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Inheritance and Allelic Relationship among Gene(s) for White Rust Resistance in Indian Mustard [*Brassica juncea* (L.) Czern & Coss]

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Abstract: White rust [*Albugo candida* (Pers. Ex. Lev) Kuntze] is an important oomycetes disease of Indian mustard [*Brassica juncea* (L.) Czern & Coss] that causes a drastic reduction in seed yield and quality when the conditions are favorable. A set of 25 Indian mustard genotypes were screened against *A. candida* Delhi isolate (Ac-Dli) under both controlled and natural epiphytotic conditions. Out of 25, only six genotypes (Pusa Karishma, PDZ-3, Heera, BEC-144, BIO YSR, and Donskaja) were found highly resistant in both environments. To study the inheritance of resistance, four resistant genotypes (BEC-144, BIO YSR, Pusa Karishma, and Donskaja) were crossed with three susceptible genotypes (PM-24, Pusa Vijay, and MSTWR-17-15) in a definite design. The parents, F_1, F_2, and BC_1F_1 (F_1 backcrossed with susceptible parent) generations were screened under both artificially controlled and natural epiphytotic conditions and the results indicated that the resistance in BIO YSR, BEC-144, and Pusa Karishma is governed by a single dominant gene, but more than one dominant gene is responsible for resistance in Donskaja. A test of an allelism conducted on the F_2 population derived by crossing resistant (BEC-144) × resistant (BIO YSR) genotypes indicated that the gene imparting resistance to Ac-Dli isolate in the resistant parents BEC-144 and BIO YSR is the same and they are allelic to each other. Therefore, in broader terms, the information generated in the present study will be useful in Indian mustard breeding programs for the development of durable resistant cultivars.

Keywords: *Albugo candida*; allelism; *Brassica juncea*; inheritance; monogenic dominance; white rust

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1. Introduction

Brassica is an economically important genus, being home to many species that are widely used as edible oilseed crops, leafy vegetables, green fodder crops, and condiments [1–3]. Globally, rapeseed mustard plays a significant role in terms of edible oilseed production and ranks third after soybean and oil palm. In India, rapeseed mustard is the second major oilseed crop in terms of area (≈22.2% of the total oilseed cultivated area) and production (≈32% of the country's oilseed production) after soybean [4]; however, as edible oil production is concerned, it ranks first [5]. Among different species of rapeseed mustard crops, Indian mustard [*Brassica juncea* (L.) Czern & Coss] is the most important and widely cultivated species in India, occupying about 90% of the area (9.168 million ha) and production (11.75 MT) of the rapeseed mustard group of crops, with a productivity of 1178 kg/ha during 2021–2022 [6]. The production of rapeseed mustard is highly affected by biotic and abiotic factors. Among biotic factors, diseases such as stem rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida*), Alternaria blight (*Alternaria brassicae*), powdery mildew (*Erysiphe cruciferarum*), downy mildew (*Hyaloperonospora parasitica*), and blackleg (*Leptoshaeria maculans*) are more devastating and significantly reduce the seed yield and

oil quality of rapeseed mustard depending on the prevailing climatic and agro-ecological conditions [7].

White rust [*Albugo candida* (Pers. Ex. Lev) Kuntze] is an obligate oomycetes pathogen of the oilseed *Brassicas* worldwide, including India, which causes localized and systemic infections in plants [8–10]. With the increase in area under mustard cultivation, the intensity and severity of white rust have increased gradually throughout the mustard-growing areas of tropical and subtropical India [11]. White rust disease in Indian mustard is favored when low temperature (15–20 °C) and high humidity (>65%) with intermittent rainfalls occur from the cotyledonary to the complete flowering stage [12]. Yield losses have been reported to the range of 23–89.9% in *B. juncea* [13]. The infected plants show localized white to pale-colored pustules on the abaxial surface of leaves, stems, and inflorescence, along with chlorosis on the adaxial surface of leaves; however, systemic infection leads to the formation of staghead and accounted for complete loss of seed formation which causes up to 90% yield losses [14]. The yield loss depends upon disease severity, which is affected by prevailing environmental conditions (temperature and humidity), planting geometry, the date of sowing [15], etc. In India, many isolates of white rust have been reported but until today systematic information is lacking about the "dominant race(s)" present in different mustard-growing regions of India [7,16].

The pathogen survives through oospores lying in the soil (Figure 1) that are formed in the hypertrophied plant tissues fallen from diseased plants or seeds, and the oospores can act as a source of inoculum after germination [14]. The oospore can survive for more than 21 years in diseased host tissue even under dry storage conditions. In natural conditions, oospore germinates once favorable environmental factors (temperature 10–20 °C and RH > 70%) occur and cause primary infection in the host leaves directly or indirectly (by entering via stomata or natural openings). However, the secondary infection takes place through sporangia and/or zoospores, which develop symptoms in the form of pustules [17]. The sporangia move away by air current from one place to another after being released from the matured and dehiscence pustules. The germination and infection process of zoospores and sporangia essentially require moisture on the host leaf surface. Later, oospores are formed in the hypertrophied tissues such as roots, stems, leaves, inflorescence, and even in the siliquae of diseased plants [12].

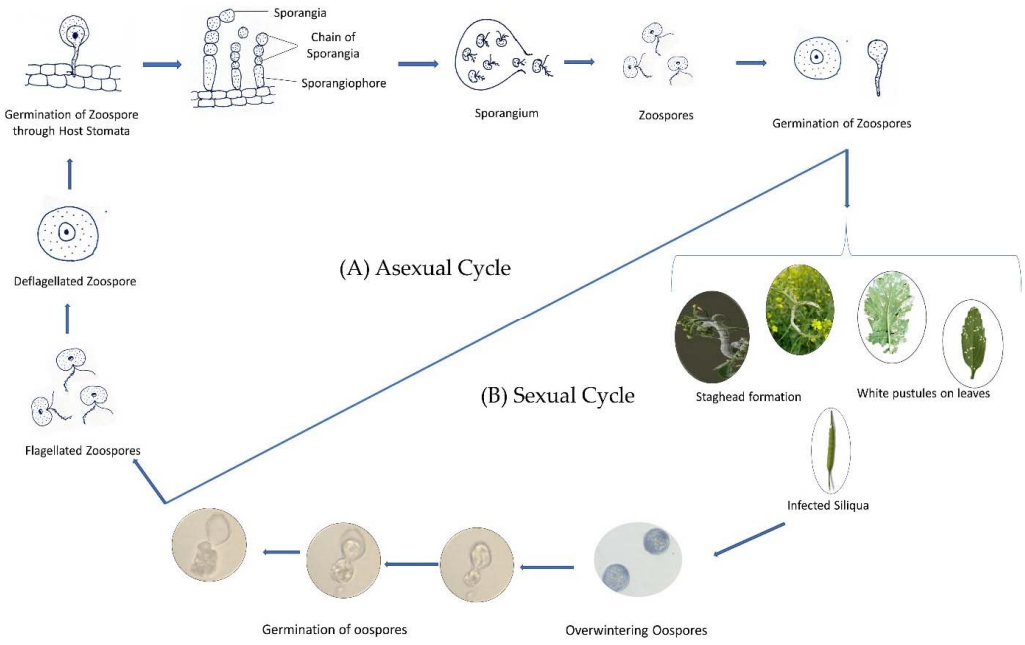

Figure 1. Life cycle of white rust (*Albugo candida*) on Indian mustard. (**A**) Asexual phase and (**B**) Sexual phase.

Disease resistance plays a major role in crop production, quality assurance, environmental safety and ultimately crop yield. Disease resistance can be controlled by a single gene, viz., R-gene, or by many genes with small effects [18,19]. A wide range of genotypic variability exists among *Brassica* species for white rust resistance. The genotypes of *B. juncea* and *B. rapa* are comparatively less tolerant to white rust than *B. napus*, but few susceptible genotypes in *B. napus* were also reported [20,21]. *B. juncea* germplasm belonging to the Indian gene pool is highly susceptible to white rust, whereas the east European germplasm is highly resistant [22,23]. In addition, genotypes of *B. juncea* accessed from Australia and China were more resistant than Indian genotypes at the leaf and/or inflorescence stage [24,25]. Earlier, many donor sources such as Donskaja IV, Heera, BIO YSR, BEC-144, and NRCDR 515 were reported and registered as resistant genetic stocks for different isolates of white rust occurring in various states of India. Wild Brassicaceae members such as *B. fruticulosa* and *Thlaspi arvense* have also been found to be resistant to white rust, whereas few species of genus *Diplotaxis* and *Sinapis* are reported as moderately resistant [26,27]. Although several *Brassica* species have been reported to carry white rust resistance genes, rapid evolutionary pathways of pathogen overcome the prevailing host resistance due to the occurrence of a high level of selection pressure in the present day mustard based cropping system, therefore searching for new resistance gene(s) is always a necessary basic and continuous process [16,18]. In the unpredictable climatic change and global warming, inbuilt resistance became imperative to stabilize and sustain the yield potential of Indian mustard cultivars under different growing conditions in India. With this background, in the present study, a set of 25 genotypes of *B. juncea* were screened under both artificial epiphytotic and field conditions, and four stable resistant and three susceptible genotypes were studied to understand the inheritance pattern of white rust resistance, and the allelic relationship among resistance conferring genes present in resistant genotypes.

2. Materials and Methods

2.1. Plant Materials

The plant material used in the present investigation includes 25 genotypes of Indian mustard of both indigenous and exotic origin. The details of genotypes, their pedigree, and disease reaction against *A. candida* Delhi isolate (Ac-Dli) under both controlled and natural field conditions were presented in Table 1. The genotypes were maintained as pure lines by continuous selfing at ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India.

Table 1. Screening of Indian mustard genotypes against Delhi isolate (Ac-Dli) of *Albugo candida* under artificial epiphytotic and natural conditions.

				Cotyledonary Stage *			True Leaf Stage @
S.N.	**Genotypes**	**Parentage**	**Genotypic Class**	**Percent Disease Index (PDI)**			**Pdi**
				2016–2017	**2017–2018**	**Combined Mean**	**2020–2021**
1.	Pusa Karishma	Pusa Barani/ZEM 1	Released cultivar	0.00 (HR)	0.00 (HR)	0.00 (HR)	0.00 (HR)
2.	PDZ-1	LES-27/NUDHYJ-3	Advanced elite line	11.11 (MS)	46.67 (S)	28.89 (S)	3.50 (R)
3.	PDZ-3	Pusa Karishma/EC597325	Advanced elite line	0.00 (HR)	0.00 (HR)	0.00 (HR)	0.00 (HR)
4.	PDZ-4	Pusa Mustard -21/EC597325	Advanced elite line	13.73 (MS)	50.56 (HS)	32.14 (S)	4.90 (R)
5.	PDZ-5	Pusa Mustard -21/EC597325	Advanced elite line	5.25 (MR)	39.22 (S)	22.23 (MS)	2.90 (R)
6.	JM-1	Pusa Bold/L 6	Released cultivar	7.14 (MR)	56.57 (HS)	31.85 (S)	26.67 (S)
7.	JM-2	Mutant of RL 9	Released cultivar	15.46 (MS)	48.15 (S)	31.80 (S)	19.44 (MS)
8.	JM-3	Varuna/YRT-3	Released cultivar	20.14 (MS)	55.00 (HS)	37.57 (S)	41.94 (S)
9.	PM-24	Pusa Bold/LEB-15//LES-29	Released cultivar	16.43 (MS)	54.81 (HS)	35.62 (S)	61.11 (HS)
10.	BIO-YSR	A somaclonal variant of *B. juncea*	Registered germplasm	0.00 (HR)	0.00 (HR)	0.00 (HR)	0.00 (HR)
11.	EC-399299	Exotic collection from China	Exotic collection	31.88 (S)	60.78 (HS)	46.33 (S)	0.83 (R)
12.	Heera	Derived from East European germplasm line	Registered germplasm	0.00 (HR)	0.00 (HR)	0.00 (R)	0.00 (HR)
13.	NPJ-181	BCEF-1-00-18-1-6/NPJ-119//NPC-9	Advanced elite line	15.20 (MS)	58.12 (HS)	36.66 (S)	10.28 (MS)
14.	BEC-144	Exotic collection from Poland	Exotic collection	0.00 (HR)	0.00 (HR)	0.00 (HR)	0.00 (HR)
15.	Donskaja	Exotic collection from the Russian Federation	Exotic collection	0.00 (HR)	0.00 (HR)	0.00 (HR)	0.00 (HR)
16.	RL-1359	RLM 514/Varuna	Released cultivar	28.40 (S)	30.86 (S)	29.63 (S)	54.44 (HS)
17.	Rohini	Selection from the natural population of Varuna	Released cultivar	44.44 (S)	60.49 (HS)	52.47 (HS)	39.72 (S)
18.	Durgamani	Local collection from Rajasthan	Released cultivar	15.25 (MS)	18.25 (MS)	15.25 (MS)	64.17 (HS)
19.	Pusa Vijay	Synthetic *B. juncea*/VSL 5	Released cultivar	56.30 (HS)	71.98 (HS)	64.14 (HS)	54.72 (HS)
20.	Varuna	Selection from Varanasi local	Released cultivar	65.27 (HS)	68.50 (HS)	66.88 (HS)	56.82 (HS)

Table 1. *Cont.*

S.N.	Genotypes	Parentage	Genotypic Class	Cotyledonary Stage *			True Leaf Stage @
				Percent Disease Index (PDI)			Pdi
				2016–2017	2017–2018	Combined Mean	2020–2021
21.	Rust 17-304	Pusa Vijay/BIO YSR//Pusa Vijay	Advanced elite line	47.86 (S)	59.93 (HS)	53.90 (HS)	34.96 (S)
22.	Rust 17-305	BEC-286/BIO YSR	Advanced elite line	38.52 (S)	54.58 (HS)	46.55 (S)	20.58 (MS)
23.	Rust 17-306	PM-30/BIO YSR//Bio YSR	Advanced elite line	17.46 (MS)	35.42 (S)	26.44 (S)	16.39 (MS)
24.	MSTWR 17-13	Pusa Vijay/BIO YSR//Pusa Vijay	Advanced elite line	11.56 (MS)	26.34 (S)	18.95 (MS)	22.50 (MS)
25.	MSTWR 17-15	BEC-286/BIO YSR	Advanced elite line	34.81 (S)	52.38 (HS)	43.60 (S)	23.89 (MS)
	C.D.			1.40	2.06	-	1.498
	C.V.			4.29	3.31	-	4.07

Letters in parentheses represent disease reaction; HR—highly resistant; R—resistant; MR—moderately resistant; MS—moderately susceptible; S—susceptible; HS—highly susceptible; * cotyledonary stage screening under artificial epiphytotic condition; @ true leaf stage screening under field condition; C.D.—critical difference at 5% level of significance; C.V.—coefficient of variation.

2.2. Inoculum Collection, Purification, and Multiplication

The inoculum of white rust zoosporangia (primary inoculum) was collected from highly infected fresh leaves of the decidedly susceptible *B. juncea* cv. Varuna grown at the experimental farm of IARI, New Delhi. Further, the single pustule method was used for inoculum purification [27] and multiplied on the genetically pure highly susceptible plants of variety Varuna.

2.3. Pathogen Inoculation and Disease Development

White rust/blisters from fresh leaves were scrapped by sterile scalpel into petriplates containing sterile double distilled water and allowed to germinate for 3–4 h at 4 °C. Hemocytometer mounted on a simple microscope was used to count the density of zoospores and a population of 7–8 $\times$ 10^4 zoospores were maintained for inoculation [28]. In artificial epiphytotic conditions, 8–10 days old seedlings at two cotyledon stage were used and 5 µL zoospore suspension was drop inoculated on the adaxial surface of each lobe of cotyledon manually employing a micropipette. The inoculated trays were kept in a humid chamber covered tightly by a thin polythene sheet (700-gauge thickness) for maintenance of relative humidity. The dark condition was maintained inside the plastic chamber for the first 24 h after inoculation by covering the polythene sheet with a light-blocking thick cloth made sheet to enable the onset of disease. Low temperature and high humidity are prerequisites for congenial white rust disease development in Indian mustard cultivars. Therefore, low temperature and high humidity inside the polythene chamber were maintained by supplying water (up to 2–3 cm height) at regular intervals once in 2–3 days and a hand automizer was used to wet the surrounding area of the chamber during the entire period of disease development.

For screening under the natural field conditions, the seeds of parents and three generations, (F_1, F_2, BC_1F_1) of four crosses (PM-24 $\times$ BEC-144; Pusa Vijay $\times$ Donskaja; PM-24 $\times$ Pusa Karishma; MSTWR-17-15 $\times$ BIO YSR) were raised at the experimental farm area of IARI, New Delhi, where the white rust occurs regularly. All the cultural practices were followed to raise a healthy crop and plants were irrigated manually to maintain soil moisture as and when required.

2.4. Development of Different Breeding Populations

The selected three susceptible (PM-24, MSTWR-17-15, Pusa Vijay) and four resistant (BEC-144, BIO YSR, Donskaja, and Pusa Karishma) genotypes (Figure 2) were crossed in specific combination to develop four F_1s (PM-24 $\times$ BEC-144; Pusa Vijay $\times$ Donskaja; PM-24 $\times$ Pusa Karishma; MSTWR-17-15 $\times$ BIO YSR). From the four F_1s, respective F_2s, and BC_1F_1 (F_1 backcrossed with susceptible parent) populations were developed at the experimental farm of ICAR-IARI, New Delhi, India during 2016–2021. To study the allelic relationship, F_1 and F_2 generations were also developed by crossing resistant genotypes (BEC-144 and BIO YSR).

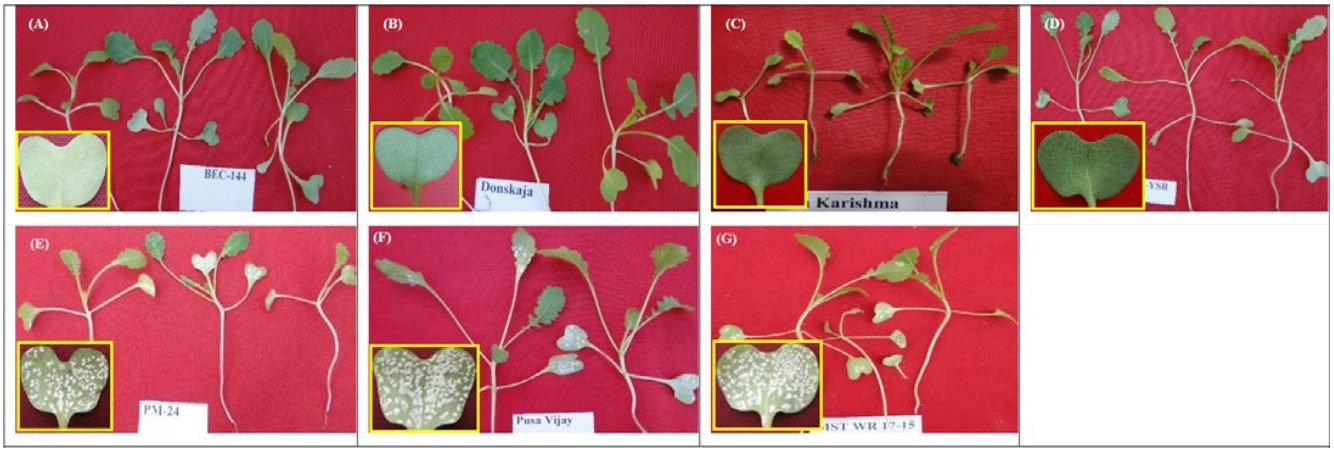

Figure 2. Symptoms caused by *Albugo candida* isolate Ac-Dli on the abaxial surface of cotyledonary leaves of resistant and susceptible genotypes under artificial epiphytotic conditions. Resistant genotypes: BEC-144 (**A**), Donskaja (**B**), Pusa Karishma (**C**), and BIO YSR (**D**), whereas susceptible genotypes: PM-24 (**E**), Pusa Vijay (**F**), and MSTWR-17-15 (**G**).

2.5. Experimental Design and Crop Maintenance

The experiments on disease screening were conducted under both artificially controlled epiphytotic and natural field conditions at IARI, New Delhi, India. Initially, 100 plants from each of 25 genotypes were screened at cotyledonary stage in a complete randomized design (CRD) with three replications for two consecutive years (2016–2018) against Ac-Dli isolate of *A. candida* under controlled conditions. In each year, two sets of genotypes were screened after 10–15 days intervals and scored, and mean values of percent disease index (PDI) were used for classification of disease reaction (Table 1). Additionally, the same set of 25 genotypes was evaluated under field conditions in a randomized complete block design (RCBD) with three replications during 2020–2021. Resistant and susceptible genotypes were identified and selected for further study. In addition, two rows of each parent (PM-24, MSTWR-17-15, Pusa Vijay, Pusa Karishma, BEC-144, BIO YSR, and Donskaja) and F_1s, three rows of BC_1F_1s and fifteen rows of F_2s were sown and evaluated at true leaf stage under field conditions during rabi 2019–2020 and all the cultural practices were followed to raise a healthy crop stand.

Under artificial epiphytotic conditions, 2–3 untreated seeds each of 25 genotypes were sown in plastic trays (34 × 22 × 9 cm) each having 40 pores of 4 cm diameter. The parents and three generations, (F_1, F_2 and BC_1F_1) of four crosses (PM-24 × BEC-144; Pusa Vijay × Donskaja; PM-24 × Pusa Karishma; MSTWR-17-15 × BIO YSR); parents and two generations (F_1 and F_2) of the single cross (BEC-144 × BIO YSR) were raised and screened in two separate experiments conducted at 20-day intervals with two replications in each experiment. Different population size was maintained for each of parent, F_1, BC_1F_1 and F_2 generations depending on the optimum criteria required for the segregation of mendelian genes. Soil mixed with FYM (3:1) was autoclaved (at 121 °C temperature and at a pressure of 106 kPa for 30–60 min) before it was filled in plastic trays for raising the seedlings. In each pore, seeds were sown at the depth of 2–3 cm and lightly covered by sand. The trays were kept in the glasshouse and watered on a regular basis for uniform and vigorous germination.

2.6. Disease Scoring

Under field conditions, 60 plants each from P_1, P_2, F_1, BC_1F_1 and 350 plants in F_2 population were selected and tagged at the seedling stage; however, scoring was initiated at true leaf stage during flowering. In artificial epiphytotic conditions, disease scoring was performed 12–15 days after inoculation. The disease scoring was conducted using 0–9 scale at both cotyledonary and true leaf stages modified from Fox and Williams [29]

and the detailed procedure is highlighted in Table 2 and Figure 3. After scoring, the percent disease index (PDI) was calculated, which indicates the percentage of host tissue or plant part covered by lesions or symptoms or damaged by the disease. Disease severity is the result of the number and size of the lesions and expresses the extent of damage caused by the disease.

Table 2. Rating scale (0–9) for measuring disease severity of white rust disease at cotyledonary and true leaf stage in Indian mustard (modified from Fox and Williams) [29].

Rating Score	Disease Symptoms		PDI	Disease Reaction
	Cotyledonary Stage	True-Leaf Stage		
0	No symptoms on abaxial (lower) and adaxial (upper) leaf surfaces	Absence of pustules	0%	Highly resistant
1	Minute pinpoint to larger brown necrotic flecks under inoculation point on the cotyledonary leaves	<5% leaf area covered by pustules	<5%	Resistant
3	Very sparse sporulation, one to few pustules on the abaxial surface, and absence of any pustules on the adaxial leaf surface	5–10% leaf area covered by pustules	5–10%	Moderately resistant
5	Few to many dispersed pustules with good sporulation on the abaxial surface, and 0 to few pustules on the adaxial surface	11–25% leaf area covered by pustules	11–25%	Moderately susceptible
7	Several pustules with copious sporulation on the abaxial surface with none to few pustules on the adaxial surface	26–50% leaf area covered by pustules	26–50%	Susceptible
9	Countless large coalescing pustules on the abaxial surface with scarce to several pustules on the adaxial surface of the cotyledonary leaves	>50% leaf area covered by pustules	>50%	Highly susceptible

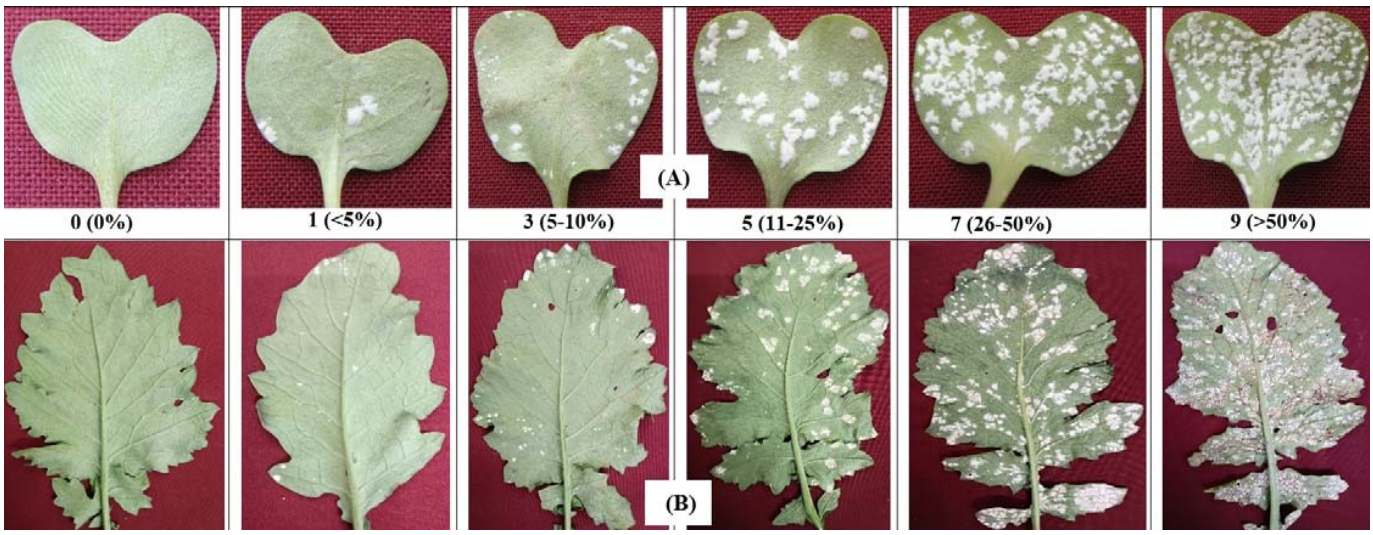

Figure 3. Photographs depicting the disease rating scales (0–9 scale) used for screening parental genotypes and segregating populations against white rust at the cotyledonary (**A**) and true leaf stage (**B**). Values in parentheses denote the percent area of leaf covered by white blisters of disease.

The PDI can be calculated using the following formula:

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

2.7. Statistical Analysis

Disease scores obtained from the artificial epiphytotic and field conditions at the cotyledonary and true-leaf stages were analyzed using Microsoft Excel. The mean values of selected and tagged plants at each replication were used for statistical analysis. Genotypic mean values were also compared using critical differences at a 5% level of significance. The Chi-square test was used for the analysis of goodness of fit in different breeding populations by comparing observed and expected frequencies [29].

3. Results

3.1. Screening of Genotypes under Artificial and Natural Epiphytotic Conditions

In the present investigation, 25 Indian mustard genotypes were initially screened for the Delhi isolate (Ac-Dli) of *A. candida* for disease response at the cotyledonary stage under artificial epiphytotic conditions for two consecutive years (Table 1). The PDI values ranged from 0 (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144, Donskaja) to 65.27% (Varuna) and 0 (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144, Donskaja) to 71.98% (Pusa Vijay) during the crop season rabi 2016–2017 and 2017–2018, respectively. In 2016–2017, six genotypes (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144 and Donskaja) were categorized as highly resistant, two genotypes (PDZ-5 and JM-1) as moderately resistant, nine genotypes (PDZ-1, PDZ-4, JM-2, JM-3, PM-24, NPJ-181, Rust-17-306, MSTWR-17-13 and Durgamani) as moderately susceptible, six genotypes (EC-399299, RL-1359, Rohini, Rust-17-304, MSTWR-17-305 and MSTWR-17-15) as susceptible and two (Pusa Vijay and Varuna) as highly susceptible. In 2017–2018, one genotype (Durgamani) was reported as moderately susceptible, six genotypes (PDZ-1, PDZ-5, JM-2, RL-1359, Rust-17-306 and MSTWR-17-13,) as susceptible, twelve genotypes (PDZ-4, JM-1, JM-3, PM-24, EC-399299, NPJ-181, Rohini, Pusa Vijay, Rust 17-304, Rust 17-305, MSTWR-17-15 and Varuna) as highly susceptible; however, six highly resistant genotypes were observed to be same as in the previous year. The mean PDI of both years categorizes the genotypes into four groups viz., highly resistant (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144 and Donskaja), moderately susceptible (PDZ-5, Durgamani and MSTWR-17-13), susceptible (PDZ-1, PDZ-4, JM-1, JM-2, JM-3, PM-24, EC-399299, NPJ-181, RL-1359, Rust 17-305, Rust-17-306 and MSTWR-17-15) and highly susceptible (Rohini, Pusa Vijay, Varuna and Rust-17-304).

In the screening of the same 25 genotypes at true leaf stage under natural epiphytotic conditions, the PDI ranged from 0 (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144 and Donskaja) to 64.17% (Durgamani). Further, the disease phenotypes were categorized into five groups viz., highly resistant (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144 and Donskaja), resistant (PDZ-1, PDZ-4, PDZ-5 and EC-399299), moderately susceptible (JM-2, NPJ-181, Rust-17-305, Rust-17-306, MSTWR-17-13 and MSTWR-17-15), susceptible (JM-1, JM-3, Rohini and Rust-17-304), and highly susceptible (Varuna, Pusa Vijay, Durgamani, RL-1359 and PM-24). Six genotypes (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144 and Donskaja) and two genotypes (Pusa Vijay and Varuna) were classified as highly resistant and highly susceptible, respectively under both the natural and controlled epiphytotic conditions. However, four genotypes (PDZ-1, PDZ-4, PDZ-5 and EC-399299) were found resistant at true leaf stage in field conditions but were susceptible at cotyledonary stage in controlled epiphytotic conditions. Other genotypes including PM-24 and MSTWR-17-15 were categorized into different disease classes from moderately susceptible to highly susceptible either in controlled or natural epiphytotic conditions.

3.2. Inheritance of White Rust Disease Resistance

Among the six genotypes which were highly resistant under both natural and artificial screening, only the four genotypes (BEC-144, Donskaja, Pusa Karishma and BIO YSR) were taken for inheritance studies. The results of disease reaction in the different parents and breeding generations (F_1, F_2 and BC_1F_1) of four crosses (PM-24 × BEC-144, Pusa Vijay × Donskaja, PM-24 × Pusa Karishma and MSTWR-17-15 × BIO YSR) against *A. candida* Ac-Dli isolate in natural and artificial epiphytotic conditions are illustrated in Table 3. In these crosses, PM-24, Pusa Vijay and MSTWR-17-15 were used as susceptible parents.

Table 3. Segregation pattern of resistance to *Albugo candida* isolate Ac-Dli in the crosses involving susceptible and resistant parents under natural and artificial epiphytotic conditions at IARI, New Delhi, India, during 2019–2022.

Crosses	Locations	Generations	Total Plants	Observed		Expected Ratio	Expected		χ^2 Value	*p*-Value	R-Gene
				R	S	-	R	S			
PM-24 (P_1) × BEC-144 (P_2)	Field Cond.	P_1	60	0	60	-	-	-	-	-	1 dominant
		P_2	60	60	0	-	-	-	-	-	
		F_1	60	60	0	-	-	-	-	-	
		F_2	350	277	73	3:1	262.5	87.5	3.204	0.073	
		BC_1F_1	60	35	25	1:1	30	30	1.667	0.197	
	Exp-1	P_1	41	0	41	-	-	-	-	-	
		P_2	43	43	0	-	-	-	-	-	
		F_1	30	30	0	-	-	-	-	-	
		F_2	592	434	158	3:1	444	148	0.901	0.343	
		BC_1F_1	265	137	128	1:1	132.5	132.5	0.306	0.580	
	Exp-2	P_1	48	0	48	-	-	-	-	-	
		P_2	45	45	0	-	-	-	-	-	
		F_1	47	47	0	-	-	-	-	-	
		F_2	725	530	195	3:1	543.8	181.3	1.391	0.238	
		BC_1F_1	108	64	45	1:1	54	54	3.352	0.067	
Pusa Vijay (P_1) × Donskaja (P_2)	Field Cond.	P_1	60	0	60	-	-	-	-	-	>1 dominant
		P_2	60	60	0	-	-	-	-	-	
		F_1	60	60	0	-	-	-	-	-	
		F_2	350	280	70	3:1	262.5	87.5	4.667	0.031	
		BC_1F_1	60	38	22	1:1	30	30	4.267	0.039	
	Exp-1	P_1	38	0	38	-	-	-	-	-	
		P_2	42	42	0	-	-	-	-	-	
		F_1	45	45	0	-	-	-	-	-	
		F_2	787	658	129	3:1	590.3	196.8	31.106	0.000	
		BC_1F_1	101	70	40	1:1	50.5	50.5	9.713	0.002	
	Exp-2	P_1	39	0	39	-	-	-	-	-	
		P_2	47	47	0	-	-	-	-	-	
		F_1	43	43	0	-	-	-	-	-	
		F_2	660	550	110	3:1	495	165	24.444	0.000	
		BC1F1	95	62	33	1:1	47.5	47.5	8.853	0.003	
PM-24 (P_1) × Pusa Karishma (P_2)	Field Cond.	P_1	60	0	60	-	-	-	-	-	1 dominant
		P_2	60	60	0	-	-	-	-	-	
		F_1	60	60	0	-	-	-	-	-	
		F_2	350	250	100	3:1	262.5	87.5	2.381	0.123	
		BC_1F_1	60	28	32	1:1	30	30	0.267	0.606	
	Exp-1	P_1	37	0	37	-	-	-	-	-	
		P_2	39	39	0	-	-	-	-	-	
		F_1	42	42	0	-	-	-	-	-	
		F_2	348	264	84	3:1	261	87	0.138	0.710	
		BC_1F_1	99	55	44	1:1	49.5	49.5	1.222	0.269	
	Exp-2	P_1	51	0	51	-	-	-	-	-	
		P_2	37	37	0	-	-	-	-	-	
		F_1	45	45	0	-	-	-	-	-	
		F_2	715	550	165	3:1	536.3	178.8	1.410	0.235	
		BC_1F_1	115	67	48	1:1	57.5	57.5	3.139	0.076	
MSTWR-17-15 (P_1) × BIO YSR (P_2)	Field Cond.	P_1	60	60	0	-	-	-	-	-	1 dominant
		P_2	60	60	0	-	-	-	-	-	
		F_1	60	60	0	-	-	-	-	-	
		F_2	350	260	90	3:1	262.5	87.5	0.095	0.758	
		BC_1F_1	60	25	35	1:1	30	30	1.667	0.197	
	Exp-1	P_1	35	0	35	-	-	-	-	-	
		P_2	39	39	0	-	-	-	-	-	
		F_1	34	31	3	-	-	-	-	-	
		F_2	751	580	167	3:1	563.3	187.8	2.791	0.095	
		BC_1F_1	117	60	55	1:1	58.5	58.5	0.248	0.619	
	Exp-2	P_1	33	0	33	-	-	-	-	-	
		P_2	39	39	0	-	-	-	-	-	
		F_1	43	43	0	-	-	-	-	-	
		F_2	787	610	177	3:1	590.3	196.8	2.643	0.104	
		BC_1F_1	105	60	45	1:1	52.5	52.5	2.143	0.143	

3.2.1. Cross-I: PM-24 × BEC-144

Under the field conditions, 60 plants used for recording disease reaction of each parent viz., PM-24 and BEC-144 were found susceptible and resistant, respectively and 60 F_1 plants of this cross exhibited resistance to the disease (Table 3). The complete susceptibility of PM-24 against Ac-Dli isolate suggested the presence of susceptible allele in this genotype. All the F_1 plants were resistant to Ac-Dli isolate, indicating the dominant nature of resistance over susceptibility. Among 350 F_2 plants, 277 were resistant and 73 were susceptible with

the best fit for a 3:1 R/S ratio (χ^2 = 3.204; p = 0.073), indicating that resistant donor BEC-144 carries a single dominant gene. Further, this monogenic dominant nature of resistance gene was also confirmed from the result of the backcross population as well. The 60 BC_1F_1 plants were segregated into 35 resistant and 25 susceptible plants, which was a good fit for 1R:1S ratio (χ^2 = 1.667; p = 0.197), suggesting that trait is controlled by monogenic dominance gene. In two independent experiments under artificial epiphytotic conditions also both the parents and F_1 plants responded similarly to Ac-Dli isolate as under the field condition. In experiment-I, 434 plants were resistant, and 158 plants were susceptible in F_2 generation, while 530 and 195 plants were resistant and susceptible, respectively in experiment-2. In the BC_1F_1 generation, 137 and 128 plants in experiment-1, 64 and 45 plants in experiment-2 were resistant and susceptible, respectively. Therefore, the segregation in F_2 and BC_1F_1 was best fit to 3:1 R/S and 1:1 R/S ratio, respectively in experiment-1 (χ^2_{F2} = 0.901; χ^2_{BC1F1} = 0.306) and experiment-2 (χ^2_{F2} = 1.391; χ^2_{BC1F1} = 3.352) imparting that the resistance is governed by single dominant gene in the resistant genotype BEC-144.

3.2.2. Cross-II: Pusa Vijay × Donskaja

Under the field conditions, 60 plants each of Pusa Vijay and Donskaja were found susceptible and resistant, respectively; 60 F_1 plants were resistant at true leaf stage, imparting that resistance is governed by dominant gene(s) in Donskaja (Table 3). In the F_2 generation, 280 plants were resistant, and 70 plants were susceptible to Ac-Dli isolate and do not fit to 3:1 R/S ratio (χ^2 = 4.667; p = 0.031), inferring that resistance is governed by more than one dominant gene. In BC_1F_1, 38 plants were resistant, and 22 plants were susceptible to Ac-Dli isolate and do not fit to 1:1 R/S ratio (χ^2 = 4.267; p = 0.039), confirming the earlier finding based on segregation in F_2. Under artificially controlled epiphytotic conditions, in the experiments 1 and 2, both parents and F_1 plants showed similar response to Ac-Dli isolate as it was found under the field condition. In F_2 population, 658 and 129 plants were resistant and susceptible in experiment-1 and 550 and 110 plants were resistant and susceptible in experiment-2, respectively; however, the segregating F_2 population does not fit the 3:1 R/S ratio ($\chi^2_{Exp\text{-}1}$ = 31.106; $\chi^2_{Exp\text{-}2}$ = 24.444). In BC_1F_1 generation also, 70 plants were resistant, and 40 plants were susceptible in experiment-1; and 62 plants were resistant and 33 plants were susceptible and do not fit to1:1 R/S ratio ($\chi^2_{Exp\text{-}1}$ = 9.713; $\chi^2_{Exp\text{-}2}$ = 8.853). Based on the segregation pattern of resistance in F_2 and BC_1F_1 it can be inferred that the resistance is governed by more than one dominant gene in the resistant genotype Donskaja.

3.2.3. Cross-III: PM-24 × Pusa Karishma

Under the field conditions, 60 plants of PM-24 were susceptible, and 60 plants of Pusa Karishma were resistant to Ac-Dli isolate of *A. candida* at true leaf stage (Table 3). The 60 F_1 plants were also found resistant signifying the dominant nature of resistance over susceptibility. In F_2 population, 250 plants were resistant, and 100 plants were susceptible, which were best fitted into 3:1 R/S ratio (χ^2 = 2.381; p = 0.123), highlighting that the resistance is governed by a single dominant gene. Further, 28 plants were resistant, and 32 plants were susceptible in BC_1F_1 and well-matched to 1:1 R/S ratio (χ^2 = 0.267; p = 0.606), confirming the findings of F_2 generation. Under artificially controlled epiphytotic conditions, both parents and F_1 plants exhibited similar disease reaction as under field conditions. In addition, 264 and 84 plants in F_2 generation and 55 and 44 plants in BC_1F_1 population were reported as resistant and susceptible to the isolate and were best fitted to 3:1 R/S (χ^2_{F2} = 0.138; p = 0.710) and 1:1 R/S (χ^2_{BC1F1} = 1.222; p = 0.269) ratio in experiment-1, respectively. Likewise, the F_2 and BC_1F_1 generations of the cross followed the monogenic mendelian segregation and were best fitted to 3:1 R/S (χ^2_{F2} = 1.410; p = 0.235) and 1:1 R/S (χ^2_{BC1F1} = 3.139; p = 0.076) ratio, respectively, in experiment-2, confirming that resistance is governed by a single dominant gene in the resistant genotype-Pusa Karishma.

3.2.4. Cross-IV: MSTWR-17-15 × BIO YSR

Under the field conditions, 60 plants each of parental genotypes MSTWR-17-15 and BIO YSR were found susceptible and resistant, respectively to Ac-Dli isolate of *A. candida*, suggesting that susceptible allele was present in MSTWR-17-15, whereas resistance allele was in BIO YSR (Table 3). The 60 plants of F_1 generation were found resistant, inferring the dominance nature of resistance over susceptibility. In F_2 generation, 260 plants were resistant, and 90 plants were susceptible and best fitted to 3:1 R/S ratio ($\chi^2 = 0.095$; $p = 0.758$), indicating dominant monogenic inheritance of resistance. Further, 25 plants were resistant, and 35 plants were susceptible in BC_1F_1 and best fitted to 1:1 R/S ratio ($\chi^2 = 1.667$; $p = 0.197$), confirming the dominant monogenic inheritance of resistance. Under controlled epiphytotic conditions also, the results were same as those of field conditions. In F_2 generation, 580 plants were resistant, and 167 plants were susceptible in experiment-1, and 610 plants were resistant, and 177 plants were susceptible in experiment-2 and best fitted to 3:1 R/S ratio ($\chi^2_{Exp\text{-}1} = 2.791$; $\chi^2_{Exp\text{-}2} = 2.643$), which proves that the resistance is governed by single dominant gene. Further, 60 plants of BC_1F_1 were resistant and 55 plants were susceptible in experiment-1, whereas 60 plants were resistant, and 45 plants were susceptible in experiment-2 fitting to 1:1 R/S ratio ($\chi^2_{Exp\text{-}1} = 0.248$; $\chi^2_{Exp\text{-}2} = 2.143$) and thus confirmed that the resistance is governed by a single dominant gene in the resistant genotype-BIO YSR.

3.3. Test of Allelism

In this experiment, two highly resistant genotypes (PDI = 0) viz., BIO YSR and BEC-144 were selected and used for the allelic relationship. The parents, F_1, and F_2 generations from the cross BIO YSR × BEC-144 were screened for white rust resistance under artificial epiphytotic conditions in two independent experiments with different population sizes (Table 4). In the exeperiment-1, all the 53 F_1 and 417 F_2 plants of BIO YSR × BEC-144 were found to be resistant against the Ac-Dli isolate of *A. candida*. Likewise, all the 51 F_1 and 315 F_2 plants were resistant to the same isolate in experiment-2. In the inheritance studies, both BIO YSR and BEC-144 showed monogenic dominant type of resistance. In the allelic test, all the F_1 plants from the cross of BIO YSR × BEC-144 were found resistant at cotyledonary stage, which indicated that the resistance is governed by the dominant gene(s). Further, no segregation for resistance was observed in F_2 plants indicated that the same gene is conferring resistance, i.e., allelic, to Ac-Dli isolate of *A. candida* in the selected genotypes.

Table 4. Segregation pattern of resistance to *Albugo candida* isolate-Ac-Dli in the crosses among the two resistant parents under artificially controlled epiphytotic conditions.

Cross		Generation	Total Seedlings	Observed		Expected Ratio	Expected		χ^2 Value	*p*-Value	Allelic Relationship
				R	S		R	S			
BIO YSR (P_1) × BEC-144 (P_2)	Exp.-1	P_1	38	38	0	-	-	-	-	-	Allelic
		P_2	45	45	0	-	-	-	-	-	
		F_1	53	53	0	-	-	-	-	-	
		F_2	417	417	0	15:1	390.94	26.06	27.80	0.00	
	Exp.-2	P_1	37	37	0	-	-	-	-	-	Allelic
		P_2	43	43	0	-	-	-	-	-	
		F_1	51	51	0	-	-	-	-	-	
		F_2	315	315	0	15:1	295.31	19.69	21.00	0.00	

4. Discussion

White rust affects a wide array of *Brassica* crops, having wide socio-economic importance; however, its incidence, severity, and damage are more common in Indian mustard than in *B. napus* and *B. carinata* [1,30,31]. In the present study, six genotypes (Pusa Karishma, PDZ-3, BIO YSR, Heera, BEC-144, Donskaja) of Indian mustard were found highly resistant (PDI = 0) under artificial and natural epiphytotic conditions against the *A. candida* Ac-Dli isolate, highlighting the continuous stability of resistance gene(s) present in these genotypes. In previous studies, Singh et al. [16] reported 12 genotypes, including Pusa Karishma, PDZ-3, BIO YSR, BEC-144, and Donskaja, of *B. juncea*, having immune type response

(score = 0) against Ac-Dli isolate at the cotyledonary stage under controlled epiphytotic conditions. Likewise, five genotypes, including BEC-144, Heera and BIO YSR of *B. juncea* were found highly resistant at true leaf stage under field conditions [32]. Similarly, Yadav et al. [33] reported 27 Indian mustard germplasm accessions with resistance reaction at Hisar, Ludhiana and Pantnagar under field conditions and eight of them were identified as highly resistant to "Delhi isolate" of *A. candida* at both cotyledonary and true leaf stages under artificial conditions. In the present study, four genotypes (PDZ-1, PDZ-4, PDZ-5, EC-399299) were resistant at true leaf stage under field conditions but were susceptible at cotyledonary stage under controlled conditions, highlighting that there is no chance for the seedlings to escape from the disease infection in controlled conditions where proper temperature and humidity were maintained, which is highly congenial for disease development. Similarly, it was testified at Pantnagar that the advanced breeding lines of *B. juncea* were completely free from disease (score = 0) under field conditions, whereas under glass house the same lines were found to show variable disease response (score = 0–5) at cotyledonary and true leaf stages [34]. In the present study, 19 genotypes were categorized from moderately susceptible to highly susceptible groups based on disease severity under both natural and artificial screening conditions (Table 1), highlighting that these genotypes do not possess any resistance gene(s), therefore the pathogen establishes very well due to compatible pathogen–host interaction leading to heavy incidence of disease. Similarly, Singh et al. [16] reported that 13 out of 30 genotypes of *B. juncea* studied by them have been reported to be highly susceptible (score = 6) to Ac-Dli isolate at cotyledonary stage under controlled conditions. The wide variation in susceptible disease reaction of different genotypes against pathogens might be due to the differential expression of resistance gene(s) and genetic background of genotypes that affects genotype–pathogen interaction [16]. The overall disease on the susceptible genotypes was higher in the experimental year 2017–2018 than in 2016–2017 at the cotyledonary stage in controlled conditions. Environment, both micro and macro, plays a vital role in changing the dynamics of host–pathogen interaction and ultimately affects disease severity [35]. As the inoculum from the fresh symptomatic leaves of infected plants from the first year was used to multiply the pathogen in the next year, the disease severity in the second year might be high due to adaption and acclimatization of the pathogen on the host. In addition, day and night temperature fluctuations (16–24 °C) at different growth stages of host plants might affect disease severity of *A. candida* [36,37]. Therefore, genotypes must be tested at both cotyledonary and true leaf stages in the controlled conditions before evaluating them in the field conditions to identify reliable and stable resistance source/s against white rust disease.

In the present inheritance study, all the F_1s developed from S × R crosses displayed complete resistance against the Ac-Dli isolate, inferring the dominant nature of resistance over susceptibility in all the resistant genotypes. In earlier studies also, F_1s derived from S × R crosses were found resistant to Ac-Dli isolate of *A. candida* under epiphytotic field conditions and reported dominance type of resistance in Indian mustard genotypes [32,38]. In addition, previous studies revealed that susceptible genotypes lack the resistant alleles and are thus more vulnerable to white rust disease [39–42]. Likewise, resistance alleles were described in different *B. juncea* genotypes such as Vniimik-405 [39], J90-2733 [40], BEC-144 [43], BEC-286 [44], NPC-12 [45], Heera and Donskaja IV [22], BIO YSR, EC-399301, JM-1 and JM-2 [46], JMY11 [42] and EC-399299 [32] for different isolates of *A. candida* in different environmental conditions across India. In the present study, the F_2 generations of all S × R crosses (PM-24 × BEC-144, PM-24 × Pusa Karishma, MSTWR-17-15 × BIO YSR), except Pusa Vijay × Donskaja, were best fitted to 3:1 R/S ratio ($p > 0.05$), signifying that the resistance is governed by a single dominant gene in all three resistant genotypes (BEC-144, Pusa Karishma and BIO YSR). Further, it was reconfirmed by employing the corresponding BC_1F_1 generation where resistant and susceptible plants segregated and fitted into 1:1 R/S ratio ($p > 0.05$). Likewise, Singh et al. [47] reported a single dominant gene by analyzing the different breeding generations derived by crossing susceptible and resistant genotypes against AcB1 isolate of *A. candida*. In the present study, the segregation of resistant and

susceptible plants in F_2 generation of S × R cross (Pusa Vijay × Donskaja) did not fit to 3:1 R/S ratio ($p < 0.05$), concluding that resistance is controlled by more than one dominant gene in resistant parent Donskaja. It was also reconfirmed using their BC_1F_1 generation in which resistant and susceptible plants did not segregate into 1:1 R/S ($p < 0.05$) ratio against the Ac-Dli isolate. Likewise, two duplicate dominant resistance genes for Ac-Dli isolate were reported in *B. napus* by screening the breeding populations developed by crossing S × R (BN-38 Sel. × BN-Sel.) genotypes [48]. Similarly, digenic inheritance was proposed in segregating generations that were developed by S × R cross (Varuna of *B. juncea* × ISN 706 of *B. napus*) against Ac-Dli isolate of *A. candida* [49]. However, several earlier studies have also reported that resistance to white rust has been governed by a single dominant gene in different genotypes of *B. juncea* [22,30,39–41,43,44,47].

In the present study with four resistance sources (BEC-144, Pusa Karishma, BIO YSR and Donskaja) it was revealed that the resistance against the Ac-Dli isolate was governed by a single dominant gene in BEC-144, Pusa Karishma, and BIO YSR, whereas more than one dominant gene is controlling the resistance in Donskaja, a widely used donor source. These resistance sources could be more rewarding if they carry different resistance genes and could be employed for achieving durable resistance against multiple isolates of *A. candida* by gene pyramiding through backcrossing. By keeping this view, test of allelism was conducted by crossing the two stable resistant genotypes BEC-144 and BIO YSR, identified in this study, under artificial epiphytotic conditions against Ac-Dli isolate. Interestingly, no segregation (all plants were resistant) in F_2 generation of cross R × R (BEC-144 × BIO YSR) was observed, indicating that the same gene is governing resistance (i.e., allelic to each other) in both the resistant parents, BEC-144 and BIO YSR. In similar studies, allelic relationship between resistance genes was reported in F_2 generations of direct and reciprocal crosses of R × R genotypes (BEC-144, BEC-286, EC399299, Heera and BIO YSR) which were screened against Ac-Dli isolate [32]. In contrast, two dominant genes showing duplicate gene interaction were reported in two resistant *B. juncea* genotypes, BIO YSR and NPC-12, against Ac-Dli isolate [45]. Further, two independent loci governing resistance to *A. candida* race 2V (AcB1) were also tagged in two east European *B. juncea* resistant genotypes viz., Heera and Donskaja-IV [22].

It was reported earlier that only the European genotypes of *B. juncea* showed resistance to white rust whereas genotypes from the Indian gene pool were all susceptible [22,50]. Therefore, two resistant genotypes, BEC-144 and Donskaja, exotic collection from Europe, were widely used in Indian resistant breeding programs with two other resistant Indian genotypes Pusa Karishma and BIO YSR (developed in India which may or may not have a European background). The pedigree of two resistant genotypes BEC-144 and BIO YSR highlighted that they have diverse genetic background, however, they were found to have the same gene for white rust resistance against the Ac-Dli isolate. Nevertheless, these two genotypes may not be rejected as they might have additional genes for different isolates of *A. candida,* which has to be revealed in further studies. The Donskaja (score = 0) and Pusa Karishma (score = 0) were found to be resistant for most of the isolates of *A. candida* collected from the different parts of India when screened under controlled conditions at ICAR-IARI, New Delhi, India [16], whereas, Pusa Vijay (score = 6) and PM-24 (score = 6) were found to be susceptible to Ac-Dli isolate under artificially controlled conditions [16].

It is very much essential to identify diverse resistance genes for white rust and pyramid them in elite breeding lines through breeding. In the fast-changing climatic conditions, it is imperative to identify diverse resistance genes in any crop species to battle the ever-evolving pathogens. In India, in recent times, extensive efforts are underway to horizontally expand the area under mustard cultivation in the non-traditional areas (eastern and southern parts of the country) under a rice-fallow system with a long-term objective of diversifying the existing cropping system. It, therefore, necessitates continuous availability of donor parents with a high level of resistance to white rust. This objective might be accomplished by screening a large set of diverse genotypes/germplasm accessions for different isolates/races of the pathogen under controlled conditions followed by field experiments. Pyramiding

the resistance genes would help in developing durable resistance for most of the *A. candida* races infecting the rapeseed mustard cultivars grown in different states of India. More resistance genes in the host would delay the appearance to new races of the pathogen as the pathogen needs more virulent genes to overcome the resistance level of the host due to its low fitness and reproductivity [51,52]. It is well accepted and proven throughout the world that genetically resistant genotypes are a more durable, reliable, cost effective, and environment-friendly approach to manage biotic stresses, including diseases and pests that adversely affect crop production and quality [18,53].

5. Conclusions

The presence of more than one resistance gene in the Donskaja genotype, as identified in this study, will be useful in breeding for durable resistance by discovering new genes for resistance and further pyramiding them. However, there is a dire need to study the virulence spectra and diversity in *A. candida* with respect to these host resistant genes under controlled condition. The present study also revealed that the resistance to Ac-Dli isolate of *A. candida* is monogenically inherited in both the resistant sources, i.e., BEC-144 and BIO YSR, and the resistance genes present in them are allelic. The backcross breeding program would be rewarding for easy transfer of single genes providing white rust resistance to the well-adapted, high-yielding genotypes lacking disease resistance. Therefore, the information generated in the present study could be used in mustard breeding programs for the development of cost-effective and durable resistance cultivars.

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