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PYRAMIDING OF WHITE RUST RESISTANCE AND OIL QUALITY TRAITS IN INDIAN MUSTARD THROUGH A MARKER-ASSISTED APPROACH

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SUMMARY

Indian mustard (*Brassica juncea* L.) is a major oilseed crop known for its adaptability to diverse agroclimatic conditions in South Asia. However, productivity, oil, and meal quality are often susceptible to white rust disease and the presence of high levels of antinutritional factors. With the aim of reducing both antinutritional factors (erucic acid) in oil and (glucosinolates) meal, along with resistance to white rust, this study used a marker-assisted pyramiding approach to introgress QTLs from Heera and RLC-3 into three elite Indian mustard cultivars: DRMR150-35, NRCHB101, and NRCDR-02. Using trait-specific markers helped foreground selection, while employing SSR markers assisted background selection in respective cross-combinations. In total, 34 pyramided lines (five BC_3F_5 in DRMR150-35/RLC-3, seventeen BC_3F_6 in NRCHB101/Heera, three BC_3F_5 in NRCHB101/RLC-3, and nine BC_3F_6 in NRCDR-02/RLC-3), with >90% recurrent parent genome recovery, attained development. The resulting pyramided lines underwent evaluation for agronomic performance, disease resistance, and oil quality. The study highlights the potential of MAS in breeding programs to accelerate the development of multi-trait superior cultivars, contributing to sustainable mustard production and enhanced oil quality.

Keywords: Indian mustard (*B. juncea* L.), marker-assisted pyramiding, white rust resistance, oil quality, molecular markers

Key findings: The 34 selected pyramided lines of the Indian mustard (*B. juncea* L.) exhibited significant resistance to white rust and superior oil quality without any yield penalty. These lines have potential for general cultivation in India.

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INTRODUCTION

Indian mustard (*Brassica juncea* L.) is an important oilseed crop widely cultivated in South Asia and parts of Africa, contributing significantly to the global oilseed industry. However, the crop faces challenges due to the presence of antinutritional components in oil and its vulnerability to the white rust disease. The antinutritional factors, like high erucic acid in oil and glucosinolates in seed meal, pose health risks to humans and animals alike (Priyamedha *et al.*, 2021). The erucic acid has reports of linkage with increased risk of myocardial disorders and elevated cholesterol, while glucosinolates typically impair thyroid function and diminish the palatability and nutritional value of animal feeds (Mortuza *et al*., 2006). In response to the health risks posed by erucic acid and glucosinolates, global breeding initiatives began in the 1970s to enhance the nutritional quality of oil and seed meals (Singh *et al*., 2020b; Priyamedha *et al*., 2021). In India, focused breeding efforts commenced, aiming to develop superior genotypes by reducing erucic acid to below 2% and glucosinolates to less than 30 µmol/g meal (Chauhan *et al*., 2010).

Alongside nutritional concerns, white rust poses a severe threat to mustard crops, often causing significant yield losses of up to 90% (Chand *et al.,* 2022), particularly under favorable environmental conditions (Meena *et al.,* 2010). Unlike chemical control, developing resistant crop cultivars is essential for long-term food security and environmental sustainability (Hanson *et al.,* 2016; Singh *et al.,* 2024). The combination of these traits in a single variety would significantly enhance both its nutritional value and disease resistance, fulfilling consumer demands for healthier oils and crop resilience.

Marker-assisted selection (MAS) has emerged as an effective technique in contemporary breeding initiatives. In particular, marker-assisted pyramiding is a cutting-edge breeding strategy where multiple genes for different traits through successive rounds of selection entail combining into a single genotype. This approach significantly enhances the efficiency of breeding compared with traditional methods by ensuring that the selection of plants with all targeted traits can occur early in the breeding cycle (Jiang*,* 2015; Cholin and Kulkarni*,* 2023).

The following study aimed to utilize a marker-assisted pyramiding approach to incorporate three crucial traits—low erucic acid, low glucosinolate content, and white rust resistance—into a *B. juncea* variety.

MATERIALS AND METHODS

Selection of parents and breeding strategy

In the presented study, DRMR150-35, NRCHB101, and NRCDR-02 were the cultivars used as the recurrent parents, whereas RLC-3 and Heera served as the donors for pyramiding of a low erucic acid, low glucosinolate, and white rust-resistant gene (Table 1). The experiment commenced at the experimental field of the ICAR-IIRMR (Indian Council of Agricultural Research-Indian Institute of Rapeseed-Mustard Research), Bharatpur. All the prescribed practices incurred strict implementation to raise healthy Indian mustard crops.

In the 2015 Rabi season, attempting independent crosses began by crossing recurrent parents with donor parents to produce F_1 seeds. True F_1 hybrids' confirmation used trait-specific markers and backcrossed with recurrent parents to generate BC_1F_1s. In Rabi 2017, the validation of BC_1F_1 plants for the presence of target genes succeeded. The gene-positive heterozygous plants then incurred backcrossing with the recurrent parent to generate BC_2F_1 seeds. The backcrossing attempts reached up to three (BC_3) generations to generate BC_3F_1 (Rabi 2019). Furthermore, BC_3F_1 positive plants for the target genes and exhibiting maximum recovery of the recurrent parent genome (RPG) underwent self-pollination to produce BC_3F_2s, which were then advanced through pedigree-based phenotypic selection up to $BC_3F_5/_6$ generations (2023–2024).

Table 1. A brief description of genotypes used in the study.

Recurrent/ Donor parents	Pedigree	Description	Reaction to white rust disease	Glucosinolate/Erucic acid content
DRMR150-35	RH819/Pusa Bold	High-yielding variety released for early sown rainfed conditions (Zone V).	Susceptible	High Glucosinolate (>30 µ moles/g defatted seed meal) and high erucic acid content (>2%)
NRCHB101	BL4/Pusa Bold	High-yielding variety released for late sown conditions (Zone III).		
NRCDR-02	MDOC43/NBPGR -36	High-yielding variety released for timely sown irrigated conditions (Zone II).		
RLC-3	JM06003/JM060 20	Variety with canola quality traits.	Resistant	Double low (Low erucic acid [<2%]; low glucosinolate [<30 µ moles/g defatted seed meal])
HEERA	ZYR-4/BJ-1058	Registered germplasm with canola quality traits.		

Extraction of gDNA and molecular marker analysis

Genomic DNA isolation from young and healthy leaves of 20-day-old seedlings of parents and backcross progeny used standard protocol (Doyle and Doyle 1990). Gene-specific CAPS markers (CAPS591, CAPS1265, and CAPS237) linked to the *FAE* gene (Saini *et al.,* 2016), PCR-based markers (GER1 and GER-5) linked to low glucosinolate QTLs of *B. juncea* (Pushpa *et al.,* 2015; Singh *et al.,* 2020b), and intron polymorphic (IP) markers, i.e., At5g41560 and At2g36360 (Kamboj *et al.,* 2024; Singh *et al.,* 2024), linked to white rust-resistant loci AcB1-A4.1 and AcB1-A5.1, respectively, proceeded utilization for foreground selection of the backcrossed and selfed generations. Published detail of markers utilized in this study already appeared in a previous report (Singh *et al.,* 2020a; Singh *et al.,* 2021).

The use of 310 SSR (simple sequence repeats) markers encompassing all 18 linkage groups of Indian mustard ensued for a polymorphism survey between the recurrent parent, DRMR150-35, NRCHB101, and NRCDR-02, and donor—RLC-3 and Heera. A variable set of polymorphic SSR markers 45, 43, 38, and 45 was a choice for every cross NRCHB101/HEERA, DRMR150 35/RLC-3, NRCHB101/RLC-3, and NRCDR-02/RLC-3, respectively, and employing them for background selection in backcross (BC_1F_1-BC_3F_1) generations. The details of the sequence of these polymorphic SSR, PCR amplification program for foreground and background selection, visualization of amplified products, and separation by gel electrophoresis proceeded as described in our earlier studies (Singh *et al.,* 2017; Singh *et al.,* 2020a; Singh *et al.,* 2021).

Recurrent parent genome recovery (G) calculation engaged the following formula (Sundaram *et al*., 2008):

$$G = ([X + 1/2Y] \times 100)/N$$

Where:
N = the total number of parental polymorphic markers screened;
X = the number of markers showing homozygosity for recurrent parent allele; and
Y = the number of markers showing heterozygosity for parental alleles

Biochemical evaluation for quality traits

The selected backcross progenies and advanced pyramided lines incurred evaluation biochemically for low erucic acid content and glucosinolate content with the quality checks (PM-30/RLC-3/HEERA) and non-quality check (NRCHB-101). The erucic acid and total glucosinolate content in oil and seed meal's analysis utilized the methods described in this study (Mawlong *et al.,* 2017; Singh *et al*., 2022).

Inoculation and phenotyping for white rust

The gene-positive selected pyramided lines (BC_3F_4/BC_3F_5) with quality traits also attained screening for resistance to white rust. Screening of selected lines continued along with their parental lines, susceptible (Rohini) and resistant (BioYSR) checks. The study used rust-susceptible Rohini as an infector, planting it every fourth test row and around experimental plots to maintain white rust disease pressure. The primary inoculum preparation and spray took place as described in earlier studies (Singh *et al.,* 2021; Priyamedha *et al.,* 2022). The disease scoring, as conducted under natural conditions, used a 0–9 scale at the true leaf stage.

The percent disease intensity (PDI) calculation used the following formula (AICRP-RM 2023):

$$\text{Percent disease intensity (PDI)} = \frac{(N\text{-}1\times0)+(N\text{-}2\times1)+(N\text{-}3\times3)+(N\text{-}4\times5)+(N\text{-}5\times7)+(N\text{-}6\times9)}{\text{Number of leaf samples} \times 9} \times 100$$

Where N-1 to N-6 represents frequency of leaves in the respective score.

Agromorphological evaluation of selected lines

Pyramided lines (BC_3F_5/$_6$), along with recurrent and donor parents, entailed evaluation for agromorphological traits in the Rabi 2022–2023 following randomized complete block design with three replications using standard agronomic practices (Shekhawat *et al*., 2012). Each plot had six rows of 5 m length with a spacing of 30 cm × 15 cm. Agronomic traits associated with yield and yield components incurred measuring and recording. Data collected from five randomly selected plants for 11 quantitative traits comprised plant height (cm), number of primary branches, number of secondary branches, fruiting zone length (cm), main shoot length (cm), and total number of siliquae on the main shoot. Other traits included 1000-seed weight (g), seeds per siliquae, siliqua per plant, siliqua length (cm), and seed yield (kg per hectare). The observation for seed yield came from the middle four rows on a plot basis (6 m^2) before converting into kg/ha by applying the conversion formula:

$$\text{Seed yield (kg/ha)} = (10000\ m^2 / 6\ m^2) \times \text{Seed yield per plot (kg)}.$$

RESULTS AND DISCUSSION

Development of pyramided lines using marker-assisted breeding

Out of 87 F_1 hybrids from the respective crosses analyzed using trait-specific molecular markers, 68 emerged as true hybrids and succeeded in backcrossing with respective recurrent parents to generate the BC_1F_1 population (Table 2). After the foreground selection, a total of 24/100 plants in DRMR150-35 x RLC-3, 17/80 in NRCHB-101 x HEERA, 37/83 in NRCHB-101 x RLC-3, and 31/90 plants in NRCDR-02 x RLC-3 cross combination appeared to be "positive" for all the targeted genes in BC_1F_1. Earlier, foreground selection or MAS conducted by various researchers efficiently utilized trait-specific molecular markers (Pushpa *et al*., 2015; Saini *et al*., 2016; Singh *et al.,* 2020b; Priyamedha *et al*., 2021). Similarly, Kamboj *et al.* (2024) also utilized trait-specific markers for foreground selection to develop white rust-resistant breeding lines in Indian mustard through MAS. The recurrent parent genome recovery in BC_1F_1 ranged from 51.1% to 73.9% (Table 2). Furthermore, the gene-positive plants with the highest genome recovery in each of the cross combinations were options for backcrossing with respective recurrent parents to generate BC_2F_1s.

During the BC_2F_1 generation, background selection with polymorphic markers revealed maximum RPG recovery of up to 83.3%, 84.4%, 81.6%, and 82.6% in cross combinations DRMR15035/RLC-3, NRCHB101/HEERA, NRCHB101/RLC-3, and NRCDR-02/RLC-3, respectively. Gene-positive

Table 2. Progressive selection statistics for number of plants selected in different generations and the recurrent parent genome recovery for development of pyramided lines.

Cross combination	Generation	No. of plants genotyped	No. of plants confirmed using foreground markers	Background selection		Range of RPG (%) among selected progenies	No. of plants selected based on high % RPG
				No. of plants selected with highest phenotypic similarity to RP	No. of primers deployed		
DRMR150-35 x RLC-3	F_1	21	19	*	*	*	*
	BC_1F_1	100	24	9	42	57.1-72.6	4
	BC_2F_1	130	59	13		69.0-83.3	5
	BC_3F_1	112	27	16		76.2-89.3	4
	BC_3F_2	60	49	31		82.1-94.0	21
NRCHB-101 x HEERA	F_1	20	15	*	*	*	-
	BC_1F_1	80	17	11	45	55.6-72.2	3
	BC_2F_1	96	40	14		71.1-84.4	3
	BC_3F_1	67	32	14		74.4-88.2	2
	BC_3F_2	52	43	35		86.7-92.2	23
NRCHB-101 x RLC-3	F_1	27	18	*	*	*	-
	BC_1F_1	83	37	13	38	55.3-71.0	3
	BC_2F_1	103	46	18		72.4-81.6	4
	BC_3F_1	56	31	16		76.3-87.8	3
	BC_3F_2	48	25	17		81.4-92.1	9
NRCDR-02 x RLC-3	F_1	19	16	*	*	*	-
	BC_1F_1	90	31	14	46	51.1-73.9	3
	BC_2F_1	153	78	23		69.4-82.6	3
	BC_3F_1	88	41	21		76.1-89.1	2
	BC_3F_2	43	32	25		85.9-92.4	17

* Not estimated; RPG- recurrent parent genome; RP-recurrent parent.

BC_2F_1 plants with the highest RPG% entailed selection and backcrossing to produce BC_3F_1 seeds. Similarly, the best BC_3F_1 plants attained self-pollination to generate BC_3F_2 populations. The identification of BC_3F_2 plants homozygous for the target gene totaled 108 using gene-linked markers from all cross combinations. At BC_3F_2, the study achieved RPG recovery of greater than 90% in backgrounds of recurrent parents, which was at par with the theoretical RPG recovery percentage. This was possible due to three backcrosses attempted followed by selfing, which was necessary while transferring multiple genes, as suggested by Shu (2009) and Tanweer *et al*. (2015). Singh *et al*. (2024) reported similar results of RPG% while introgressing white rust-resistant traits in elite *Brassica juncea* backgrounds. A comparable RPG% came from a work by Yathish *et al*. (2022), who introgressed the low phytic acid locus (lpa2) into elite *Zea mays* inbreds using MABB. Stringent phenotypic screening of these selected plants for recurrent parent phenotype continued until $BC_3F_{5/6}$ generations, rejecting the plants with dissimilar phenotypes.

The 58 selected homozygous $BC_3F_{5/6}$ lines sustained foreground selection for confirmation of targeted QTLs. Furthermore, after the background selection of these selected lines ($BC_3F_5/_6$), an improvement in the recurrent parent genome recovery of up to 94% was evident, which was in concordance with the findings of Zheng *et al.* (2022). After the evaluation of 58 lines ($BC_3F_5/_6$) for targeted traits and other morphophysiological traits, finalizing 34 lines proceeded for advancement. The trait-specific molecular marker confirmation and chromosome-wise introgression of genes in 34 selected pyramids appear in Figure 1 and Figure 2, respectively. Roslinsky *et al.* (2021) introgressed the *FAE*

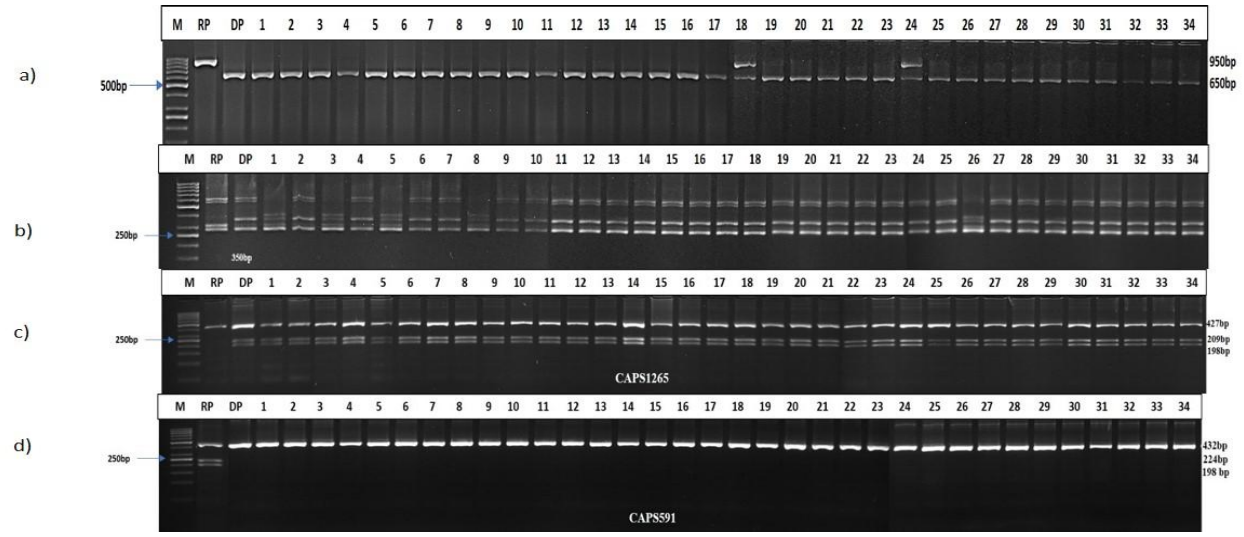

Figure. 1 Electropherogram representing confirmation of gene-pyramided lines (1–34); RP - Recurrent parent; DP - Donor parent along with recurrent and donor parents (DP) using molecular markers linked to specific trait; a) GER-1, b) GER-5, c) CAPS1265 (HEA-427bp/LEA-427bp,209bp,198bp), d) CAPS591 (LEA-432 bp/HEA -432bp, 224 and 198 bp), and M - 50bp molecular-weight size marker (DNA ladder).

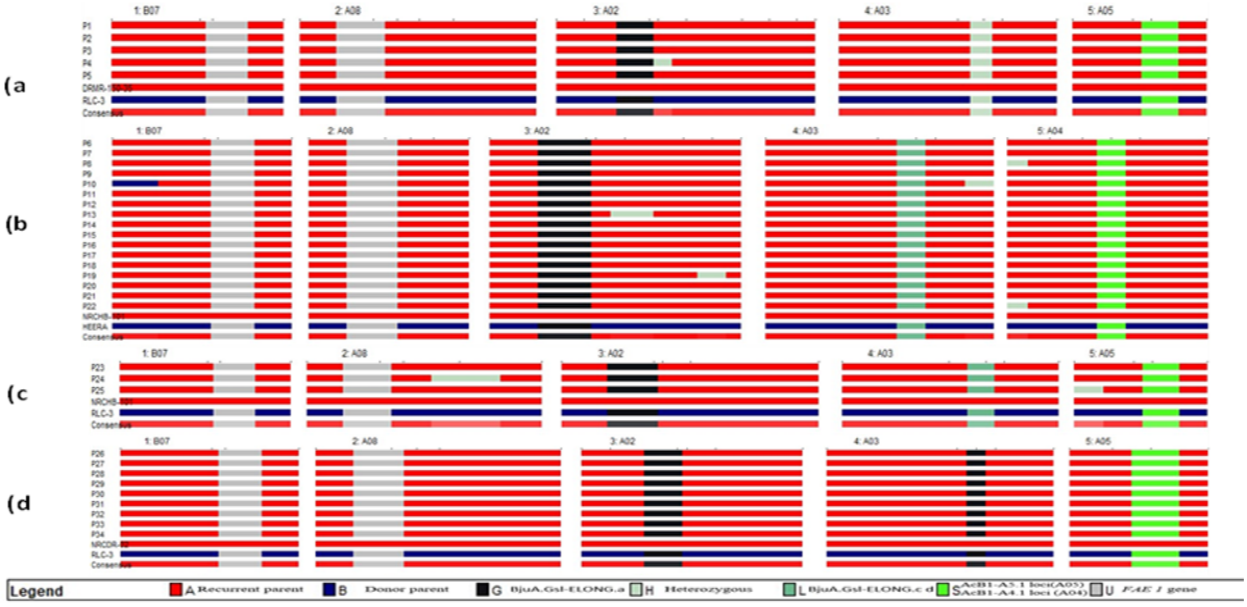

Figure 2. Graphical representation of genotyping image of the selected 34 pyramided lines. Red and blue represent the genomes of RP and DP genomic regions, respectively, and residual heterozygous regions are represented by H in faded green in each group (a, b, c, and d). 1. B07 indicates FAE 1.2 on chromosome B07, 2. A08 indicates FAE 1.1 on chromosome A08, 3. A02 indicates BjuA.Gsl-ELONG.a on chromosome A02, 4. A03 indicates BjuA.Gsl-ELONG.c d on chromosome A03, 5. A05 indicates AcB1-A5.1 loci on chromosome A05, and 5. A04 in image (b) indicates AcB1-A4.1 loci on chromosome A04.

gene from *B. rapa* into *B. carinata*, achieving super-high erucic acid content through backcross breeding, while Singh *et al*. (2024) used MABC to develop *B. juncea* introgression lines resistant to *Albugo candida.* Wang *et al.* (2022) and Baloch *et al.* (2024) developed some *B. napus* introgression lines by pyramiding multiple genes conferring resistance to clubroot, resistance to herbicide, high oleic acid content, and early maturity.

Biochemical analysis of pyramided lines

Biochemical estimation of erucic acid and glucosinolate content commenced for parents and selected backcross progenies (Table 3). Gene-positive plants at each backcross stage obtained post-genotyping analysis to confirm the expression of the introgressed *FAE* gene and low glucosinolate traits in the recurrent parent background. The erucic acid and glucosinolate concentrations for the recurrent parents, viz., DRMR150-35, NRCDR-02, and NRCHB101, were 36.93%, 40.94%, and 38.53% and 156.56, 126.93, and 142.00 µg/g of defatted seed meal, respectively. The newly developed pyramided $BC_3F_5/_6$ lines, which are homozygous for the erucic acid and glucosinolate alleles, contained lower levels of erucic acid (ranging from 0.25% to 2.81%) and glucosinolates (ranging from 28.20 to 53.53 µg/g of defatted seed meal), with an average glucosinolate content of 36.22 µg/g of defatted seed meal. The introgression of both alleles governing the erucic acid trait resulted in a significant reduction in erucic acid content, with the lowest value recorded at 0.25% in DRMRCI(Q)-73.

The study also recorded a minimum level of glucosinolate content of 28.20 µg/g of defatted seed meal in DRMRCI(Q)-92 and 29.73 µg/g of defatted seed meal in DRMRCI(Q)-38 (Table 3). However, it was higher than their respective donor parent but still falls under set standards for low glucosinolate concentration (below 30 µg/g of defatted seed meal).

Similarly, Priyamedha *et al.* (2021) evaluated 44 advanced genotypes (F_7 gen.) for quality traits and found those genotypes to be double low, i.e., low erucic acid content in oil and low glucosinolate content in seed meal. The creation of a heatmap with a dendrogram helped visualize differences and similarities in fatty acid and total glucosinolate compositions of pyramided lines and recipient and donor parents.

Two clusters identified revealed all the pyramided lines clustered together with donor parents (Cluster II) according to fatty acid composition (C16:0, C18:1, C18:2, C18:3, C20:1, C22:1, and glucosinolate). Meanwhile, the recurrent parents displayed grouping in a separate cluster (Cluster I). Cluster I exhibited higher levels, particularly for C16:0, C20:1, C22:1, and glucosinolate. Conversely, Cluster II demonstrated relatively lower levels across most parameters under study. However, a moderate increase in the level of oleic acid was also notable for some pyramided lines, suggesting their potential as sources of high-quality oil. The heatmap effectively illustrated patterns of similarity and divergence among pyramided lines.

Disease reaction against white rust

The $BC_3F_{5/6}$ pyramided genotypes succeeded in assessing their resistance to white rust in the field conditions. The resistance screening for reaction to white rust of all 34 pyramided lines along with the resistant (BioYSR) and susceptible (Rohini) controls ensued. Recurrent parents and donor parents, also included in the screening, had their reaction studied against the *Albugo candida* inoculation. The recurrent parent (DRMR150-35, NRCDR-02, NRCHB 101) was highly susceptible to the white rust disease with a disease score of 9 (HS), whereas the resistant control BioYSR was free from the disease with a disease score of 0 (HR) at the true leaf stage. Among the pyramided lines, 18 lines carrying introgressed loci for white rust resistance exhibited high resistance (HR) against white rust, while four lines displayed moderate resistance (MR). The remaining 12 lines without the introgression of white rust resistance loci showed complete susceptibility with a high score according to the disease rating scale (scored 5–9) against the white rust disease (Table 3).

Table 3. Evaluation of agronomic characters of the pyramided lines under experimental field conditions during the Rabi (2022–2023).

Pyramided lines	Plant identity	PH (cm)	PB	SB	FZL (cm)	MSL (cm)	SMS	SL (cm)	S/S	S/P	Yield (kg/ha)	TSW (g)	Glucosinolate content	Erucic acid (%)	Reaction to white rust
Checks	DRMR150-35	186.27	5.77	9.60	82.33	75.93	49.03	5.39	16.13	313.47	2160.03	4.81	156.56	36.93	S
	NRCDR-02	187.13	5.60	9.20	91.13	75.40	46.27	5.11	16.59	300.53	2212.01	4.44	126.93	40.94	S
	NRCHB101	183.40	6.13	10.93	81.73	78.47	55.73	5.58	15.07	390.33	2242.85	4.65	142.00	38.53	S
	PM30	210.00	6.00	9.20	84.53	70.53	46.93	5.42	15.11	290.00	2239.91	4.56	103.47	0.99	S
	RLC-3	187.13	5.60	5.20	73.20	59.00	39.07	4.33	15.80	255.20	1974.83	3.42	16.80	0.39	R
	HEERA	226.60	8.87	13.13	72.00	43.60	38.20	3.25	15.19	337.00	1912.07	2.14	29.53	0.44	R
DRMR15035 x RLC-3 (BC_3F_5)	DRMRCI(Q)-181	199.67	4.30	8.07	93.20	68.77	44.07	3.68	16.82	263.60	2267.97	4.42	49.14	0.87	MR
	DRMRCI(Q)-7	204.20	5.07	7.07	82.20	73.33	52.27	3.71	15.72	263.93	2022.02	4.44	39.48	0.81	MS
	DRMRCI(Q)-6	198.73	6.67	11.00	89.27	70.33	50.13	4.95	17.13	289.80	2261.50	4.19	42.74	0.47	R
	DRMRCI(Q)-180	194.90	6.67	8.93	80.13	72.93	51.20	5.23	17.40	333.67	2433.11	4.81	37.15	1.07	S
	DRMRCI(Q)-11	188.07	5.33	10.21	77.53	63.00	40.70	5.18	17.23	283.87	2138.69	4.71	37.12	0.52	S
NRCHB101 x HEERA (BC_3F_6)	DRMRCI(Q)-179	191.00	7.13	9.93	75.07	70.27	45.00	5.20	16.47	289.47	2295.57	4.29	31.17	0.42	S
	DRMRCI(Q)-114	194.20	5.53	7.53	85.83	75.80	53.87	4.66	17.37	288.53	2225.86	3.60	34.24	0.48	S
	DRMRCI(Q)-13	197.10	5.67	10.00	87.87	66.13	46.40	4.55	17.64	290.13	2309.54	3.51	42.19	2.22	S
	DRMRCI(Q)-30	191.60	6.13	8.93	82.40	66.00	51.53	3.72	18.99	308.33	2274.95	4.17	33.33	0.41	MR
	DRMRCI(Q)-75	199.93	6.87	12.47	89.07	79.00	52.07	4.28	14.87	283.73	2226.30	4.38	34.65	0.91	R
	DRMRCI(Q)-23	195.97	6.47	10.47	84.67	77.53	46.53	3.45	15.80	300.87	2278.49	4.53	37.20	0.36	R
	DRMRCI(Q)-26	197.60	6.00	8.00	84.07	77.40	47.13	3.36	13.73	268.80	2305.94	4.14	32.38	0.40	S
	DRMRCI(Q)-73	194.27	5.13	7.80	66.07	71.80	42.13	4.19	14.65	331.73	2059.65	4.37	35.28	0.25	S
	DRMRCI(Q)-47	191.27	5.73	8.67	80.27	76.53	44.60	5.28	15.17	287.13	2250.24	3.48	33.43	0.67	R
	DRMRCI(Q)-57	193.27	6.93	8.33	82.93	67.67	40.07	5.06	15.12	276.53	2085.87	3.45	34.21	0.46	R
	DRMRCI(Q)-158	201.67	5.47	6.40	87.27	73.13	41.07	4.90	17.23	272.13	2399.21	3.84	30.79	0.37	MR
	DRMRCI(Q)-17	195.67	7.00	12.87	91.47	72.33	47.60	5.06	15.49	358.20	2211.58	3.65	36.68	0.45	R
	DRMRCI(Q)-27	201.00	6.20	6.53	77.67	79.87	44.00	5.39	16.43	261.00	2197.43	4.11	38.41	0.33	R
	DRMRCI(Q)-38	197.73	6.13	7.67	74.07	69.20	45.47	4.47	15.12	283.00	2089.38	4.09	29.73	0.84	S
	DRMRCI(Q)-20	203.47	5.93	9.87	83.27	70.07	50.00	4.32	15.75	282.80	2130.93	3.49	31.34	0.65	S
	DRMRCI(Q)-18	207.40	6.13	9.47	91.00	75.93	68.07	4.52	16.05	340.13	2311.49	3.85	33.28	0.78	MS
	DRMRCI(Q)-44	204.80	8.27	8.60	92.47	75.53	62.07	4.03	15.97	324.33	2092.06	4.13	32.17	2.81	MS
NRCHB101 x RLC-3 (BC_3F_5)	DRMRCI(Q)-92	192.27	7.73	11.13	88.47	73.93	45.93	5.96	17.45	282.67	2271.98	4.12	28.20	0.47	R
	DRMRCI(Q)-32	194.33	6.60	10.60	87.87	68.27	44.53	4.72	15.22	274.07	2433.76	4.59	53.53	0.83	R
	DRMRCI(Q)-278-3	183.27	5.67	6.00	77.67	64.53	42.00	4.14	15.59	263.47	2115.57	4.87	31.99	0.84	R
NRCDR-02 x RLC-3 (BC_3F_6)	DRMRCI(Q)-119	202.00	4.67	7.93	87.20	76.67	55.87	4.87	17.71	270.00	2072.35	4.38	41.84	0.71	R
	DRMRCI(Q)-120	188.80	4.47	7.33	89.00	78.13	63.80	4.57	15.63	345.00	2183.90	4.70	39.39	1.03	R
	DRMRCI(Q)-121	197.43	5.00	8.47	89.33	82.73	69.27	4.47	14.88	312.93	2217.91	4.37	42.88	0.83	R
	DRMRCI(Q)-122-3	189.67	3.67	4.80	79.60	80.60	60.07	5.21	15.17	303.87	2200.96	4.69	42.49	0.75	R
	DRMRCI(Q)-172	191.60	3.60	6.47	93.07	82.60	55.60	5.42	16.21	293.13	2299.47	3.82	33.81	0.76	R
	DRMRCI(Q)-125	197.93	4.13	5.93	90.67	81.93	59.67	4.32	16.85	325.40	2334.50	4.47	31.64	0.59	MR
	DRMRCI(Q)-126	189.53	3.40	8.67	84.73	85.00	62.27	5.10	17.08	328.47	2339.50	4.37	34.26	0.73	R
	DRMRCI(Q)-127	187.67	4.00	9.07	78.93	78.20	55.73	4.83	16.39	330.93	2178.53	4.84	33.28	0.50	R
	DRMRCI(Q)-37	190.17	4.07	8.20	89.53	86.80	66.47	5.43	17.71	337.53	1900.56	4.21	40.26	0.73	R
	Min.	183.27	3.40	4.80	66.07	43.60	38.20	3.25	13.73	255.20	1900.56	2.14	16.80	0.25	-
	Max.	226.60	8.87	13.13	93.20	86.80	69.27	5.96	18.99	390.33	2433.76	4.87	156.56	40.94	-
	CD (P=0.05)	17.55	0.83	1.49	11.99	11.3	4.75	0.39	1.40	41.36	315.74	0.22	4.72	1.34	-
	CV (%)	5.52	8.88	10.46	8.79	9.47	5.78	5.10	5.32	8.46	8.82	3.25	6.40	23.02	-

PH = Plant height; PB = Number of primary branches; SB = Number of secondary branches; FZL = Fruiting zone length; MSL = Main shoot length; SMS = Total number of siliquae on main shoot; SL = Siliquae length; S/S = Seed per siliquae; S/P = Siliqua per plant; SY = Seed yield; TSW = thousand seed weight.

These resistant lines also received screening in Wellington, a well-known hot spot for white rust disease, and proved resistant to white rust disease. This indicated that introgression of the white rust resistance gene in this study was effective and sufficient for white rust resistance breeding practice, which was consistent with previous studies (Singh *et al.*, 2020a; Chand *et al*., 2022; Priyamedha *et al.,* 2021). Among these six pyramided lines, namely, DRMRCI(Q)-47, DRMRCI(Q)-57, DRMRCI(Q)-172, DRMRCI(Q)-179, DRMRCI(Q)-180, and DRMRCI(Q)-181, showing resistance to white rust with more than 90% RPG recovery, achieved selection for multilocation pathological trial under AICRP-RM (All India Coordinated Research Project on Rapeseed-Mustard, 2023–2024).

Agronomic characterization of the pyramided lines

All the selected 34 pyramided lines obtained evaluation for 11 agromorphological traits along with their recurrent parent, as well as commercial variety checks, during the Rabi 2022–2023 alongside their respective recurrent parents and standard commercial variety checks. The recurrent parents—DRMR150-35, NRCDR-02, and NRCHB 101—showed favorable performance for grain yield. On the other hand, the donor parents, RLC-3 and HEERA, exhibited comparatively lower yield potential. The national quality check (PM-30) maintained a consistent and competitive yield, serving as a reliable benchmark for comparison, as shown in Table 3. The pyramided lines, viz., DRMRCI(Q)-181, DRMRCI(Q)-6, DRMRCI(Q)-180, DRMRCI(Q)-179, DRMRCI(Q)-13, DRMRCI(Q)-30, DRMRCI(Q)-47, DRMRCI(Q)-158, DRMRCI(Q)-23, DRMRCI(Q)-26, DRMRCI(Q)-18, DRMRCI(Q)-92, DRMRCI(Q)-32, DRMRCI(Q)-121, DRMRCI(Q)-172, DRMRCI(Q)-125, and DRMRCI(Q)-126, showed yields at par with their respective recurrent parents (Table 3).

The agronomic assessment of 34 selected improved pyramided lines of DRMR150-35, NRCHB101, and NRCDR-02 backgrounds at the $BC_3F_5/_6$ generations revealed most of the agromorphological traits of these pyramided lines were at par with their recurrent parents. Moreover, they showed durable resistance to white rust disease. Notably, some pyramided lines were very close to the recurrent parent, and some outperformed the recurrent parent based on yield, which might be due to the inheritance of yield-contributing traits from the recurrent parents. The quality parameters, i.e., erucic acid content in the selected lines, were similar to the donor parent (RLC-3, Heera) (Table 3). Furthermore, six best-performing lines—DRMRCI(Q)-181, DRMRCI(Q)-180, DRMRCI(Q)-179, DRMRCI(Q)-47, DRMRCI(Q)-158, and DRMRCI(Q)-172—succeeded in their selection for nomination under station trial based on superior yield and successful introgression of target traits.

CONCLUSIONS

The promising work underscores the effectiveness of marker-assisted pyramiding as a precise and efficient breeding strategy to integrate white rust resistance and oil quality traits in *B. juncea*. This approach led to the development of superior lines with enhanced yield potential, high RPG recovery, better oil quality, and durable disease resistance. Not all lines met agronomic standards, but key selections showed remarkable improvement in both quality and resilience. Overall, this work demonstrates the efficiency of the marker-assisted pyramiding approach, which can be gainfully practical for introgressing genes into elite *B. juncea* varieties.

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