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Potential Source of Resistance in Introgressed, Mutant and Synthetic *Brassica juncea* L. Lines against Diverse Isolates of White Rust Pathogen, *Albugo candida*

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Abstract: The existing resistance genes against white rust disease are often ineffective due to racial variation of the causal fungal pathogen, *Albugo candida*. Therefore, new sources of resistance effective against multiple races are needed for durable resistance. Large-scale phenotyping of advanced introgressed (ILs), mutant, and resynthesized (RBJ) lines of *Brassica juncea* L., under artificial inoculation at cotyledonary and true leaf stages, against thirteen diverse isolates of *Albugo candida* and simultaneously at the adult plant stage under multi-location field evaluation from 2019–2022, revealed significant differences in white rust reactions. Amongst 194 introgressed lines, three lines, namely ERJ 39, ERJ 12, and ERJ 15, and three lines among 90 resynthesized and 9 mutant lines, including RBJ 18, DRMR 18-36-12, and DRMR 18-37-13, were identified as potential sources of resistance against multiple isolates at all three developmental stages of the plant. Furthermore, correlation and principal component analysis revealed a positive correlation between white rust resistance at true leaf and adult plant stages for ILs as well as mutant and RBJ lines. These novel sources of host resistance will play vital roles are required for the mustard improvement program and to establish a strong genetic and molecular foundation for identifying white rust resistance linked marker(s), QTLs, or gene(s) for sustainable disease management in India.

Keywords: *Albugo candida*; *Brassica juncea* L.; introgressed lines; mutant lines; resistance; resynthesized lines; screening

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1. Introduction

Indian mustard (*Brassica juncea* L.), belonging to the family brassicaceae, is one of the important oilseed crops, and is currently ranked as the world's third-most important oil seed crop in terms of production and area [1]. In India, among the nine major oilseed crops, soybean (36%), groundnut (28%), and rapeseed and mustard (28% each) contribute to more than 90% of the total oilseed production in the country [2]. Out of the total area under rapeseed and mustard production, more than 80% of the acreage is located in Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh, and Gujarat [3]. Although India has a large land area (61.24 lakh ha) under oilseed *Brassica* cultivation, with a total production of

10.11 million metric tons, it still imports 13.35 metric tons of oilseeds from other countries to meet its domestic requirements [4,5]. A major bottleneck in the productivity enhancement of Indian mustard is recurrent yield loss due to a range of biotic and abiotic stress factors. Most of the rapeseed and mustard crops in India are vulnerable to biotic constraints such as white rust, downy mildew, Alternaria blight, Sclerotinia rot, and powdery mildew [6]. Out of these, white rust disease, incited by *Albugo candida* (Pers. Ex. Lev.) Kuntze, is one of the most destructive diseases affecting brassicaceae crops globally, and has a wide host range of about 400 plant species around the world. Considering the significant yield losses in *Brassica*, it is one of the top ten Oomycete pathogens [7]. To date, around 17 distinct physiological races of *A. candida* have been reported globally. Often the virulence of races significantly differs, making some of the races devastating for a particular species; for example, race 2 in *B. juncea*, race 9 in *B. oleracea*, race 7 in *B. rapa*, race 1 in *Raphanus sativus*, and race 5 in *Sisymbrium officinale* [8–11]. The disease results in both localized and systemic infections that affect all aerial parts of the plant, including the cotyledons, leaves, stem, and inflorescence. The localized infection is characterized by the development of white to cream-colored zoosporangial pustules. On the other hand, systemic infection on meristems and inflorescences causes "stagheads", or hypertrophied or malformed racemes, leading to no seed production [10]. Stagheads (inflorescence nerves) may also appear during the latter part of the growing season as a result of meristematic host tissue contamination [12]. In India, Australia, and Canada, the pathogen has been linked to yield losses in *B. juncea* ranging from 20 to 60% [13,14]. The disease has the greatest impact in India because of the susceptibility of nearly all of the newly released commercially grown cultivars [13]. While many chemicals and cultural techniques have been proposed to control this disease [15–17], the alternative approach, such as the breeding of genetic resistance into the cultivars, is regarded as one of the most cost-effective and environmentally friendly methods for disease management.

The process of domestication and continuous selection for yield has resulted in a narrow genetic base for the *B. juncea* cultivars. However, previous studies have identified resistance sources in the gene pool of oil brassica including the Indian mustard. Arora et al. reported resistance against six isolates collected from the northern regions of India in the Indian mustard variety Donskaja-IV and identified a single CC-NB-LRR protein-coding R gene (BjuWRR1) as providing resistance against white rust in the European variety [18]. Another gene WRR12 has also been identified in *A. thaliana* which confers resistance to *A. candida* race 9 that infects *B. oleracea* [19]. Even though there are, at present, sources available for white rust resistance, the existing sources are often ineffective against new races of pathogens or multiple races of the same pathogen. To create new variability in *Brassica* sp. for genetic resistance, three major approaches include: resynthesis, which is the process of incorporating diversity from a progenitor species; introgressing variation from a related species; and mutagenesis through physical or chemical methods [20,21]. The goal is to create novel genetic or phenotypic variation due to intergenomic recombination between the parent species' chromosomes and other polyploidy-related outcomes [22]. In addition, analysis using allozymes and genetic markers has further demonstrated that resynthesized genotypes are excellent diversity conduits for the *Brassica* crop [23,24]. Prior to this, Hasan and Rahman (2018) [25] created clubroot-resistant *B. juncea* through resynthesis by mating two susceptible *B. nigra* lines with a resistant genotype of *B. rapa*. This resulted in the formation of the clubroot-resistant line of *B. juncea*. Additionally, due to an infection brought on by *A. brassicae*, resistance to the *Brassica* leaf blight has been transferred from *B. hirta* to *B. juncea* [26]. The identification of R-genes is the first and foremost step in developing disease-resistant varieties through breeding programs. Previous studies have shown that the white rust resistance in different *Brassica* species can be governed by a single dominant gene, one or two dominant genes with epistatic effects, an additive dominant gene with epistatic effects, or a partial resistant single recessive gene *wpr* [27]. As a result of environmental and pathogenic variation, some R-genes interact differently with *Brassica* genotypes in different environments. Previous work has identified a few genotypes with resistance, but dynamic

changes in pathogen race composition and scanty screening with a small number of isolates have frequently resulted in short-lived host resistance in improved varieties, necessitating the identification and characterization of novel sources of white rust resistance from diverse genetic backgrounds. Therefore, understanding the interactions of *Brassica* genotypes with *A. candida* is essential in determining resistant genotypes with specific abilities to adapt and exploit for further improvements. Understanding the variability of responses of different germplasm to *A. candida* infections is critical, as is strengthening the breeding programme to incorporate resistance genes against the white rust pathogen in oilseed *Brassica*.

Field evaluation is the most commonly used method for identifying resistant sources; however, it is resource intensive and requires repeated testing to confirm the consistency of reactions, as there is a risk of disease escape due to low or poor inoculum concentration or potential, disease pressure, and other factors. Considering these problems, the current study aimed to identify and validate resistant sources in advanced introgressed, mutant, and resynthesized lines of *B. juncea* against the most prevalent virulent pathotypes of *A. candida* collected from major mustard growing hotspot locations across India under artificially inoculated conditions. Simultaneously, rigorous multilocational field testing was conducted in open fields to identify potent sources of resistance against the highly virulent isolates and races of *A. candida*. These sources can be employed as resistant donors in the backcross transfer of white rust resistance in popular mustard varieties for a successful mustard breeding program.

2. Materials and Methods

2.1. Plant Material Acquisition

For the present study, a diverse set of 194 advanced introgressed (ILs), 90 resynthesized *B. juncea* (RBJ) and 9 advanced stable mutant lines developed by the ICAR-National Institute for Plant Biotechnology (ICAR-NIPB), New Delhi, and ICAR- Directorate of Rapeseed-Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan were systematically evaluated against 13 prevalent isolates of white rust (*A. candida*) collected from major mustard growing region of India at the cotyledonary (7–10-day old seedlings), true leaf (21–25-day old seedlings), and adult plant stages (45 days) under controlled and natural environmental conditions.

2.1.1. Development of *B. juncea* Introgression Lines

B. juncea introgression lines were developed at ICAR-NIPB, Delhi, as described by Vassupalli et al. [28], using resistant *Diplotaxis erucoides* (D^eD^e, 2n = 14) as the donor parent and susceptible *B. juncea* (RLM198) (AABB, 2n = 36) as the recurrent parent. Initially, *B. rapa* was used as a species to bridge the ploidy gap between the donor and the recurrent parent. The synthetic amphidiploid "eru-rapa" was developed through wide hybridization between *B. rapa* (AA, 2n = 20) and *D. erucoides* (D^eD^e, 2n = 14) followed by embryo rescue and colchicine treatment [28]. Finally, the synthetic amphidiploid was backcrossed twice with *B. juncea* (RLM 198) [29]. The population was advanced by repeated selfing until the BC_2F_{10} generation, which was then used in this study.

2.1.2. Development of *B. juncea* Mutant Lines

The mutant population was created using both physical and chemical mutagenesis methods. Genetically pure seeds of Indian mustard cultivar RH 749 were irradiated with different doses of γ-rays (100 kR) at the Bhabha Atomic Research Centre (BARC), Mumbai, and then treated with 0.05% of the chemical mutagen ethyl methane sulfonate (EMS) at the ICAR-DRMR, Bharatpur. The mutants were advanced by continuous selection and selfing. Populations from the M_9 generation onwards were used for the proposed study.

2.1.3. Development of Resynthesized *B. juncea* Lines

The resynthesized *B. juncea* (RBJ) lines used in this study were developed at ICAR-NIPB, New Delhi, by crossing the parental species, *B. rapa* (AA, 2n = 20) and *B. nigra* (BB,

2n = 16), followed by an in vitro (embryo rescue) and in vivo approach to develop the synthetic amphihaploid. These amphihaploids were then subjected to colchicine treatment for chromosome doubling. The selfing was performed in subsequent generations for the stability and advancement of the material till the S_8 generation, which was used for the current study (Figure 1).

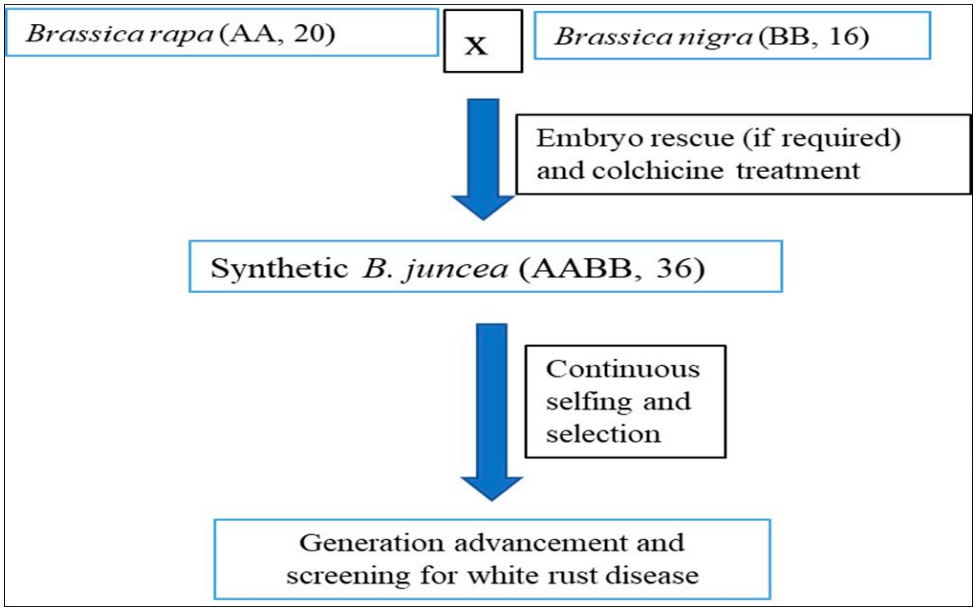

Figure 1. Schematic diagram representing the development of resynthesized *B. juncea* (RBJ) lines by crossing *B. rapa* with *B. nigra*.

2.2. White Rust Pathogen (A. candida)

2.2.1. Collection of *A. candida* Isolates

A. candida -infected leaf samples were collected from cultivated brassicaceae host species in the following mustard growing and disease hot spot locations in India's various agro-climatic regions: Punjab (*Ac-Ldh*, Ludhiana: 30.9010° N, 75.8117° E), Haryana (*Ac-Hsr*, Hisar: 29.7868° N, 77.1301° E), Gujarat (*Ac-Skn*, SK Nagar: 21.1828° N, 72.8571° E), Rajasthan (*Ac-Bpr*, Bharatpur: 27.1987° N, 77.4573° E), Delhi (*Ac-Ndl*, Pusa: 28.9000° N, 77.2114° E), Uttar Pradesh (*Ac-Ayo*, Ayodhya: 26.8202° N, 81.8845° E; *Ac-Met*, Meerut: 28.9693° N, 77.7405° E), Uttarakhand (*Ac-Pnt*, Pantnagar: 29.0229° N, 79.4879° E), Madhya Pradesh (*Ac-Mor*, Morena: 26.4795° N, 77.9890° E), Bihar (*Ac-Smt*, Samastipur: 27.7984° N, 85.4582° E), Jharkhand (*Ac-Ran*, Ranchi: 23.3602° N, 85.3413° E), Tamil Nadu (*Ac-Wlg*, Wellington: 11.3798° N, 76.7738° E) and Karnataka (*Ac-Dha*, Dharwad: 15.4891° N, 74.9814° E). The isolates were chosen based on their geographical distribution, and preliminary screening studies revealed variability in their virulence. Using GPS technology, the latitude, longitude, and altitude of each sampled location were recorded and geotagged.

2.2.2. Genetic Variability of the Isolates

To investigate the genetic diversity of the selected isolates DNA sequence analysis of the Internal Transcribed Spacer (ITS) region of rDNA was done. DNA was isolated using the fungal DNA isolation kit (Quick-DNA™ Fungal/Bacterial Miniprep Kit, Zymo research, Irvine, CA, USA) following the standard protocol. The PCR reaction for ITS was performed using the forward primer ITS1 (5′-TCC-GTA-GGT-GAA-CCT-GCG-G 3′) and reverse primer ITS4 (5′ TCC-TCC-GCT-TAT-TGA-TAT-GC 3′) [30] The 1.2 µL template DNA (50 ng), 1.0 µL of the forward and reverse primers each, 1.25 µL of 10 mM dNTP (Thermo fisher Scientific™, Waltham, MA, USA), 0.2 µL of Taq DNA polymerase (Thermo fisher Scientific™), 1.25 µL of 10X Buffer A (With 17.5 mM $MgCl_2$) (Thermo fisher Scientific™), and 23.6 µL of Nuclease free water. The PCR protocol for ITS primer was as 5 min initial

denaturation step at 94 °C followed by 35 cycles of amplification consisting of 1 min denaturation at 95 °C, 1 min of annealing at 55 °C and 2 min of extension at 72 °C, with an extra extension step of 7 min at 72 °C and storage at 4 °C. The PCR product was then resolved on 2% agarose gel and purified from the gel using a PCR clean up kit (PureLink PCR Purification Kit, Thermo fisher Scientific™, Waltham, MA, USA). The purified product was then sequenced through sanger sequencing (Eurofins and Barcode biosciences, Bengaluru, India) and the sequences were compared to the other sequences in NCBI database through BLAST (http://blast.ncbi.nlm.nih.gov). Further, sequences were aligned and phylogenetic tree was constructed using MEGA11 (Tamura, Stecher, and Kumar 2021) along with the sequences downloaded from the ncbi database (GQ328840, GQ328843, AY929829, MK067078, AY929834, KJ941074).

2.3. In Vitro and In Vivo Screening for the Identification of Resistant Genotypes

2.3.1. Preparation of Inoculum and Artificial Screening

The inoculation technique for the white rust (*A. candida*) pathogen [31,32] was standardized for large-scale screening of the introgressed, mutant and resynthesized lines in glasshouse under controlled environmental conditions with minor modifications. Zoosporangial powder collected from a single pustule of the infected leaf was used to inoculate susceptible *B. juncea* cv. "Pusa Jai Kisan" seedlings and maintained under sterile conditions in phytotron chamber to obtain purified cultures of the pathogenic isolate. As the disease progressed the pure cultures were collected into empty gelatin capsules (Patco Pharma, Mumbai, India) with a sterile scalpel from mature pustules of freshly collected infected leaves, then packed in a falcon tube wrapped in parafilm and stored at −20 °C to ensure the availability of inoculum throughout the year.

For artificial screening of germplasm seeds were planted in three replications in plastic trays of 4.0 cm diameter and 9.0 cm depth, filled with a double-sterilized mixture of soil + compost + sand (3:1:1), and stored in the glasshouse. One set is for the inoculation of cotyledons, and another is for the true leaf inoculation. Simultaneously, susceptible *B. juncea* cultivars "Pusa Jai Kisan" and "Varuna" were sown in each tray as controls for inoculation and comparing the disease reactions. Prior to inoculation, 50 mg of zoosporangial powder was dissolved in 100 mL of sterile, double-distilled water by stirring with a glass rod in a 500 mL beaker to disperse the sporangia. The inoculum concentration was adjusted to 2×10^4 zoosporangia mL^{-1} using a hemocytometer. The inoculum suspension was incubated at 13 °C for 2 h and then kept at room temperature (20 °C) for 15 min to trigger zoospores release. The presence of motile zoospores was confirmed using a microscope (Leica DM750/Carl Zeiss Axiolab5). Seedlings were sprayed with sterile distilled water to eliminate any soil particles from their surface and allowed to dry at room temperature for 1 h. The inoculum was then carefully applied to the adaxial surface of each lobe on 7-day-old cotyledonary leaves at growth stage 1 (GS 1) and 21-day-old true leaves using micropipettes (15 µL each droplet) [33]. During the mustard growing seasons from 2019 to 2022, inoculated plants were kept inside separate moist chambers in a glasshouse at ICAR-NIPB in New Delhi at 16/15 ± 1 °C Day and night temperature and >90% relative humidity, with a 16-h photoperiod and 8-h of darkness. Furthermore, for successful infection and disease development, the moist chamber was covered with a light-coloured tarpaulin sheet after inoculation and filled with water up to 2–3 cm height to maintain humidity (>99%) (Figure 2). After 15 days of inoculation, data on terminal disease severity was recorded on both crop stages.

Figure 2. Figure showing: (**A**) in vitro evaluation in moist chamber; (**B**) using drop inoculation technique; (**C**) evaluation of white rust disease under natural field conditions and (**D**) white rust infected leaf.

2.3.2. Natural Field Screening

Field screening was carried out under natural epiphytic conditions at various mustard growing sites during the crop seasons from 2019 to 2022 at eight white rust hotspot locations namely Delhi (Ndl), Bharatpur (Bpr), Pantnagar (Pnt), Ludhiana (Ldh), Wellington (Wlg), Samastipur Bihar (Bih), SK Nagar (Skn), and Ranchi (Ran). The field experiments were planted on the 15th to 20th of November every year at all eight locations from 2019 to 2022 under late-sown conditions in anticipation of favourable weather conditions for the development of early white rust disease. Line Sowing was done with three replications per treatment, with two rows of each germplasm 3 m long and a 15 cm plant-to-plant distance. Susceptible checks were sown after every five rows of test germplasm lines, along with border rows and infector rows (Pusa Jai Kisan and Varuna). After 15 days of germination, the plants were thinned out. Regular agronomical practices, including recommended fertilizer doses and four irrigations, were followed. To create a heavy inoculum load under field conditions, a zoosporangial suspension of the pathogen was prepared and sprayed twice directly on plants in the field at 10-day intervals at the time of flowering initiation in the evening. Irrigation was performed immediately following inoculation, and water was sprayed on a regular basis to maintain high humidity for three days after inoculation. The appearance of disease symptoms on test plants was monitored on a regular basis (Figure 2).

2.4. White Rust Evaluation

The white rust severity and disease reaction observations were recorded at the peak of disease pressure. In each line, 10 plants were chosen at random from each germplasm and tagged to record visual disease ratings. For both controlled and natural conditions, the disease index was calculated based on the percentage of leaf area infected on a scale of 0 to 9 (Table 1). Plants with a disease rating of 0 were considered immune; plants with a disease rating of 1–3 were considered resistant; and plants with a disease rating of >5 were considered susceptible. The percent disease index (PDI) for white rust was calculated on a 0–9 scale as approved by AICRP-RM plant pathologists [34], where N1 to N6 represent the frequency of leaves in the respective scores.

$$\text{Percent disease index} = \frac{(N1 \times 0) + (N2 \times 1) + (N3 \times 3) + (N4 \times 5) + (N5 \times 7) + (N6 \times 9)}{\text{Number of leaf samples} \times \text{Maximum disease rating } (9)} \times 100 \quad (1)$$

Table 1. Phenotypic observations as a rating (0–9) scale for measuring disease severity and reaction to *A. candida* as approved by AICRP-RM, 2012.

Grade	Description	Disease Reaction
0	Immune (I)	There is no sign of infection or pustules
1	Highly Resistant (HR)	Less than 5% leaf area covered by lesions, light necrotic flecks, no sporulation.
3	Moderately Resistant (MR)	Pustules cover 5–10% of the leaf area; there is heavy necrotic flecking but no sporulation; and the infection is limited to the plant's lower leaves.
5	Susceptible (S)	Disease symptoms cover 11–25% of the leaf area, with numerous pustules appearing on lower surface of the leaves
7	Moderately Susceptible (MS)	Disease symptoms cover 26–50% of the leaf area, with large, scattered pustules coalescing on the lower surface leaves.
9	Highly Susceptible (HS)	White rust pustules cover more than half of the leaf area. Large coalescing pustules on the lower surface of leaves with staghead formation

2.5. Data Analysis

Experimental data from three years of disease scoring from artificial as well as open field evaluations of germplasms were generated, pooled (after checking for the homogeneity of error variances), and analysed to find consistency in immunity and resistance reactions in white rust entries. Data obtained (angular transformation) under controlled environmental conditions were analysed using ANOVA (SPSS) in respect of disease reaction at the cotyledonary, and true-leaf stage. The mean values of plants within a replication were used for statistical analysis. Critical differences (CD) were calculated at the 5% probability level of significance for comparison of genotype means. For field trials individual ANOVA was performed for each location to study the effect of germplasm. After checking the homogeneity of error variances of the different trial location and applying suitable transformations (Aitkin's transformations) in case of heterogeneous error variances, combined analysis was performed to study the effect of location and interaction of genotype and location along with the effect of genotype. Using R studio, correlation analysis and PCA were used to decipher the relationship between the cotyledonary, true leaf, and adult plant stages, as well as to understand the genotype and environment interaction.

3. Results and Discussion

In the present study, a total of 194 introgression lines (ERJ) and 9 mutants, as well as 90 resynthesized *B. juncea* lines, were screened at the cotyledonary leaf (CL) and true leaf (TL) growth stages under artificial conditions and at the adult plant stage in the field under natural conditions. As the expression of host resistance is reflected through the severity of white rust disease, based on the percent disease index of white rust, promising germplasm exhibiting immune or highly resistant reactions against thirteen isolates of *A. candida* was identified. Among the tested germplasm (194 ILs, 90 RBJs, and 9 mutant lines), a wide range of reactions were observed, which varied from being immune or fully free from the disease with the NN type interaction phenotype (no infection and no sign of pustules on either side of the leaf surface) to being highly susceptible (Table 1).

3.1. Genetic Variability of the Selcted Isolates

ITS sequences of thirteen isolates from major mustard growing locations with a nucleotide length of 542 bases were used for sequence alignments. The evolutionary history was inferred by using the Neighbour Joining method based on the Tamura-Nei model [35]. Based on the phylogenetic tree Ac-Ayo, Ac-Ran and Ac-Mor were clubbed together to form a monophyletic group (Figure 3). Similarly, Ac-Wlg and Ac-Ndl were clustered together in a monophyletic group and formed another cluster. However, Ac-Skn, Ac-Bpr, Ac-Met, Ac-Smt, Ac-Pnt, Ac-Hsr, Ac-Ldh and Ac-Dha formed different clusters. According to the phylogenetic tree, Ac-Ldh was found out to be the most diffferent as compared to the other clusters that have derived from the same node. The molecular characterization of these 13 A. candida isolates with the ITS gene clearly showed significant variability among

different selected isolates. This also indicates association of geographical regions and the variability amid A. candida isolates. Such variability in the population might be the result of different selection pressure on pathogen due to availability of a host species for survival and infection and difference in agro-climatic conditions of 13 diverse mustard growing regions of India. Therefore, identification of the genetic diversity provides a good resolution in the differentiation of A. candida isolates.

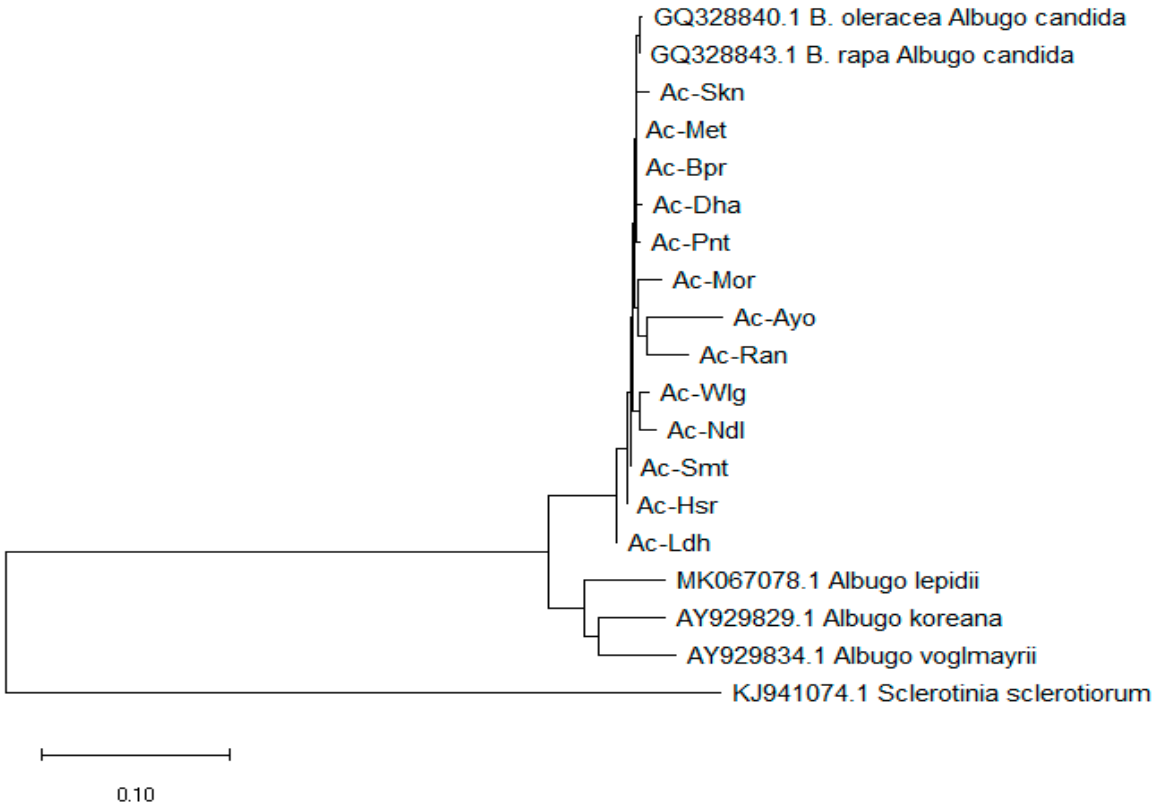

Figure 3. Neighbour Joining phylogenetic tree based on the ITS gene showing the relationship of thirteen Indian A. candida isolates collected from major mustard growing regions.

3.2. Screening of Introgressed Lines

Introgressed lines of *B. juncea* were developed through interspecific-crossing between the wild plant, *Diplotaxis erucoides* (D^eD^e, 2n = 14) which shows immunity against multiple isolates of *A. candida* and therefore is used as the donor parent, and the highly susceptible cultivar, RLM 198 (AABB, 2n = 36) as the recurrent parent (Table 2). Among the 194 ILs of *B. juncea*, ERJ 39, ERJ 12, and ERJ 15 showed significant potential for white rust resistance against the highest number of isolates of the pathogen (Figure 4). Both ERJ 39 and ERJ 12 demonstrated an immune response (PDI = 0) characterized by NN type interaction phenotypes (no infection and no sign of pustules on either side of the leaf surface) across all the growth phases, including the cotyledonary and true leaf stages under artificial infection and the adult plant stage in field trials, against five isolates of *A. candida*: *Ac-Ndl*, *Bpr*, *Pnt*, *Ldh*, and *Ran* (Tables 3 and 4). Because white rust resistance is governed by a single gene, such genetic resistance at all the growth stages is practically ideal for breeding approaches, which is highly desirable for further introgression and commercial cultivation of *B. juncea* L. varieties in India.

Table 2. Percent disease index for the donor parents used to develop introgressed and resynthesized lines against pan Indian white rust pathogen (*A. candida*) isolates at cotyledonary leaf (CL) and true leaf (TL) plant growth stages under controlled conditions.

Parent Details	*Ac-Ndl*		*Ac-Met*		*Ac-Bpr*		*Ac-Pnt*		*Ac-Ldh*		*Ac-Hsr*		*Ac-Ayo*		*Ac-Wlg*		*Ac-Dha*		*Ac-Smt*		*Ac-Mor*		*Ac-Skn*		*Ac-Ran*	
	CL	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**
Parents used for the development of Introgression lines																										
D. erucoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RLM 198	61.8	83.5	39.2	54.5	36.7	50.3	47.7	71.8	72.3	44.2	62.0	78.8	30.8	52.5	36.5	74.8	55.3	82.3	90.2	59.8	33.5	56.2	77.5	62.8	20.5	44.3
Parents used for the development of Resynthesized *B. juncea* lines																										
Rapa 9	0	0	0	0	3.0	0	9.0	19.7	9.7	22.0	30.3	18.8	34.7	7.3	3.5	10.0	17.9	4.0	2.0	10.0	3.5	4.8	3.7	8.3	5.0	27.8
YSH401	3.5	2.7	32.5	37.7	8.3	23.3	45.3	59.8	23.2	9.5	4.7	6.2	1.7	0	0	0	0	3.3	7.6	3.5	21.2	4.8	18.2	30.7	7.2	22.0
IC0623820	4.5	8.3	39.3	55.5	9.2	18.3	7.7	32.8	3.3	0	54.7	38.8	15.7	4.5	8.8	2.7	18.7	0	2.5	20.5	8.1	20.3	4.3	16.8	4.8	3.2
Pusa Gold	49.3	70.5	36.3	54.2	55.8	21.2	39.8	94.0	8.7	42.8	54.3	83.2	56.7	41.8	32.5	61.3	44.8	78.7	20.5	27.3	14.2	51.2	65.5	22.0	24.8	30.7
Tobin-1	3.0	9.0	0	0	2.5	19.5	2.8	9.7	8.2	21.2	4.7	7.5	17.7	18.8	17.3	7.7	3.0	8.2	4.1	9.3	0	0	0	0	4.7	8.3
Tobin-2	0	0	1.7	0	9.2	6.8	0	0	11.5	9.7	0	0	2.3	0	20.3	12.8	14.5	20.7	5.0	9.3	11.3	4.2	0	9.2	0	0
IC-257	21.5	10.0	17.7	8.2	42.5	17.8	18.7	9.5	0	5.7	8.3	7.7	14.5	35.2	18.7	26.3	8.3	0	6.7	29.8	5.0	9.8	0	7.7	4.7	0
BN-PI-459012	15.8	21.5	21.2	36.6	52.3	22.7	8.0	4.5	0	0	29.8	2.8	19.3	8.2	6.5	9.5	27.7	48.3	9.3	2.7	35.2	8.3	22.5	43.2	0	0
EC426390	0	2.7	0	2.7	15.7	0	24.3	39.2	3.2	8.3	16.0	9.3	4.0	19.8	14.5	2.8	49.8	7.5	8.2	4.5	32.8	23.6	19.2	4.5	2.5	4.5
EC472704	0	18.7	3.3	15.3	12.7	38.2	21.2	40.8	22.5	4.3	0	6.5	52.3	27.0	39.7	21.2	24.8	27.5	33.3	6.8	3.7	7.7	37.8	27.2	24.5	57.5

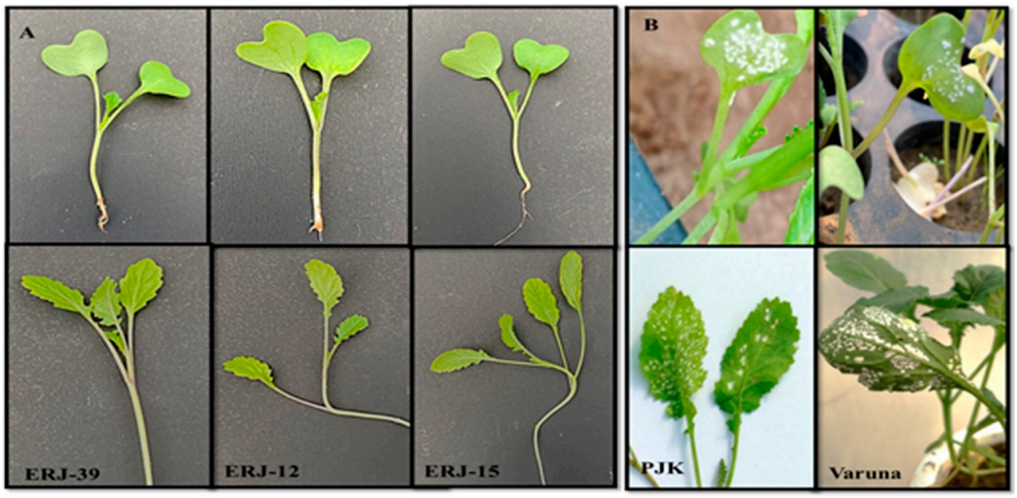

Figure 4. Figure showing (**A**) immune response in introgressed lines (ERJ) at cotyledonary and true leaf stages under artificially inoculated conditions and (**B**) susceptible reaction in cv., 'Pusa Jai Kisan' and 'Varuna'.

Introgressed line ERJ 39 also expressed an immune response against *Ac-Hsr* and *Ac-Met* at both cotyledonary and true leaf stages. For *Ac-Mor* and *Skn* isolates, immunity was only observed for the cotyledonary phase, and moderate resistance was observed with PDI values of 7.2 and 7.5 at true leaf stage, respectively (Table 3). Furthermore, immunity to *Ac-Smt* was also found in ERJ 39 at the cotyledonary and adult plant stages but not at the true leaf stage, where moderate resistance was seen (PDI = 8.7%). Such an immune response in the cotyledonary phase to *A. candida* infection is highly desired because the plants can avoid systemic spread of the disease and staghead formations caused by hypertrophy or hyperplasia, which can lead to significant yield losses [13]. This introgressed line showed immunity to resistance responses at all plant growth stages to a range of isolates obtained from different agro-climatic zones, thus making it superior to all of the other introgression lines evaluated.

In the case of ERJ-12, immunity was recorded at the cotyledonary and true leaf stages against *Ac-Met* and *Ac-Mor* when tested artificially. However, for *Ac-Hsr*, *Ayo*, *Dha*, *Smt*, and *Skn*, immunity was only seen at the cotyledonary stage, but high (PDI = 1–5%) to moderate resistance (PDI = 6–10%) was demonstrated at the true leaf and mature plant stages. This type of shift in resistance to plant diseases linked to the transition from the juvenile to the adult phase is demonstrated in *A. thaliana* as well [36]. Coelhoe et al. [37] made similar observations in resistance at different growth stages in *B. oleracea* against downy mildew, suggesting that the age of the plant is also one of the factors on which resistance levels can depend based on the ability of the test pathogen to infect.

ERJ 15, another potential germplasm, expressed a complete immune reaction against four isolates, *Ac-Bpr*, *Pnt*, *Ldh*, and *Ran*, at all three growth stages of the plant, i.e., cotyledonary, true leaf, and adult plant stages.

This germplasm also demonstrated immune responses for isolates *Ac-Met*, *Hsr*, and *Ayo* in artificial trials at both the cotyledonary and true leaf stages. In the case of *Ac-Smt* and *Ac-Ndl* isolates, immunity was observed at the cotyledonary stage. However, at the true leaf stage, the same lines reacted as highly resistant (PDI = 1–5%). Under field experiments, however, PDI scores of 0 (immune) and 3.3 (high resistance) were reported for *Ac-Smt* and *Ndl*, respectively. For *Ac-Dha*, which was sourced from the southern part of India, a susceptible reaction was seen at the cotyledonary stage (PDI = 11.2) and moderate resistance (PDI = 9.3) at the true leaf stage. Moreover, in the case of *Ac-Wlg*, susceptible to moderately susceptible responses were observed across all the plant growth stages (Tables 3 and 4). This illustrates the contrast in virulence between the northern and southern Indian races of the test pathogen.

Table 3. Percent disease index for introgressed lines against white rust pathogen (*A. candida*) isolates obtained from major mustard growing locations in India at cotyledonary leaf (CL) and true leaf (TL) plant growth stages under controlled conditions (pooled data).

Genotype	*Ac-Ndl*		*Ac-Met*		*Ac-Bpr*		*Ac-Pnt*		*Ac-Ldh*		*Ac-Hsr*		*Ac-Ayo*		*Ac-Wlg*		*Ac-Dha*		*Ac-Smt*		*Ac-Mor*		*Ac-Skn*		*Ac-Ran*	
	CL	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**
ERJ 3	19.7	0	9.5	3.5	20.7	3.5	9.5	2.7	20.8	3.0	22.5	7.3	8.5	8.3	12.2	10.0	21.8	7.8	19.8	3.8	50.8	24.5	40.5	0	28.2	4.5
ERJ 5	0	0	3.3	9.3	22.5	57.7	0	4.3	28.7	54.2	4.2	0	8.8	4.2	9.3	41.8	8.7	8.7	4.2	9.0	33.7	41.2	0	0	4.7	21.2
ERJ 7	9.3	3.8	8.7	8.2	42.7	22.3	31.8	8.2	31.7	21.5	8.3	19.2	4.2	9.5	4.5	34.3	7.2	14.3	3.3	8.2	20.3	15.3	16.7	21.3	2.5	0
ERJ 9	29.8	15.7	8.3	8.3	19.8	40.2	22.3	55.5	22.3	45.7	9.7	18.5	0	0	0	9.2	0	0	38.7	9.3	19.2	32.8	38.5	30.7	54.3	72.8
ERJ 12	0	0	0	0	0	0	0	0	0	0	0	4.3	0	9.3	3.7	15.7	0	7.5	0	8.7	0	0	0	4.5	0	0
ERJ 13	0	0	0	4.7	0	0	0	0	16.2	22.3	0	8.7	3.2	9.8	9.2	0	4.3	12.7	0	4.2	17.8	26.2	7.3	32.8	3.8	17.3
ERJ 14	0	6.3	0	4.0	0	7.3	18.7	0	0	0	2.8	9.2	4.3	13.2	22.3	20.5	14.8	26.3	0	5.0	6.7	0	0	14.2	0	0
ERJ 15	0	4.0	0	0	0	0	0	0	0	0	0	0	0	0	18.7	43.2	11.2	9.3	0	4.3	23.2	31.8	12.8	9.8	0	0
ERJ 16	8.5	17.7	4.5	7.8	8.3	7.7	8.7	0	23.3	7.7	0	0	46.7	72.8	10.0	50.3	12.7	18.2	16.2	3.2	32.8	2.7	33.2	8.3	34.7	9.2
ERJ 17	14.7	9.3	9.3	8.0	9.2	21.8	0	7.7	18.8	42.5	0	0	12.8	22.3	23.3	12.8	15.5	27.7	28.5	9.3	47.2	6.3	0	9.2	0	0
ERJ 19	16.5	10.0	17.7	23.2	6.8	28.2	32.5	40.8	15.2	27.7	8.3	7.7	14.5	20.5	6.8	0	8.3	0	43.8	2.8	6.7	0	0	7.7	4.7	0
ERJ 20	32.2	6.8	8.2	0	4.2	21.3	14.2	4.2	18.3	4.3	0	6.8	9.2	16.2	8.5	0	6.7	0	37.7	4.2	14.8	8.2	0	26.3	27.2	34.3
ERJ 32	19.8	8.7	24.2	39.8	0	0	19.3	10.0	17.5	9.2	3.2	4.2	16.7	7.7	0	8.7	0	6.7	44.3	12.8	23.5	9.8	0	24.5	3.3	8.8
ERJ 33	0	4.5	3.3	4.3	3.3	17.8	18.7	21.2	3.3	12.5	0	3.5	15.3	9.3	59.7	21.2	24.8	27.5	13.3	3.3	3.7	7.7	37.8	27.2	57.5	16.5
ERJ 38	4.2	0	3.8	8.7	0	0	3.3	12.8	0	0	9.5	20.7	8.2	10.0	0	3.8	4.7	0	0	2.5	4.2	9.3	24.3	21.3	4.2	14.2
ERJ 39	0	0	0	0	0	0	0	0	0	0	0	0	4.8	3.2	4.2	2.7	2.3	9.2	0	8.7	0	7.2	0	7.5	0	0
ERJ 40	0	0	0	0	4.2	0	0	0	0	0	0	4.3	9.3	7.8	72.8	22.3	43.8	18.8	9.2	15.2	2.8	9.5	7.7	7.7	0	0
ERJ 41	8.3	7.7	7.8	4.2	8.7	3.2	24.2	7.0	34.7	8.3	11.7	7.8	3.2	6.3	66.3	42.5	51.5	28.7	3.7	12.7	0	0	6.3	8.8	6.7	8.5
ERJ 44	24.2	12.3	4.2	17.7	0	12.7	44.2	3.2	4.3	7.7	4.2	12.2	8.8	11.5	33.7	5.0	26.7	8.2	36.3	9.3	5.0	6.8	62.5	34.2	3.2	4.3
ERJ 47	0	3.3	8.2	14.3	0	9.5	8.8	4.7	15.7	27.5	6.8	14.5	12.7	9.2	3.5	18.8	17.3	12.8	29.8	8.2	0	9.0	57.2	38.7	1.8	3.2
ERJ 54	8.7	8.8	0	2.7	2.3	26.8	9.3	2.8	26.3	35.2	7.8	17.8	9.5	23.7	4.2	9.3	6.2	7.7	6.7	19.8	4.7	2.3	65.7	78.5	3.5	32.8
ERJ 76	23.8	7.2	0	3.5	8.2	14.7	0	4.2	14.2	21.8	9.5	3.2	10.0	16.8	20.8	36.2	18.5	12.2	4.3	16.3	3.3	3.2	24.3	42.7	12.7	15.3
ERJ 78	14.3	3.7	9.7	4.0	7.5	2.7	3.7	17.8	6.3	16.7	8.3	18.7	17.8	9.3	3.3	45.7	7.7	19.3	14.8	35.2	0	4.5	0	16.2	19.3	9.2
ERJ 90	20.5	0	8.3	24.2	7.8	22.3	4.0	0	39.7	32.8	9.2	0	7.2	9.5	0	20.5	14.8	7.5	59.7	46.8	0	0	19.2	36.8	54.2	69.7
ERJ 99	0	0	0	8.8	9.8	13.2	0	8.2	8.2	8.5	0	19.3	8.3	13.7	30.2	74.8	41.2	65.7	8.2	10.0	8.2	18.3	12.8	8.2	38.5	47.8
ERJ 103	12.2	8.2	0	8.2	7.2	4.5	22.8	0	13.8	8.2	13.7	8.2	11.5	24.2	3.7	28.7	13.7	16.2	35.5	13.7	3.5	0	26.7	28.3	14.8	0
ERJ 108	27.8	52.8	16.3	9.2	24.7	42.3	8.3	36.7	0	0	29.2	16.7	14.7	43.3	15.3	21.2	16.3	42.3	16.2	8.5	18.8	26.8	7.8	6.7	9.2	17.7
ERJ 109	8.8	0	0	0	6.3	9.2	0	0	0	0	3.3	0	9.5	12.8	12.8	18.3	20.5	34.8	0	4.2	0	3.2	0	0	0	0
ERJ 110	9.7	0	0	0	0	7.7	0	0	0	0	3.2	7.8	8.2	13.7	0	68.3	12.7	46.2	24.5	17.3	2.3	17.7	53.2	33.2	0	0
ERJ 125	13.3	17.7	14.8	7.3	2.3	4.2	0	8.5	2.8	7.7	4.7	13.7	7.3	15.5	22.3	58.8	15.2	47.8	8.7	9.2	0	4.3	4.7	3.5	0	6.3
ERJ 127	0	8.3	0	6.7	0	0	16.7	9.3	8.0	13.2	14.8	19.5	25.0	17.8	41.5	70.2	21.3	51.3	0	4.7	23.2	9.5	26.5	40.2	0	0
ERJ 157	11.8	6.7	0	3.8	14.8	2.3	0	2.3	0	0	3.7	7.3	12.8	9.2	4.2	7.3	3.7	4.2	8.2	7.8	0	3.2	3.3	15.5	12.7	4.5
ERJ 158	0	0	0	3.5	0	0	0	8.0	9.2	33.3	7.8	0	4.2	7.3	0	6.8	0	2.7	0	8.7	0	4.7	0	23.8	0	0
ERJ 159	18.7	2.3	5.0	0	4.2	6.8	2.7	12.7	7.5	2.8	0	6.7	10.0	12.8	21.5	46.5	12.8	49.3	0	7.2	0	2.8	31.8	64.3	0	0
ERJ 160	12.8	9.5	3.2	7.2	41.7	23.2	0	0	0	0	5.0	9.2	13.5	14.7	27.7	36.3	14.3	54.8	4.3	13.8	9.2	4.3	33.7	17.7	36.3	9.7
ERJ 161	12.3	3.8	7.3	9.8	37.8	23.8	4.5	20.8	9.3	8.7	4.3	0	6.7	9.3	42.3	30.7	54.5	68.3	0	17.3	7.8	2.7	40.2	56.5	16.5	23.2
ERJ 165	16.5	8.7	0	2.5	24.3	4.8	23.2	7.7	33.2	38.5	8.5	13.8	14.8	23.5	36.8	44.2	23.3	55.0	12.8	16.2	36.3	2.3	28.3	61.2	5.0	9.3
ERJ 182	0	9.2	2.7	9.3	8.2	3.8	0	6.8	5.0	9.0	6.7	0	3.7	4.2	29.2	23.7	17.7	36.8	3.3	3.8	12.7	19.2	3.7	8.3	3.3	9.2

Table 4. Percent disease index for introgression lines against white rust pathogen (*A. candida*) at adult plant stage under multi-locational field trials (Pooled data).

Genotype	*Ac-Ndl*	*Ac-Bpr*	*Ac-Pnt*	*Ac-Ldh*	*Ac-Wlg*	*Ac-Smt*	*Ac-Skn*	*Ac-Ran*
ERJ 3	9.2	4.2	40.8	3.2	8.5	50.3	5.0	4.7
ERJ 5	4.3	48.5	4.2	52.8	42.8	8.2	2.3	18.3
ERJ 7	8.5	19.3	12.3	24.0	31.7	0	16.7	0
ERJ 9	21.8	37.8	20.5	36.7	9.3	60.5	37.8	44.2
ERJ 12	0	0	0	0	7.8	9.3	4.2	0
ERJ 13	3.7	8.2	0	18.8	9.7	4.2	16.3	21.5
ERJ 14	10.0	9.5	9.2	0	17.5	9.2	24.2	0
ERJ 15	0	0	0	0	27.3	3.3	8.0	0
ERJ 16	9.3	15.3	0	7.7	54.2	16.0	9.7	8.3
ERJ 17	9.7	23.7	4.0	32.2	15.8	18.8	8.2	0
ERJ 19	8.8	22.2	51.7	35.5	4.3	19.7	6.3	6.8
ERJ 20	8.7	12.5	7.3	7.8	3.7	57.3	14.8	29.2
ERJ 32	7.7	0	9.2	12.7	8.2	21.2	17.5	7.7
ERJ 33	4.5	13.8	16.5	19.3	37.8	7.5	33.7	23.3
ERJ 38	0	0	19.7	0	3.3	0	21.8	11.5
ERJ 39	0	0	0	0	4.2	0	9.5	0
ERJ 40	3.2	0	0	0	42.7	24.2	8.3	0
ERJ 41	7.3	3.2	28.3	8.2	57.0	8.3	13.2	11.8
ERJ 44	29.5	26.0	3.2	9.3	4.8	40.7	46.7	2.3
ERJ 47	8.0	36.8	1.7	38.0	28.5	7.8	43.8	4.2
ERJ 54	6.8	32.7	8.5	46.8	8.7	20.5	65.2	35.7
ERJ 76	15.3	22.3	4.3	27.5	51.3	21.2	58.3	12.8
ERJ 78	4.2	4.5	24.2	12.8	48.2	22.3	23.8	10.2
ERJ 90	0	17.2	4.8	44.2	15.5	23.7	48.2	72.8
ERJ 99	7.7	15.7	3.7	11.3	81.8	8.5	8.7	53.7
ERJ 103	9.5	8.0	0	6.7	33.2	31.8	37.5	4.3
ERJ 108	7.2	53.8	30.2	0	30.3	21.7	9.0	13.8
ERJ 109	0	7.7	0	0	25.0	0	0	0

In addition to the aforementioned introgression lines, which displayed total immunity against the majority of the *A. candida* isolates at all growth stages, 35 genotypes were discovered to produce an immune resistance level of responses against specific pathotypes of white rust under artificial as well as natural field conditions (Table 5). These genotypes can be exploited in mustard improvement programs for developing resistance cultivars against white rust disease. Among these 35 genotypes, ERJ 108, and ERJ 157 showed immunity against *Ac-Ldh* specifically; furthermore, ERJ 159, ERJ 13, and ERJ 32 were also shown to be immune against *Ac-Ran*, *Pnt*, and *Bpr*, respectively (Table 5). Such a response to the disease can be used to study the differences between host and pathogen and to comprehend the biological specialization that exists among the isolates collected from diverse plant hosts in various places.

Screening available ILs for resistance revealed a higher percentage of susceptibility to the white rust pathogen. Out of 194 germplasm samples tested under *B. juncea*, 39.69% expressed a highly susceptible reaction at the cotyledonary stage, while 40.48% and 40.08% showed high susceptibility at the true leaf and adult plant stages, respectively. Only 5.37% of the total ERJ displayed immunity at the cotyledonary stage, 3.68% at the true leaf stage, and 3.41% at the mature plant stage. Under artificial inoculation, high resistance (PDI = 1–5%) was observed in 7.09% of total ILs at the cotyledonary stage and 5.37% at the true leaf stage, while only 6.52% of ILs demonstrated high resistance at the adult stage in fields. Additionally, restricted sporulation occasionally supplemented with necrosis or chlorosis with a FN interaction phenotype was found in 7.52% of ERJs at the cotyledonary stage, 7.05% at the true leaf stage, and 6.83% at the adult plant stage (Figure 5), indicating moderate resistance (PDI 6–10%) in the introgressed lines.

Table 5. Introgressed lines showing immune response (PDI = 0) against different number of *A. candida* isolates under both artificial inoculation and natural field conditions.

Accessions	Artificial Condition		Natural Field Condition	
	Name of Isolates	No. of Isolates	Name of Isolates	No. of Isolates
ERJ 39	*Ac-Ndl, Met, Bpr, Pnt, Ldh, Hsr, Ran*	7	*Ac-Ndl, Bpr, Pnt, Ldh, Smt, Ran*	6
ERJ 12	*Ac-Ndl, Met, Bpr, Pnt, Ldh, Mor, Ran*	7	*Ac- Ndl, Bpr, Pnt, Ldh, Ran*	5
ERJ 15	*Ac-Met, Bpr, Pnt, Ldh, Hsr, Ayo, Ran*	7	*Ac-Ndl, Bpr, Pnt, Ldh, Ran*	5
ERJ 109	*Ac-Met, Pnt, Ldh, Skn, Ran*	5	*Ac-Ndl, Pnt, Ldh, Smt, Skn, Ran*	6
ERJ 40	*Ac-Ndl, Met, Pnt, Ldh, Ran*	5	*Ac-Bpr, Pnt, Ldh, Ran*	4
ERJ 110	*Ac-Met, Pnt, Ldh, Ran*	4	*Ac-Ndl, Ldh, Ran*	3
ERJ 158	*Ac-Ndl, Bpr, Ran*	3	*Ac-Ndl, Bpr, Ran*	3
ERJ 13	*Ac-Ndl, Bpr, Pnt*	3	*Ac-Pnt*	1
ERJ 38	*Ac-Bpr, Ldh*	2	*Ac-Ndl, Bpr, Ldh, Smt*	4
ERJ 14	*Ac-Ldh, Ran*	2	*Ac-Ldh, Ran*	2
ERJ 127	*Ac-Bpr, Ran*	2	*Ac-Bpr, Ran*	2
ERJ 160	*Ac-Pnt, Ldh*	2	*Ac- Pnt, Ldh*	2
ERJ 17	*Ac-Hsr, Ran*	2	*Ac-Ran*	1
ERJ 32	*Ac-Bpr*	1	*Ac-Bpr, Skn, Pnt*	3
ERJ 16	*Ac-Hsr*	1	*Ac-Pnt*	1
ERJ 90	*Ac-Mor*	1	*Ac-Ndl*	1
ERJ 108	*Ac-Ldh*	1	*Ac-Ldh*	1
ERJ 157	*Ac-Ldh*	1	*Ac-Ldh*	1
ERJ 159	*Ac-Ran*	1	*Ac-Ran*	1

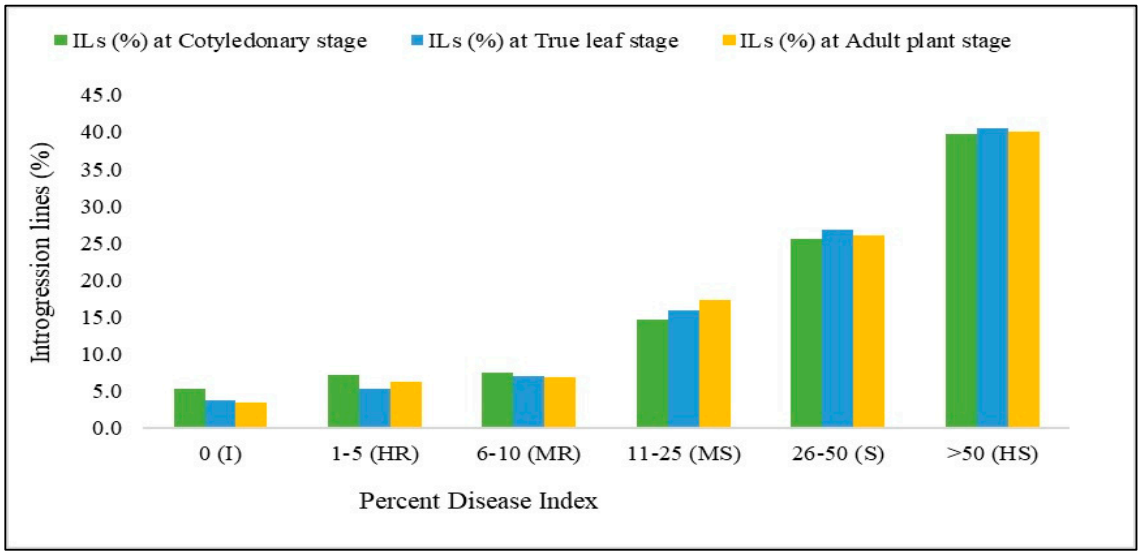

Figure 5. Percentage of introgressed (ILs) lines under the different classes of percent disease index reactions at cotyledon, true leaf and adult plant growth stages.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.3. Screening of Mutant and Resynthesized B. juncea Lines

In the present study, 90 resynthesized and 9 advanced mutant lines of *B. juncea* developed at ICAR-NIPB, New Delhi, and ICAR-DRMR, Bharatpur, were also evaluated for identifying novel host resistance sources against the white rust (*A. candida*) pathogen. The resynthesized lines were developed by crossing *B. rapa* (AA, 2n = 20) and *B. nigra* (BB, 2n = 16), which showed various levels of resistance for different *A. candida* isolates,

followed by the colchicine treatment to develop amphidiploid *B. juncea* (AABB, 2n = 36) (Table 2). Meanwhile, mutant lines were derived from the Indian mustard cultivar RH 749 following treatment with different doses of gamma rays and ethyl methane sulfonate (EMS). Among the tested germplasms, the mutant lines DRMR 18-36-12 and DRMR 18-37-13 and the resynthesized line RBJ 18 were found to express immunity and showed no sign of disease against multiple races of the *A. candida* pathogen (Figure 6).

Figure 6. Figure showing (**A**) immune response in mutant and resynthesized (RBJ) lines at cotyledonary and true leaf stages under artificially inoculated conditions and (**B**) susceptible reaction in cv., 'Pusa Jai Kisan' and 'Varuna'.

DRMR 18-36-12 showed complete immunity to four isolates, *Ac-Ndl*, *Bpr*, *Pnt*, and *Ran*, at all three growth stages of plants, including cotyledonary, true leaf, and adult plant growth stages. Additionally, under artificial inoculation with *A. candida*, no immune reaction (PDI = 0%) was observed for *Ac-Met*, *Hsr*, and *Mor* isolates at both cotyledonary and true leaf stages. For the isolate *Ac-Ldh*, immunity was expressed at the cotyledonary stage, while the true leaf and adult plant stages both had high resistance, with PDI scores of 3.2 and 3.8, respectively. This was in contrast to isolates *Ac-Smt* and *Skn*, where immunity was observed at the true leaf and adult plant stages but not at the cotyledonary stage, as PDI scores of 4.3% and 4.0% were observed in these genotypes, respectively (Tables 6 and 7). Another mutant line, DRMR 18-37-13, also expressed complete immunity against *Ac-Ndl*, *Bpr*, *Ldh*, and *Ran* at cotyledonary, true leaf, and adult plant stages. Moreover, under controlled conditions, an immune response was observed against *Ac-Met*, *Hsr*, and *Mor*. For isolates *Ac-Pnt*, *Ayo*, *Smt*, and *Skn*, immunity (PDI = 0) was also found at true leaf stage but not at cotyledonary stage, as high to moderate resistance was expressed at this phase of seedling growth. This germplasm, on the other hand, demonstrated susceptible (formation of numerous pustules on the lower surface of leaves covering 11–25% of the leaf area) to moderately susceptible (coalescing large, scattered pustules on the lower surface of leaves covering 26–50% of the leaf area) reactions for *Ac-Wlg* and *Dha* at all the three stages of plant development.

Table 6. Percentage disease index for mutant and resynthesized *B. juncea* lines against white rust pathogen (*A. candida*) isolates obtained from major mustard growing locations in India at cotyledonary leaf (CL) and true leaf (TL) plant growth stages under controlled conditions (Pooled data).

Genotype	*Ac-Ndl*		*Ac-Met*		*Ac-Bpr*		*Ac-Pnt*		*Ac-Ldh*		*Ac-Hsr*		*Ac-Ayo*		*Ac-Wlg*		*Ac-Dha*		*Ac-Smt*		*Ac-Mor*		*Ac-Skn*		*Ac-Ran*	
	CL	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**	**CL**	**TL**
RBJ 8	13.3	24.2	5.0	9.3	16.8	8.2	18.0	4.8	32.3	44.0	4.0	9.5	12.7	27.8	32.7	20.2	25.0	41.3	19.8	9.5	7.3	4.3	38.7	10.0	22.7	32.2
RBJ 9	21.7	7.8	0	0	23.7	7.8	19.2	12.7	42.2	16.2	0	16.8	9.2	26.7	40.8	62.3	28.8	72.5	24.2	8.3	12.2	3.7	36.8	47.8	55.0	12.8
RBJ 10A	4.2	9.3	4.5	0	8.2	21.3	18.8	0	46.5	24.3	0	0	0	0	7.3	9.5	7.7	6.7	14.3	10.0	13.5	5.0	21.3	16.7	32.8	51.7
RBJ 10C	13.8	4.5	3.7	8.7	18.5	14.7	24.3	5.0	35.7	47.8	20.8	17.7	32.8	19.0	22.2	8.7	14.5	8.3	12.8	0	3.7	0	19.5	34.5	23.3	15.2
RBJ 11	11.3	6.7	7.3	4.2	15.7	22.2	23.5	8.7	36.8	9.2	0	0	24.5	20.2	31.0	4.3	19.3	9.2	24.3	9.2	4.3	8.2	57.8	26.3	24.5	13.8
RBJ 12	12.7	27.0	14.8	6.5	19.3	9.3	8.2	3.2	39.2	61.3	9.5	7.3	26.3	41.8	20.3	37.2	8.2	9.0	17.7	11.7	9.2	3.3	69.2	56.2	14.2	32.7
RBJ 14	17.8	3.2	3.2	3.8	14.8	26.8	17.7	7.5	30.5	21.8	11.3	8.2	37.0	30.3	19.7	14.0	25.7	40.7	37.0	7.8	6.8	8.8	42.0	17.8	18.8	9.3
RBJ 17	20.5	2.3	4.3	7.3	12.3	9.5	22.8	0	47.3	23.5	3.7	10.0	24.3	15.5	38.5	77.7	27.8	62.0	31.2	9.3	7.0	9.2	21.7	4.5	9.0	16.5
RBJ 18	0	0	4.2	0	0	0	0	0	0	0	4.2	9.2	4.2	7.2	3.3	17.8	4.2	9.3	0	0	4.2	0	8.3	0	0	0
RBJ 19	4.3	20.7	3.7	14.7	18.7	9.2	19.3	4.2	37.8	14.7	9.3	24.3	12.8	29.7	40.8	56.5	65.3	22.2	27.8	8.5	9.3	2.7	35.5	0	15.8	36.2
RBJ 26	64.7	8.8	9.3	4.5	15.5	28.7	43.5	59.3	21.0	8.3	2.5	9.8	30.7	47.8	20.7	8.3	24.0	8.7	8.7	9.0	0	6.3	4.2	7.3	0	3.8
RBJ 32	19.2	7.5	9.5	3.3	16.2	12.8	14.7	22.8	18.7	25.0	12.8	22.7	15.3	23.0	40.2	26.7	16.8	32.8	22.3	7.3	8.7	33.0	44.3	56.7	35.2	20.3
RBJ 34	8.8	0	3.8	4.7	12.7	8.3	16.2	13.7	8.2	26.2	0	8.2	9.0	22.3	12.3	33.0	0	18.3	4.2	6.8	11.8	2.8	3.7	9.8	0	15.2
RBJ 35	55.5	7.2	2.2	9.2	20.8	7.5	36.3	8.2	21.8	8.5	6.3	13.0	2.7	27.8	30.5	43.2	28.5	12.7	30.5	8.2	4.8	12.2	38.3	24.2	8.3	54.0
RBJ 37	9.2	10.0	6.7	8.8	21.3	18.0	4.2	19.0	13.3	41.3	8.7	33.8	17.8	14.2	19.8	26.0	12.7	7.2	21.2	9.8	9.7	6.7	18.8	23.3	9.2	9.3
RBJ 38	0	4.3	1.8	0	4.2	0	11.7	7.8	4.0	9.2	0	0	3.8	8.3	21.2	8.8	8.8	3.8	0	0	3.2	7.8	32.0	47.0	12.7	8.2
RBJ 40	0	4.7	4.7	0	0	0	22.0	3.7	0	0	0	0	4.7	9.5	9.0	4.3	3.3	0	3.3	2.7	4.5	0	9.2	7.5	8.8	4.5
RBJ 42	12.3	8.3	0	0	9.7	4.3	0	7.3	21.5	7.8	0	18.2	29.2	36.7	20.7	12.7	13.2	8.3	24.5	8.3	0	9.3	14.7	26.8	10.0	7.7
RBJ 60	9.7	2.8	3.3	4.3	10.0	22.2	32.3	52.5	23.8	40.7	9.2	7.3	8.3	2.2	8.5	5.0	4.3	7.5	23.7	12.2	0	6.2	0	2.2	0	1.8
RBJ 66	7.8	7.2	4.0	8.5	8.3	7.7	52.8	9.2	12.7	30.0	45.3	8.8	17.2	28.3	7.3	36.5	19.5	12.8	35.2	7.7	4.7	9.8	41.5	9.3	13.2	3.2
RBJ 73	4.3	3.5	2.8	9.7	20.5	16.5	48.5	39.3	16.2	23.3	0	2.7	7.7	4.7	6.8	6.7	6.7	18.2	21.3	4.2	3.8	7.2	37.8	53.7	18.5	39.3
RBJ 89	40.5	6.8	13.7	2.8	29.2	6.8	22.7	4.5	37.3	14.5	17.8	7.5	42.5	31.0	16.7	23.8	25.0	9.3	14.2	7.5	20.2	0	34.7	0	41.7	26.2
RBJ 90	70.7	38.3	2.5	8.2	45.7	15.3	30.2	46.7	55.0	28.2	15.7	45.3	22.2	39.2	12.2	27.3	9.3	32.7	0	0	8.3	20.7	23.3	8.8	0	3.7
DRMR 18-37-13	0	0	0	0	0	0	0	4.0	0	0	0	0	0	4.3	11.8	18.7	16.8	27.8	0	6.8	0	0	0	7.2	0	0
DRMRSJ 4	0	1.7	4.8	5.0	0	0	9.0	4.3	3.3	7.7	0	3.2	0	2.8	35.7	0	27.2	0	16.8	5.0	12.8	16.8	0	2.8	0	0
DRMRDJ 1	0	8.2	0	7.8	12.8	21.2	40.3	7.8	8.2	12.8	0	20.0	14.7	9.2	7.8	0	12.7	0	14.3	23.2	9.2	3.8	8.2	11.3	15.0	22.7
DRMRSJ 1	0	0	0	3.7	9.3	22.5	0	0	11.7	13.3	0	7.3	0	7.3	4.3	4.2	3.3	6.2	5.0	8.3	11.7	14.2	12.3	21.7	4.3	7.8
DRMR 18-35-11	0	0	0	0	0	0	8.7	9.3	0	21.0	0	0	0	6.5	9.5	3.3	9.0	9.8	6.7	9.2	15.5	29.5	5.2	0	0	0
DRMR 18-36-12	0	0	0	0	0	0	0	0	0	3.2	0	0	8.3	0	9.2	4.5	7.7	9.3	4.3	0	0	0	4.0	0	0	0

Table 7. Percent disease index for mutant and resynthesized *B. juncea* lines against white rust pathogen (*A. candida*) at adult plant stage under multi-locational field trials (Pooled data).

Genotype	*Ac-Ndl*	*Ac-Bpr*	*Ac-Pnt*	*Ac-Ldh*	*Ac-Wlg*	*Ac-Smt*	*Ac-Skn*	*Ac-Ran*
RBJ 8	30.8	9.3	7.8	53.2	24.2	19.8	9.2	38.3
RBJ 9	20.0	14.0	22.2	12.8	78.7	10.2	36.3	19.8
RBJ 10A	6.2	17.7	0	32.3	10.0	18.3	20.0	46.5
RBJ 10C	7.3	23.8	3.8	56.5	9.7	0	44.7	21.2
RBJ 11	15.8	34.5	9.3	8.2	24.2	9.7	38.2	18.7
RBJ 12	42.7	13.2	3.2	74.0	42.8	14.8	67.8	27.3
RBJ 14	2.5	29.3	16.0	19.3	23.7	7.2	22.7	11.2
RBJ 17	9.3	7.7	0	37.5	81.3	12.3	4.3	23.5
RBJ 18	0	0	0	0	12.5	0	0	0
RBJ 19	38.2	8.8	4.3	18.8	69.2	0	0	35.8
RBJ 26	30.7	10.0	67.5	9.3	9.3	9.2	8.2	3.3
RBJ 32	9.2	15.2	25.2	28.7	37.8	7.7	70.7	23.2
RBJ 34	8.5	9.5	10.3	41.5	48.2	6.3	8.0	20.7
RBJ 35	10.2	6.3	12.7	10.2	56.3	8.8	17.3	64.0
RBJ 37	9.7	22.0	8.8	49.8	32.0	10.0	19.2	10.3
RBJ 38	7.2	0	10.3	7.3	40.5	0	55.5	9.2
RBJ 40	4.3	0	9.0	0	8.7	4.3	7.8	4.5
RBJ 42	10.7	4.3	6.7	10.0	24.2	10.2	34.7	8.3
RBJ 60	9.0	14.7	68.2	43.0	5.0	19.7	4.3	2.7
RBJ 66	4.8	7.8	12.8	38.8	43.8	12.8	7.7	4.8
RBJ 73	5.3	24.2	47.7	17.7	8.3	8.2	68.5	45.5
RBJ 89	8.2	8.7	7.0	22.2	35.2	11.3	0	34.0
RBJ 90	48.0	23.3	55.8	40.8	39.3	0	12.8	3.2
DRMR 18-37-13	0	0	2.2	0	23.7	8.7	6.7	0
DRMRSJ 4	6.8	0	4.7	4.2	0	5.3	2.3	0
DRMRDJ 1	5.0	18.2	14.8	11.8	0	15.2	10.2	30.5
DRMRSJ 1	2.7	16.8	0	16.3	2.3	7.5	23.8	5.3
DRMR 18-35-11	0	0	27.0	14.5	13.8	9.8	15.0	0
DRMR 18-36-12	0	0	0	3.8	10.2	0	0	0

Under both in vitro and in vivo field trials, one of the most promising resynthesized lines, RBJ 18, demonstrated total immunity to six isolates of *A. candida*: *Ac-Ndl*, *Bpr*, *Pnt*, *Ldh*, *Smt*, and *Ran*. This line demonstrated an immune response with *Ac-Met*, *Mor*, and *Skn* at true leaf stage but a high (1–5%) to moderate (6–10%) resistance response when tested at cotyledonary leaf stage.

Moreover, under natural field conditions, RBJ 18 also displayed an immune response at the adult plant stage against *Ac-Skn*. For isolates *Ac-Dha* and *Ac-Wlg*, a highly resistant response was observed at the cotyledonary stage; however, at the true leaf stage, moderate resistance (PDI = 9.3) and a susceptible reaction (PDI = 17.8) were found, respectively (Table 6). Resynthesized lines such as RBJ 38 and RBJ 90 expressed immunity specifically against *Ac-Smt*, while mutant line DRMRSJ 1 was found immune only to *Ac-Pnt* at the cotyledonary, true leaf, and adult plant stages (Table 8). Such germplasm can be explored for resistance exclusive to one race and for host differential-related studies. Unexpectedly, none of the mutant and RBJ lines showed complete immunity against *Ac-Wlg* and *Dha*, which shows a limited variability in resistance of these resynthesized lines to varying degrees of virulence as shown by different isolates across India. A total of 29 genotypes were discovered among the resynthesized lines of *B. juncea* that displayed good levels of resistance, of which the immune reaction was represented by only 4.55% of the total germplasm in the field, 5.05% at true leaf stage, and 5.67% at cotyledonary stage. Immunity aside, 11.97% of germplasm exhibited high resistance (PDI = 5%) at the cotyledonary stage and 9.17% at the true leaf stage under artificial inoculation. However, at the adult stage in fields, 10.98% of the germplasm was found to be highly resistant. In addition, 12.59%

of genotypes were categorized as moderately resistant (PDI = 6–10%) at the cotyledonary stage, 10.80% at the true leaf stage, and 11.62% at the adult plant stage (Figure 7).

Table 8. Mutant and resynthesized *B. juncea* lines showing immune response (PDI = 0) against different numbers of *A. candida* isolates under both artificial inoculation and natural field conditions.

Accessions	Artificial Condition		Natural Field Condition	
	Name of Isolates	No. of Isolates	Name of Isolates	No. of Isolates
DRMR 18-36-12	*Ac-Ndl, Met, Bpr, Pnt, Hsr, Mor, Ran*	7	*Ac- Ndl, Bpr, Pnt, Smt, Skn, Ran*	6
DRMR 18-37-13	*Ac- Ndl, Met, Bpr, Ldh, Hsr, Mor, Ran*	7	*Ac- Ndl, Bpr, Ldh, Ran*	4
RBJ 18	*Ac-Ndl, Bpr, Pnt, Ldh, Smt, Ran*	6	*Ac-Ndl, Bpr, Pnt, Ldh, Smt, Skn, Ran*	7
DRMR 18-35-11	*Ac- Ndl, Met, Bpr, Hsr, Ran*	5	*Ac-Ndl, Bpr, Ran*	3
RBJ 40	*Ac-Bpr, Ldh, Hsr*	3	*Ac-Bpr, Ldh*	2
DRMRSJ 4	*Ac-Bpr, Ran*	2	*Ac-Bpr, Wlg, Ran*	3
RBJ 38	*Ac-Hsr, Smt*	2	*Ac-Bpr, Smt*	2
DRMRSJ 1	*Ac-Ndl, Pnt*	2	*Ac-Pnt*	1
RBJ 10A	*Ac-Hsr, Ayo*	2	*Ac-Pnt*	1
RBJ 90	*Ac-Smt*	1	*Ac-Smt*	1

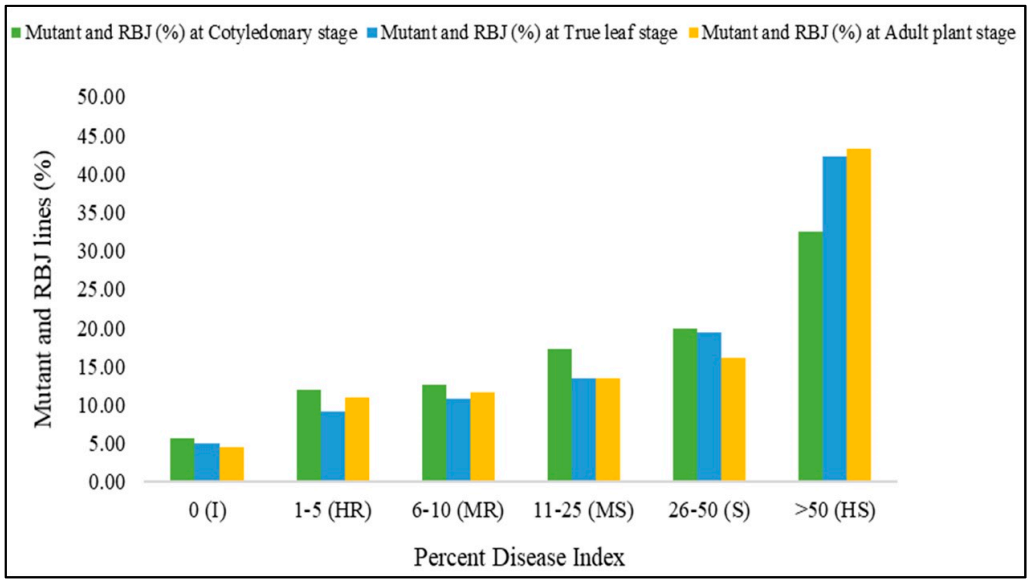

Figure 7. Percentage of mutant and resynthesized (RBJ) lines under different classes of percent disease index reactions at cotyledon, true leaf and adult plant growth stages.

3.4. Relation between Cotyledonary, True Leaf Stage, and Adult Plant Resistance

In the trial run under artificial inoculation in the growth chamber and under natural field conditions, the relationship between cotyledonary, true leaf, and adult plant resistance was also investigated. Therefore, to ascertain this relation between resistance at the cotyledonary, true leaf, and adult growth phases of plants, a correlation analysis was conducted. Cotyledonary stage resistance is highly valued in *A. candida* infections, as plants can become infected during the early stages of growth, which ultimately results in systemic infections that lead to hypertrophy, hyperplasia, extensive distortion of the affected tissues, and staghead formations [14]. However, cotyledon resistance and adult plant resistance are not well correlated and vary with germplasm and the screening method used [38].

In the current study, 17 genotypes out of a total of 194 introgressed lines expressed complete immunity against one or more isolates at all three plant growth stages, including cotyledonary, true leaf, and mature plant. This form of resistance at all stages is typically ideal in plant breeding because the individual genes provide high levels of resistance against a larger range of races and have a tendency to be robust [39]. Despite the similar

trend between the resistance levels at the three growth stages, four ILs, ERJ 5, ERJ 13, ERJ 40, and ERJ 110, showed moderate (PDI = 6–10%) to high resistance responses (PDI = 1–5%) at the adult stage under field conditions, which is a slightly lower level of resistance as compared to the seedling stage in greenhouse conditions, where they exhibited immunity against different isolates of the pathogen (Table 3). This might be because a larger mixed inoculum builds up occurs in outdoor settings as opposed to growth chambers, where tests are conducted with a single isolate under controlled environmental circumstances. It is therefore advisable to test the resistance in the field as well as in controlled conditions with prominently virulent isolates of the pathogen to identify the robust sources of host resistance. Four ILs, namely, ERJ 15, ERJ 38, ERJ 39, and ERJ 109, were found to express immunity at the cotyledonary stage and adult plant stage but not at the true leaf stage. On the contrary, eight germplasms, ERJ 7, ERJ 16, ERJ 38, ERJ 40, ERJ 90, ERJ 103, ERJ 109, and ERJ 110, were identified to express immunity (PDI = 0) at true leaf and adult plant stages but not at cotyledonary stages (Tables 3 and 4). This shows there is a considerably larger association between resistance at the true leaf and adult plant stages than there is between resistance at the cotyledonary stages. Correlation studies have further supported this, as the true leaf stage and adult growth stage of plants in introgression lines showed a significantly strong correlation for disease resistance against *A. candida* (r = 0.734, n = 912, $p < 0.001$), followed by the correlation between adult plant stage and cotyledonary stage (r = 0.444, n = 912, $p < 0.001$) and the true leaf stage and cotyledonary stage (r = 0.402, n = 912, $p < 0.001$).

Among mutant and resynthesized lines, nine genotypes were shown to exhibit complete immunity at all three plant growth stages, including cotyledonary, true leaf, and mature plant. Only one mutant line, DRMRSJ 1, was found to express high resistance (PDI = 2.7%) at the adult stage under field conditions; however, under greenhouse conditions, it exhibited immunity against the various isolates of the pathogen. Immunity (PDI = 0) was found in 10 genotypes, including three mutant lines (DRMR 18-36-12, DRMRDJ 1, and DRMRSJ 4) and seven resynthesized lines (RBJ 18, RBJ 10A, RBJ 38, RBJ 19, RBJ 10C, RBJ 17, and RBJ 89), at true leaf and adult plant stages, while high resistance (PDI = 1–5%) to susceptible reaction (PDI = 11–25%) was observed at cotyledonary stage (Tables 6 and 7). There was no genotype that expressed immunity at the cotyledonary and adult plant stages but not at the true leaf stage, implying that most genotypes that were immune at the adult, cotyledonary or both stages were also immune at the true leaf stage. This probably suggests a strong correlation between the true leaf stage and the adult plant stage. This was further confirmed by the correlation studies, as a significantly strong correlation for disease resistance against *A. candida* was found for true leaf stage and adult plant stage (r = 0.797, n = 696, $p < 0.001$), followed by correlation between the adult plant stage and cotyledonary stage (r = 0.441, n = 696, $p < 0.001$) and the true leaf stage and cotyledonary stage (r = 0.399, n = 696, $p < 0.001$).

It could therefore be concluded from the correlation analysis of ERJ, mutant, and RBJ lines that the genotype responses during the early growth stages, particularly the true leaf stage of the young seedling, could be dependably employed as a quick assay for determining genetic resistance against *A. candida* pathogenesis. Although this makes it possible to lower the expense of multi-location trials and eliminates the possibility of weather-related variability when screening adult plant resistance in the field, it is not reliable all the time, as depending on the conditions, there are always chances for disease escape. Moreover, a shift in plant developmental stage from juvenile to adult might also trigger a different kind of resistance response. Therefore, for a conclusive result, rigorous testing of the genotypes under both controlled and natural conditions at all growth stages is preferred.

3.5. Analysis of Variance

Under both field and in vitro conditions, the fit test results for the GGE model for *B. juncea* revealed a very significant main impact of environment (E) and genotype (G) and

genotype by environment interaction (GEI) ($p < 0.001$). (Tables 9 and 10). An analysis of variance (ANOVA) for introgressed lines indicated that under controlled conditions, 15.8% of the total sum of squares (SS) was explained by the effect of genotype and 14.8% and 69.4% was attributable to the environment (E) main effects and genotype by environment interaction (GEI), whereas under field conditions, 18.6% was represented by genotype, 17.7% by environment, and 63.7% of the total SS by GEI. Meanwhile, for mutants and resynthesized *B. juncea* under artificial conditions, 28.2% of the total SS was explained by genotype, while 16.6% and 55.2% were represented by environment and the interaction between genotype and environment, respectively. Similarly, under field trials, 21.4% of total SS was represented by genotype, and the rest, 11.9% and 66.8%, were denoted by environment and GEI, respectively. These total SS show the variation in genotypes for the white rust disease index across isolates and locations. While the environmental component (locations) coupled with weather conditions influence the genotypes' performance at various locations, variation due to G or GE interactions is a measure for the strains' response across the environments and locations. The higher percentage of GE shows that adaptabilities are preferred by the genotype. Therefore, because there is significantly more refinement in the variances for G and GE than in a single location, multi-environment trials (METs) realize the virtue of germplasm for both temporal and geographic stability [40].

Table 9. Summary of ANOVA representing total percentage of variation attributed to Environment (E), Genotype (G) and Genotype x Environment interaction (GEI) for reaction of introgressed (ERJ) lines tested against *A. candida* isolates under artificial inoculation and natural conditions.

Source	DF	MS	F	*P*	SS (%)
Artificially inoculated conditions for Introgression lines					
Environment (E)	12	2209.194	97.89	<0.001	14.8
Genotype (G)	37	760.087	760.08	<0.001	15.8
GEI	444	279.071	279.07	<0.001	69.4
Natural field conditions for Introgression lines					
Environment (E)	7	2701.334	2012.09	<0.001	17.7
Genotype (G)	37	534.873	398.40	<0.001	18.6
GEI	259	262.238	195.32	<0.001	63.7

DF = Degree of freedom, MS = Mean sum of squares, SS = Sum of Squares.

Table 10. Summary of ANOVA representing total percentage of variation attributed to Environment (E), Genotype (G) and Genotype x Environment interaction (GEI) for reaction of mutant and resynthesized (RBJ) lines tested against *A. candida* isolates under artificial inoculation and natural conditions.

Source	DF	MS	F	*P*	SS (%)
Artificially inoculated conditions for Resynthesized *B. juncea* lines					
Environment (E)	12	1705.448	73.78	<0.001	16.6
Genotype (G)	28	1240.206	53.65	<0.001	28.2
GEI	336	202.709	8.77	<0.001	55.2
Natural field conditions for Resynthesized *B. juncea* lines					
Environment (E)	7	1464.08	1292.05	<0.001	11.9
Genotype (G)	28	660.13	582.56	<0.001	21.4
GEI	196	294.33	259.74	<0.001	66.8

DF = Degree of freedom, MS = Mean sum of squares, SS = Sum of Squares.

3.6. Interaction Studies between Genotypes and Hotspot Locations

Principal component analysis (PCA) was carried out for the multi-locational trials to comprehend the impact of location-specific environments in terms of the resistance testing against *A. candida* isolates exclusively for the introgression, mutant, and resynthesized lines of *B. juncea*. The pattern of environments in connection to genotypes for white rust

severity was visualized using GGE biplots based on symmetric scaling of genotype and environment. The GGE biplot graph used to evaluate white rust severity in the present study accounted for 57.9% of the total variation due to G + GE for ERJ and 55.3% for RBJ. In GGE biplot, environment vector lines connect the plot origin and markers for the environments. The correlation coefficient between two environments or genotypes is related to the angle between their vectors. While a perpendicular angle denotes no association and an obtuse angle demonstrates a negative correlation, an acute cosine angle suggests a positive correlation between the environment(s) or genotype(s). Therefore, according to the cosine of angles of environment vectors for ERJ, Wellington and S. K. Nagar (SKN) showed a positive correlation, while Wellington and Pantnagar were negatively correlated, suggesting they both have differing agro-climatic conditions for the development of the white rust disease (Figure 8A). However, in the case of RBJ and mutant lines, no correlation could be seen between Wellington and SKN as the environment vectors in this case formed a right cosine angle; instead, a positive correlation was seen between Ranchi and Wellington, while, similarly to ERJ, a negative correlation was found between Wellington and Pantnagar (Figure 8B).

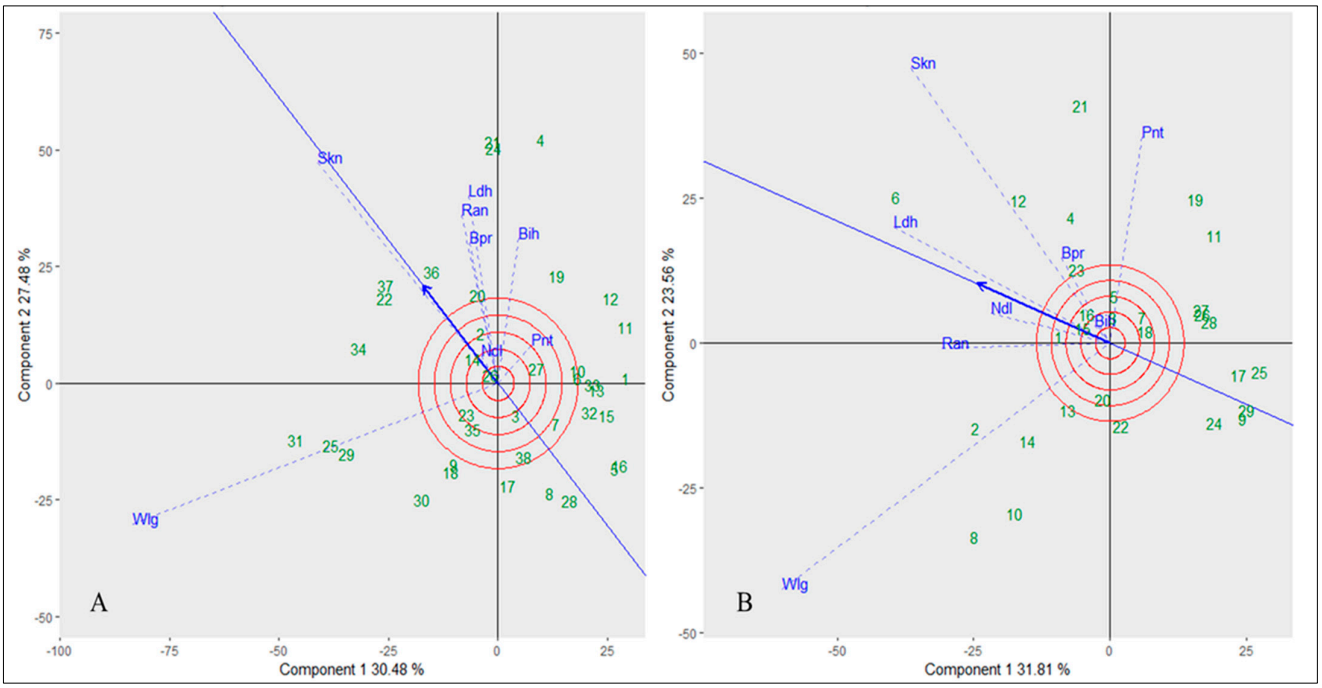

Figure 8. GGE biplot representing genotypes by environment interaction in terms of reaction of (**A**) introgressed lines and (**B**) mutant and resynthesized *B. juncea* lines to *A. candida* isolates under natural field conditions.

In order to choose widely adaptable genotypes, an ideal habitat should have both the power of discrimination (the capacity of the environment to discriminate among genotypes) and representativeness (how well the location represents the mega-environment). The discriminating power of the location is directly correlated with the length of the location vector, whereas the power of representativeness is directly correlated with the angle between the location vector and AEC abscissa, with smaller angles being preferred. As indicated in Figure 7, Wellington and SKN were the most discriminating environments for both introgression and resynthesized lines, whereas Delhi and Bihar were the least discriminating for ERJ, mutants, and RBJ. In terms of representativeness, Ludhiana was the most representative of the test environment for mutants and RBJ, while SKN formed the smallest angle with the AEC abscissa, thus representing the mega environment for ERJ. Usually for genotypes, the interpretation in the GGE biplot is that the performance of a genotype in an environment is better than average if the angle between its vector and the

environment's vector is <90°; it is poorer than average if the angle is >90°; and it is near average if the angle is about 90°. However, in this study, the opposite trend was followed, as lower values were considered to be resistant because a higher disease score indicates greater susceptibility. Therefore, in the case of introgressed lines, genotypes 5, 8, 16, and 28 representing ERJ 12, ERJ 15, ERJ 39 and ERJ 109 were the most resistant germplasms that performed better as compared to other genotypes and showed better stability across the environments, especially ERJ 39 which showed multiple resistance across the trials. Meanwhile for resynthesized lines, genotypes 9, 17, 25 representing RBJ 18, RBJ 40, DRMRSJ 4 and mutants 24 and 29 represented by DRMR 18-37-13 and DRMR 18-36-12 were found to be more stable and perform well against *A. candida* isolates at diverse locations, especially resynthesized and mutant line RBJ 18 and DRMR 18-36-12, respectively, which showed complete immunity and stability across multiple isolates and locations (Figure 8).

4. Conclusions

Albugo candida is known to be a notorious biotroph responsible for significant economical and yield losses in *B. juncea* L. throughout the world. Recommended management strategies, such as employing systemic fungicides to combat this disease, not only seem to be ineffective against this disease but also affect the environment negatively. As a result, identifying host resistance may be the best alternative approach to combating this major pathogenic threat. Resistant sources can be further utilized to introgress the resistant genes and produce durable resistance. Mutagen-induced novel variations also add to the scope of screening for resistance. The present investigation was focused on the generation of putative resistance sources that would be suitable for Indian conditions among introgressed, resynthesized, and mutant *B. juncea* L. lines against prominently virulent isolates of *A. candida*. To the best of our knowledge, introgressed lines (ERJ 39, ERJ 12, and ERJ 15), resynthesized line RBJ 18, and mutant lines (DRMR 18-36-12 and DRMR 18-37-13) have all been identified as novel sources of resistance against multiple isolates of *A. candida* at all plant growth stages, including cotyledonary, true leaf, and adult plant stages. Among the others, ILs such as ERJ 108, ERJ 157, ERJ 159, ERJ 13, and ERJ 32 specifically showed resistance against single isolates. Similarly, mutant lines DRMRSJ 1 and RBJ, RBJ 38, and RBJ 90 expressed specific immunity to a single race of *A. candida.* The study also revealed a positive correlation between true leaf and adult plant stages; however, with the cotyledonary stage, both true leaf and adult plant stages showed a weak correlation. Moreover, one cannot rely solely on artificial or field testing for a conducive result as there is sometimes the possibility of disease escape and, therefore, the germplasm might show resistant reactions. GGE biplot analysis in this study allowed efficient assessment of the resistance of ERJ, mutants, and RBJ lines to white rust disease across environments. The method allowed for the selection of ideal genotypes based on their adaptability and stability to various agro-climatic zones and environments. The value of such genetic resistances in terms of yield advantage under varying environmental conditions, which could have the yield penalty caused by white rust, is very high. More such studies on the tripartite interaction between genotype, isolate, and environment will be much more useful in minimizing yield loss due to white rust in a particular area. The potential sources of resistance among *Brassica* germplasms identified in our study will have a practical impact on further identification and molecular mapping of the resistance gene(s) or QTLs and their marker-assisted incorporation into the leading cultivars. These lines also could be introgressed into commercial cultivars which have already broken down their resistance and sustainable management of white rust in Indian mustard.

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