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ORIGINAL PAPER

# Molecular mapping reveals two independent loci conferring resistance to *Albugo candida* in the east European germplasm of oilseed mustard *Brassica juncea*

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**Abstract** White rust caused by *Albugo candida* (Pers.) Kuntze is a major disease of the oilseed mustard *Brassica juncea*. Almost all the released varieties of *B. juncea* in India are highly susceptible to the disease. This causes major yield losses. Hence, there is an urgent need to identify genes for resistance to white rust and transfer these to the existing commercial varieties through marker-assisted breeding. While the germplasm belonging to the Indian gene pool is highly susceptible to the disease, the east European germplasm of *B. juncea* is highly resistant. In the present study, we have tagged two independent loci governing resistance to *A. candida* race 2V in two east European lines, Heera and Donskaja-IV. Two doubled haploid populations were used; the first population was derived from a cross between Varuna (susceptible Indian type) and Heera (partially resistant east European line) and the second from a cross between TM-4 (susceptible Indian type) and Donskaja-IV (fully resistant east European line). In both the resistant lines, a single major locus was identified to confer resistance to white rust. In Heera, the resistance locus AcB1-A4.1 was mapped to linkage group A4, while in Donskaja-IV, the resistant locus AcB1-A5.1 was mapped to linkage group A5. In both the cases, closely linked flanking markers were developed based on synteny between Arabidopsis and *B. juncea*. These flanking markers will assist introgression of resistance-conferring loci in the susceptible varieties.

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## Introduction

White rust caused by *Albugo candida* (Pers. ex Lev.) Kuntze is a destructive disease in many agronomically important *Brassica* species and also affects *Brassica juncea*, a major oilseed crop of the Indian subcontinent. Both the vegetative and flowering phases of the plant can be affected by the fungal pathogen. This leads to massive yield losses in *Brassica* crops (Singh et al. 1990; Saharan and Verma 1992; Bisht et al. 1994), with the maximum damage occurring due to staghead formation (Lakra and Saharan 1989). Infection during the vegetative phase results in the appearance of white pustules, predominantly on the abaxial surface of leaves and cotyledons. The pathogen can spread

systemically and cause severe malformation of the inflorescence through hypertrophy and hyperplasia resulting in staghead formation. At least 13 races of *A. candida* have been identified on the basis of their specificity to different crucifer species (Verma et al. 1999), of which race 2 predominantly infects *B. juncea* (Petrie 1988; Rimmer et al. 2000). However, this species-specific race pathogenicity is not absolute. Most races can also infect related *Brassica* species, especially those sharing their genome with the hosts from which they were originally collected (Liu et al. 1996).

In a number of studies, resistance to white rust has been shown to be governed by a single dominant gene in the germplasm of *B. rapa* (Kole et al. 1996), *B. napus* (Ferreira et al. 1995) and *B. juncea* (Tiwari et al. 1988; Sachan et al. 1995; Prabhu et al. 1998; Mukherjee et al. 2001). Verma and Bhowmik (1989), however, described a digenic mode of inheritance of resistance in *B. napus* against *A. candida* race 2, while Fan et al. (1983) and Liu et al. (1996) reported three dominant genes involved with resistance in *B. napus* against *A. candida* race 7.

Almost all the major varieties of *B. juncea* belonging to the Indian gene pool are highly susceptible to white rust. Sources of resistance to white rust are available in the east European gene pool of *B. juncea*. Genetic analysis of white rust resistance in *B. juncea* has been undertaken at the molecular level to locate the gene/s and to identify markers for marker-assisted introgression of the trait. All these previous studies have mapped a single locus for white rust resistance using RFLP (Cheung et al. 1998), RAPD (Prabhu et al. 1998; Mukherjee et al. 2001), AFLP (Somers et al. 2002) or CAPS (Varshney et al. 2004) markers. It is not possible to determine whether the loci governing the resistance identified in these independent studies are the same or are different as no common set of markers were used in these studies.

In the present study, we identified two resistance loci against an *A. candida* isolate (designated as AcB1) collected from a severely infected *B. juncea* field at Bharatpur, Rajasthan, one of the areas of most intensive cultivation of mustard in India. These two independent loci were mapped in two different doubled haploid (DH) mapping populations. The first population (VH) was derived from a cross between a susceptible Indian cultivar Varuna and a partially resistant east European line Heera (Pradhan et al. 2003). The second population (TD) was derived from a cross between a susceptible Indian-type variety TM-4 and a completely resistant east European line Donskaja-IV. The presence of two independent loci for white rust resistance within the east European germplasm will allow gene stacking for more durable resistance to white rust in *B. juncea*.

## Materials and methods

### Plant material and mapping populations

Different oil-yielding Brassica species consisting of eight varieties of *B. juncea*, four varieties of *B. carinata*, five varieties of *B. rapa*, a single variety of *B. nigra* and two varieties of *B. napus* were used for studying their disease response against *A. candida* isolate AcB1 (Table 1). All these Brassica species were maintained for several generations by selfing for self-compatible and by sib-mating for self incompatible types. Two doubled haploid (DH) mapping populations were used; one mapping population consisted of 123 DH lines that were derived from a cross between the Indian cultivar Varuna and a '00' line Heera (Pradhan et al. 2003). This population is termed VH in the text. The second population of 100 DH lines, termed TD, was derived from a cross between an Indian-type cultivar TM-4 and an east European line Donskaja-IV.

### Pathogen isolation and plant infections

White pustules of *A. candida* were collected at the peak flowering stage (in the month of January) from infected leaves of *B. juncea* growing at a field in Bharatpur, Rajasthan. Mixed pustules were given four passages of inoculations on susceptible line Varuna to develop a single pustule isolate (SPI). In each passage, zoosporangia from a single pustule were suspended in 200 µl of sterilized double-distilled water and cotyledons of 7-day-old seedlings were drop inoculated. At 15 days after inoculation, mature zoosporangia were scrap collected in plastic vials with the help of a scalpel and frozen at −80°C till required for the next inoculation. The strain isolated after repeated inoculations is described as AcB1 (*Albugo candida* Bharatpur-1).

For screening susceptibility/resistance, the stored zoosporangial mass was rehydrated in autoclaved double-distilled water. The inoculum was adjusted to $2 \times 10^4$ sporangia/ml and was incubated in the dark at 8°C for 2 h before inoculation. Seeds were germinated in pots containing soilrite in Conviron growth chambers maintained at 20°C with a 10-h light/14-h dark photoperiod and 70% relative humidity. Seven-day-old seedlings were drop inoculated by placing 10 µl of inoculum on each of the cotyledons. Inoculated seedlings were transferred to a mist chamber and kept in the dark at 18°C for 48 h. Post-dark incubation, inoculated seedlings were shifted to a growth chamber maintained at 20°C with 16-h light/8-h dark photoperiod and 80% relative humidity. Each experiment consisted of five to six seedlings from each entry and at least three independent disease assay experiments were performed.

**Table 1** Reaction of *Brassica* germplasm against *Albugo candida* isolate AcB1

| *Brassica* species | | | Percent disease index (PDI) | | | |
|---|---|---|---|---|---|---|
| | | Variety | Experiment 1 | Experiment 2 | Experiment 3 | Mean |
| *B. juncea* | 1 | *Varuna | 75.56 | 80.00 | 82.22 | 79.26 |
| | 2 | Kranti | 68.89 | 77.78 | 77.78 | 74.82 |
| | 3 | Pusa Bold | 62.22 | 64.44 | 71.11 | 65.92 |
| | 4 | *Heera | 0.00 | 10.00 | 17.78 | 9.26 |
| | 5 | *TM-4 | 50.00 | 58.90 | 64.40 | 57.77 |
| | 6 | Cutlass | 68.89 | 75.56 | 82.22 | 75.56 |
| | 7 | *Donskaja-IV | 0.00 | 0.00 | 0.00 | 0.00 |
| | 8 | Skorospieka | 77.78 | 82.22 | 71.11 | 77.04 |
| *B. carinata* | 9 | HC-25 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 10 | HC-17 | 3.33 | 3.33 | 6.67 | 4.44 |
| | 11 | CAR-3-1 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 12 | BC-2-2 | 0.00 | 0.00 | 0.00 | 0.00 |
| *B. rapa* | 13 | R-500 | 50.00 | 55.56 | 55.56 | 53.71 |
| | 14 | Torch | 18.44 | 25.89 | 25.89 | 23.41 |
| | 15 | Candle | 8.89 | 13.33 | 33.33 | 18.52 |
| | 16 | Tobin | 8.89 | 10.00 | 10.00 | 9.63 |
| | 17 | Chiffu | 0.00 | 0.00 | 0.00 | 0.00 |
| *B. nigra* | 18 | IC-257 | 8.33 | 27.78 | 55.56 | 30.56 |
| *B. napus* | 19 | GN-48 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 20 | Regent | 0.00 | 0.00 | 3.33 | 1.11 |

*Germplasm used for tagging of the resistance-conferring loci

## Disease scoring

Disease scoring was carried out post-inoculation at different time intervals. Interaction phenotypes were scored on a 0–9 scale (Williams 1985), based on the disease manifestation on the abaxial surface of cotyledons 10 days after inoculation. The interaction phenotypes were rated as 0 (no symptoms or necrosis), 1 (hypersensitive response), 3 (one or few isolated pustules on the abaxial or the adaxial surface, sometimes chlorosis/necrosis), 5 (moderate pustule density on the abaxial and/or adaxial surface, sometimes chlorosis or necrosis), 7 (high pustule density on the abaxial surface and moderate density on the adaxial surface) and 9 (heavy pustule density on both the abaxial and the adaxial surfaces). A percent disease index (PDI) was calculated following the formula: PDI = [Σ numerical ratings/(number of samples scored x maximum score) × 100] (McKinney 1923).

## Histological studies

Histological studies of disease development were carried out on the inoculated cotyledons by microscopic observations. Cotyledons of inoculated seedlings were collected 1–7 and 11 days post-inoculation (dpi). Inoculated cotyledons were stained with trypan blue at different time intervals, as described by Koch and Slusarenko (1990), and boiled for 2 min in an ethanol: lactophenol solution (1:1) containing 250 µg/ml trypan blue. Cotyledons were then cleared in a chloral hydrate solution (1 g/ml water) and mounted in 50% glycerol for microscopic observations under bright field conditions. Hydrogen peroxide ($H_2O_2$) detection was done by the DAB (3,3-diaminobenzidine) staining method following the protocol of Thordal-Christensen et al. (1997).

## QTL mapping

QTL mapping of the white rust resistance trait in the VH population was carried out using a previously developed linkage map (Panjabi et al. 2008). This map consisted of 486 intron polymorphic (IP) markers. For QTL analysis of white rust resistance in the TD population, a linkage map consisting of 838 markers (AFLP, SSR and IP) was constructed (details of this map will be reported elsewhere).

The trait phenotype used for analysis was the mean PDI based on three and five independent experiments from VH and TD populations. Composite interval mapping (CIM; Zeng 1993; Zeng 1994) using the software package WinQTL Cartographer version 2.5 (Wang et al. 2005) was carried out following the mapping criteria described by Ramchiary et al. (2007). The corresponding linkage groups

between the two maps (VH and TD) were determined by the common mapped markers between the two maps.

## Results

### Reaction of isolate AcB1 on crucifer host differentials and *B. juncea* germplasm

The infectivity of *A. candida* isolate AcB1 was studied on 20 lines belonging to five different oil-yielding *Brassica* species (Table 1). Resistance reaction on *B. napus*, *B. carinata*, *B. nigra* types and reaction of the standard host differentials of *B. rapa* such as Torch, Candle and Tobin indicated that isolate AcB1 belongs to race 2 of *A. candida*. Of the eight lines of *B. juncea* that were tested, Donskaja-IV was rated as completely resistant, as no pustule formation was observed after repeated inoculations. Heera occasionally showed formation of one to a few pustules on the abaxial surface of a few cotyledons (Fig. 1a) and was considered partially resistant. All the Indian genotypes including Varuna (Fig. 1b) and TM-4 were highly susceptible to isolate AcB1. However, extensive sporulation of AcB1 isolate on the *B. juncea* host differential Cutlass indicated that the AcB1 isolate of India could be a variant of race 2 and in all probability belonged to race 2V (Rimmer et al. 2000).

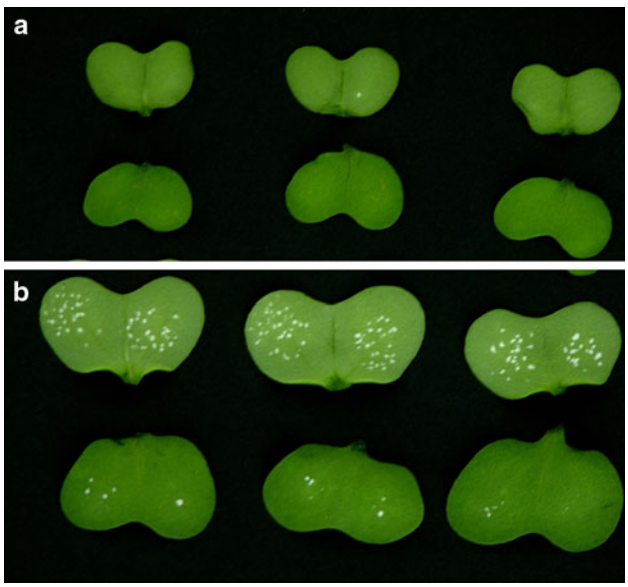


**Fig. 1** Disease response with white rust isolate AcB1 on the cotyledons of *B. juncea* cultivars (**a**) Heera and (**b**) Varuna. Each row represents the disease reaction on the abaxial and adaxial surfaces of the cotyledonary leaves of the respective cultivar 10 days post-inoculation with the *A. candida* isolate AcB1; (**a**) Reaction on the partially resistant line Heera shows occasional presence of pustules; (**b**) reactions on the highly susceptible line Varuna shows profuse sporulation on the abaxial surface and few pustules on the adaxial surface. Donskaja-IV was completely resistant, with no pustule formation, and TM-4 showed a reaction similar to Varuna; hence, these cultivars are not shown

Typical disease reaction and disease progression as revealed by the histological studies in the resistant parents (Donskaja-IV and Heera) and the susceptible parents (TM-4 and Varuna) used for mapping white rust resistance has been shown in Fig. 2. One day post-inoculation, germination of zoospores was observed in all the four lines used in the study. Numerous appresoria were observed and germ tubes were found entering the leaf tissue through the stomata (Fig. 2a1, b1 and c1). In the susceptible lines Varuna and TM-4, 2 days post-inoculation, intercellular mycelium growth with haustoria formation was observed (Fig. 2a2). The mycelial spread progressed profusely (Fig. 2a3) culminating in extensive formation of pustules on the abaxial surface by the 11th day after inoculation (Fig. 2a4). In the case of the partially resistant line Heera, initiation of intercellular mycelium growth and haustoria formation was observed in many microscopic frames. However, in a number of cases, hypersensitive response was observed at the points of fungal entry into the leaf tissue at 2 days post-inoculation (Fig. 2b2). Therefore, in contrast to the susceptible lines of the Indian gene pool, Heera showed both penetration and spread and also a hypersensitive response, the latter resulting in cell death with no further fungal growth. The mycelial growth, wherever it progressed, showed restricted spread in comparison to the highly susceptible lines (Fig. 2b3). After 11 days, further mycelial growth could be observed with occasional formation of pustules on some of the cotyledons (Fig. 2b4). In case of the resistant line Donskaja-IV, an extensive hypersensitive response was observed 2 days post-inoculation (Fig. 2c2). No further development of fungal germ tube was observed (Fig. 2c3, c4).

To further confirm the hypersensitive response in Heera and Donskaja-IV, DAB staining of 3-day post-inoculated cotyledonary leaves of Varuna, Heera and Donskaja-IV was undertaken. In Donskaja-IV, many of the stomatal openings and the adjacent cells showed dark brown staining indicating the presence of hydrogen peroxide (Fig. 1Sa of ESM). Heera also showed a similar response at many spots (Fig. 1Sb of ESM). However, many fungal entry points in Heera did not show DAB staining. No hypersensitive response was observed in Varuna following DAB staining (Fig. 1Sc of ESM).

### Inheritance of resistance and QTL mapping of resistance

Since the resistant parents (Heera and Donskaja-IV) varied in their degree of resistance to isolate AcB1, the criteria used for defining the resistant and susceptible lines were different for the two DH populations. For the VH population, a minimum PDI cutoff of 30 was considered. All lines exhibiting a PDI higher than 30 were considered to be susceptible. Those with PDI lower than 30 were considered to be resistant. For the TD population, individuals with no

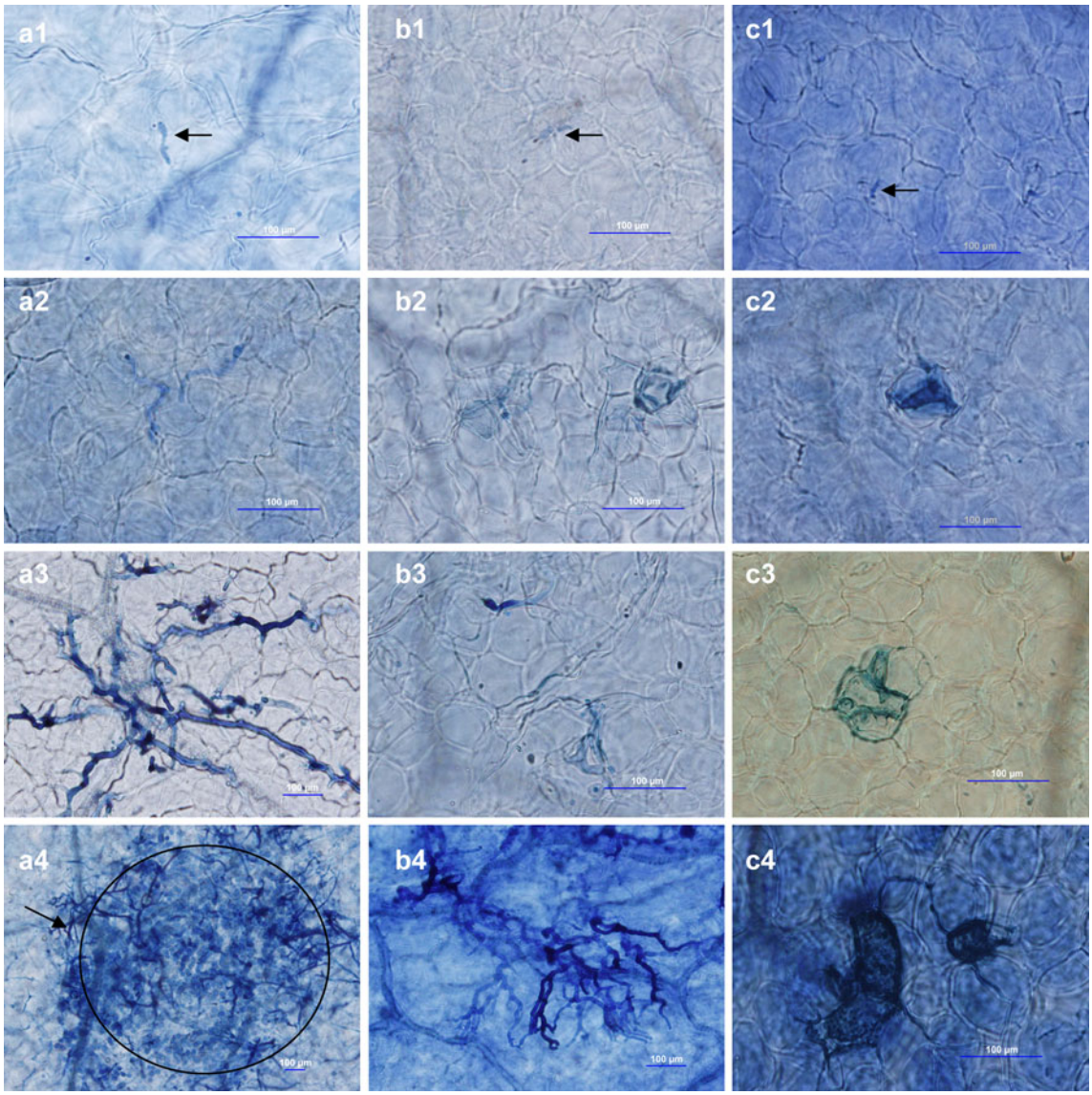


**Fig. 2** Light microscopic study of the progression of *A. candida* isolate AcB1 on cotyledonary tissues of three lines of *B. juncea* used in this study: susceptible line Varuna (**a**), partially resistant line Heera (**b**) and completely resistant line Donskaja-IV (**c**). The disease progression as observed one (1), two (2), six (3) and eleven (4) days post-inoculation. Day 1 post-inoculation germination and penetration of zoospores at the stomatal openings (indicated by *arrow*; **a1**, **b1** and **c1**). Varuna: initiation of mycelial growth and no hypersensitive response (**a2**). The mycelial growth progressed (**a3**) subsequently leading to profuse pustule formation (**a4**). Heera: intercellular mycelium with occasional hypersensitive response (**b2**), subsequently resulted in further mycelial growth in a restricted manner (**b3**) leading to occasional deeply penetrated mycelial spread (**b4**). Donskaja-IV: hypersensitive response (**c2**) with no further development of mycelia observed on six (**c3**) and eleven (**c4**) days post-inoculation

sporangia were considered to be resistant, while lines supporting any sporulation were scored as susceptible. With the above described criteria, a bimodal frequency distribution was observed (Fig. 3) for both the DH populations.

In the VH population of 123 lines, 51 lines were scored as resistant (PDI = 0–30) and 65 were susceptible (PDI > 30). Hence the segregation pattern for disease resistance did not deviate significantly from the 1:1 ratio (with a $\chi^2$ value of 1.68 at 95% confidence limit and $p < 0.05$) pointing to the presence of a single major locus imparting resistance to white rust in the partially resistant line Heera. QTL mapping of white rust resistance in this population detected one major QTL localized to the linkage group A4 within the genetic interval of 7–17 cM, with the maximum peak at 11.4 cM at an LOD value of 47, accounting for 76% of the phenotypic variation (Fig. 4a). This locus was designated AcB1-A4.1.

In the TD population, of the 100 tested lines, 46 were found to be resistant (PDI = 0) and 54 susceptible (PDI > 0) to the isolate AcB1. The chi square values did not deviate

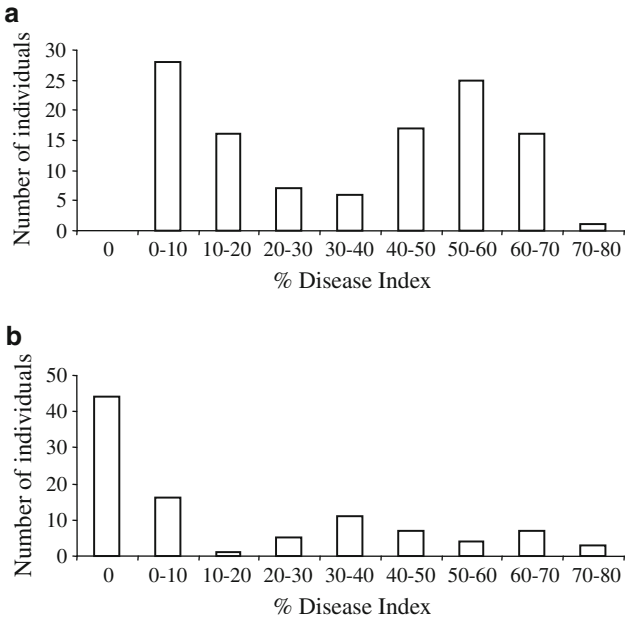


**Fig. 3** Frequency distribution of (**a**) VH and (**b**) TD based on disease reaction after inoculating with white rust isolate AcB1. Mean PDI was estimated based on three independent experiments from VH and five independent experiments from TD populations

significantly from the 1:1 ratio ($\chi^2 = 0.64$ at 95% confidence limit and $p < 0.05$) signifying that in this case also a single gene governs the resistance trait. QTL analysis revealed a single major locus mapping in the linkage group A5 within the genetic interval of 18–24 cM (Fig. 4b). The maximum peak, accounting for 70% of the phenotypic variance was found at 23 cM at an LOD value of 25. This locus was designated AcB1-A5.1.

Use of comparative mapping with *Arabidopsis thaliana* (At) to generate markers tightly linked to the white rust resistance loci

The AcB1-A4.1 white rust resistance locus in the VH *B. juncea* map (Fig. 4a) is homologous to the 'S block' of chromosome 5 of At (Panjabi et al. 2008). To further saturate this QTL region in the *B. juncea* map, IP primers with the criteria defined previously (Panjabi et al. 2008) were designed from the S block of At. Of the 54 primer pairs tested, 8 markers (At5g36950, At5g37580, At5g40670a, At5g40390, At5g40950, At5g40200, At5g41360 and At5g41940) mapped to the AcB1-A4.1 region (Fig. 4a). To find the markers closely linked with the resistance locus, a genotype–phenotype co-segregation analysis of the markers spanning the QTL region 6.4–25.2 cM was performed. Except for the four individuals, the phenotype of the rest of the population showed perfect co-segregation with the markers, At5g41560 and At5g41940, spanning a distance of 1.2 cM (Fig. 4a).

The resistance locus AcB1-A5.1 in the TD *B. juncea* map flanked by markers At2g46390 and At2g29530 (Fig. 4b) is homologous to the 'J' block of chromosome 2 of At (Panjabi et al. 2008). Although 15 AFLP markers were present in this region (unpublished data), a large region of 27.3 cM was still devoid of markers. An attempt was made to saturate this region with more IP markers from the J block syntenous region. A total of 147 IP primers were designed from the J block of At, of which 38 primer pairs were polymorphic between the two parents, TM-4 and Donskaja-IV. Four of these (At2g45790b, At2g36360, At2g34510 and At2g32920) were mapped to the QTL region. With the addition of these new IP markers, the QTL was localized to the region of 18–24 cM, flanked by markers At2g45790b and At2g34510 (Fig. 4b). Co-segregation analysis of the disease phenotype with the markers available in the region revealed that the AcB1-A5.1 locus lies between the markers At2g34510 and At2g36360. Marker At2g36360 was found to be tightly linked with the resistance locus since no recombinants were seen, while three recombinants were found between the marker At2g34510 and AcB1-A5.1.

## Discussion

Verma et al. (1999) distinguished an Indian isolate of *A. candida* infecting *B. juncea* from race 2 of Canadian isolate on the basis of the differential interaction of these two isolates on *B. rapa* var. yellow sarson R-500. This Indian isolate was designated race 12. However, the above study did not include *B. juncea* host differentials such as Domo or Cutlass, which are resistant to race 2A but show susceptibility to its variant race 2V (Rimmer et al. 2000). In the present study, we observed that the *B. juncea* host differential Cutlass (Table 1) showed severe susceptibility reaction against the Indian isolate AcB1, indicating that AcB1 could be similar to race 2V.

Among the *B. juncea* lines, Donskaja-IV was identified as a source of complete resistance with extensive hypersensitive response and no mycelial growth on infection. On the other hand, Heera was identified as a source of partial resistance, supporting the growth of the fungal mycelia, albeit in a restricted manner, leading to occasional pustule formation. The resistance trait in both these lines was found to be simply inherited, controlled in each case by a single dominant gene. However, these two sources were found to be non-allelic as they mapped to two different linkage groups. The Donskaja-IV source (AcB1-A5.1) mapped to linkage group A5 and the Heera source (AcB1-A4.1) mapped to the linkage group A4 of the *B. juncea* genome. Both Donskaja-IV and Heera belong to the east European germplasm. The presence of different loci governing resistance to the same

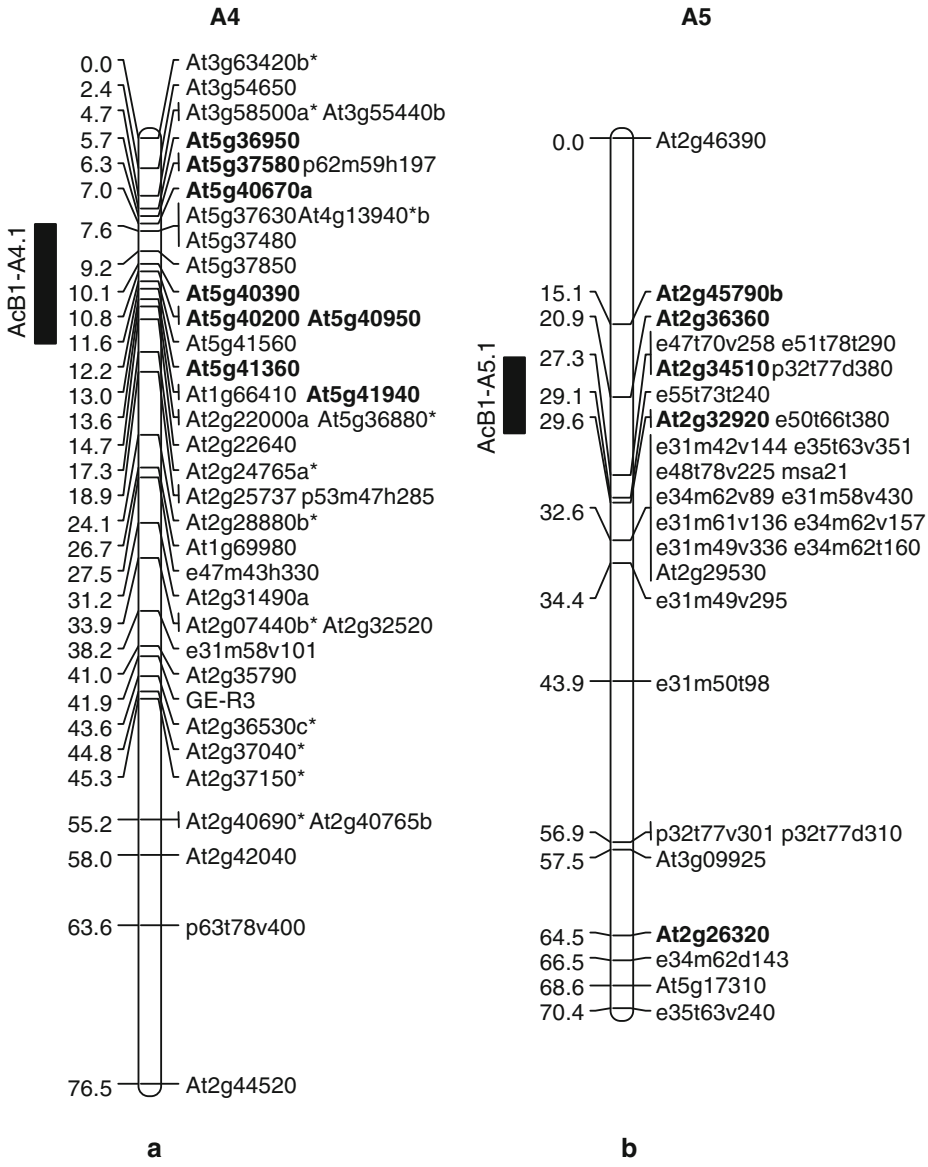


**Fig. 4** QTL mapping of white rust resistance in two DH populations of *Brassica juncea*. (**a**) In the VH population one major QTL (AcB1-A4.1; *black bar*) was mapped in the linkage group A4 at a genetic interval of 7–17 cM and (**b**) in the TD population, a major QTL (AcB1-A5.1; *black bar*) was detected in the linkage group A5 at a genetic interval of 18–24 cM. The markers highlighted in *bold* represent the new IP markers mapped using syntenic relationship with *Arabidopsis*

isolate of *A. candida* suggests that the two loci have evolved independently.

Based on the syntenic relationships between *A. thaliana* and *B. juncea* (Panjabi et al. 2008), additional IP markers could be mapped to saturate both the regions in the *Brassica* map where the resistance-conferring QTLs were localized. With these additional IP markers, AcB1-A4.1 locus in Heera was localized to a 1.2-cM region flanked by markers At5g41560 and At5g41940 (Fig. 4a), and these markers were found to be closely linked with the resistance phenotype. In Donskaja-IV, the resistance loci AcB1-A5.1 was localized between the markers At2g34510 and At2g36360, covering a 6.4-cM region (Fig. 4b), and the marker At2g36360 was found to be closely linked with the resistance loci. This study therefore shows that syntenic relations between the *Brassica* species and At can be used to generate IP markers for fine mapping of important loci. These closely linked markers could be used for marker-assisted pyramiding of the two loci.

Genes present in the syntenic regions in At (TAIR; http://www.arabidopsis.org/) were analyzed to search for candidate genes involved in disease resistance/pathogenesis. Syntenic region in At corresponding to AcB1-A4.1 locus flanked by markers, At5g41560 and At5g41940, revealed the presence of at least three disease resistance genes (At5g41550, At5g41740 and At5g41750). All the three genes are annotated to be coding for TIR-NBS-LRR class of disease resistance protein. In the syntenic region in At corresponding to the region between markers At2g34510 and At2g36360 (where locus AcB1-A5.1 was mapped in Donskaja-IV), four genes, At2g34690, At2g34930, At2g35960 and At2g35980, have been described as genes

that could be involved with disease resistance. None of the genes in this region belong to the TIR-NBS-LRR class. Further studies will focus on deciphering if any of these putative candidate genes are involved in governing white rust resistance in Brassica or new genes altogether have evolved.

Earlier mapping work on resistance to white rust in *B. rapa* (Kole et al. 1996) and *B. napus* (Ferreira et al. 1995) have also shown the involvement of a major locus (in addition to minor loci involved in the extent of disease expression) in conferring resistance to white rust. In *B. rapa*, a single major locus (ACA1) has been mapped to linkage group 4 (LG A6 in *B. juncea*), while in *B. napus* ACA1 locus has been localized to linkage group 9 (A2 in *B. juncea*). Hence the resistant loci identified by molecular mapping in the previous reports are different from the ones reported in the present study (linkage group A4 and A5), suggesting that all these loci have evolved independently and could be used either for stacking or sequential deployment. Single dominant gene conferring resistance to *A. candida* race 2 in *B. juncea* has also been reported in some other studies (Cheung et al. 1998; Prabhu et al. 1998; Mukherjee et al. 2001). Since the linkage groups in these studies have not been identified, no correlation can be made between the genes mapped in the previous studies and those found in the present study.

Many wild and related species/genera of Brassica also show resistance to *A. candida*. Moderate resistance has been reported in certain species of *Diplotaxis* (Gupta et al. 1995) and *Sinapis alba* (Saharan et al. 1988). All the accessions of *Eruca sativa* have been reported to be resistant to race 2, which infects *B. juncea* (Bansal et al. 1997). Additionally, as a distant relative, Arabidopsis also holds great potential for identification of useful sources of resistance for crop plants. Three loci (*RAC1*, *RAC2* and *RAC3*) conferring resistance to *A. candida* isolate Acem1 have been mapped in Arabidopsis (Borhan et al. 2001), of which *RAC1* has been cloned and was found to code for a TIR-NB-LRR protein (Borhan et al. 2004). More recently, a white rust resistance gene, *WRR4* (a TIR-NB-LRR encoding gene), was cloned from Arabidopsis that conferred broad-spectrum resistance to four races of *A. candida*- 2, 4, 7 and 9, when inoculated on susceptible Arabidopsis ecotypes. These races happen to be the most destructive on different Brassica crop species (Borhan et al. 2008). It will be useful to see if *WRR4* will confer resistance to *A. candida* strain used in this study.

We had earlier reported that there are two major gene pools in oilseed mustard, the Indian and the east European (Srivastava et al. 2001). Hybrids between the two gene pools are heterotic for yield (Pradhan et al. 1993). Based on this earlier work, a hybrid, DMH-1, was developed and released in India based on the combiners belonging to the two heterotic gene pools (Sodhi et al. 2006). DMH-1 shows field resistance to white rust, as observed for almost 7 years, in which period the hybrid was extensively tested at multiple locations in the mustard growing areas of India. The resistance is inherited from the east European parent Heera containing the locus AcB1-A4.1. Since, under epiphytotic conditions the east European parent is only partially resistant to white rust, this hybrid could very soon become vulnerable to it. It is therefore necessary to stack multiple genes to breed for more durable resistance in the hybrid DMH-1. The identification of the resistance locus (AcB1-A5.1) in the completely resistant parent Donskaja-IV and mapping of the locus between markers At2g34510 and At2g36360 will allow marker-assisted transfer of this locus to the east European or Indian gene pool combiner of DMH-1. Through our earlier mapping efforts, it is also known that no other major QTL for any of the yield traits is present in the region in which AcB1-A5.1 is located (Ramchiary et al. 2007). The work described here will have its immediate application in protecting hybrid DMH-1 from vulnerability to *A. candida*. Introgression of locus AcB1-A5.1 into highly susceptible Indian-type lines would also be beneficial to yield stability in the pure lines currently grown extensively in the northwestern parts of India. Marker-assisted introgression of Donskaja-IV locus AcB1-A5.1 is currently underway. It will be worthwhile to analyze the east European germplasm more extensively to discover if there are more independent loci for resistance to white rust. A more detailed isolation of *A. candida* strains from different geographical locations in India is also necessary.

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## References

Bansal VK, Tewari JP, Tewari I, Gomez-Campo C, Stringam GR (1997) Genus *Eruca*: a potential source of white rust resistance in cultivated Brassicas. Plant Genet Res Newslett 109:25–26

Bisht IS, Agrawal RC, Singh R (1994) White rust (*Albugo candida*) severity in mustard (*Brassica juncea*) varieties and its effects on seed yields. Plant Var Seeds 7:85–89

Borhan MH, Brose E, Beynon JL, Holub EB (2001) White rust (*Albugo candida*) resistance loci on three Arabidopsis chromosomes are closely linked to downy mildew (*Peronospora parasitica*) resistance loci. Mol Plant Pathol 2:87–95

Borhan MH, Holub EB, Beynon JL, Rozwadowski K, Rimmer SR (2004) The Arabidopsis TIR-NB-LRR gene *RAC1* confers resistance to *Albugo candida* (white rust) and is dependent on *EDS1* but not *PAD4*. Mol Plant-Microbe Interact 17:711–719

Borhan MH, Gunn N, Cooper A, Gulden S, Tör M, Rimmer SR, Holub EB (2008) *WRR4* encodes a TIR-NB-LRR protein that confers

broad-spectrum white rust resistance in *Arabidopsis thaliana* to four physiological races of *Albugo candida*. Mol Plant-Microbe Interact 6:757–768

Cheung WY, Gugel RK, Landry BS (1998) Identification of RFLP markers linked to the white rust resistance gene (*Acr*) in mustard (*Brassica juncea* (L.) Czern. and Coss.). Genome 41:626–628

Fan Z, Rimmer RS, Stefansson BR (1983) Identification of resistance to *Albugo candida* in rape (*Brassica napus* L.). Can J Genet Cytol 25:420–424

Ferreira ME, Williams PH, Osborn TC (1995) Mapping of a locus controlling resistance to *Albugo candida* in *Brassica napus* using molecular markers. Phytopathology 85:218–220

Gupta S, Sharma TR, Chib HS (1995) Evaluation of wild allies of Brassica under natural conditions. Cruc Newslett 17:10–11

Koch E, Slusarenko A (1990) *Arabidopsis* is susceptible to infection by a downy mildew fungus. Plant Cell 2:437–445

Kole C, Teutonico R, Mengistu A, Williams PH, Osborn TC (1996) Molecular mapping of a locus controlling resistance to *Albugo candida* in *Brassica rapa*. Phytopathology 86:367–369

Lakra BS, Saharan GS (1989) Correlation of leaf and staghead infection intensities of white rust with yield and yield components of mustard. Indian J Mycol Plant Pathol 19:279–281

Liu JQ, Parks P, Rimmer SR (1996) Development of monogenic lines for resistance to *Albugo candida* from a Canadian *Brassica napus* cultivar. Phytopathology 86:1000–1004

McKinney HH (1923) Influence of soil temperature and moisture on the infection of wheat seedling by *Helminthosporium sativum*. J Agric Res 26:195–217

Mukherjee AK, Mohapatra T, Varshney A, Sharma R, Sharma RP (2001) Molecular mapping of a locus controlling resistance to *Albugo candida* in Indian mustard. Plant Breed 120:483–487

Panjabi P, Jagannath A, Bisht NC, Padmaja KL, Sharma S, Gupta V, Pradhan AK, Pental D (2008) Comparative mapping of *Brassica juncea* and *Arabidopsis thaliana* using intron polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C Brassica genomes. BMC Genomics 9:113

Petrie GA (1988) Races of *Albugo candida* (white rust and staghead) on cultivated Cruciferae in Saskatchewan. Can J Plant Pathol 10:142–150

Prabhu KV, Somers DJ, Rakow G, Gugel RK (1998) Molecular markers linked to white rust resistance in mustard *Brassica juncea*. Theor Appl Genet 97:865–870

Pradhan AK, Sodhi YS, Mukhopadhyay A, Pental D (1993) Heterosis breeding in Indian mustard (*Brassica juncea* L. Czern & Coss): analysis of component characters contributing to heterosis for yield. Euphytica 69:219–229

Pradhan AK, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D (2003) A high-density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor Appl Genet 106:607–614

Ramchiary N, Bisht NC, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D, Pradhan AK (2007) QTL analysis reveals context-dependent loci for seed glucosinolate trait in the oilseed *Brassica juncea*: importance of recurrent selection backcross scheme for the identification of 'true' QTL. Theor Appl Genet 116:77–85

Rimmer SR, Mathur S, Wu CR (2000) Virulence of isolates of *Albugo candida* from western Canada to *Brassica* species. Can J Plant Pathol 22:229–235

Sachan JN, Kolte SJ, Singh B (1995) Genetics of resistance to white rust (*Albugo candida* race-2) in mustard (*Brassica juncea* (L.) Czern & Coss). In: GCIRC ninth International Rapeseed Congress, Cambridge, UK, pp 1295–1297

Saharan GS, Verma PR (1992) White rusts-A review of economically important species. International Development Research Centre, Ottawa, p 65

Saharan GS, Kaushik CD, Kaushik JC (1988) Sources of resistance and epidemiology of white rust of mustard. Indian Phytopathol 41:96–99

Singh NR, Devi NS, Singh NI (1990) Yield loss on Indian mustard in Manipur caused by *Albugo candida* Lev. Kunze. New Agriculturist 1:39–42

Sodhi YS, Chandra A, Verma JK, Arumugam N, Mukhopadhyay A, Gupta V, Pental D, Pradhan AK (2006) A new cytoplasmic male sterility system for hybrid seed production in Indian oilseed mustard *Brassica juncea*. Theor Appl Genet 114:93–99

Somers DJ, Rakow G, Rimmer SR (2002) *Brassica napus* DNA markers linked to white rust resistance in *Brassica juncea*. Theor Appl Genet 104:1121–1124

Srivastava A, Gupta V, Pental D, Pradhan AK (2001) AFLP-based genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of *Brassica juncea*. Theor Appl Genet 102:193–199

Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB (1997) Subcellular localization of $H_2O_2$ in plants. $H_2O_2$ accumulation in papillae and hypersensitive response during the barley–powdery mildew interaction. Plant J 11:1187–1194

Tiwari AS, Petrie GA, Downey RK (1988) Inheritance of resistance to *Albugo candida* race 2 in mustard [*Brassica juncea* (L). Czern and Coss]. Can J Plant Sci 68:297–300

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

Verma U, Bhowmik TP (1989) Inheritance of resistance to a *Brassica juncea* pathotype of *Albugo candida* in *B.* napus. Can J Plant Pathol 11:443–444

Verma PR, Saharan GS, Bartaria AM, Shivpuri A (1999) Biological races of *Albugo candida* on *Brassica juncea* and *B.* rapa var. toria in India. J Mycol Pl Pathol 29:75–82

Wang S, Basten CJ, Gaffney P, Zeng ZB (2005) Windows QTL Cartographer version 2.5. North Carolina State University, Raleigh

Williams PH (1985) Crucifer genetics cooperative resource book. Department of Plant Pathology, University of Wisconsin, Madison

Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468