however, they did not always exhibit the highest SCA effects or heterosis. Therefore, breeding decisions should integrate not only combining ability but also the genetic divergence among promising parents. Molecular diversity analysis provides an objective tool to assess parental relatedness. Accordingly, SSR-based diversity analysis was conducted to evaluate genetic relationships among the parental genotypes and to complement the combining ability findings.

Simple sequence repeat (SSR)-based molecular diversity and cluster analysis

To complement our combining ability findings, the genetic diversity among the 14 parental genotypes was assessed using 20 SSR markers. All primers produced clear and scorable amplification profiles, revealing polymorphism across the genotypes. A total of 40 alleles were detected across the 20 loci, with an average of 2.0 alleles per locus (ranging from 1 to 4 alleles). Allele sizes varied approximately from 100 bp to 700 bp across loci. The polymorphic information content (PIC) values ranged from 0.07 to 0.79, indicating varying levels of marker informativeness. Markers sORA43 and Ni02-D08a were the most informative (PIC = 0.79), whereas BRMS-017 showed low polymorphism (PIC = 0.07). The mean PIC value across all loci was 0.38, reflecting moderate genetic diversity among the parental genotypes. Rare alleles were also observed at certain loci, a unique allele at NA10-C01c was detected only in PRD-14-13, highlighting the presence of genotype-specific variation within the collection.

The Jaccard similarity matrix (Fig. 5) provided pairwise genetic similarity coefficients among the 14 genotypes, with values ranging from 0.45 to 0.85. The lowest similarity (0.45) was observed between PR-2019-9 and PRD-14-13, indicating that these 2 genotypes are the most genetically divergent within the panel. In contrast, the highest similarity (0.85) was recorded between PR-2016 -4 and PRL-2017-7 and between RGN-73 and RB-101, suggesting

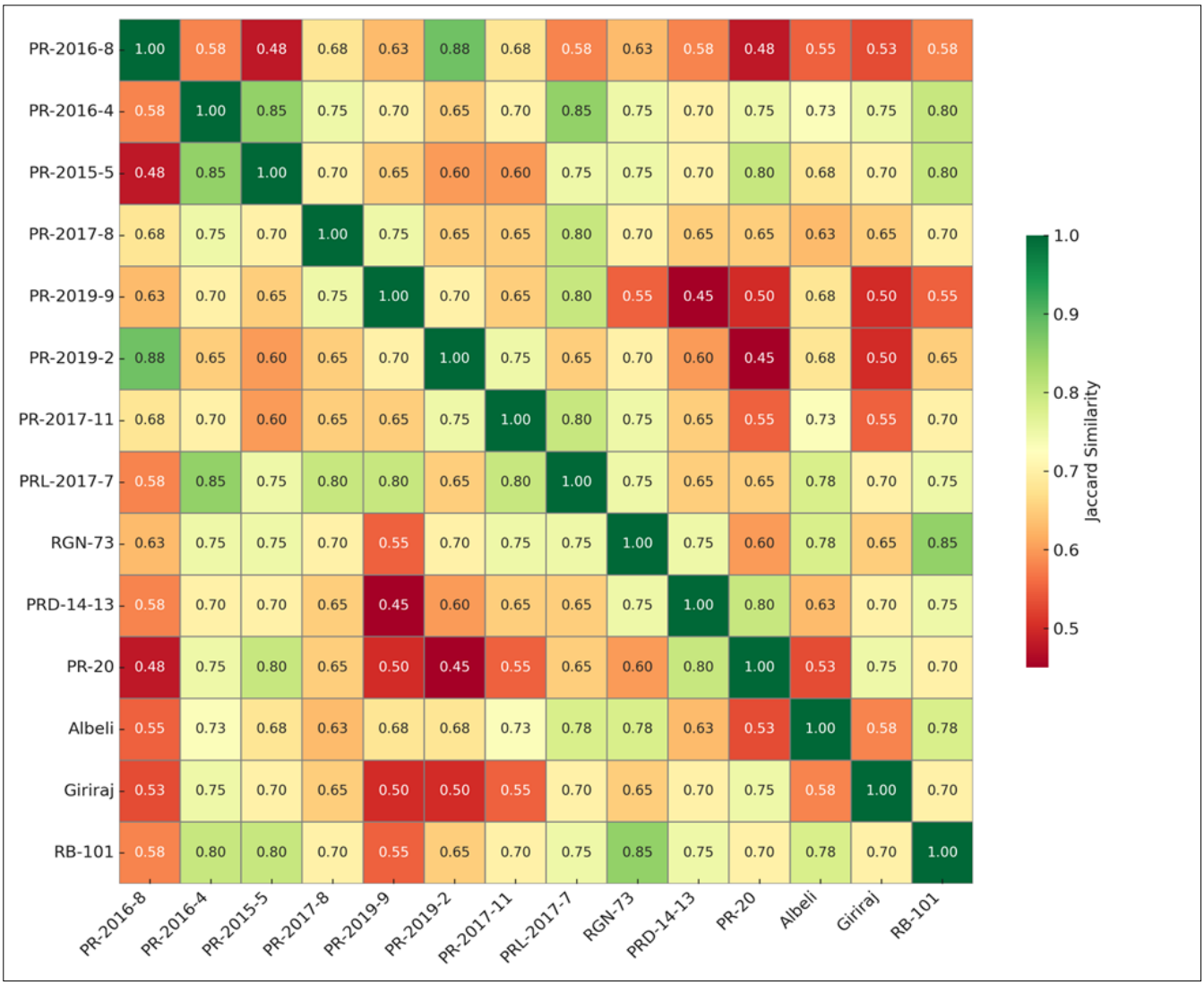

Fig. 5. Genetic distance among the genotypes based on Jaccard's similarity matrix.

close genetic relatedness within these pairs. Several other pairs also showed relatively high similarity (≥0.80), indicating shared genetic background among certain lines. Conversely, combinations such as PR-20 and PR-2019-2 exhibited comparatively lower similarity (~0.50), reflecting greater genetic divergence.

The presence of both closely related and genetically divergent genotypes within the panel is advantageous for breeding, as related lines can be used for trait stabilisation, while divergent lines may enhance heterosis. For instance, PR-2015-5, PR-2016-4 and PRL-2017-7 formed a genetically close group, likely reflecting shared Varuna/Pantnagar lineage, whereas lines such as PR-2019-2, derived from a background involving NRCHB-101, were more distinct. The UPGMA cluster analysis grouped the 14 genotypes into 3 major clusters (I, II and III), as summarised in Table 7 and the phylogenetic dendrogram (Fig. 6) clearly illustrated the genetic distances among genotypes. This clustering pattern was consistent with the similarity matrix and was further supported by the principal coordinate analysis (PCoA) shown in Fig. 7.

From a breeding perspective, SSR-based clustering provides a useful framework for selecting diverse parental combinations. Inter-cluster crosses generally involve greater genetic divergence than intra-cluster crosses and may enhance the likelihood of heterosis. In the present study, several high-performing hybrids involved parents from different clusters. For example, PR-2016-8 (Cluster I) crossed with PR-20 or Giriraj (Cluster II) and PR-2019-2 (Cluster I) × Albeli (Cluster III), exhibited strong heterosis for seed yield. Similarly, PRL-2017-7 (Cluster III) × PR-20 (Cluster II) showed substantial heterotic response. In contrast, crosses among closely related genotypes within the same cluster, such as PR-2015-5 × PR-2016-4, generally displayed lower heterosis. Like previous diversity studies in mustard (22). Several highly heterotic hybrids involved parents from different clusters, suggesting that greater genetic divergence may contribute to improved hybrid performance. However, the relationship between molecular marker-based genetic distance and heterosis is not always consistent, as neutral markers may not fully represent variation at yield-related genes (23). Therefore, SSR-based diversity should be used as a supportive tool, along with combining ability and per se performance, for selecting parents and prioritising crosses for further evaluation.

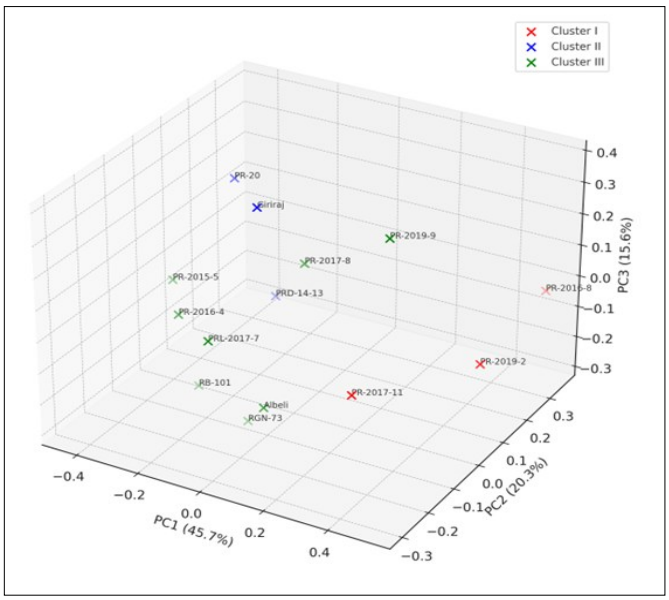

Fig. 7. Principal coordinate analysis revealing the relation among 14 mustard genotypes.

Table 7. Cluster summary table

Cluster	Genotypes	Breeding Origin	Interpretation
Cluster I	PR-2016-8, PR-2019-2, PR-2017-11,	Likely Krishna lineage; both share NRCHB/PR backgrounds	Genetically distinct group: PR-2016-8 may carry rare alleles (possibly from Maya); outliers in factorial analysis
Cluster II	Giriraj, PRD-14-13, PR-20	Linked via Kranti/Varuna ancestry (traditional Indian mustard lines)	Represents "Varuna–Kranti gene pool"; moderate divergence from Cluster I; high-yielding testers
Cluster III	PR-2017-8, PR-2019-9, PR-2015-5, PR-2016-4, PRL-2017-7, Albeli, RGN-73, RB-101	Pantnagar lines + diverse selections (Albeli, RGN-73, RB-101)	Largest and most diverse cluster; internal subgroups include a Pantnagar core varieties; Albeli genetically close to Rajasthan-origin genotypes

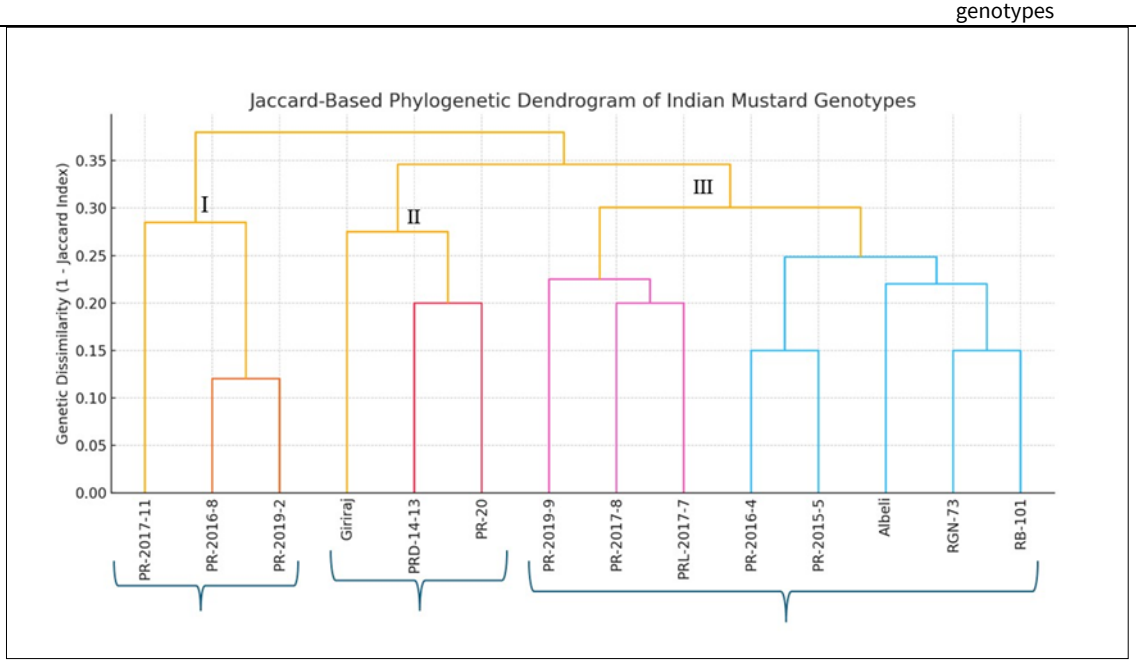

Fig. 6. Phylogenetic dendrogram for the 14 genotypes under study.

Conclusion

The present study demonstrates that integrating combining ability analysis, heterosis evaluation and SSR-based molecular diversity assessment provides a comprehensive framework for Indian mustard improvement. The line × tester analysis identified superior general combiners, particularly PR-2019-9, PR-2019-2 and PR-20, along with specific cross combinations such as PR-2019-2 × Albeli and PRL-2017-7 × PR-20 that exhibited high SCA effects and substantial heterosis for seed yield under the present experimental conditions. The results indicate the contribution of both additive and non-additive gene action in governing yield and related traits.

The SSR-based molecular analysis grouped the parental genotypes into 3 major clusters and revealed moderate genetic divergence among lines. Several high-performing hybrids involved parents from different clusters, suggesting that molecular diversity can complement combining ability analysis in guiding parental selection. However, molecular distance alone should not be considered a definitive predictor of hybrid performance.

Overall, the combined use of phenotypic and molecular approaches enhances breeding efficiency by enabling informed parent selection and strategic hybrid development. The superior crosses identified in this study require further multi-environment evaluation to assess their stability and potential for commercial exploitation in Indian mustard breeding programs.

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Authors' contributions

SP executed the research work; conducted all field and laboratory experiments; collected and curated the complete phenotypic and molecular datasets; performed the preliminary analyses; and drafted the original manuscript. BSR assisted with statistical data analysis, including line × tester and heterosis analyses, and contributed to the correction and refinement of the data analysis and Python codes. AS assisted in PCR optimisation and SSR marker amplification and contributed to the generation of molecular marker data. UP supervised the entire research as the major advisor, provided conceptual guidance and experimental planning, and critically reviewed and edited the manuscript for important intellectual content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None

References

1. USDA. Oilseeds and products update: New Delhi, India. Washington, DC: USDA Foreign Agricultural Service; 2025.
2. Tyagi P, Singh R. Rapeseed and Mustard: Breeding Objectives of Rapeseed and Mustard. Saarbrücken: LAP LAMBERT Academic Publishing; 2016.
3. Mehta BV. Global Vegetable Oil Market: India's Perspective. In: 2nd Sustainable Vegetable Oils Conference / CPOPC; 2023; Mumbai, India.
4. Lallu L, Baghel R, Srivastava S. Assessment of mustard genotypes for thermo tolerance at seed development stage. 2010.
5. Sodhi YS, Pradhan AK, Verma JK, Arumugam N, Mukhopadhyay A, Gupta V, et al. DMH-1: The first mustard hybrid in India based on a novel CMS-restorer system. Bull Int Rapeseed Mustard Counc. 2008;23:29–34.
6. Kumar A, Verma S, Yadav R, Verma S, Kumar M. Heterosis for seed yield and its component traits in Indian mustard [*Brassica juncea* (L.) Czern. & Coss.]. J Andaman Sci Assoc. 2024;29(2):165–71.
7. Fasahat P, Rajabi A, Rad JM, Derera J. Principles and utilization of combining ability in plant breeding. Biometrics Biostat Int J. 2016;4 (1):1–24. https://doi.org/10.15406/bbij.2016.04.00085
8. Kempthorne O. An introduction to genetic statistics. New York: John Wiley & Sons; 1957.
9. Sprague GF, Tatum LA. General vs. specific combining ability in single crosses of corn. Agron J. 1942;34(10):923–32. https://doi.org/10.2134/agronj1942.00021962003400100008x
10. Patel A, Arha M, Khule A. Combining ability analysis for seed yield and its attributes in Indian mustard [*Brassica juncea* (L.) Czern and Coss]. Asian J Bio Sci. 2013;8(1):11–2. https://doi.org/10.56739/jor.v31i1.142111
11. Younas M, Xiao Y, Cai D, Yang W, Ye W, Wu J, et al. Molecular characterization of oilseed rape accessions collected from multi continents for exploitation of potential heterotic group through SSR markers. Mol Biol Rep. 2012;39:5105–13. https://doi.org/10.1007/s11033-011-1306-0
12. Baghel R, Sharma A, Tiwari S, Tripathi M, Tripathi N. Genetic diversity analysis of Indian mustard (*Brassica* spp.) germplasm lines using SSR molecular markers. Int J Curr Microbiol App Sci. 2020;9(12):137–43. https://doi.org/10.20546/ijcmas.2020.912.018
13. Singh K, Singh L, Parmar N, Kumar S, Nanjundan J, Singh G, et al. Molecular characterization and genetic diversity analysis in Indian mustard (*Brassica juncea* L. Czern & Coss.) varieties using SSR markers. PLoS One. 2022;17(8):e0272914. https://doi.org/10.1371/journal.pone.0272914
14. Vinu V, Naveen Singh NS, Sujata Vasudev SV, Giri S, Rajendra Singh RS, Dass B, et al. Relationship of molecular and phenotypic divergence with hybrid performance in Indian mustard (*Brassica juncea*). Indian J Agric Sci. 2016;86(9):1153–9. https://doi.org/10.56093/ijas.v86i9.61502
15. Murray M, Thompson W. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980;8(19):4321–6. https://doi.org/10.1093/nar/8.19.4321
16. Rout S, Roy SK, Mandal R, Singla S, Rahimi M, Sur B, et al. Genetic analysis and heterosis breeding of seed yield and yield-attributing traits in Indian mustard (*Brassica juncea* (L.) Czern & Coss.). Sci Rep. 2025;15(1):2911. https://doi.org/10.1038/s41598-025-86621-8
17. Saroj R, Soumya S, Singh S, Sankar SM, Chaudhary R, Yashpal, et al. Unraveling the relationship between seed yield and yield-related traits in a diversity panel of *Brassica juncea* using multi-traits mixed model. Front Plant Sci. 2021;12:651936. https://doi.org/10.3389/fpls.2021.651936
18. Yadava DK, Singh N, Vasudev S, Singh R, Singh S, Giri SC, et al. Combining ability and heterobeltiosis for yield and yield-contributing traits in Indian mustard (*Brassica juncea*). Indian J Agric Sci. 2012;82 (7):563–7. https://doi.org/10.56093/ijas.v82i7.21632

19. Meena H, Kumar A, Ram B, Singh VV, Meena PD, Singh BK, et al. Combining ability and heterosis for seed yield and its components in Indian mustard (*Brassica juncea* L.). J Agric Sci Technol. 2015;17 (7):1861–71.

20. Griffing BR. Concept of general and specific combining ability in relation to diallel crossing systems. Aust J Biol Sci. 1956;9(4):463–93. https://doi.org/10.1071/BI9560463

21. Lamkey KR, Edwards JW. Quantitative genetics of heterosis. In: Coors JG, Pandey S, editors. Genetics and exploitation of heterosis in crops. Madison: ASA-CSSA-SSSA; 1999. p. 31–48. https://doi.org/10.2134/1999.geneticsandexploitation.c4

22. Pratap P, Thakur AK, Meena PD, Meena HS, Sharma P, Singh D, et al. Genetic diversity assessment in Indian mustard (*Brassica juncea* L.) for Alternaria blight tolerance using SSR markers. J Oilseed Brassica. 2015;6(1):175–82.

23. Diers BW, McVetty PB, Osborn TC. Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). Crop Sci. 1996;36(1):79–83. https://doi.org/10.2135/cropsci1996.0011183X003600010014x

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