Indian J. Genet. Plant Breed., (2024); 84(2): 232-241
https://doi.org/10.31742/ISGPB.84.2.11
ISSN: 0975-6906
www.isgpb.org

ARTICLE RESEARCH

Marker-assisted introgression lines of elite Indian mustard (*Brassica juncea*) cultivars for resistance against white rust (*Albugo candida*)

V.V. Singh*, Monika, Priyamedha[1], Balbeer, M.L. Meena, Pankaj Sharma and R.K. Rai

Abstract

White rust caused by *Albugo candida* is a major biotic constraint responsible for significant yield loss in mustard production across all mustard-growing regions. This experiment was conducted with the aim of developing WRR lines of Indian mustard using the MABB approach. The elite mustard cultivars of *Brassica juncea viz*., DRMR150-35, DRMRIJ-31 and NRCDR-02 were used as the recurrent parent, whereas BioYSR, BEC-144 and Donskaja were taken as donors. A parental polymorphism survey using 315 microsatellites (SSR) was done among recurrent and donor parents. To confirm introgression of white rust resistance gene foreground selection was performed using *Arabidopsis*-derived IP markers that are linked to the white rust loci AcB1-A5.1 (At2g36360) for Donskaja and BioYSR and AcB1-A4.1 (At5g41560) for BEC-144coupled with background selection and phenotypic selection for highest recurrent parent genome recovery. Finally, 19 BC_3F_3 lines were obtained, which led to the identification of 5 highly white rust-resistant lines with RPG recovery percentage of >90%. These selected improved lines showed a resistance response with mean %WR severity (100 DAS) below 5% and possess the agro-morphological characters at par to their respective recurrent parent. These lines have good potential for future release and demonstrated MABB as a valuable tool for expediency, enrichment and precision in accelerating the development of new disease-resistant Indian mustard varieties.

Keywords: White rust, Indian mustard, Foreground selection, background selection, marker-assisted backcross breeding, SSR markers.

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is the most significant and widely cultivated species among India's six cultivated species of rapeseed mustard group of crops. Indian mustard accounts for about 90% of the area (9.17 mha) and production (11.75 mt) of the rapeseed-mustard with productivity of 1178 kg/ha (Anonymous 2022). According to the USDA's 2020 report, India accounts for 9.8% of global rapeseed mustard production and owns 19.8% of the world's arable land. Biotic and abiotic stresses profoundly influence the production of rapeseed mustard. Among Biotic stresses, diseases like white rust (*Albugo candida*), stem rot (*Sclerotinia sclerotiorum*), Alternaria blight (*Alternaria brassicae*), powdery mildew (*Erysiphe cruciferarum*),downy mildew (*Hyaloperonospora parasitica*) and blackleg (*Leptoshaeria maculans*) are the more devastating that significantly reduce the seed yield and oil quality of rapeseed mustard (Dev et al. 2020). The expansion of the mustard-growing areas in tropical and subtropical India has increased the severity and intensity of the white rust disease (Saharan et al. 2014).

White rust is caused by an obligate parasite [*Albugo candida* (Pers.) Kuntze.] is one of the most widespread and destructive diseases of Brassicas, especially for Indian mustard and turnip rape, *Brassica rapa* L. (Saharan and Verma 1992; Kole et al. 1996). The favorable environmental conditions for the development of white rust disease in

ICAR-Directorate of Rapeseed–Mustard Research, Sewar 321 303, Bharatpur, Rajasthan, India

[1]National Rice Research Institute, CRURRS, Hazaribagh 825 302, Jharkhand, India

***Corresponding Author:** V. V. Singh, ICAR-Directorate of Rapeseed-Mustard Research, Sewar 321 303, Bharatpur, Rajasthan, India, E-Mail: singhvijayveer71@gmail.com

How to cite this article: Singh V.V., Monika, Priyamedha, Balbeer M.L., Meena, Sharma P. and Rai R.K. 2024. Marker-assisted introgression lines of elite Indian mustard (*Brassica juncea*) cultivars for resistance against white rust (*Albugo candida*). Indian J. Genet. Plant Breed., **84**(2): 232-241.

Source of support: ICAR, Govt. of India under CRP-Molecular breeding.

Conflict of interest: None.

Received: July 2023 **Revised:** March 2024 **Accepted:** April 2024

Indian mustard include low temperature (15–20°C) and high humidity (>65%) with intermittent rainfalls during the cotyledonary to the complete flowering stage (Mehta et al. 2021). The infected plants exhibit chlorosis on the adaxial surface of leaves and localized white to pale-colored pustules on the abaxial surface of leaves, stems and inflorescence. However, systemic infection leads to stag heads forming, resulting in non-formation of seed, which causes up to 90% yield losses (Chand et al. 2022; Lakra and Saharan 1989). Donors like Donskaja IV, Heera, BIO-YSR, BEC-144 and NRCDR 515 are the registered R genetic stocks for resistance against different isolates of white rust from various mustard-growing states of India. Wild species or relatives of such as *B. fruticulosa* and *Thlaspiarvense* have also been found to be resistant to white rust, whereas few species of genus Diplotaxis and Sinapis were reported as moderately resistant (Saharan et al.1988; Dang et al. 2012). Previous genetic studies on white rust resistance have predicted that a single dominant gene controls resistance to white rust (Singh et al. 2020; Yadava et al. 2012; Singh et al. 2012; Vignesh et al. 2011), which have been identified and mapped in *B. juncea* (Cheung et al. 1998; Prabhu et al. 1998; Varshney et al. 2004; Massand et al. 2010), *B. napus* (Somers et al. 2002), *B. rapa* (Kole et al. 1996) and *A. thaliana* (Borhan et al. 2008, 2010). As reported by earlier mapping studies, two independent loci govern resistance to *A. candida* race2Vintwo East European lines (Heera and Donskaja-IV) and information on tightly linked markers to these loci has been reported by Panjabi et al. (2010).

Introgression of the white rust-resistant gene in elite Indian mustard cultivars is one of the most effective approaches for its management, as other strategies like the application of fungicides, crop rotation and sanitation practices have only been moderately effective. The development of resistant cultivars through a conventional breeding approach is time-consuming and labor-intensive. However, the molecular marker-assisted backcross breeding approach (MABB) is a more precise and quick approach for the development of white rust resistant (WRR) introgression lines of elite cultivars (Varshney et al. 2010). Molecular marker-assisted breeding has been successfully employed in the introgression of genes conferring resistance to white rust (Somers et al. 2002, Arora et al. 2019, Singh et al 2020), downy mildew (Saha et al 2021), Sclerotinia stem rot (Rana et al. 2017), blackleg (Saal et al. 2005; Kalia et al. 2017), clubroot (Kawasaki et al. 2021; Hirani et al. 2016; Kaur et al. 2022) disease affecting *Brassica* species. Considering all these facts, the present study was, therefore, conducted to develop WRR lines in the background of elite mustard cultivars, namely, DRMR150-35, NRCHB101, DRMRIJ-31 and NRCDR-02 having high yielding potential, better seed quality, oil content and good market price using marker-assisted backcross breeding approach. These cultivars became susceptible to *A. candida* with time, considerably affecting their productivity.

Materials and methods

Plant materials and MABB strategy

Elite cultivars of Indian mustard *viz.,* DRMR150-35, DRMRIJ-31 and NRCDR-02 were taken as recipient/recurrent parents, while BIO-YSR, BEC-144 and Donskaja were taken as donors for introgression of white rust resistance gene. The crosses, DRMR150-35 × BioYSR, DRMR150-35 × BEC-144, NRCDR-02 × Donskaja and DRMRIJ-31 × Donskaja were generated to produce F_1 seeds during *rabi* 2015. Hybridity in F_1 plants was checked using Intron polymorphic (IP) markers, At5g41560 and At2g36360 (Panjabi et al. 2010) linked with white rust resistance loci AcB1-A4.1 and AcB1-A5.1, respectively. After hybridity confirmation of the plants, true hybrid heterozygous plants were backcrossed with recurrent parents to generate BC_1F_1 seeds during *rabi* 2016. Foreground selection using tightly linked markers and background selection using respective parental polymorphic SSR markers have been performed in subsequent generations (BC_1F_1 to BC_3F_1) during *rabi* 2017-2018 for confirmation of the white rust resistance gene and genome recovery of the recurrent parent. Simultaneously, phenotypic selection was also performed for recurrent parent similarity among the confirmed hybrid plants in BC_1F_1 to BC_3F_1 generation. The gene-introgressed plants with the highest genotypic recovery and maximum phenotypic similarity to the recurrent parent were selfed up to BC_3 - F_{5-6} during crop *rabi* of 2019-2020 and 2020-2021 including off-season nursery at IARI, Regional Station, Wellington, Tamil Nadu, India. In the BC_3F_{5-6} population, homozygous lines with introgressed white rust-resistant genes along with a maximum genome recovery of recurrent parent were finally selected.

Genotyping

DNA was extracted from young and healthy leaves of 20-day old seedlings of using the standard Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle 1990), followed by DNA purification by removing protein impurities and RNA. Then the purified DNA was analyzed for quality and quantity using 0.8% agarose gel and the final concentration of DNA was normalized to 30 ng/µl. Polymerase chain reaction for IP markers used for foreground selection and SSR for background selection of complete recurrent parent genome recovery, visualization of amplified products, and separation by gel electrophoresis was performed as described in the earlier studies (Singh et al. 2020, Singh et al. 2019).Foreground selection employed previously described Arabidopsis-derived IP markers that are linked to the white rust loci AcB1-A5.1 (At2g36360) and AcB1-A4.1 (At5g41560), respectively (Panjabi et al. 2010). The marker's locus and sequence details used for FGS are listed in Table 1.A total of 315 SSR markers that encompass all 18 linkage groups of Indian mustard were used for a polymorphism survey between the recurrent parent *viz.*

Table 1. Details of IP markers used for foreground selection for development of WRR introgression lines in *B. juncea*

S. No.	Locus code	Resistance locus mapped in genotype*	Linkage group	IP Marker	Primer sequence (5′→3′)	Markers validated in genotypes	Reference
1	AcB1-A4.1	Heera	A4	At5g41560F At5g41560R	GAGGTGGAAGAGTACGGTTGTG CCTCACAATTTCAGTCAACATCGT	Heera, BEC-144	Singh et al. 2015, Singh et al. 2020
2	AcB1-A5.1	Donskaja	A5	At2g36360F At2g36360R	GCCACCTCCTAGATGTGGTCATA GTCCATCCAGGTGTTTCACG	Donskaja, BioYSR	

*Panjabi et al. (2010)

DRMR150-35, DRMRIJ-31, NRCDR-02 and their respective donor, BioYSR, BEC-144 and Donskaja. A variable set of polymorphic SSR markers identified for every cross (Table 2), was used in background selection for recovery of recurrent parent genome in BC_1F_1, BC_2F_1 and BC_3F_1 generations.

Single bands were scored either as the homozygous allele for the recurrent/female parent represented by the letter 'A' or the homozygous allele for the donor/male parent represented by letter 'B'. Double bands were scored as heterozygous alleles (heterozygotes) carrying the genes of both recurrent and donor parents and represented by the letter' H.' The respective parental polymorphic SSR markers estimated the amount of recurrent parent genome contribution. Recurrent parent genome recovery (RPGR) represented as 'G' was calculated as per Sundaram et al. (2008).

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

where,

N = total number of parental polymorphic markers screened

X = number of markers showing homozygosity for recurrent parent allele

Y = number of markers showing heterozygosity for parental alleles

Phenotyping

The selected WRR introgressed lines (BC_3F_2 progenies) along with their parental lines, susceptible check (Rohini) and resistant checks (BioYSR, Donskaja and BEC-144) were phenotyped for resistance to white rust at offseason nursey in Wellington, India during offseason in year 2018 under natural condition. Rohini was interplanted after every four test rows to maintain constant disease pressure. The superior lines identified in BC_3F_2 progenies were evaluated under AICRP-RM pathology trials consecutively for three years 2019-21. The superior lines were identified based on high resistance against white rust disease.

Disease pressure was created in the field by primary inoculum collection of white rust zoosporangia of the strain AcB1 (*A. candida*, Bharatpur 1) from highly infected fresh leaves of the susceptible *B. juncea* cv. Varuna grown at the experimental farm of ICAR-DRMR, Bharatpur, India. With the help of a hemocytometer slide and the proper dilution with sterilized distilled water, the inoculum's concentration was adjusted to 2×10^4 zoosporangia per milliliter. Before inoculation, the suspension was incubated for 2 hours in the dark at 80°C and then kept at room temperature (20–25°C) to cause the release of zoospores. At two stages (2/3 leaf and the beginning of flowering), the plants were sprayed directly with the sporangial suspension of the AcB1 strain. A high humidity level was maintained in the experimental fields through frequent irrigation as per Fox and Williams(1984). After 10 days of inoculation or when the disease manifested itself, the genotypes were assessed for disease infestation on the ventral surface of the leaf. The disease was graded using a 0–9 scale (Williams 1985).

The percent disease incidence (PDI) can be calculated using the following formula:

$$PDI = \frac{\text{sum of all numerical rating}}{\text{(number of cotyledons or leaves scored} \times \text{maximum grade of scale)}} \times 100$$

Agro-morphological Evaluation

The agro-morphological performance of advanced stable WRR introgressed lines was evaluated in the field during *rabi* 2021-22 along with recurrent and donor parent for yield and attributing traits in randomized complete block design with three replications in 6 rows of 5 m length with a distance of 15, 30 cm between plants and between rows respectively. Standard packages and practices were followed for the growing of good crop. Observations were recorded on randomly selected five competitive plants for 11 quantitative traits, *i.e.* plant height (cm), number of primary branches, the number of secondary branches, fruiting zone length (cm), main shoot length (cm), total number of siliquae on main shoot; 1000 seed weight (g), seed per siliquae; siliquae per plant, seed yield (kg per hectare) and siliquae length (cm). The observation for seed yield has been taken from middle four rows on a plot basis (6 square meters) and converted into kg/ha by applying the conversion formula: Seed yield (kg/ha) = (10000 m^2 /6m^2) × Seed yield per plot (kg).

Result and discussion

Development of WRR introgression lines

Introgression of white rust resistant gene in elite background of Indian mustard cultivars (DRMR150-35, DRMRIJ-31 and NRCDR-02) was carried out as per the MABB programme (Fig.1). Foreground selection for white rust resistant gene

Table 2. Polymorphic SSR markers used for background selection for development of WRR introgression lines in Brassica juncea

Chromosome location	DR-02/Donskaja	DRMRIJ-31/Donskaja	DRMR150-35/BioYSR	DRMR150-35/BEC-144
A genome specific (*B. rapa*)				
A01	Ra2-E04,nia_m091a	Ra2-E04	Ra2-E04	Ra2-E04
A02	BrgMS383, BrgMS4539, BrgMS638, BrgMS397, BrgMS787, ENA23,cnu_m611a	BrgMS383, BrgMS4539, BrgMS638, BrgMS397, BrgMS787, ENA23	BrgMS383, BrgMS4539, BrgMS638, BrgMS397, BrgMS787, ENA23	BrgMS383, BrgMS4539, BrgMS638, BrgMS397, BrgMS787, ENA23
A03	BrgMS2766	BrgMS2766	BrgMS2766, BrgMS216	BrgMS2766, BrgMS216
A04	BrgMS638, BrgMS841, BrgMS233, BrgMS36, cnu_m585a,	BrgMS638, BrgMS841, BrgMS233, cnu_m585a	BrgMS638, BrgMS841, BrgMS233	BrgMS638, BrgMS841
A05	BrgMS166, ENA17	BrgMS166, ENA17	ENA17, BrgMS166	BrgMS166, ENA17
A06	BrgMS787, Ra1-F06,Ra2-D04	BrgMS787, Ra1-F06	BrgMS787, Ra1-F06	BrgMS787
A07	BrgMS10, BrgMS502,BRMS-036	BrgMS10, BrgMS502	BrgMS10, BrgMS502	BrgMS10, BrgMS502
A08	BrgMS465, BrgMS66, BrgMS732, cnu_m584a,	BrgMS465, BrgMS732, cnu_m584a	BrgMS465, BrgMS66, BrgMS732, cnu_m584a, Ra2-E12	BrgMS465, BrgMS66
A09	BrgMS639, Ra2-E11, Ra2-A11,Ra2-A02,cnu_m625a	BrgMS639, BrgMS3322, Ni4D09, cnu_m626a, Ra2-A11	BrgMS639, Ni4D09, cnu_m626a, cnu_m619a	Ni4D09, cnu_m626a
A10	BrgMS383, BrgMS713	BrgMS383, BrgMS713	BrgMS383	BrgMS383
B genome specific (*B. nigra*)				
B01	SJ4933, Ni2H03	Ni2H03	SJ4933	
B02	BrgMS383, SJ3302RI	BrgMS383, SJ3302RI	BrgMS383, SJ3302RI, Ni4A03	BrgMS383
B03	BrgMS465	BrgMS465, BRMS-006	BrgMS465, BRMS-006	BrgMS465
B04	SB1935A, SJ8033, SB0372	SB1935A, SJ8033, SB0372	SA0306, SJ8033, SB0372, cnu_m619a	SJ8033, SB0372
B05	SJ3874I, Ni2C12,Ni2C06	SJ6842, Ni2C12	SJ6842, SJ3874I, Ni2C12	SJ3874I
B06	BrgMS10, SJ1505, Ra1-F06	BrgMS10, SJ1505, Ra1-F06	BrgMS10, SJ1505, Ra1-F06	BrgMS10
B07	BrgMS2766, BrgMS639, Ni4C09	BrgMS2766, BrgMS639	BrgMS2766, BrgMS639	BrgMS2766, Ni4C09
B08	Ni2A08	Ni2A08	Ni2A08, Ni4D10	Ni2A08

was done by using IP markersAt2g36360 linked to allele AcB1- A5.1 and At5g41560 linked to AcB1-A4.1 (Table 1), which were already validated among resistant and susceptible genotypes of *B. juncea* in our previous studies (Singh et al. 2020). Previously, these markers were also validated in 25 *B. juncea* genotypes (Singh et al. 2015). The AcB1-A4.1 and AcB1-A5.1 loci confer the resistance to against an *A. candida* isolate (designated as AcB1; race 2V) and were found located on different linkage groups A4 and A5 in Heera and Donskaja- IV respectively. These

Table 3. Details of genotyping and RPG recovery in different generations for development of WRR introgression lines in *B. juncea*

Cross No.	Cross Name	Generation	No. of plants genotyped	No. of heterozygotes/ homozygotes identified for target loci	No. of plants selected for backcrossing/selfing with higher RPG recovery	Range of RPG recovery (%)
1	DRMR150-35x BioYSR	F_1	19	19	-	-
		BC_1F_1	140	59	4	49.16-70.88
		BC_2F_1	121	65	3	54.16-85.41
		BC_3F_1	167	71	4	77.08-91.66
		BC_3F_2	130	119	5	82.29-94.79
2	DRMR150-35x BEC-144	F_1	20	20	-	-
		BC_1F_1	105	48	3	48.33-71.6
		BC_2F_1	158	71	3	66.66-83.33
		BC_3F_1	123	68	2	70.00-94.00
		BC_3F_2	167	123	8	76.66-93.33
3	DRMRIJ-31x Donskaja	F_1	23	19	-	-
		BC_1F_1	113	47	3	38.63-72.72
		BC_2F_1	158	57	4	60.22-82.95
		BC_3F_1	144	70	3	68.18-88.63
		BC_3F_2	106	89	5	77.27-95.15
4	NRCDR-02x Donskaja	F_1	27	24	-	-
		BC_1F_1	121	62	3	42.30-71.15
		BC_2F_1	158	57	4	52.88-83.65
		BC_3F_1	179	81	2	75.00-92.30
		BC_3F_2	87	63	5	84.61-94.23

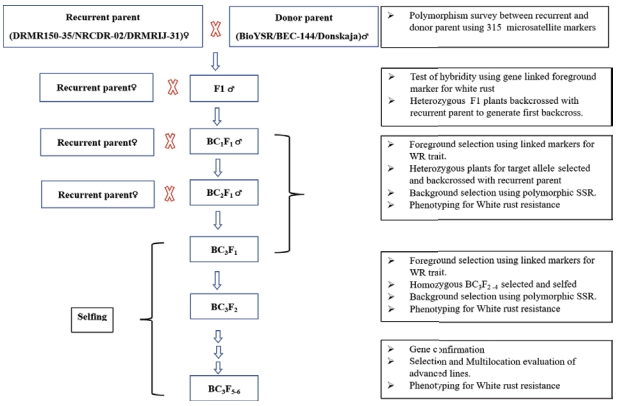


Fig. 1. Details of MABB strategy followed in development of WRR introgression lines of *B. juncea*

loci were mapped and linked IP markers, At2g36360 and At5g41560 reported by Panjabi et al (2010). These IP markers were also utilized for genotyping 44 genotypes developed for double zero and WRR trait following a conventional pedigree breeding approach by Priyamedha et al.(2021). The IP marker At2g36360 (AcB1-A5.1 locus) was used in foreground selection of progenies of the crosses NRCDR-02 × Donskaja, DRMR 150-35 × BIO-YSR and DRMRIJ-31 × Donskaja, while, At5g41560 (AcB1-A4.1 locus) was used in foreground selection of progenies of the cross DRMR150-35 × BEC-144. Screening for polymorphism using genomic SSR markers between recurrent and donor parents lead to the selection of 22-25 SSR markers for each cross combination (Table 2). These polymorphic SSR markers were used during background selection for genome recovery of the

Table 4. Disease reaction of advanced WRR introgression lines of *Brassica juncea*

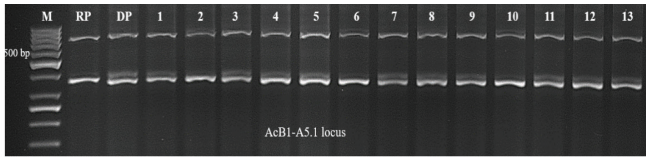
Entry	Per cent WR severity (100 DAS)				Per cent staghead incidence			Offseason at Wellington			Generation	Pedigree	Locus present
	2019-20	2020-21	2021-22	Mean	2019-20	2020-21	2021-22	Mean	Code Kharif 2021	Code Kharif 2022	Reaction to white rust			
DRMRCI 125	7.9	3.4	0.4	3.9	10.0	8.4	0.0	6.1	WR89	–	Resistant	BC_3F_6	NRCDR-02 × Donskaja	AcB1-A5.1
DRMRCI 126	8.3	16.4	5.9	10.2	10.0	6.5	0.0	8.25	WR111	R82	Resistant	BC_3F_5	NRCDR-02 × Donskaja	AcB1-A5.1
DRMRCI131	–	10.9	0.0	5.45	–	10.3	0.0	5.15	WR104	R37	Resistant	BC_3F_5	DRMR150-35 × BEC-144	AcB1-A4.1
DRMRCI132	–	8.4	0.0	4.2	–	8.4	0.0	4.2	WR73	R27	Resistant	BC_3F_5	DRMR150-35 × BioYSR	AcB1-A5.1
DRMRCI139	–	10.9	0.0	5.45		6.5	0.0	3.25	WR79	R76	Resistant	BC_3F_6	DRMRIJ-31 × Donskaja	AcB1-A5.1
BioYSR (Resistant Check)	4.4	27.9	4.2	12.1*/16.05**	11.2	33.4	25.0	23.2*/29.2**	WR111	R73	–	–	–	AcB1-A5.1
Rohini (Susceptible Check)	35.2	35.1	39.7	36.6*/37.4**	13.6	33.8	20.0	22.4*/26.9**	WR113	R71	–	–	–	–

**Mean of two years (2020-21 and 2021-22); *Mean of three years (2019-20,2020-21 and 2021-22)

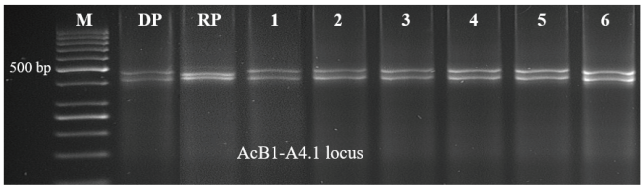
recurrent parent. The detail of number of plants used in foreground and background selection in each generation with a percentage of recurrent parent genome recovery is represented in Table 3. In the present study,a total of 19 WRR introgressed lines from all four crosses in the BC_3F_3 generation showed more than 90% RPG recovery (Table 3). As per our study, three backcross cycles were sufficient to recover lines that closely resembled recurrent parent with maximum RPG recovery and morphological similarity to recurrent parent with white rust resistance gene carried from WRR donor. A higher RPG recovery was obtained because of selection of plants phenotypically similar to recurrent parent in each backcross generation. The current phenotypic and marker-based selection strategies are consistent with the earlier findings (Joseph et al. 2004; Gopalakrishnan et al. 2008; Tanweer et al. 2015; Patroti et al. 2019). Introgression of white rust resistant gene in elite background of Indian mustard cultivars in selected advanced WRR introgression lines has been further confirmed by IP markers linked with white rust resistance (Fig. 2)

Disease reaction WRR introgression lines

The selected BC_3F_3 lines (19) were subjected to phenotyping against white rust disease and advanced to F6 generation using an off-season nursery in Wellington, India. These lines were also screened in Wellington, a hot spot for white rust disease. Among these 19 advanced introgressed lines, total five lines namely, DRMRCI 125 (NRCDR-02 × Donskaja), DRMRCI 126 (NRCDR-02 × Donskaja), DRMRCI131 (DRMR150-35 × BEC-144), DRMRCI132 (DRMR150-35 × BIO-YSR) and DRMRCI139 (DRMRIJ-31 × Donskaja) showing resistance to white rust (Fig. 3)with more than 90% RPG recovery were



Agarose gel showing gene confirmation of stable white rust resistant lines using markers linked to white rust A12g36360 (AcB1-A5.1) loci, with product size of 800bp (white rust susceptible) and 850 bp (white rust resistant) M-50bp ladder. RP=Recurrent parent (DRMR150-35, NRCDR-02 and DRMRIJ-31) DP=Donor Parent (BioYSR, Donskaja) 1-DRMRCI-168 2-DRMRC1-169 3-DRMRC1-170 4-DRMRCI-171 5-DRMRCI-132 6-DRMRCI-655 7-DRMRCI-653 8-DRMRCI-651 9-DRMRCI-139 10-DRMRCI-125 11-DRMRCI-126 12-DRMRCI-125-1 13-DRMRCI-126-2



Agarose gel showing gene confirmation of stable white rust resistant lines using markers linked to white rust At5g41560 (AcB1-A4.1) loci, with product size 445bp (white rust susceptible) and 465bp (white rust resistant) with 420bp common in both susceptible and resistant. M-50bp ladder. RP= Recurrent parent (DRMR150-35) DP= Donor Parent (BEC-144) 1. DRMRCI-182 2. DRMIRCI-192 3. DRMRCI-131 4. DRMRCI-160 5. DRMRCI-143 6. DRMRCI-191

Fig. 2. Agarose gel showing confirmation of WRR introgression lines of *B. juncea* using IP markers

Table 5. Agro-morphological characters of backcross derived advanced lines at BC3F5/BC3F6 generation under late sown condition (2021-22).

Pedigree/ Generation	Entry	PH(cm)	PB	SB	FZL (cm)	MSL (cm)	SMS	SL (cm)	S/S	S/P	Yield (kg/ha)	Twt. (gm)
Checks	PM30	170.8	4.60	9.13	71.40	66.33	42.93	5.08	15.10	236.06	1825.45	4.86
	DRMR150-35	168.06	4.93	10.53	76.46	74.53	48.60	5.15	16.17	282.60	1751.52	4.87
	DRMRIJ31	199.33	5.06	9.00	71.33	63.20	43.13	4.90	15.74	224.66	1986.34	5.45
	NRCDR-02	194.6	5.06	10.26	89.60	72.00	45.93	5.12	16.16	248.33	1931.52	5.19
	BIOYSR	169.46	5.33	10.80	73.06	65.20	48.40	5.32	16.49	367.60	1558.18	3.87
	Donskaja	188.86	3.80	8.73	68.86	63.40	47.13	4.24	13.73	273.00	1622.25	3.60
DRMR150-35 × BioYSR (BC_3F_5)	DRMRCI-168	172.2	4.06	6.06	70.20	66.20	41.40	5.34	15.69	241.46	1666.87	4.35
	DRMRCI-169	177.13	4.13	8.00	68.13	69.86	43.66	5.24	13.81	259.60	1816.96	6.18
	DRMRCI-170	186.00	4.60	10.13	75.40	68.13	40.40	4.96	15.46	266.00	1688.21	3.86
	DRMRCI-171	176.06	5.93	10.80	69.00	57.66	35.93	5.18	15.32	245.73	1710.20	3.42
	DRMRCI-132	162.66	4.53	5.60	69.26	63.06	31.60	3.99	15.42	200.33	1704.65	3.34
DRMR150-35 × BEC-144 (BC_3F_5)	DRMRCI-22 (DRMRCI-182)	155.53	3.86	8.13	68.26	64.00	34.80	4.72	14.49	198.20	1722.06	3.97
	DRMRCI-25 (DRMRCI-192)	169.53	4.40	7.80	76.53	71.46	36.73	4.85	16.44	214.86	1703.19	3.69
	DRMRCI-131	185.53	6.20	13.13	82.93	71.80	47.80	5.04	12.33	392.53	1714.12	5.97
	DRMRCI-160	198.60	5.06	10.06	85.80	76.46	46.40	5.39	15.37	307.86	1935.04	5.05
	DRMRCI-143	198.80	5.33	10.06	87.40	77.86	37.06	4.86	14.56	285.13	1763.25	4.86
	DRMRCI-104 (DRMRCI-191)	185.73	4.46	10.53	71.20	63.33	38.13	4.98	13.48	258.33	1740.68	3.78
DRMRIJ-31 × Donskaja (BC_3F_6)	DRMRCI-655	179.53	5.53	12.00	81.80	75.26	48.93	5.31	15.40	273.40	1964.64	5.94
	DRMRCI-653	178.26	5.20	9.06	75.73	73.60	47.46	4.94	15.21	219.93	1900.79	4.47
	DRMRCI-651	176.06	5.33	10.60	75.60	72.33	49.26	4.82	14.66	241.60	1857.28	5.08
	DRMRCI-139	178.60	5.73	9.13	75.86	75.33	50.46	5.16	14.65	212.06	2021.74	6.37
NRCDR-02 × Donskaja (BC_3F_6)	DRMRCI-125	197.86	4.40	8.60	86.06	86.20	46.60	5.36	16.30	215.00	1941.10	4.53
	DRMRCI-126	191.20	3.93	9.00	81.53	70.20	40.87	4.00	13.73	224.93	1674.05	3.55
	DRMRCI-125-1	195.93	4.26	10.20	85.73	75.06	44.46	5.22	16.10	263.66	1839.32	4.48
	DRMRCI-126-2	194.43	4.00	8.60	81.48	78.40	42.88	4.97	15.34	252.13	1867.38	3.86
	Range	155.53-199.33	3.8-6.2	5.6-13.13	68.1-89.6	57.66-86.2	31.6-50.46	3.99-5.39	12.33-16.49	198.20-392.53	1558.18-2021.75	3.34-6.37
	CD (.05)	16.72	0.50	0.96	9.61	6.89	5.68	0.49	1.26	22.84	344.90	0.44
	CV %	5.60	6.30	6.20	7.63	5.96	8.00	6.01	5.11	5.45	11.70	5.78

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; Twt = 1000-seed weight

selected for multilocation pathological trial under AICRP-RM (All India Coordinated Research Project on Rapeseed-Mustard). All five lines were also found resistant to white rust in an off-season nursery at Wellington, India for two consecutive years (2021-22). The multilocation pathological trial of these lines was conducted at 6 locations: Morena, Pantnagar, Hisar, Ludhiana, Jagdalpur, and Bharatpur consecutively for three years from 2019 to 2021. Among these lines, DRMRCI 125, DRMRCI131, DRMRCI139 and DRMRCI132 were found highly resistant to white rust disease with almost 0% WR severity (100 DAS). Staghead incidence in year 2021-22, whereas, the resistant check (BioYSR) showed 4.2% WR severity (100 DAS) and 25% Staghead incidence. The mean %WR severity (100 DAS) of DRMRCI 125 and DRMRCI132 were found to be below 5% for three and two years, respectively in multilocation trials (Table 4).

Agro-morphological characterization of WRR introgression lines

All the selected 19 introgression lines were evaluated for eleven agro-morphological traits along with their recurrent parent as well as commercial variety checks during *rabi* 2021-22. The recurrent parents *viz.* DRMR150-35, NRCDR-02, and DRMRIJ-31 recorded an overall mean grain yield of 1751.52, 1931.52, 1986.34 kg/ha while the donor parent viz; BioYSR and Donskaja recorded 1558.18 and 1622.25 kg/ha under late sown conditions, respectively. Commercial variety check, PM-30 recorded an overall mean grain yield of 1825.45kg/ha. The test introgression lines *viz.,* DRMRCI-169, DRMRCI-160, DRMRCI-143, DRMRCI-191, DRMRCI-655, DRMRCI-653,

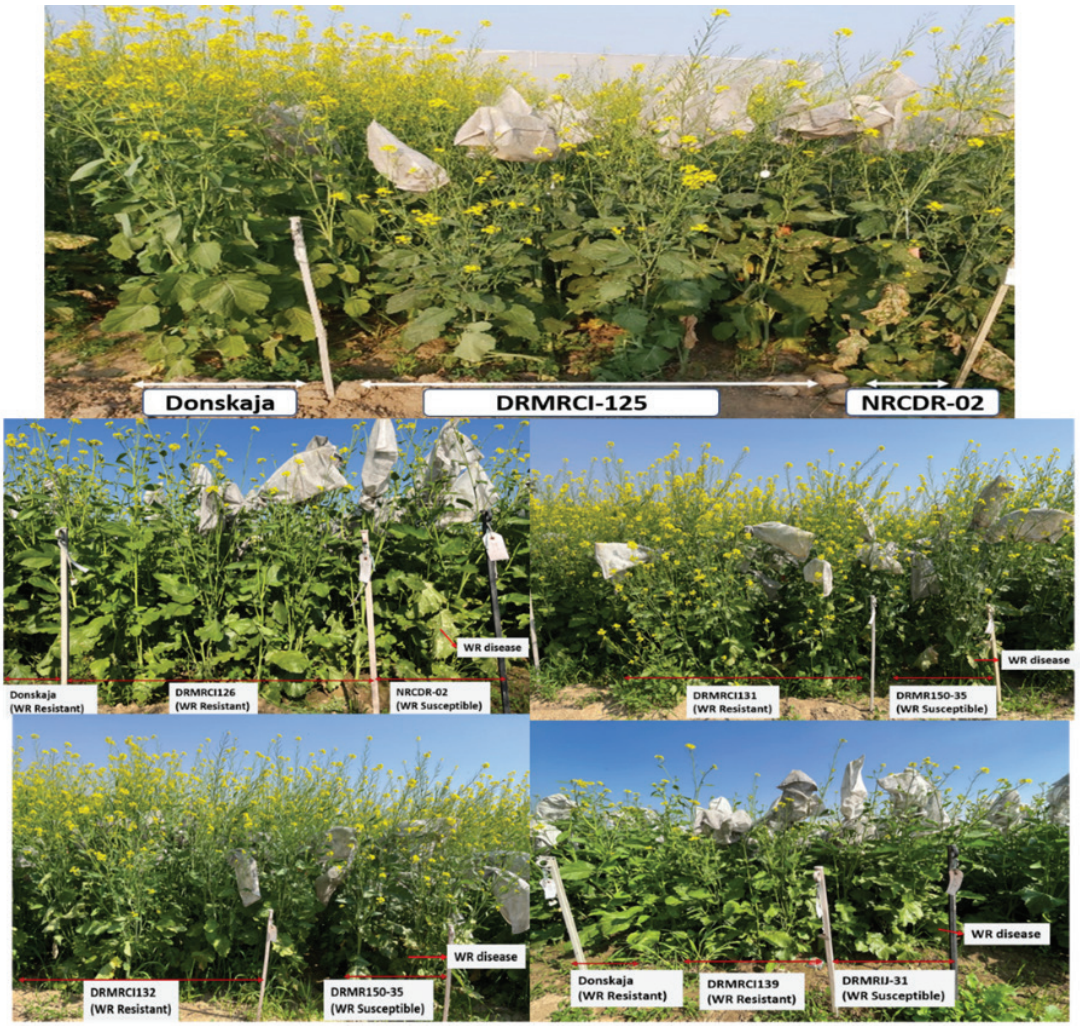


Fig. 3. Phenotypic screening of WRR introgression lines of B. juncea. for White Rust resistance at experimental farm ICAR-DRMR, Bharatpur, India

DRMRCI-139andDRMRCI-125showed grain yields at par with their respective recurrent parents (Table 5). A total of 8 lines out of 19 showed grain yields at par when compared with commercial variety check, PM-30. Other traits under study in the selected lines were found to be comparable to their respective recurrent parent. Advanced introgression lines exhibited an almost identical agro-morphological performance in the field, with their respective recurrent parents viz. DRMR150-35, NRCDR-02 and DRMRIJ-31.The introgression of resistant genes for WR diseases and higher recovery of the genome of the recurrent parents in advanced lines (DRMRCI-125, DRMRCI-126, DRMRCI-131, DRMRCI-132 and DRMRCI-139) having at par yield with respect to their recurrent parents were significant achievements of the present study. The introgressed lines may be released as improved white rust-resistant varieties having comparable agro-morphological traits of elite cultivars. It will go a long way in reducing the use of chemicals, thus protecting the environment from being polluted and reducing pesticide residues in the produce, making it safe for human consumption.

Author's contribution

Conceptualization of research (VVS); Designing of the experiments (VVS,); Contribution of experimental materials (VVS,P); Execution of field/lab experiments and data collection (VVS, M, B, MLM, PS, PKR); Analysis of data and interpretation (VVS, M, P); Preparation of the manuscript (VVS, M, P).

Acknowledgments

Authors sincerely acknowledge ICAR, New Delhi for providing funds under CRP-Molecular breeding. Thanks are also due to IARI, Regional Station, Wellington, Tamil Nadu for providing off season nursery facilities for white rust screening.

References

Anonymous. 2022. Third Advance Estimates of Production of Oilseeds and Commercial Crops 2021-22; Ministry of Agriculture and Farmers Welfare, Government of India: New Delhi, India, 2022; pp. 1–2.

Arora H., Padmaja K.L., Paritosh K., Mukhi N., Tewari A.K., Mukhopadhyay A., Gupta V., Pradhan, A.K. 2019.*BjuWRR1*, a CC-NB-LRR gene identified in *Brassica juncea*, confers resistance to white rust caused by *Albugo candida*. Theor. Appl. Genet., **132**: 2223–2236.

Borhan M. H., Gunn N., Cooper A., Gulden S., Tor M., Rimmer S.R. and Holub E. B. 2008. WRR4 encodes a TIR-NB-LRR protein that confers broad-spectrum white rust resistance in *Arabidopsis thaliana* to four physiological races of *Albugo candida*. Mol. Plant Microbe, **21**: 757–768. https://doi.org/10.1094/MPMI-21-6-0757

Borhan M. H., Holub E. B., Kindrachuk C., Omidi M., Bozorgmanesh-Frad G., and Rimmer S. R. 2010. WRR4, a broad-spectrum TIR-NB-LRR gene from *Arabidopsis thaliana* that confers white rust resistance in transgenic oilseed Brassica crops. Mol. Plant Pathol., **11**: 283–291.https://doi.org/10.1111/j.1364-3703.2009.00599.

Chand S., Singh N.,PrasadL.,NanjundanJ., Meena V.K., Chaudhary R., Patel M.K.,TaakY., Saini N., Vasudev S and Yadava D.K. 2022.Inheritance and Allelic Relationship among Gene(s) for White Rust Resistance in Indian Mustard [*Brassica juncea* (L.) Czern&Coss]. Sustainability, **14**: 11620.

Cheung W. Y., Gugel R. K. and Landry B. S. 1998. Identification of RFLP markers linked to the white rust resistance gene (Acr) in mustard [*Brassica juncea* (L.) Czern. and Coss.]. Genome,**41**: 626–628.doi: 10.1139/gen-41-4-626.

Dang J.K., Sangwan M.S., Mehta N. and Kaushik C.D. 2012. Multiple Disease Resistance against Four Fungal Foliar Diseases of Rapeseed-Mustard. Indian Phytopath., **53**: 455–458.

Dev D., Tewari A.K., Upadhyay P. andDanielG.R. 2020. Identification and Nomenclature of *Albugo candida* Pathotypes of Indian Origin Causing White Rust Disease of Rapeseed-Mustard. Eur. J. Plant Pathol., **158**: 987–1004.

Doyle J. J. and Doyle J. L. 1990. Isolation of plant DNA from fresh tissue. Focus, **12**: 13-15.

Fox D. T. and Williams P. H. 1984.Correlation of sporeproduction by *Albugo Candida* on *Brassica campestris* and a visual white rust rating scale. Can.J. Plant Pathol., **6**: 175-178.

Gopalakrishnan S., Sharma R.K., Rajkumar K.A., Joseph M., Singh V.P., Singh A.K., Bhat K.V., Singh N.K. and Mohapatra T. 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breed., **127**:131–139.

Hirani A.H., Gao F., Liu J., Fu G., Wu C., Yuan Y., Li W., Hou J., Duncan R. and Li G. 2016. Transferring clubroot resistance from Chinese cabbage (*Brassica rapa*) to canola (*B. napus*). Can. J. Plant Pathol., **38**: 82–90.doi:10.1080/07060661.2016.1141799.

Joseph M., Gopalakrishnan S., Sharma R.K., Singh V.P., Singh A.K., Singh N.K. and Mohapatra T. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. Mol. Breed., **13**:377–387.

Kalia P., Sharma B. B. and Singh D. 2017. Marker-assisted introgression of black-rot resistance gene Xca1bc into cauliflower through interspecific hybridization. In IV International Symposium on Molecular Markers in Horticulture,**1203**: 51-58.

Kaur K., Liu Y. and Rahman H. 2022. Introgression of Resistance to Multiple Pathotypes of *Plasmodiophora brassicae* from Turnip (*Brassica rapa* ssp. *rapifera*) into Spring *B. napus* Canola. Agronomy, **12**: 1225. https://doi.org/10.3390/agronomy12051225.

Kawasaki M., Ohara T., Ishida M., Takahata Y. and Hatakeyama K. 2021. Development of novel clubroot resistant rapeseed lines (*Brassica napus* L.) effective against Japanese field isolates by marker assisted selection. Breed Sci., **71**: 528-537.

Kole C., Teutonico R., Mengistu A., Williams P. H., and Osborn T. C.1996. Molecular mapping of a locus controlling resistance to *Albugo candida* in *Brassica rapa*. Phytopathol., **86**: 367–369.

Lakra B.S. and Saharan G.S.1989. Correlation of leaf and stag head infection intensities of white rust with yield and yield components of mustard. Indian J. Mycol. Plant Pathol., **19**: 279–281.

Massand P. P., Yadava S. K., Sharma P., Kaur A., Kumar A. and Arumugam N. 2010. Molecular mapping reveals two independent loci conferring resistance to *Albugo candida* in the east European germplasm of oilseed mustard *Brassica juncea*. Theor. Appl. Genet., **121**: 137–145.

Mehta N. 2021. Epidemiology and Prediction Models for Themanagement of Rapeseed–Mustard Diseases: Current Status and Future Needs. Indian Phytopathol., **74**: 437–452.

Panjabi-Massand P., Yadava S. K., Sharm P., Kaur, A., Kumar A., Arumugam N., Sodhi Y. S., Mukhopadhyay A., Gupta V., Pradhan A. K. and Pental D. 2010. Molecular mapping reveals two independent loci conferring resistance to *Albugo candida* in the east European germplasm of oilseed mustard *Brassica juncea*. Theor. Appl. Genet., **121**: 137–145. https://doi.org/10.1007/s00122-010-1297-6.

Patroti P., Vishalakshi B., Umakanth B., Suresh J., Senguttuvel P. and Madhav M.S. 2019. Marker-assisted pyramiding of major blast resistance genes in Swarna-Sub1.,an elite rice variety (*Oryza sativa* L.). Euphytica, **215**: 1–11.

PrabhuK. V., Somers D. J., Rakow G. and Gugel R. K.1998. Molecular markers linked to white rust resistance in mustard *Brassica juncea*. Theor. Appl. Genet., **97**: 865–870.

Priyamedha, Singh V.V., Dubey M., Kumar P., Ram B., Sujith M.S. and Rai P.K.2021. Deployment of double zero and white rust resistance trait and validation of known linked molecular markers in Indian mustard (*Brassica juncea* L.). Indian J. Genet. Plant Breed., **81**: 422-430. DOI: 10.31792/ISGPB.81.3.9

Rana K., Atri C., Gupta M., Akhatar J., Sandhu P.S., Kumar N., Jaswal R., Barbetti M.J. and Banga S.S. 2017. Mapping resistance responses to Sclerotinia infestation in introgression lines of *Brassica juncea* carrying genomic segments from wild Brassicaceae, *B. fruticulosa*. Sci Rep., **7**: 5904.

Saal B. and Struss D. 2005. RGA- and RAPD-derived SCAR markers for a *Brassica* B-genome introgression conferring resistance to blackleg in oilseed rape. Theor. Appl. Genet., **111**: 281–290.

Saha P., Ghoshal C., Saha N.D., Verma A., Srivastava M., Kalia P. and Tomar B.S. 2021. Marker-Assisted Pyramiding of Downy Mildew-Resistant Gene *Ppa3* and Black Rot-Resistant Gene *Xca1bo* in Popular Early Cauliflower Variety Pusa Meghna. Front. Plant Sci., **12**: 603600

Saharan G.S., Kaushik C.D. and Kaushik J.C. 1988. Sources of Resistance and Epidemiology of White Rust of Mustard.

Indian Phytopathol., **41**: 96–99.

Saharan G. S. and Verma P. R. 1992. White Rusts: Areview of economically important species.International Development Research Center (IDRC), Ottawa. Canada MR 315e, 65.

Saharan G.S., Verma P.R., Meena P.D., Borhan M.H. and Singh D. 2014. Analysis of White Rust Research Progress through Bibliography. J. Oilseed Brassica, **5**: 42–115.

Singh V.K., Singh A., Singh S.P., Ellur R.K., Choudhary V., Sarkel S., Singh D., Krishnan S.G., Nagarajan M., Vinod K.K., Singh U.D., Rathore R., Prashanthi S.K., Agrawal P.K., BhattJ.C., Mohapatra T., Prabhu K.V. and Singh A.K. 2012. Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding. Field Crops Res., **128**: 816

Singh B. K., Nandan D., Ambawat S., Ram B., Kumar A., Singh T., Meena H. S., Kumar V., Singh V. V., Rai P. K. and Singh D. 2015. Validation of molecular markers for marker-assisted pyramiding of white rust resistance loci in Indian Mustard (*Brassica juncea*L.). Can. J. Plant Sci., **95**: 939-945.

Singh A.K., Krishnan S.G., Vinod K.K., Ellur R.K., Bollinedi H., Bhowmick P.K. and Nagarajan M. 2019. Precision breeding with genomic tools: a decade long journey of molecular breeding in rice. Indian J. Genet. Plant Breed., **79**(Suppl): 191

Singh V.V., Dubey M., Gurjar N., Meena M.L., Sharma P. and Rai P.K. 2020. Genetics of White Rust Resistance in Indian Mustard (*Brassica juncea* L.) and Its Validation Using Molecular Markers. Indian J. Genet. Plant Breed., **80**: 275–281. DOI: 10.31742/IJGPB.80.3.6

Somers D. J., Rakow G. and Rimmer S. R. 2002. *Brassica napus* DNA markers linked to white rust resistance in *Brassica juncea*. Theor. Appl. Genet., **104**: 1121–1124.

Sundaram R.M., Vishnupriya M.R., Biradar S.K., Laha G.S., Reddy G.A., Rani N.S., Sarma N.P. and Sonti R.V. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica, **160**: 411–422.

Tanweer F.A., Rafii M.Y., Sijam K., Rahim H.A., Ahmed F.,AshkaniS. and Latif M.A. 2015. Introgression of Blast Resistance Genes (Putative *Pi-b* and *Pi-kh*) into Elite Rice Cultivar MR219 through Marker-Assisted Selection. Front. Plant Sci., **6**: 1002.

USDA 2020. Foreign Agriculture Service. United States Department of Agriculture. Available online at: https://www.fas.usda.gov/regions/india.

Varshney A., Mohapatra T. and Sharma R. P. 2004. Development and validation of CAPS and AFLP markers for white rust resistance gene in *Brassica juncea*. Theor. Appl. Genet.,**109**: 153–159.

Varshney R,K., Thudi M., May G.D. and Jakson S.A. 2010. Legume genomics and breeding. Plant Breed. Rev., **33**: 257–304.

Vignesh M., Yadava D.K., Sujata V., Yadav A.K., Mohapatra T. and Prabhu K.V.2011.Characterization of an Indian mustard (*Brassica juncea*) indigenous germplasm line Bio-YSR for white rust resistance. Indian J. Plant Genet. Resour., **71**: 40–42.

Williams P. H. 1985. White rust [*Albugo candida* (Pers. ex.Hook.) Kuntze] In: Crucifer Genetics Cooperative(CRGC) Resource Book. University Wisconsin USA: 1-7.

Yadava D.K., Vignesh M., Sujat V., Singh, N., Singh R., Dass B., Yadav M.S., Mohapatra T. and Prabhu K.V. 2012. Understanding the Genetic Relationship among Resistant Sources of White Rust, a Major Fungal Disease of *Brassica juncea*. Indian J. Genet. Plant Breed., **72**: 89–91.