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CONTENTS

Improving *Brassica juncea* performance through hybrid breeding strategies: a focus on combining ability and heterosis analysis

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ABSTRACT: Seven advanced lines/varieties of Indian mustard (*Brassica juncea* L.) were selected for building the experimental materials. It consisted of 3 lines and 4 testers, mated in Line×Tester design during rabi season 2021-22. Fifteen parameters, including seed yield per plant (g), glucosinolate content (µmole/g), oil content (%), and erucic acid content (%), were assessed in RBD during the rabi season of 2022–2023 for parental lines and their 12 F_1s. Analysis of variance for combining ability showed significant differences amid treatments for all the characters; except for seeds per siliqua and siliqua density. Tester PWR 13-8 was a good combiner for a maximum of eight traits, including seed yield per plant and oil content. Lines NRCHB-101 and Kranti were good combiners for seed yield per plant. The cross-combination Giriraj × Heera for seed yield per plant, NRCHB-101 × PWR 13-8 for glucosinolate content, Kranti × PWR 13-8 for oil content, and Kranti × Donskaja for erucic acid content had the highest significant SCA effect. Cross combination Kranti × PWR 13-8 showed the highest mid-parent and better-parent heterosis for seed yield per plant. Cross-combination NRCHB-101 × PWR 13-8 showed the highest economic heterosis (222.81^{**}) for seed yield per plant along with high estimates, i.e., -17.24^{**} for mid-parent heterosis, -22.06^{**} for better-parent heterosis and -26.88^{**} for economic heterosis, for glucosinolate content. Cross Kranti × PWR 13-8 showed the highest mid-parent heterosis (5.97^{**}), and Giriraj × Bio YSR showed the highest better-parent (5.07^{**}) and economic heterosis (9.11^{**}) for oil content.

Key words: Combining ability, Heterosis, Indian mustard, Line×Tester, Seed yield

The Indian subcontinent is perfectly suited for growing all of the major annual oilseed crops because of its extensive agro-ecological diversity. Rapeseed mustard is the third-most significant annual oilseed crop in India among the nine edible and non-edible oilseed crops, behind soybeans and groundnuts, which make up one-fourth of the country's total area and provide one-third of its total oil production. In India, Rapeseed-mustard seed production has reached 11.10 million tonnes in 2021–2022 with 7.99 million hectares covered and a productivity of 1.39 tonnes/ha (Kamboj *et al*., 2023). It provides a significant source of income, particularly for marginal and small farmers who make up around 25% of all cultivable land in rain-fed areas.

Brassica juncea, or Indian mustard, is a key oilseed crop with origin in central Asia, and is a vital part of agriculture in India. This study focuses on improving several important traits to boost the crop's performance and resilience. We're looking at factors like the days to flowering initiation, which affects the total duration of the crop; plant height, which impacts yield and how prone the plant is to lodging; and the length and number of siliquae on the main raceme, which are crucial for seed production. Branching patterns, siliqua length, and seed count per siliqua also play a role in determining how much seed the plant can produce. Other key traits include seed yield per plant, test weight, and quality aspects like glucosinolate content, oil content, and erucic acid levels. By focusing on these traits, the study aims to develop mustard varieties that are more productive, and better suited for commercial use. Based on observation recorded for these traits, this study was aimed to identify superior parent lines and crosses with desirable combining abilities and heterosis effects. Lines identified superior could then be used as potential donors in a hybridization programme aimed at improving crops in multiple dimensions and could be further commercially exploited (Singh *et al.,* 2013). Additionally, because Indian mustard is largely self-pollinating, the Line×Tester mating system suggested by Kempthorne (1957) for GCA and SCA analysis was employed to quickly screen lines. The results of this study may have significant implications in heterosis breeding aimed at improving mustard seed yield and related traits.

MATERIALS AND METHODS

The experimental materials for this study comprised of 7 advanced lines/varieties; of Indian mustard, comprised of 3 lines (Giriraj, NRCHB-101, and Kranti) and 4 testers (Donskaja, PWR 13-8, Bio-YSR, and Heera). Kranti was used as a standard check for the estimation of standard heterosis. Seeds of these materials were obtained from the Department of Genetics and Plant Breeding at the G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Seven parents and their twelve single crosses (F_1s) were developed *via* selfing and hand emasculation- pollination, respectively, using Line×Tester mating design in rabi, 2021-22. On 1-11-2022 (rabi 2022-23) this experimental material was sown in Randomized Block Design (RBD) with three replications in single environment. Parents along with their F_1s were sown in single rows of 3m each and were evaluated for 15 traits, namely days to flowering initiation (DFI), days to 50 % flowering (DF), plant height (PH), length of main raceme (LMR), siliquae on main raceme (SMR), number of primary branches (NPB), number of secondary branches (NSB), siliqua length (SL), seed per siliqua (SS), siliqua density (SD), seed yield per plant (SY), test weight (TW), glucosinolate content (GC), oil content (OC) and erucic acid content (EC). Quality traits like OC, GC and EC were measured using FT-NIR. The collected data were subsequently examined using the Kempthorne (1957) method for GCA and SCA effects, the Shull (1908) method for relative heterosis, the Briggle (1963) method for standard heterosis, and the Fonesca and Patterson (1968) method for heterobeltiosis, in R_4.3.0. software. The percentages that lines, testers, and line × tester interactions contributed to the overall variation were computed as:

Percent contribution of lines= $\frac{\text{S.S due to lines}}{\text{S.S due to crosses}} \times 100$

Per cent contribution of testers = $\frac{\text{S.S due to testers}}{\text{S.S due to crosses}} \times 100$

Per cent contribution of line × tester interaction = $\frac{\text{S.S due to lines} \times \text{testers}}{\text{S.S due to crosses}} \times 100$

In order to pick the best parents and produce excellent F_1 hybrids, heterosis which acts as a foundation and guide was computed using following formula:

Relative Heterosis (%) = $\frac{(\overline{F_1} - \overline{MP})}{\overline{MP}} \text{ X } 100$

Better Parent Heterosis (%) = $\frac{(\overline{F_1} - \overline{BP})}{\overline{BP}} \text{ X } 100$

Standard Heterosis (%) = $\frac{(\overline{F_1} - \overline{SC})}{\overline{SC}} \text{ X } 100$

Where, MP = mid parent; BP = better parent; SC = standard check.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for the design of experiment, i.e., simple RBD, for fifteen characters were calculated and according to the results adequate variation was found for all the characters under study. Further ANOVA for CA showed the variance due to lines and testers which were highly significant for SY. Variances due to testers were also significant for traits SS and TW. Nonetheless, variations resulting from the line × tester were found to be highly significant for characters examined except for PH, SS and SD (Table 1).

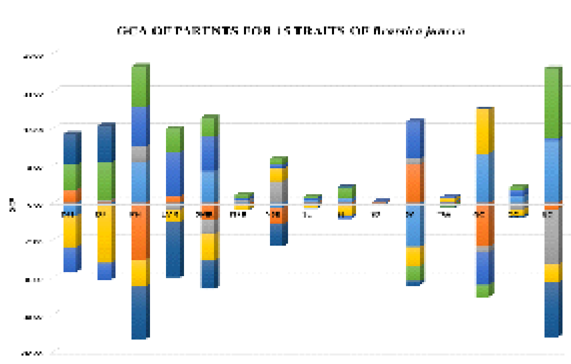

Fig 1: GCA of parents for 15 traits of Brassica juncea under study

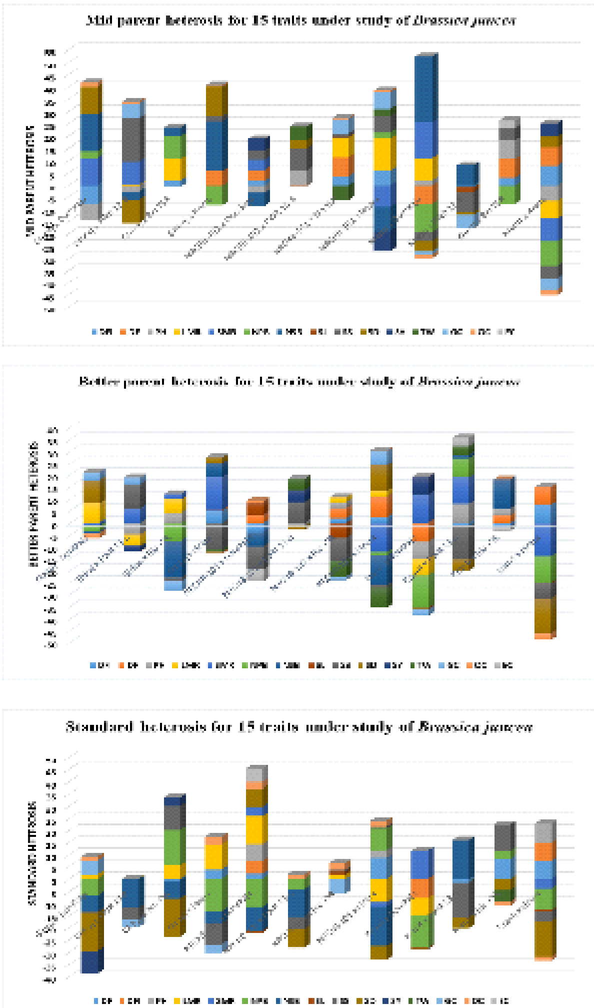

Fig 2: Heterosis for 15 traits of Brassica juncea under study

Lines Giriraj, NRCHB-101 and tester Donskaja were found to be a good combiner for four traits each, whereas, line Kranti and tester Heera and Bio-YSR were found to be a good combiner for three, two and one trait under study, respectively. Tester PWR 13-8 was found to be a good combiner for maximum number of traits under study, i.e., eight compared to other parents taken (Table 2). Fig.1 showed the GCA effects in parents for all 15 characters under study. The importance of additive effects in the development of seed yield and its component traits is indicated by the presence of significant GCA variation (Mamun *et. al.,* 2022). Breeders can further choose parents with good combining ability for hybridization programmes based on GCA effect. Even while it's true that not all heterotic crossings result from mating of high GCA parents, these preparatory procedures nonetheless aid in assembling parents into various groups to create original recombinants (Dahiya *et al.* 2018).

SCA effects in the negative direction were preferred for traits like DFI, DF, PH, GC, and EC since they contributed traits like earliness, dwarfism, and improved genotypes meal quality. Positive SCA effects were desired in other characters under study. Cross-combination NRCHB-101 × PWR 13-8 for DFI, DF and GC; Giriraj × Heera for LMR, SMR, NSB and SY; Kranti × PWR 13-8 for NPB and OC; Kranti × Bio YSR for SL; Kranti × Donskaja for TSW and EC expressed highest significant SCA effects in the desirable direction. For all the traits under study, with the exception of SS, SY, and TW, it was discovered that the interaction between line and tester contributed more towards overall variation than either the line or the tester did. The tester's contribution to overall variation was found to be largest in SS and TW. However, for SY, lines contributed the most to the overall variance.

Superior crosses on the

Table 1: ANOVA for combining ability for 15 characters in Brassica juncea

Sources of variation	D.f.	Mean sum of squares														
		DFI	DF	PH	LMR	SMR	NPB	NSB	SL	SS	SD	SY	TW	GC	OC	EC
Lines	2	36.75	1.00	545.99	7.69	159.92	1.13	83.65	1.56	2.19	0.11	372.55**	0.24	475.19	9.13	735.14
Testers	3	168.67	342.67	368.29	304.59	162.93	1.79	37.28	0.55	12.95*	0.01	107.85**	0.12*	171.41	4.77	449.56
Line × Tester	6	360.19**	287.89**	208.94	239.58**	89.12**	5.38**	40.69**	0.83**	3.25	0.04	9.21**	0.29**	184.83**	5.50**	292.84**
Error	36	18.79	25.53	105.09	23.88	24.34	0.64	7.02	0.03	2.30	0.02	2.06	0.02	22.16	0.68	1.02

Table 2: Parents with GCA values in the desired direction for the traits of *Brassica juncea* under study

S. No.	Parents	Traits with significant GCA values in desired direction
Lines		
01.	Giriraj	SMR (4.2*), SL (0.38**), TW (0.16**), OC (0.84**).
02.	NRCHB-101	PH (-7.54*), SY (5.14**), GC (-5.9**), EC (-0.87**).
03.	Kranti	NSB (2.9**), SY (0.78**), EC (-7.35**).
Tester		
01.	Donskaja	DFI (-4.22**), DF (-7.67**), TW (0.37**), EC (-2.49**).
02.	PWR 13-8	DFI (-3.22*), LMR (5.96**), SL (0.31**), SMR (4.61*), SY (5.03**), TW (0.22**), GC (-4.3**), OC (0.75**).
03.	Bio YSR	SS (1.6**).
04.	Heera	PH (-7.2*), EC (-7.32**).

Table 3: Superior crosses on the basis of SCA effects for seed yield per plant, glucosinolate content and oil content with suitable breeding strategy for crop improvement

S.No.	Character	Crosses with highest SCA effect	SCA effects	Heterosis: Mid Parent	Heterosis: Better Parent	Heterosis: Standard Heterosis	Other characters showing highest SCA effect	Parent combination	Suitable breeding method
1	Seed yield	Giriraj × Heerayield	2.08** Heera	-15.07*	-41.1**	63.16**	LMR, SMR, NSB	P × P	Heterosis breeding/diallel selective mating with selection pressure on LMR, SMR, NSB and SD.
		NRCHB-101× Donskaja	1.03	4.75	-14.92**	161.57**	SD	G × P	
2	Oil content	Kranti× PWR 13-8	2.02**	5.97**	4.96**	7.01**	NPB	P × G	Heterosis breeding/diallel selective mating with selection pressure on NPB.
		Giriraj× Bio YSR	1.41**	5.49**	5.07**	9.11**	——	G × A	
3	Gluco-sinolate content	NRCHB-101× PWR 13-8	-8.54**	-17.24**	-22.06**	-26.88**	DFI, DF	G × G	Heterosis breeding/diallel selective mating with selection pressure on DFI, DF and SL.
		Kranti× Bio YSR	-6.13*	-15.22**	-17.02**	-17.02**	SL	G × P	
4	Erucic acid content	Kranti× Donskaja	-13.85**	-71.27**	-83.68**	-83.68**	TW	G × G	Heterosis breeding/diallel selective mating with selection pressure on DFI, DF and TW.
		NRCHB-101× PWR 13-8	-8.96**	-37.76**	-38.57**	-31.98**	DFI, DF	G × P	

basis of SCA effects in the desired direction for SY, GC, OC and EC with suitable breeding strategy for crop improvement is given in Table 3. The potential crossings that have good×good GCA effects respond favourably to traditional breeding techniques like pedigree approaches in terms of improvement. Whereas, in the cases where crosses involving good×average and poor×poor general combiners, heterosis breeding is preferred. The character estimates of GCA variations were higher than SCA variance for quality traits like OC and EC, showing dominance variance predominated. In contrast, it was discovered that the SCA variance estimate for GC was higher than the GCA variance, indicating that the dominant variance is stronger than the additive variance. Studies with similar findings with greater dominance variance than the additive variance for traits under study have been reported earlier (Mandal *et. al.,* 2023a). Further evidence that non-additive gene activity predominated over additive gene action was discovered in the ratio of variance resulting from general and specific combining abilities, which was

Table 4: Two cross combination having highest SCA effects, better parent heterosis, relative heterosis and economic heterosis in the desired direction

S. No.	Characters	Cross with high SCA	Relative heterosis	Better parent heterosis	Economic heterosis
1	DFI	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**
		Giriraj × Bio YSR**	Kranti × Donskaja**	Giriraj × Donskaja**	Giriraj × Donskaja**
2	DF	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**
		Giriraj × Donskaja**	Giriraj × Bio YSR**	Giriraj × Bio YSR**	Giriraj × Donskaja**
3	PH	Giriraj × PWR 13-8	NRCHB-101 × Heera**	NRCHB-101 × Heera**	Giriraj × Bio YSR
		Kranti × Bio YSR	Giriraj × Heera**	Kranti × Heera**	Kranti × PWR 13-8
4	LMR	Giriraj × Heera**	NRCHB-101 × Donskaja**	NRCHB-101 × Donskaja**	Kranti × Bio YSR**
		Kranti × Bio YSR**	Giriraj × Heera**	Kranti × Bio YSR**	NRCHB-101 × PWR 13-8**
5	SMR	Giriraj × Heera**	Kranti × Bio YSR**	Kranti × Bio YSR**	Giriraj × Heera**
		NRCHB-101 × PWR 13-8	NRCHB-101 × Bio YSR**	NRCHB-101 × Bio YSR**	NRCHB-101 × PWR 13-8**
6	NPB	Kranti × PWR 13-8**	Kranti × PWR 13-8*	Kranti × PWR 13-8	Kranti × PWR 13-8**
		Giriraj × Bio YSR**	Giriraj × Bio YSR	NRCHB-101 × Heera	Giriraj × Bio YSR
7	NSB	Giriraj × Heera*	Kranti × Donskaja **	Kranti × Donskaja**	Kranti × Donskaja**
		Kranti × Bio YSR	Kranti × Bio YSR**	Kranti × Bio YSR	Kranti × Bio YSR**
8	SL	Kranti × Bio YSR**	Giriraj × PWR 13-8**	Giriraj × PWR 13-8**	Giriraj × PWR 13-8**
		NRCHB-101 × PWR 13-8**	Giriraj × Heera**	Giriraj × Donskaja**	NRCHB-101 × PWR 13-8**
9	SS	Giriraj × Donskaja	Giriraj × Donskaja**	Giriraj × Donskaja**	Kranti × Bio YSR
		NRCHB-101 × Heera	Giriraj × Bio YSR**	Giriraj × PWR 13-8	Giriraj × Bio YSR
10	SD	NRCHB-101 × Donskaja	NRCHB-101 × Donskaja**	NRCHB-101 × Donskaja**	NRCHB-101 × Donskaja
		Kranti × Bio YSR	NRCHB-101 × Heera**	NRCHB-101 × Heera	Kranti × Bio YSR
11	SY	Giriraj × Heera**	Kranti × PWR 13-8**	Kranti × PWR 13-8**	NRCHB-101 × PWR 13-8**
		NRCHB-101 × Donskaja	Kranti × Donskaja**	Kranti × Donskaja	Kranti × PWR 13-8**
12	TW	Kranti × Donskaja**	Kranti × Donskaja**	Kranti × Donskaja**	Kranti × Donskaja**
		NRCHB-101 × Bio YSR**	Giriraj × Donskaja**	Giriraj × Donskaja**	Giriraj × Donskaja**
13	GC	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**
		Kranti × Bio YSR**	Kranti × Bio YSR**	Kranti × Bio YSR**	Kranti × Bio YSR**
14	OC	Kranti × PWR 13-8**	Kranti × PWR 13-8**	Giriraj × Bio YSR**	Giriraj × Bio YSR**
		Giriraj × Bio YSR**	Giriraj × Bio YSR**	Kranti × PWR 13-8**	Kranti × PWR 13-8**
15	EC	Kranti × Donskaja**	Kranti × Donskaja**	Kranti × Donskaja**	Kranti × Donskaja**
		NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**	NRCHB-101 × PWR 13-8**

shown to be smaller than unity for these quality traits. The study looked at how different plant crosses performed in terms of heterosis, which can be expressed as percent increase or decrease in the mean value of F_1 hybrid over mid parent (relative heterosis), standard check (economic heterosis) and better parent (heterobeltiosis). For traits like DFI, DF and GC, the cross-combination NRCHB-101 × PWR 13-8 performed well, showing the earliest flowering compared to other crosses. For PH, NRCHB-101 × Heera showed highest estimates of heterosis in the desired direction, which can result in shorter plants. For LMR, NRCHB-101 × Donskaja had the highest estimate in the desired direction, indicating the presence of plants with longer length of main raceme which can contribute towards higher seed yield. Cross Kranti × Bio YSR performed better than the standard check Kranti for LMR. For SMR, Kranti × Bio YSR estimates indicates that it can mature the fastest, while Giriraj × Heera performed better than the check for the trait. In terms of NSB, TW, and EC, Kranti × Donskaja performed the best overall, which could be great for improving these yield-related traits. Kranti × PWR 13-8 showed a higher estimate of mid-parent and standard heterosis for NPB, which indicates higher number of primary branches in the cross. For SL and SD, Giriraj × PWR 13-8 and NRCHB-101 × Donskaja had the highest estimate in the desired direction for all three heterosis under study. Giriraj × Donskaja also had the highest estimate for seeds per siliqua among all the cross combinations under study which can further increase the seed yield per plant. For SY, Kranti × PWR 13-8 had the highest estimates in the desired direction for mid-parent and better-parent heterosis, and NRCHB-101 × PWR 13-8 showed the highest standard heterosis, suggesting better yield potential. Lastly, for OC, Kranti × PWR 13-8 had the highest mid-parent heterosis, while Giriraj × Bio YSR showed the best results for both better-parent and standard heterosis, indicating high oil content. Earlier reports from numerous studies have also reported a relatively higher magnitude of heterosis for SY and its component traits (Meena *et al*., 2015; Kaur *et al*., 2021; Mandal *et. al.,* 2023b). Thus, these findings suggest that certain cross combinations have the potential to significantly enhance various traits, which could be really beneficial for improving crop performance and yield.

CONCLUSION

Combinations with high yield, considerable positive SCA, better parent, mid parent, and economic heterotic responses for yield per plant can be used to assess the genotype's commercial value. At that point, the genotype can be deemed excellent for usage in yield improvement programmes (Table 3). Crosses with good × good GCA parents are best improved with traditional breeding methods, while good × average GCA crosses benefit from heterosis or mass selection. Poor × poor GCA crosses require heterosis breeding and other advanced mating techniques. Conventional breeding techniques can be used to improve these crossings, with selection pressure on traits such as DFI, DF, LMR, SMR, NPB, NSB, and TW, among others.

Numerous genotypes and cross combinations of Indian mustard with notable heterosis and combining ability for important traits are identified in this study as promising. High economic and mid-parent heterosis is demonstrated by the cross NRCHB-101 × PWR 13-8, which stands out for its higher SY and GC. Kranti × PWR 13-8 exhibits significant mid-parent heterosis for SY and the highest estimations of heterosis for OC. Furthermore, the higher estimates of SCA for SY and EC was obtained for Giriraj × Heera and Kranti × Donskaja, respectively. These findings suggest that NRCHB-101 × PWR 13-8, Kranti × PWR 13-8, Kranti × Donskaja, and Giriraj × Heera can be used in hybrid development and varietal improvement programs aimed at enhancing yield, and other qualitative traits in Indian mustard.

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