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ORIGINAL ARTICLE

Fine mapping of a powdery mildew resistance gene *MlIW39* derived from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*)

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Abstract

Key message **Powdery mildew resistance gene *MlIW39*, originated from wild emmer wheat accession IW39, was mapped to a 460.3 kb genomic interval on wheat chromosome arm 2BS.**

Abstract Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is destructive disease and a significant threat to wheat production globally. The most effective way to control this disease is genetic resistance. However, when resistance genes become widely deployed in agriculture, their effectiveness is compromised by virulent variants that were previously minor components of the pathogen population or that arise from mutation. This necessitates continual search for new sources of resistance in both wheat and its near relatives. In this study, we produced a common wheat line 8D49 (87-1/IW39//2*87-1), which has all-stage immunity to *Bgt* isolate E09 and many other Chinese *Bgt* isolates, by transferring powdery mildew resistance from Israeli wild emmer wheat (WEW) accession IW39 to the susceptible common wheat line 87-1. Genetic analysis indicated that the powdery mildew resistance in 8D49 was controlled by a single dominant gene, temporarily designated *MlIW39*. Genetic linkage analyses with molecular markers showed that *MlIW39* was located in a 0.7 cm genetic region between markers *QB-3-16* and *7Seq546* on the short arm of chromosome 2B. Fine mapping using three large F_2 populations delimited *MlIW39* to a physical interval of approximately 460.3 kb region in the WEW reference genome (Zavitan v1.0) that contained six annotated protein-coding genes, four of which had gene structures similar to known disease resistance genes. This provides a foundation for map-based cloning of *MlIW39*. Markers *7Seq622* and *7Seq727* co-segregating with *MlIW39* can be utilized for marker-assisted selection in further genetic studies and wheat breeding.

Introduction

Wheat powdery mildew, caused by the biotrophic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a destructive foliar disease that threatens wheat production, especially in regions with humid and cool climates (Juroszek and von Tiedemann 2013). It is more severe in dense crops of semi-dwarf cultivars grown under irrigated conditions with high inputs of fertilizer (Singh et al. 2016). In China, the areas affected by powdery mildew range from 6 to 8 million hectares leading to grain losses of 300,000 metric tons each year in the past decade (Ma et al. 2016a; Sun et al. 2018; Chen et al. 2019). The most effective, economical and environment-friendly method to control powdery mildew is resistant cultivars. However, new introductions with race-specific resistance are usually rapidly overcome by virulent races of the pathogen, especially when a single resistance gene is deployed across a wide area (Luo et al. 2009; Li et al. 2020a). Consequently, a search for new sources of resistance for breeding is an ongoing task.

To date, more than 100 *Pm* resistance genes/alleles, including 68 that are formally named, have been identified and mapped on all wheat chromosomes except for 3D and 4D (https://shigen.nig.ac.jp/wheat/komugi/genes/symbo

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lClassList.jsp; He et al. 2020; Kang et al. 2020). Plant resistance genes tend to be clustered rather than evenly distributed across wheat genome (McHale et al. 2006). For instance, several genes are located in wheat chromosome arm 2BS, indicating a complex cluster that includes *pm26* (Rong et al. 2000), *pm42* (Hua et al. 2009), *MlIW170* (Liu et al. 2012), *Ml5323* (Piarulli et al. 2012), *PmL962* (Shen et al. 2015), *pmWE99* (Ma et al. 2016b) and *Pm68* (He et al. 2020).

Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*, WEW, AABB, $2n = 4x = 28$), the progenitor of both cultivated tetraploid and hexaploid wheats (Nevo et al. 2002), carries a significant number genes for resistance to powdery mildew (Xie and Nevo 2008). Some of these genes have been transferred to durum and/or common wheat, including *Mlzec1* (Mohler et al. 2005) and *MlAB10* (Maxwell et al. 2010) on chromosome arm 2BL; *PmG16* (Ben-David et al. 2010), *MlIW72* (Ji et al. 2008) and *MlIW172* (Ouyang et al. 2014) on 7AL; *Ml3D232* (Zhang et al. 2010) on 5BL; *MlIW30* (Geng et al. 2016) and *MlNFS10* (Yin et al. 2021) on 4AL; and *PmG3M* (Xie et al. 2012) on 6BL. Other WEW derived *Pm* genes include *Pm16* (Reader and Miller 1991) and *Pm30* (Liu et al. 2002) that are possibly allelic on chromosome arm 5BS (Chen et al. 2005), *Pm36* (Blanco et al. 2008) on 5BL, *Pm41* (Li et al. 2009) on 3BL and *Pm64* (Zhang et al. 2019) on 2BL. Among all these genes, only *Pm41* has been cloned and it encodes a protein with a coiled-coil, nucleotide-binding site and leucine-rich repeat structure (CNL) (Li et al. 2020b), which is typical of many disease resistance genes in the plant kingdom.

Molecular markers, especially the simple sequence repeat (SSR), are highly efficient for gene localization (Zhang et al. 2019). However, SSR markers are not uniformly distributed on chromosomes and their numbers are insufficient for fine mapping of target genes (https://wheat.pw.usda.gov/GG3/). The releases of reference genome sequences of common wheat (*Triticum aestivum* cv. Chinese Spring RefSeq v.1.0) (IWGSC 2018) and whole-genome-shotgun assembly of WEW accession Zavitan (Avni et al. 2017) have greatly assisted marker development and map-based cloning of important genes. In addition to SSR markers, re-sequencing of mapping parents allows identification of single nucleotide polymorphisms (SNP) and insertion/deletion (InDel) variations in target regions providing further relevant markers for fine mapping (Li et al. 2020c).

In the present study, we report: (1) identification and fine mapping of powdery mildew resistance gene *MlIW39* that was introgressed from WEW accession IW39 into common wheat, and (2) development of co-segregating markers allowing efficient selection of the resistance allele in genetic studies and wheat breeding.

Materials and methods

Plant materials

Powdery mildew resistant line 8D49 (87-1/IW39//2*87-1) is a second backcross selection from a wild emmer wheat (WEW) accession IW39 and susceptible Chinese common wheat line 87-1. WEW accession IW39 was kindly provided by Dr. T. Fahima, University of Haifa, Israel. 8D49 was highly resistant to *Bgt* isolate E09 both at the seedling and adult growth stages (all-stage resistance). 8D49 was crossed with the susceptible spring wheat Apogee and 152 $F_{2:3}$ lines were used for genetic analyses and mapping. To fine map the target gene, three populations of 2303 F_2 plants were generated from crosses of 8D49 with susceptible cultivars Shi4185, Liaochun10 and Apogee, respectively. Xuezao, a highly susceptible common wheat line, was used as the susceptible control and to multiply *Bgt* isolate E09, which was provided by Dr. Xiayu Duan, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing, China. Five hundred and forty-five Chinese varieties/landraces and 279 accessions from other countries were used to test the efficacy of the markers co-segregating with *MlIW39* in further research and breeding (genotype list not shown).

Powdery mildew evaluations

The parental lines 8D49, Apogee, Shi4185, Liaochun10, and the corresponding F_1, F_2, $F_{2:3}$ materials and recombinant families from the fine mapping populations were evaluated for response to powdery mildew under greenhouse conditions. In brief, the F_2 seeds were planted in rectangular seedling trays with 128 cells (8×16), 1 seed per cell. Thirty seeds for each $F_{2:3}$ family were planted in 50-cell trays (5×10) and each family was sown in two cells (15 seeds per cell). The resistant and susceptible parents, as well as susceptible control, were planted in the middle of each tray as the resistant and the susceptible controls, respectively. Seedlings with unfolded first leaves were inoculated with *Bgt* E09 by dusting and brushing of conidiospores. Infection types (ITs) were evaluated after 15 days and re-confirmed two days later on a scale of 0–4, in which, 0, 0; 1, 2, 3, 4 represented immune, necrotic flecks, high resistance, moderate resistance, moderate susceptibility and high susceptibility, respectively. Phenotypes were classified into two groups, resistant (R, IT 0–2) and susceptible (S, IT 3–4) (Liu et al. 1999). 8D49 was also challenged by 25 *Bgt* isolates collected from different regions of China, and the susceptible Zhongzuo9504 was the control.

Bulked segregant analysis

The leaves of individual F_2 plants were used to extract the genomic DNA by using a modified cetyltrimethylammonium bromide (CTAB) method (Devi et al. 2013). Based on F_2 genotypes deduced from powdery mildew responses of corresponding F_3 families, resistant and susceptible DNA pools were constructed by bulking 10 homozygous resistant and 10 homozygous susceptible F_2 plants, respectively. The DNA pools were genotyped with the Illumina 90 K iSelect SNP Chip by China Golden Marker Biotech Co. (CGMB, Beijing, China).

Marker development and genotyping

SSR markers were designed according to the Chinese Spring reference genome sequence (RefSeq v1.0) (https://wheat-urgi.versailles.inra.fr/) using BatchPrimer3 with minim of 12, 10, 8, 6 and 4 di-nucleotide, tri-nucleotide, tetra-nucleotide, penta-nucleotide and hexa-nucleotide SSR pattern repeats, respectively (http://batchprimer3.bioinformatics.ucdavis.edu/). To improve the efficiency of marker development, the parental lines 8D49 and Apogee were re-sequenced. Briefly, high quality genomic DNA of the parents was used to construct paired-end-sequencing libraries with insert sizes of approximately 500 bp (Chai et al. 2018). The average sequencing depth was 6× of the Chinese Spring reference genome. Sequence reads were generated using the Illumina HiSeq X Ten platform with 150-bp paired-end reads and mapped to the Chinese Spring reference genome (RefSeq v1.0) with the Burrows-Wheeler Aligner program (BWA,ver.0.7.15) (Li and Durbin 2009). The HaplotypeCaller module of the Genome Analysis Toolkit (GATK) was used to identify single-nucleotide polymorphisms (SNPs) and insertion/deletions (InDels) sequences. We searched the InDel variations between pairs of parents in the target interval to design InDel markers as described in Qiu et al (2020). The SSR and InDel markers used to fine map the powdery mildew resistance locus are listed in Table S3.

The PCR system contained 5 µl 2 × *Taq* PCR StarMix, 1.5 µl primer (mixture of left and right primers, 2 µM), 1.5 µl DNA template (50–100 ng/µl) and 2 µl ddH_2O. PCR cycling was as follows: 94 °C for 5 min; 35 cycles of 94 °C denaturation for 30 s, 56 °C primer annealing for 30 s, 72 °C extension for 30 s; and a final extension of 72 °C for 5 min. PCR products were separated in 10% non-denaturing polyacrylamide gels (39 acrylamide:1 bisacrylamide), that were silver stained and photographed after electrophoresis.

Data analysis

SSR and InDel markers polymorphic between the resistant and susceptible pools, along with the two parents, were used to genotype the 152 F_2 individuals derived from the cross Apogee × 8D49 for linkage analysis. The linkage map was constructed with JoinMap4.0 (Van Ooijen 2006) and the Kosambi mapping function was used to convert recombination values into map distance (Kosambi 1943). Mapdraw was used to draw the linkage map (Liu and Meng 2003). Chi-squared tests for goodness-of-fit were used to evaluate the deviations of observed segregation ratios from the theoretical Mendelian ratios.

Comparative genomics analysis

The nearest flanking markers were used as queries in BLASTn search against the genomes of durum wheat cv. Svevo (https://www.interomics.eu/durum-wheat-genome; Maccaferri et al. 2019) and hexaploid wheat cv. Chinese Spring (http://www.wheat-urgi.versailles.inra.fr/; IWGSC 2018), Norin61, Mace, CDC Stanley, Jagger, Julius_MAGIC3 and spelt wheat PI 190962 (https://webblast.ipk-gatersleben.de/wheat_ten_genomes/; Walkowiak et al. 2020) to confirm the corresponding physical interval in these genomes and the annotated genes in the target interval (Tables S4, S5). Collinearity analysis among different genomes was performed on Triticeae-Gene Tribe (TGT) (http://wheat.cau.edu.cn/TGT/; Chen et al. 2020).

Sequence comparison and RT-PCR analysis of annotated genes in the target interval

Annotated genes within the mapped interval were amplified from the resistant and susceptible parents 8D49 and Shi4185 using TKS Gflex™ DNA polymerase (TAKARA, Dalian, China) using the primers listed in Table S6. Primers were designed using Primer3Plus (http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi) according to the information in the WEW accession Zavitan reference genome. PCR products were separated by 1% agarose gel electrophoresis, and target DNA fragments were recycled from the gel and sequenced by the TsingKe Biological Technology in Beijing. The sequencing results were analyzed with the DNAMAN version 6.0 (Gao et al. 2019).

Leaves of 8D49 and Shi4185 at two-leaf seedling stage were collected at 24 hours post inoculation (hpi) with *Bgt* isolate E09 for total RNA isolation with TRIzol reagent (Invitrogen, Shanghai, China). First-strand cDNA was synthesized from 1 ug total RNA using a FastKing RT Kit following the manufacturer's instructions (TIANGEN Biotech Co. Ltd, Beijing, China). Specific primers used for RT-PCR analysis are listed in Table S6.

Results

Genetic analysis of powdery mildew resistance in 8D49

The parental lines, F_1 and F_2 populations derived from crosses Apogee × 8D49, Liaochun10 × 8D49 and Shi4185 × 8D49 were tested for response to *Bgt* isolate E09. 8D49 was highly resistant (IT = 0;), whereas Apogee, Liaochun10 and Shi4185 were highly susceptible (IT = 4) (Fig. 1). F_1 plants were resistant (IT = 0;), indicating dominance of resistance. Resistant plants produced a very thin layer of mycelia on infected leaves, then the infection sites necrosed with expansion over time, whereas the leaves of susceptible plants remained green with heavy sporulation. The segregations of resistant and susceptible F_2 plants as well as the pooled data fitted 3:1 ratio (Table 1). These results indicate that the powdery mildew resistance in 8D49 is governed by a single dominant gene, provisionally designated as *MlIW39*.

Bulked segregant analysis (BSA) and molecular mapping of the *MlIW39* locus

To determine the genomic position of the gene for the powdery mildew resistance, we constructed DNA pools from 10 homozygous resistant and 10 homozygous susceptible F_2 plants and genotyped them with the 90 K iSelect SNP Chip. One hundred and ninety-two SNPs were polymorphic between the pools of the detected 79,058 SNP markers, 67 of them (about 34.90%) were anchored on chromosome 2B; 62 (92.54%) were concentrated in the 0–40 Mb chromosomal region (Fig. 2; Tables S1, S2), suggesting the resistance gene was distally located on the short arm of chromosome 2B.

To map *MlIW39* more precisely, we designed 82 SSR primers in the candidate region based on Chinese Spring reference genome sequence (RefSeq v1.0) and screened the polymorphic markers in the two pools and parents. Six polymorphic loci, *IWB12-4*, *QB-3-16*, *SSR12*, *SSR34*, *SSR43*, *SSR48*, were identified and used to genotype the 152 F_2 individuals derived from Apogee × 8D49 and a genetic linkage map (Fig. 3a) was constructed from the data. To further saturate the genetic linkage map, an additional 23 InDel primers were developed according to re-sequenced data from the parents. This added a further four markers, *7Seq247*, *7Seq546*, *7Seq270*, *7Seq288*, to the linkage map (Fig. 3a). Finally, *MlIW39* locus was localized to a 0.7 cM genetic interval between flanking markers *7Seq546* and *QB-3-16* at genetic distances of 0.4 cM (distal) and 0.3 cM (proximal) (Fig. 3a).

Fig. 1 Phenotypes of resistant parent 8D49 and susceptible parents, including Apogee, Shi4185 and Liaochun10, at two weeks post-inoculation with *Bgt* isolate E09

Fine mapping of powdery mildew resistant gene *MlIW39*

To further narrow down the genomic region harboring the *MlIW39* locus, 48 InDel markers were developed according to the InDel polymorphisms in the *MlIW39* genetic interval between 8D49 and Apogee; 10 InDel markers were polymorphic between the resistant and three susceptible parents. The flanking markers *7Seq546* and *QB-3-16*, also polymorphic between 8D49 and Shi4185 and Liaochun10, were used to genotype all 2,303 F_2 individuals from the three mapping populations, and 38 recombinants were identified (Fig. 3b). The 10 InDel markers located between markers *7Seq546* and *QB-3-16* were used to genotype all recombinants which were classified into 20 categories (Table S7). Genotyping of the 38 recombinants was performed by progeny testing with *Bgt* isolate E09. Finally, *MlIW39* was placed in an approximate 460.3 kb interval flanked by markers *7Seq610* and *7Seq705* (Fig. 3c and d). According to the WEW Zavitan (v1.0) reference genome sequence (https://wheat.pw.usda.gov/jb/?data=/ggds/whe-zavitan2017), this genomic

Table 1 Responses of F_2 plants from crosses 8D49 with three susceptible wheat varieties to the *Bgt* isolate E09

Cross	Generation	Resistant	Susceptible	$\chi^2_{3:1}$	*P*-value
Shi4185/8D49	F_2	137	41	0.3674	0.5446
Liaochun10/8D49	F_2	276	97	0.2011	0.6539
Apogee/8D49	F_2	111	42	0.4902	0.4838
Total	F_2	524	180	0.1212	0.7277

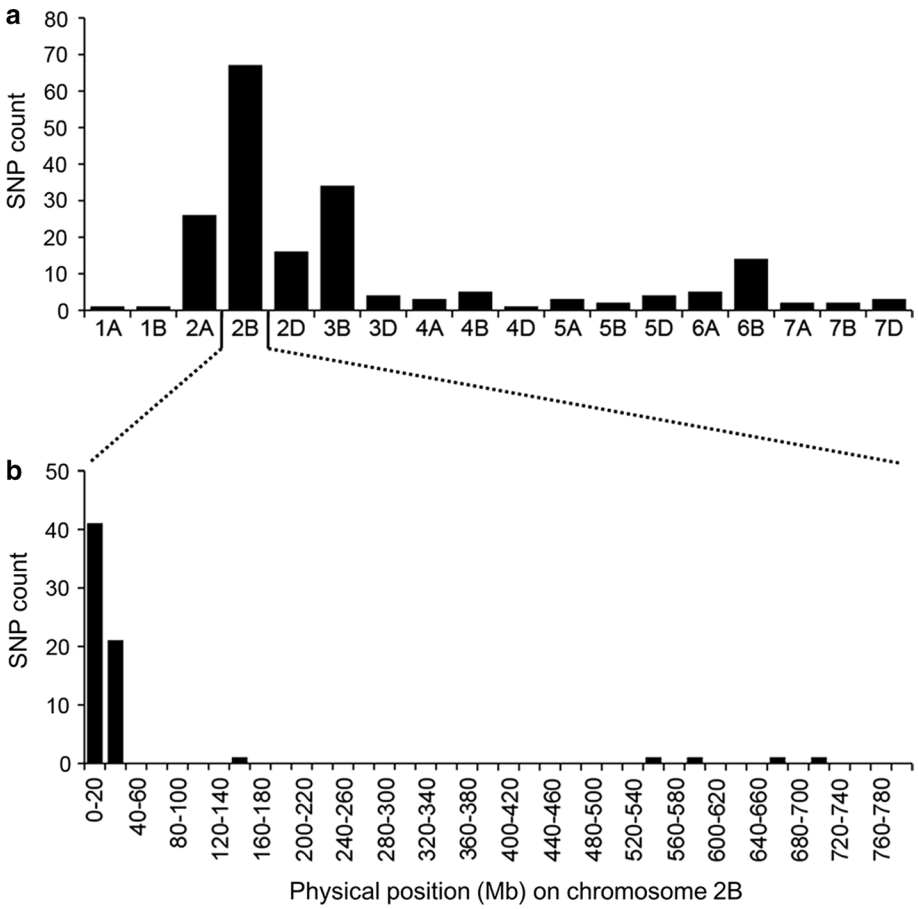

Fig. 2 Number of SNP markers between the resistant and susceptible pools of the F_2 population derived from cross 8D49 × Apogee. **a** Distribution of SNPs per chromosome; **b** The physical positions of SNPs on chromosome 2B based on the Chinese Spring reference sequence v1.0

region contained six high-confidence protein-coding genes (Fig. 3d), among which four were related to disease resistance genes. These included *TRIDC2BG003930* which encodes putative disease resistance protein homologous to At3g14460, *TRIDC2BG003950* encoding a G-type lectin S-receptor-like serine/threonine-protein kinase B120, *TRIDC2BG003970* encoding RPM1-like disease resistance protein and *TRIDC2BG003990* encoding a putative disease resistance RPP13-like protein 1.

Comparative mapping of *MlIW39* among durum and hexaploid wheat genomes

The *7Seq610* and *7Seq705* markers flanking *MlIW39* were used to search against the durum wheat cv. Svevo reference genome and hexaploid wheat genomes, including Chinese Spring, Norin61, Mace, CDC Stanley, Jagger, Julius_MAGIC3 and spelt wheat PI 190962 to define the interval in these genomes (Table S4). Protein-coding genes annotated in the corresponding interval were used to perform collinearity analysis between the tetraploid and hexaploid genomes (Table S5). Sequence analysis revealed that three genes, *TRIDC2BG003930*, *TRIDC2BG004000* and *TRIDC2BG004010*, were syntenic among different genomes, whereas three disease resistance-related genes, namely, *TRIDC2BG003950*, *TRIDC2BG003970* and *TRIDC2BG003990*, were present on chromosome arm 2BS only in WEW. On the other hand, there were three added genes in the durum and hexaploid wheat genomes and a further two only in hexaploid wheat (Fig. 4).

Analysis of the annotated genes in the candidate interval

To compare the six annotated genes in the candidate interval, we amplified *TRIDC2BG003930*, *TRIDC2BG004000* and *TRIDC2BG004010* from the resistant and susceptible parents. However, we obtained *TRIDC2BG003950*, *TRIDC2BG003970* and *TRIDC2BG003990* only from the resistant parent (Fig. S1a), a result that coincided with the comparative mapping showing these three genes present only in the WEW genome. Sequences alignment revealed that there were 7 and 10 SNPs between 8D49 and Shi4185 in genes *TRIDC2BG003930* and *TRIDC2BG004010*, respectively (Table S8). There was considerable variation in *TRIDC2BG004000* between 8D49 and Shi4185 (data not shown). RT-PCR analysis showed that all 6 predicted genes were expressed in seedlings of 8D49 after *Bgt* infection (Fig.

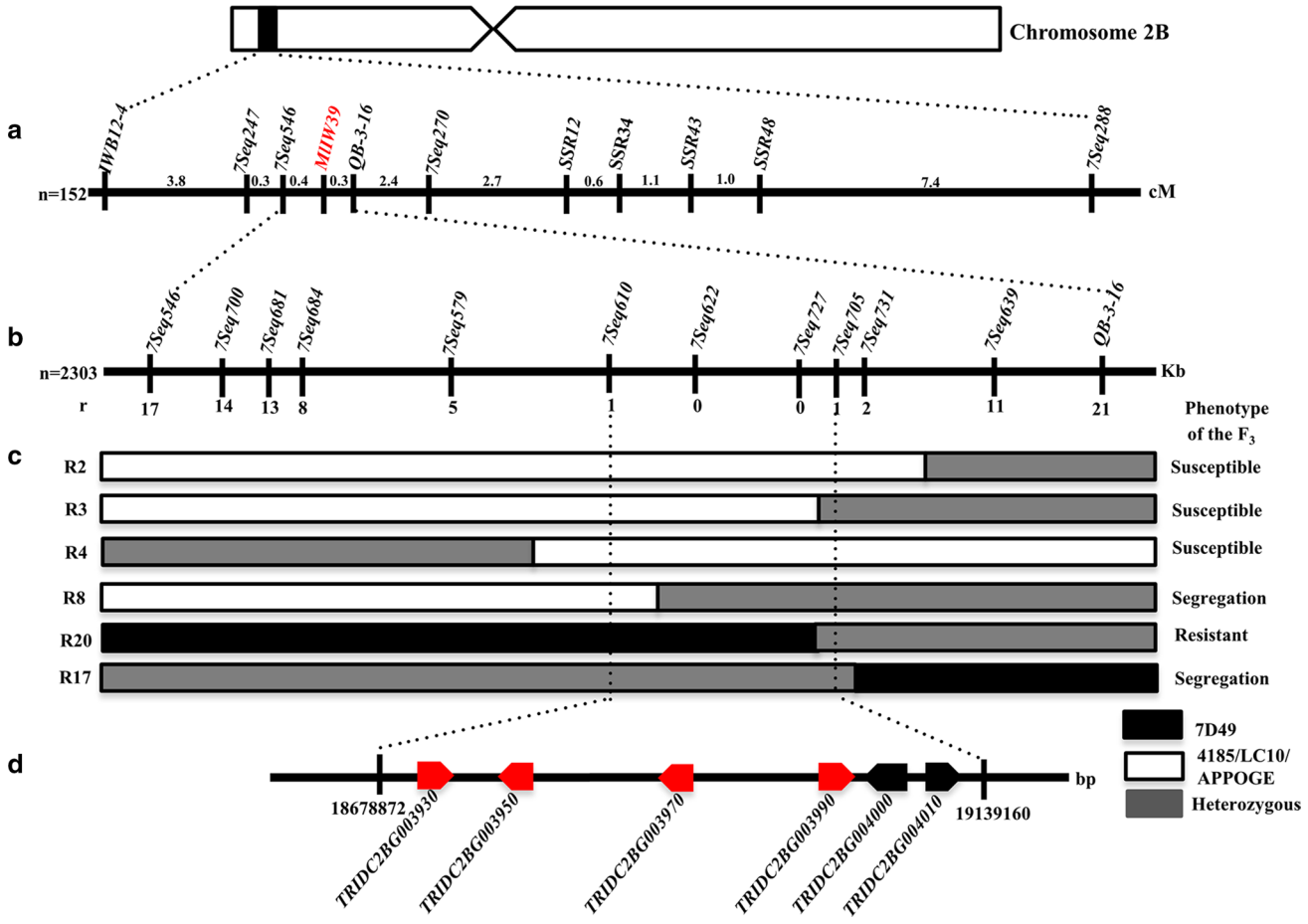

Fig. 3 Fine mapping of the powdery mildew resistance gene *MlIW39*. **a** Genetic linkage map of *MlIW39*. Numbers above the line are genetic distances between adjacent markers in cM. **b** Physical positions of molecular markers used for fine mapping of *MlIW39* according to the WEW accession Zavitan reference genome v1.0, r indicates the number of recombinants. **c** Genotypes and phenotypes of the six relevant recombinant events. Recombinant events and phenotypes are indicated at the left and right, respectively. Black, white and gray blocks indicate homozygous segments from 8D49, homozygous segments from susceptible parents and heterozygous segments, respectively. **d** Annotated genes in the target region of the *MlIW39* locus. Red pentagons represent genes related to disease resistance

S1b). Further research is needed to identify the candidate gene for *MlIW39*.

Marker assessment and response of 8D49 to a panel of *Bgt* isolates

To evaluate the usefulness of the markers flanking *MlIW39* in wheat research and breeding, two markers *7Seq622* and *7Seq727* that co-segregated with the resistance allele were tested in 545 Chinese cultivars/landraces and 279 accessions from other countries. The PCR product sizes of the two markers in 8D49 were unique and not detected in any of the 824 varieties (data not shown). Hence, these markers should be diagnostic for marker-assisted selection of *MlIW39*.

When inoculated with 25 additional *Bgt* isolates collected from different regions in China line 8D49 was resistant to almost all the isolates showing IT 0 or 0; to 6 isolates, IT 1 to 15 isolates, IT 2 to 3 isolates and IT 2^+ to 1 isolate (Table 2; Fig. S2). This result indicates that *MlIW39* confers a wide spectrum resistance and thus is a potentially valuable source of resistance for wheat breeding.

Discussion

Comparison of *MlIW39* with other powdery mildew resistance genes on 2BS

In this study, we identified and fine mapped a dominant powdery mildew resistance allele *MlIW39*, transferred to common wheat from wild emmer to a 460.3 kb genomic region on short arm of chromosome 2B according to the reference genome of WEW accession Zavitan (v1.0) (https://wheat.pw.usda.gov/jb/?data=/ggds/whe-zavitan2017) (Fig. 3d). To date, a total of seven powdery mildew resistance genes had been mapped on wheat chromosome arm 2BS, including

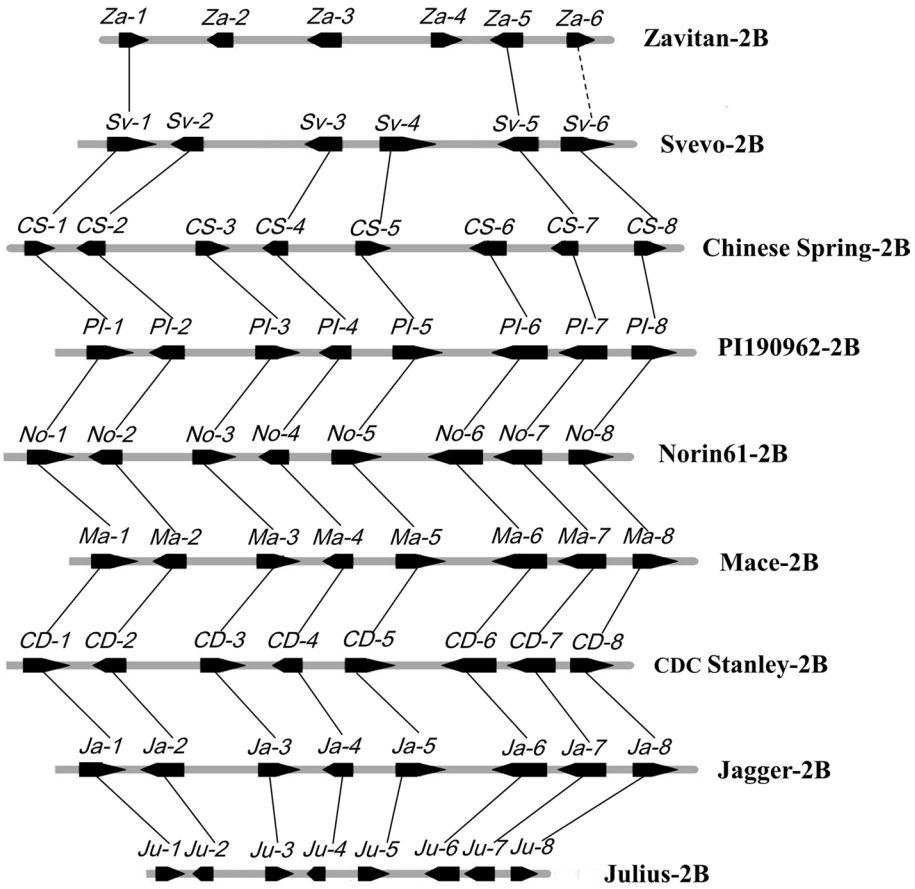

Fig. 4 Collinearity of the genomic region of *MlIW39* between durum and hexaploid wheat. Orthologous genes are linked by lines; solid and dashed lines indicate match scores of 70–100 and 50–70, respectively. *Za-1*, *TRIDC2BG003930*; *Za-2*, *TRIDC2BG003950*; *Za-3*, *TRIDC2BG003970*; *Za-4*, *TRIDC2BG003990*; *Za-5*, *TRIDC2BG004000* and *Za-6*, *TRIDC2BG004010*; *Sv-1*, *TRITD2Bv1G010240*; *Sv-2*, *TRITD2Bv1G010260*; *Sv-3*, *TRITD2Bv1G010300*; *Sv-4*, *TRITD2Bv1G010330*; *Sv-5*, *TRITD2Bv1G010390* and *Sv-6*, *TRITD2Bv1G010400*; *CS-1*, *TraesCS2B02G045700*; *CS-2*, *TraesCS2B02G045800*; *CS-3*, *TraesCS2B02G045900*; *CS-4*, *TraesCS2B02G046000*; *CS-5*, *TraesCS2B02G046100*; *CS-6*, *TraesCS2B02G046300*; *CS-7*, *TraesCS2B02G046400* and *CS-8*, *TraesCS2B02G046500*; *PI-1* to *PI-8*; *No-1* to *No-8*; *Ma-1* to *Ma-8*; *CD-1* to *CD-8*; *Ja-1* to *Ja-8* and *Ju-1* to *Ju-8* represent genes *TraesTSP2B01G048000* to *TraesTSP2B01G048700*; *TraesNOR2B01G051100* to *TraesNOR2B01G051800*; *TraesMAC2B01G050700* to *TraesMAC2B01G051400*; *TraesSTA2B01G053300* to *TraesSTA2B01G054000*; *TraesJAG2B01G047100* to *TraesJAG2B01G047800* and *TraesJUL2B01G046900* to *TraesJUL2B01G047600* in the genomes of spelt wheat PI 190962 and common wheat cultivars Norin 61, Mace, CDC Stanley, Jagger and Julius_MAGIC3, respectively

pm26 (Rong et al. 2000), *pm42* (Hua et al. 2009), *MlIW170* (Liu et al. 2012), *Ml5323* (Piarulli et al. 2012), *PmL962* (Shen et al. 2015), *pmWE99* (Ma et al. 2016b) and *Pm68* (He et al. 2020). Among these genes, *pm26*, *pm42* and *MlIW170* were from *T. dicoccoides* (Rong et al. 2000; Hua et al. 2009; Liu et al. 2012), *pm26* and *MlIW170* were reported to be allelic or closely linked loci (Liang et al. 2015). *PmL962* and *pmWE99* were from *Thinopyrum intermedium* (Shen et al. 2015; Ma et al. 2016b), *Ml5323* from *T. turgidum* ssp. *dicoccum* and *Pm68* from *T. turgidum* ssp. *durum* (Piarulli et al. 2012; He et al. 2020). *MlIW39* differs from the three known genes *pm26*, *pm42* and *MlIW170* derived from WEW according to their difference in genomic positions and effects. Based on the genetic maps of these four genes, *MlIW39* was located on the distal side of *pm26*, *MlIW170* and *pm42* (Fig. 5a, d, e, f). For their effects on resistance phenotype, *MlIW39* is a dominant gene, whereas *pm26* and *pm42* were recessive and *MlIW170* was incomplete dominant. The genes *Ml5323* and *PmL962* are proximal to marker *Xcau516*, and the genetic distances between the markers and the genes were 7.2 cM and 16.06 cM, respectively (Fig. 5g, i), while *MlIW39* was positioned at the distal part to marker *Xcau516*. The gene *pmWE99* was 10.4 cM distal to SSR marker *Xgwm148*, which mapped to the 107.9 Mb region in the WEW accession Zavitan reference assembly. The genetic distance between marker *7Seq288* and *MlIW39* was 15.5 cM, and the marker was anchored at the position 27.8 Mb in the reference assembly (Fig. 5a, b, h), indicating the

Table 2 Infection types produced by 8D49 and control Zhongzuo9504 to 25 *Bgt* isolates collected from different locations in China

Bgt isolates	Origin (city, province)	8D49	Zhongzuo9504
A5	Baoding, Hebei	1	4
A6	Baoding, Hebei	1	4
A8	Cangzhou, Hebei	0;	4
A11	Handan, Hebei	2	4
A21	Liaocheng, Shandong	2^+	4
A22	Tai'an, Shandong	1	4
A29	Beijing	1	4
A31	Anyang, Henan	1	4
A37	Jinzhong, Shanxi	1	4
A38	Wenjiang, Sichuan	1	4
A40	Wenjiang, Sichuan	0;	4
A43	Tianjin	2	4
A44	Linfen, Shanxi	2	4
A48	Beijing	1	4
A49	Shijiazhuang, Hebei	1	4
A70	Yangling, Shaanxi	0;	4
A71	Linfen, Shanxi	0;	4
B4	Handan, Hebei	1	4
B12	Shijiazhuang, Hebei	1	4
B22	Tai'an, Shandong	1	4
B23	Zibo, Shandong	1	4
B34	Xinxiang, Henan	1	4
B39	Sichuan	1	4
B41	Tianshui, Gansu	0	4
B50	Cangzhou, Hebei	0;	4

pmWE99 is far from *MlIW39*. Moreover, *pmWE99* gene was recessive, originated from *Th. intermedium*. Based on above analysis, we concluded that *MlIW39* was different from these six genes.

He et al. (2020) reported a powdery mildew resistance gene *Pm68* in durum wheat and located it on the physical interval 21.59–23.37 Mb in the reference genome of durum wheat cv. Svevo, which corresponded to 17.69–19.56 Mb in the reference assembly of WEW accession Zavitan (v1.0) (Fig. 5b and c). The physical interval of *Pm68* overlaps the region harboring *MlIW39* (18.7–19.1 Mb) (Fig. 5a and b), suggesting the two genes might be at the same locus or closely linked loci. The origin of the two genes was different. *Pm68* was identified from durum wheat, while *MlIW39* was from WEW. However, genetic maps are not entirely reliable and dominance might vary with background, we can't rule out the possibility that *MlIW39* may be identical or allelic to these *Pm* genes. Only after cloning of some of the genes can we determine their relationships.

Candidate genes for *MlIW39*

MlIW39 was delimited to a 460.3 kb genomic region containing 6 high-confidence protein-coding genes (*TRIDC2BG003930* to *TRIDC2BG004010*). Among the six predicted loci, four (*TRIDC2BG003930*, *TRIDC2BG003950*, *TRIDC2BG003970* and *TRIDC2BG003990*) encode disease resistance-related proteins. Moreover, the latter three genes *TRIDC2BG003950*, *TRIDC2BG003970* and *TRIDC2BG003990* were only present in the WEW genome

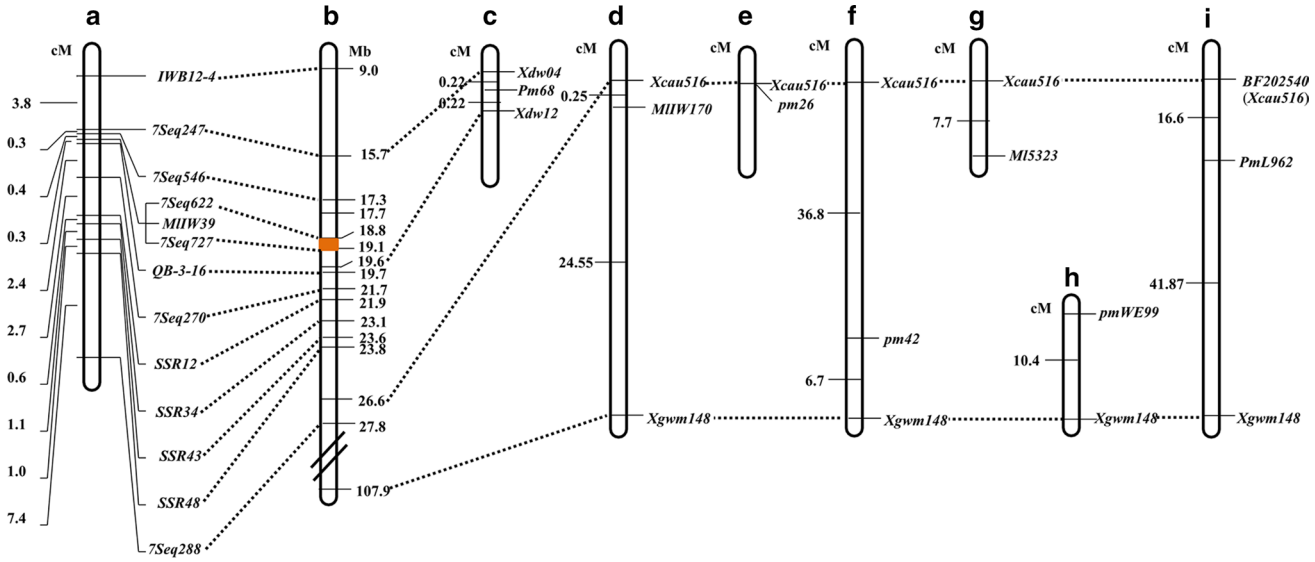

Fig. 5 Comparison of the current linkage map of *MlIW39* (**a**) with those reported for *Pm68* (**c**), *MlIW170* (**d**), *pm26* (**e**), *pm42* (**f**), *Ml5323* (**g**), *pmWE99* (**h**) and *PmL962* (**i**) using the linked markers *Xcau516/BF202540* and *Xgwm148* as anchors. **b** Physical map of the *MlIW39* region based on the WEW accession Zavitan reference genome sequence (v1.0), physical locations in Mb are shown at the right

(Fig. 4). To date, the most of cloned powdery mildew resistance genes are nucleotide-binding leucine-rich repeat (NLR) genes, including *Pm3* (Yahiaoui et al. 2004), *Pm8* (Hurni et al. 2013), *Pm2* (Sánchez-Martín et al. 2016), *Pm17* (Singh et al. 2018), *Pm60* (Zou et al. 2018), *Pm21* (He et al.2018; Xing et al. 2018), *Pm5e* (Xie et al. 2020), *Pm41* (Li et al. 2020b) and *Pm1a* (Hewitt et al. 2020). One of these four genes might be the candidate gene for *MlIW39* and cloning of the gene is underway to identify the real candidate gene.

Potential of *MlIW39* for wheat breeding

Many genes for resistance to different pathogens have been identified in wild emmer wheat, including genes for resistance to stripe rust (Klymiuk et al. 2018), leaf rust (Moseman et al. 1985), stem rust (Anikster et al. 2005), powdery mildew (Nevo et al. 1985) and Fusarium head blight (Buerstmayr et al. 2003). *Yr15* for resistance to stripe rust has been successfully used in agriculture (Yaniv et al. 2015; Chen and Kang 2017). Further explorations of WEW will unravel additional favorable genes useful for wheat improvement (Xie and Nevo 2008).

Up to now, more than 100 powdery mildew resistance genes have been reported (https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp), nearly half of them are derived from wheat wild relatives. Some resistance genes in wild relatives are associated with undesirable traits and a time-consuming procedure is required to reduce or eliminate the linkage drag (Summers and Brown 2013; Li et al. 2016). Since WEW and cultivated wheat are closely related, the chromosomes from WEW can cross over readily with their homologues in common wheat; therefore, the linkage drag will not be a problem for the utilization of genes from WEW (Nevo 2014). In this study, a powdery mildew resistance gene *MlIW39* was transferred from WEW into common wheat and resulted in an all-stage resistant line 8D49. In addition to its excellent powdery mildew resistance, 8D49 showed longer spike and larger seed size than its recurrent parent 87-1 (data not shown), indicating no detrimental effects with *MlIW39*. 8D49 is highly effective against some *Bgt* isolates collected from different regions of China (Table 2; Fig. S2). Because the powdery mildew pathogen is highly variable, one major resistance gene will not be enough to control the disease if deployed alone. *MlIW39* must be used in combination with other *Pm* genes to broaden the genetic basis and to increase the durability of any resistance that is deployed. The co-segregating molecular markers *7Seq622* and *7Seq727* of *MlIW39* should be useful in building effective combinations of resistance genes by marker-assisted selection.

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Author contribution statement CX conceived the project; LQ performed the experiments; NL, HW, QZ, WW assisted in phenotyping and genotyping of the populations; XS performed inoculation of twenty-five *Bgt* isolates; FL analyzed the SNP data; WG analyzed the re-sequenced data; ZH, HL, JM, QS assisted in revising the manuscript; LQ and CX analyzed the experimental results and wrote the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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