Fine Mapping of Powdery Mildew Resistance Gene MlWE74 Derived From Wild Emmer Wheat (Triticum Turgidum ssp. Dicoccoides) in An NBS-LRR Gene Cluster

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Fine mapping of powdery mildew resistance gene *MlWE74* derived from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) in an NBS-LRR gene cluster

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Abstract

Key message

Powdery mildew resistance gene *MlWE74*, originated from wild emmer wheat accession G-748-M, was mapped in an NBS-LRR gene cluster of chromosome 2BS.

Abstract

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a globally devastating disease. Wild emmer wheat (*Triticum turgidum* var. *dicoccoides*) is a valuable genetic resource for improving disease resistance in common wheat. A powdery mildew resistance gene was transferred to hexaploid wheat line WE74 from wild emmer accession G-748-M. Genetic analysis revealed that the powdery mildew resistance in WE74 is controlled by a single dominant gene, herein temporarily designated *MlWE74*. Bulked segregant analysis (BSA) and molecular mapping delimited *MlWE74* to the terminal region of chromosome 2BS flanking by markers *WGGBD412* and *WGGBH346* within a genetic interval of 0.25 cM and corresponding to 799.9 kb genomic region in the Zavitan reference sequence. Sequence annotation revealed two phosphoglycerate mutase-like genes, an alpha/beta-hydrolases gene, and five NBS-LRR disease resistance genes that could serve as candidates for map-based cloning of *MlWE74*. The geographical location analysis indicated that *MlWE74* is mainly distributed in Rosh Pinna and Amirim regions, in the northern part of Israel, where environmental conditions are favorable to the occurrence of powdery mildew. Moreover, the co-segregated marker *WGGBD425* is helpful in marker-assisted transfer of *MlWE74* into elite cultivars.

Introduction

Powdery mildew, caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the devastating diseases of wheat (*Triticum aestivum* L.) in areas with temperate climates. Breeding for resistance is the most economical and effective strategy to control powdery mildew. Up to now, more than a hundred powdery mildew resistance genes/alleles have been documented, and some of them have played important roles in stabilizing wheat yield, such as *Pm21* (He et al. 2018; Xing et al. 2018), *Yr18/Lr34/Pm38/Sr57* (Krattinger et al. 2009), and *Pm46*/*Yr46*/*Lr67*/*Sr55* (Moore er al. 2015). However, the emergence of new virulent pathotypes of *Bgt* reduces the resistance conferred by resistance (*R*) genes (Singh et al. 2016). Recent studies indicated that *Pm2*, *Pm3a*, *Pm3b*, *Pm3f*, *Pm4a*, *Pm6*, *Pm8*, and *Pm17* have been overcome in part or all of the USA, while *Pm1a*, *Pm3a*, and *Pm8* were defeated in Australia, China, and Egypt (Parks et al. 2008; Cowger et al. 2018). Therefore, in response to the newly evolved *Bgt* virulent isolates, it is necessary to continuous search for new powdery mildew resistance genes.

Among the powdery mildew resistance genes that were currently reported, more than half of them are derived from diploid or tetraploid wild relatives of wheat including *Aegilops squarrosa*, *Ae. speltoides*, *Ae. longissima*, *Ae. ovata*, *Dasypyrum villosum*, *T. urartu*, *T. turgidum* var. *dicoccoides, T. turgidum* var. *dicoccum*, *T. turgidum* var. *durum*, *T. timopheevii*, *T. monococcum*, *Thinopyrum intermedium*, and cereal rye (*Secale cereale* L.) (http://wheat.pw.usda.gov/). The wild relatives of wheat are important sources for discovering wheat powdery mildew resistance genes. Transfer of alien genes is still an effective strategy for increasing the genetic diversity of powdery mildew resistance in wheat breeding.

Wild emmer wheat (WEW), *T. turgidum* ssp. *dicoccoides* (2n = 4x = 28; genome AABB), is the wild progenitor of both cultivated tetraploid and hexaploid wheats. It carries many agronomically important traits that can be exploited for wheat improvement, e.g., quality attributes and disease resistances (Moseman et al. 1984; Nevo et al. 1991, 2014). Many powdery mildew resistance genes derived from wild emmer wheat have been discovered, for example, *Pm26* (Rong et al. 2000), *Pm42* (Hua et al. 2009), *MlIW170* (Liu et al. 2012), and *MlIW39* (Qiu et al. 2021) on 2BS; *Mlzec1* (Mohler et al. 2005), *MlAB10* (Maxwell et al. 2010), and *Pm64* (Zhang et al. 2019) on 2BL; *Pm41* (Li et al. 2009) on 3BL; *MlIW30* (Geng et al. 2016) and *MlNFS10* (Yin et al. 2021) on 4AL; *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) and *Ml3D232* (Zhang et al. 2010) on 5BL; *PmG3M* (Xie et al. 2012) on 6BL; *PmG16* (Ben-David et al. 2010), *MlIW72* (Ji et al. 2008), *MlIW172* (Ouyang et al. 2014), and *MlWE18* (Wu et al. 2021) on 7AL. Among them, only *Pm41* has been cloned, which encodes a typical CC-NBS-LRR protein (CNL) (Li et al. 2020).

Wild emmer wheat accession G-748-M is resistant to *Bgt* isolate E09. The powdery mildew resistance gene from this accession was transferred to hexaploid wheat by crossing and backcrossing with susceptible common wheat cultivars, resulting in common wheat line WE74 (YD1817/G-748-M//7*ND015). Line WE74 conferred highly resistance to *Bgt* isolate E09 at the seedling stage in the greenhouse and the adult plant stage in fields. The objectives of this study is to fine map the powdery mildew resistance gene in WE74, with an ultimate goal of cloning the powdery mildew resistance gene and providing breeders with friendly markers that can be used in marker-assisted breeding.

Materials and methods

Plant materials

Wild emmer wheat accession G-748-M was kindly provided by Dr. ZK Gerechter-Amitai, Agricultural Research Organization, The Volcani Centre, Israel. G-748-M was resistant, with infection type (IT) 0, to *Bgt* isolates E09. Yanda 1817 and ND015 were used as susceptible parental lines for crossing and backcrossing to transfer resistance gene from G-748-M to common wheat, resulting in the powdery mildew resistant line WE74 (YD1817/G-748-M//7*ND015). WE74 was highly resistant to *Bgt* isolate E09 both at the seedling and adult growth stages. WE74 was crossed to the susceptible wheat Xuezao (XZ), developing 165 F_2 plants and their $F_{2:3}$ families for genetic analyses and genetic mapping. A large F_2 population including 2,107 plants from a cross between WE74 and XZ was used to construct a high-density linkage map. A collection of 461 wild emmer wheat accessions from different geographical collections were used to test the distribution of the powdery mildew resistance gene identified in WE74.

Powdery mildew evaluations

The parental lines WE74, XZ and the corresponding F_1, F_2 and $F_{2:3}$ materials and the recombinant families from the mapping populations were evaluated for response to powdery mildew at two-leaf stage. The inoculated plants were grown under a daily cycle of 16 h of light and 8 h of darkness at 22±2 °C in a greenhouse. The resistant and susceptible parents 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 of conidiospores.

Infection types (ITs) were evaluated after 15 d on a scale of 0-4, in which, 0, 0; 1, 2, 3, and 4 represented immune, necrotic fecks, 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). WE74, IW170 and Pm26, carrying *MlWE74*, *MlIW170* and *Pm26* respectively, were also challenged by 10 *Bgt* isolates collected from different regions of China.

Genomic DNA isolation and marker analysis

Genomic DNA was extracted from parental lines, F_2 plants, $F_{2:3}$ families and wild emmer wheat accessions following the CTAB method (Devi et al. 2013). For bulked segregant analysis, separate DNA bulks were assembled using equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible F_2 plants, respectively. Wheat microsatellite markers (*Xgwm*, *Xwmc*, *Xbarc*, *Xcfa*, *Xcfd* and *Xcau* series) mapped on A and B genome chromosomes (Graingenes, http://wheat.pw.usda.gov/) were chosen for marker analyses. Polymorphic markers indicative of linkage with the powdery mildew resistance gene were further used to genotype the entire $F_{2:3}$ mapping population to determine genetic linkage between the gene and the markers. Based on genomic locations of the identified SSR markers in the Zavitan reference sequence (Zhu et al. 2019), SSR, STS and InDel markers in the target region of the powdery mildew resistance gene were developed for linkage analysis.

PCR was performed in a 10 µl reaction mixture containing 5 µl 2× Rapid Taq Master Mix (Vazyme, Nanjing, China), 1 µl primer (mixture of left and right primers, 2 µM), 1 µl DNA template (50-100 ng/µl) and 3 µl ddH_2O. Amplification of DNA was performed at 95 °C for 3 min, followed by 35 cycles at 95 °C for 15 s, 55-60 °C for 15 s depending on the annealing

temperatures of primer pairs, and 72 °C for 15 sec/kb, with a final extension at 72 °C for 5 min. The PCR products (3 μl) mixed with 2 μl loading buffer were separated on 8% non-denaturing polyacrylamide gels (39 acrylamide: 1 bisacrylamide). Gels were silver stained and photographed.

Data analysis

Genetic analysis was performed to examine the expected segregation ratios in the F_2 and $F_{2:3}$ from WE74 × XZ populations using a Chi-squared ($\chi 2$) test. MAPMAKER 3.0 (Lander et al. 1987) was used to construct a linkage map, with a LOD score of 3.0 as the threshold. The genetic map was drawn with the software Mapdraw V2.1 (Liu et al. 2003).

Micro-collinearity analysis

The nearest flanking markers of the powdery mildew resistance gene were used to obtain the genomic region of Chinese Spring (http://www.wheat-urgi.versailles.inra.fr/; IWGSC 2018) and durum wheat Svevo (https://www.interomics.eu/durum-wheat-genome; Maccaferri et al. 2019), hexaploid wheat cv. Fielder (https://shigen.nig.ac.jp/wheat/komugi/genome/download.jsp; Sato et al. 2021), Julius_MAGIC3, Landmark, Jagger, ArinalFor, Mattis, Longreach Lancer, Mace, Norin61 and spelt wheat PI 190962 (https://webblast.ipk-gatersleben.de/wheat_ten_genomes/; Walkowiak et al. 2020). The annotated genes of corresponding physical interval in these genomes were used for micro-collinearity analysis.

Geographical distribution analysis

A total of 461 accessions of wild emmer wheat collected from natural populations, representing a wide range of ecogeographic distribution of wild emmer wheat in Israel, Lebanon, Syria, Turkey and its vicinity were used to profile the distribution of the powdery mildew resistance gene. The

co-segregating markers were used to detect the collection of wild emmer wheat.

Results

Inheritance of powdery mildew resistance in WE74

Genetic analysis was carried out to investigate the inheritance mode of powdery mildew resistance in WE74. WE74, XZ and F_1, F_2, and $F_{2:3}$ progenies from the WE74 × XZ cross were challenged by *Bgt* isolate E09 at the two-leaf stage. WE74 was resistant (IT 0), XZ was susceptible (IT 4), and the F_1 plants were resistant (IT 0;), indicating dominance of the resistance (Fig. 1). We initially phenotyped 165 F_2 plants, of which 120 were resistant and 45 were susceptible. The powdery mildew resistance was shown to segregate as a single dominant trait in the F_2 population (Table 1). To verify this result, the corresponding $F_{2:3}$ progenies segregated as 38 homozygous resistant : 82 segregating : 45 homozygous susceptible families, fitting to the ratio 1:2:1. Therefore, these results indicate that the powdery mildew resistance in WE74 was controlled by a single dominant gene, temporarily designated *MlWE74*.

Chromosomal location of *MlWE74*

A total of 352 wheat SSR markers (*Xgwm*, *Xwmc*, *Xbarc*, *Xcfa*, *Xcfd* and *Xcau*) mapped to A and B genomes were screened for polymorphism between the resistant and susceptible F_2 DNA bulks for bulked segregant analysis. Polymorphic SSR markers were selected to genotype the 165 F_2 plants from WE74 × XZ cross DNA samples. Nine polymorphic SSR markers, *Xgwm210*, *Xgwm614*, *Xbarc297*, *Xcau357*, *Xwmc243*, *Xgwm257*, *Xbarc7*, *Xbarc55*, and *Xwmc477* were detected linked to *MlWE74* (Table S1) and a genetic linkage map was constructed. All the 9 SSR markers were located on chromosome 2B (Fig. 2a). Gene *MlWE74* was localized to a 1.8 cM genetic interval between flanking markers *Xcau357* and *Xwmc243* at genetic distances of 1.2 cM

177 (distal) and 0.6 cM (proximal), respectively (Fig. 2b). These results verified that the powdery
178 mildew resistance gene *MlWE74* was located on chromosome arm 2BS.

179 **Fine mapping of *MlWE74***

180 In order to construct a high-resolution genetic linkage map for the powdery mildew resistance
181 gene *MlWE74*, a large segregating population including 2,107 F_2 plants derived from the cross
182 between WE74 and XZ was developed. The two flanking molecular markers *Xcau357* and
183 *Xwmc243* were used to genotype the entire F_2 population, resulting in 76 F_2 recombinants
184 between them. The $F_{2:3}$ families of these recombinants were tested for powdery mildew resistance
185 and then used for genotyping by the newly developed polymorphic markers. From 102 SSR and
186 InDel primer pairs designed according to the WEW Zavitan (v2.0) and Chinese Spring reference
187 genomes, nine polymorphic markers were developed and integrated into the genetic linkage map
188 of *MlWE74* after genotyping all the 76 recombinants (Tables S1 and S2). Finally, *MlWE74* was
189 narrowed down into a 0.25 cM genetic interval franked by markers *WGGBD412* and
190 *WGGBH346* (Fig. 2c and d). Molecular marker *WGGBD425* co-segregated with *MlWE74* in the
191 WE74 and XZ population (Table S1; Fig. 2c and d; Fig. S1a).

192 **Gene annotation of the *MlWE74* genomic region**

193 The flanking markers *WGGBD412* and *WGGBH346* were used to identify the corresponding
194 genomic region of the WEW Zavitan (v2.0) reference genome sequence. Finally, *MlWE74* was
195 delimited to an approximate 799.9 kb physical interval. Eight protein coding genes in this
196 physical interval were predicted with gene density of 10 genes Mb^{-1} (Fig. 2e). These predicted
197 genes included 2 phosphoglycerate mutase-like proteins (*TRIDC2BG005010* and
198 *TRIDC2BG005040*), 5 NBS-LRR disease resistance proteins (*TRIDC2BG005090*,

TRIDC2BG005100, *TRIDC2BG005110*, *TRIDC0UG006500* and *TRIDC2BG005230*) and an alpha/beta hydrolases protein (*TRIDC2BG005120*). It has an obvious NBS-LRR gene cluster in the mapping region of *MlWE74*.

Micro-collinearity analysis of the *MlWE74* locus among tetraploid and hexaploid wheat genomes

The two flanking markers *WGGBD412* and *WGGBH346* were used to search against the durum wheat cv. Svevo reference genome and hexaploid wheat genomes, including Chinese Spring, Fielder, Julius_MAGIC3, Landmark, Jagger, ArinalFor, Mattis, Longreach Lancer, Mace, Norin61 and spelt wheat PI 190962, to define the corresponding physical intervals of locus *MlWE74* in these genomes. The collinear relationship of the protein-coding genes in these intervals revealed highly micro-collinearity among tetraploid and hexaploid wheat genomes (Table S3). Six genes, *TRIDC2BG005010*, *TRIDC2BG005040*, *TRIDC2BG005090*, *TRIDC2BG005100*, *TRIDC2BG005110*, *TRIDC2BG005110*, and *TRIDC2BG005230*, in Zavitan genome were syntenic among different genomes, whereas *TRIDC2BG005120* and *TRIDC0UG006500* were only present in Zavitan (Fig. 3; Table S4).

Geographical distribution analysis of *MlWE74*

The distribution of *MlWE74* locus was investigated by screening the geographically diverse accessions of wild emmer wheat using the co-segregating marker *WGGBD425*. The positive amplicon was found in 15 WEW accessions that were mainly present in the region of Rosh Pinna and Amirim in Israel belong to the northern WEW population of Israel (Fig. 4; Table S5). The frequency of *MlWE74* is only 3.3% in 461 wild emmer wheat accessions tested. Powdery mildew tests confirmed that all these WEW accessions carrying *MlWE74* co-segregated marker were

resistant to *Bgt* E09 (Table S6).

Comparative analysis of *MlWE74*, *Pm26* and *MlIW170*

Two powdery mildew resistance genes, *Pm26* and *MlIW170* derived from wild emmer wheat were located on chromosome 2BS (Rong et al. 2000; Liu et al. 2012). *MlIW170*, an incompletely dominant resistance gene, was identified and mapped to the distal region of chromosome 2BS by flanking markers *WGGC1323* and *WGGC9140* covering the physical interval Chr2B_Zavitan v2.0: 26.41-27.25 Mb (Liang et al. 2015), whereas the dominant gene *MlWE74* was mapped on Chr2B_Zavitan v2.0: 25.48-26.28 Mb. The physical intervals of *MlWE74* and *MlIW170* are different, but overlapping. The recessive gene *Pm26*, co-segregating with marker *Xcau516*, is considered to be located in the same genomic region or be allelic to *MlIW170* (Liu et al. 2012; Liang et al. 2015). We compared three resistant parents, WE74, IW170 and Pm26 lines by inoculating with 10 *Bgt* isolates collected from different regions of China. The results demonstrated that IW170 conferred highly resistant to all the 10 isolates at the level of IT 0; and IT 1. We also found that isolates 9-43, 12-82, E21 and 46-30 virulent to wheat line WE74 and isolates 9-43, 12-50, 12-82, and E21 virulent to the wheat line Pm26 (Table S7). Isolates 12-50, 12-82, and 46-30 showed differential infection type between lines WE74 and Pm26. In addition, the *MlWE74* co-segregated marker *WGGBD425* failed to amplify the target DNA band on IW170 and Pm26 lines (Fig. S1b).

Discussion

Comparison of *MlWE74* with known powdery mildew resistance genes on 2BS

A dominant powdery mildew resistance gene *MlWE74* derived from wild emmer wheat was identified and finely mapped to a 799.9 kb genomic region on chromosome 2BS according to the

reference genome of WEW cv. Zavitan (v2.0) (Fig. 2). Up to now, eight powdery mildew resistance genes in wheat are reported on chromosome arm 2BS, including three permanently designated genes *Pm26* (Rong et al. 2000), *Pm42* (Hua et al. 2009), *Pm68* (He et al. 2020) and five temporarily designated loci *MlIW170* (Liu et al. 2012), *Ml5323* (Piarulli et al. 2012), *PmL962* (Shen et al. 2015), *pmWE99* (Ma et al. 2016b), and *MlIW39* (Qiu et al. 2021). The minimum mapping interval of *MlWE74* corresponds to the physical region of Chr2B_Zavitan v2.0: 25.48-26.28 Mb in the reference genome of WEW cv. Zavitan (v2.0) (Fig. 5a and c). The smallest physical intervals of the other six powdery mildew resistance genes, *Pm42*, *Pm68*, *Ml5323*, *PmL962*, *pmWE99* and *MlIW39* are Chr2B_Zavitan v2.0: 62.24-118.92 Mb, 20.73-22.24 Mb, 22.00-25.47 Mb, 7.03-23.09 Mb, distal terminal-118.92 Mb, 21.95-22.24 Mb, respectively (Fig. 5c-k). *Ml5323* derived from *Triticum turgidum* ssp. *dicoccum* has different origin with *MlWE74*. Moreover, allelism test showed that resistance genes *Pm26* and *Ml5323* are not allelic (Piarulli et al. 2012). The recessive gene *pmWE99*, originated from wheat-*Thinopyrum intermedium*, was mapped between chromosome 2BS distal terminus to marker *Xgwm148*. *MlWE74* is a dominant powdery mildew resistance gene derived from wild emmer wheat. Hence, *MlWE74* is different from those known genes on chromosome 2BS according to their difference in physical locations, effects and origins.

Powdery mildew resistance gene *MlIW170* derived from wild emmer wheat was located on the physical interval Chr2B_Zavitan v2.0: 26.41-27.25 Mb in the reference genome (Fig. 5h). The physical interval of *MlIW170* is different from the region of *MlWE74*, Chr2B_Zavitan v2.0: 25.48-26.28 Mb, but overlapping (Fig. 5h). The *Pm26*-derived from wild emmer wheat was mapped to 2BS, co-segregating with RFLP marker *Xwg516* (Rong et al. 2000). The STS marker

Xcau516 was developed from *Xwg516* co-segregated with *MlIW170* in an F_2 population. These results indicate that *Pm26* and *MlIW170* are likely to be identical, or allelic (Liang et al. 2015). *MlIW170* is an incomplete dominant gene, and *Pm26* is a recessive gene, whereas *MlWE74* is a dominant gene. The reactions of the three genes to 10 *Bgt* isolates revealed different infection types (Table S7). The *MlWE74* co-segregated marker *WGGBD425* also detected different amplification pattern in *Pm26* and *MlIW170*, indicating sequence divergence in the genomic intervals harboring the three genes (Fig. S1b). However, those differences may be resulted from variation in genetic background and polyploidy level difference between IW170 and WE74. We suppose that *MlWE74*, *MlIW170* and *pm26* may be identical or allelic, which needs future clarification by gene cloning.

Genomic structure and micro-collinearity analysis of the *MlWE74* locus

The genomic region of *MlWE74* (799.9 kb) according to the reference genome of WEW cv. Zavitan (v2.0) corresponds to the physical intervals from 430.1 kb to 491.5 kb in the genomes of Svevo, Chinese Spring, Fielder, Julius_MAGIC3, Landmark, Jagger, ArinalFor, Mattis and PI 190962, and from 1,663.9 kb to 1,669.8 kb in the genomes of Longreach Lancer, Mace and Norin61 (Table S3; Fig 3). Since the protein-coding genes are relatively conserved in those genomes, the physical region difference from 400 kb to 800 kb, and from 800 kb to 1,600 kb may mainly resulted from transposons and retrotransposons amplifications in those genomes, together with some NBS-LRR gene duplications.

Five genes (*TRIDC2BG005090*, *TRIDC2BG005100*, *TRIDC2BG005110*, *TRIDC0UG006500*, and *TRIDC2BG005230*) encode NBS-LRR resistance proteins have been annotated in the genomic interval of *MlWE74*. This indicates that *MlWE74* is located in an NBS-LRR gene cluster.

Up to date, 33 of 42 cloned race-specific resistance genes against fungal pathogens in wheat, barley, rye, and wild relatives are NBS-LRR resistance genes (Sánchez-Martín et al. 2021). Nine out of the 13 cloned powdery mildew resistance genes, *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. 2020), and *Pm1a* (Hewitt et al. 2020), belong to the NBS-LRR resistance genes family. Therefore, the NBS-LRR resistance genes in the physical interval could serve as candidates of *MlWE74* for further characterization.

Geographical distribution of *MlWE74* and potential value in wheat breeding

Wild emmer wheat is a valuable source in improving both durum and common wheat due to its direct ancestry and rich genetic diversity (Nevo et al. 2014). It is mainly distributed in Israel, Syria, Jordan, Lebanon, south-east Turkey, northern Iraq, and western Iran (Nevo et al. 2014). We used 461 accessions of wild emmer wheat, representing a wide range of ecogeographic distribution in Near-Eastern Fertile Crescent natural populations, to profile the distribution of *MlWE74*. A low frequency of *MlWE74* was detected in the region of Rosh Pinna and Amirim in Israel, that were assigned to narrow area in Mount Hermon region, which is sometimes considered to be the southernmost extension with higher and cooler elevation (up to 1600-1900 m) and favorable climatic conditions for disease development (Li et al. 2020b). The pathogens environment of high selective pressure facilitates the co-evolution of disease-resistant genes. The absence of *MlWE74* in the WEW natural populations from southeastern Turkey, where wheat is believed to be domesticated, suggests that *MlWE74* may not participate in the process of gene transfer into cultivated wheat during wheat domestication and polyploidization. Therefore,

MlWE74 derived from WEW would be a valuable resource for disease resistance. The co-segregating molecular marker *WGGBD425* could serve as an efficient, unique and convenient tool for marker assisted selection in wheat breeding program.

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Author contribution statement

WH, ZYL and MML designed the experiments. KYZ, MML, HBW, DYZ, LLD, QHW, YXC, JZX, PL, GHG, HZZ, PPZ, BBL, WLL, LD, QFW, JHZ, WLH, LQG, RGW, and CGY performed the experiments, conducted fieldwork, analyzed data, and performed *Bgt* inoculation. KYZ, MML, WH, HJL and ZYL wrote the paper. All authors read, revised, and approved the manuscript.

Compliance with ethical standards

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

Data availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

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480

Tables and Figures legend

Table 1 Segregation ratios of *MlWE74* in WE74 × XZ populations

Cross	Generation	Number of the F_2 plants or $F_{2:3}$ families	Observed ratio			Expected ratio	χ^2
			HR	Seg	HS		
WE74/XZ	F_2	165	120		45	3:1	0.455
WE74/XZ	$F_{2:3}$	165	38	82	45	1:2:1	0.515

HR: homozygous resistant, Seg: segregating (heterozygous resistant), and HS: homozygous susceptible

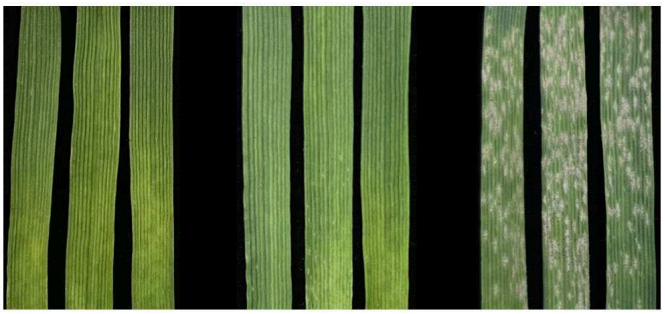


Figure 1 Phenotypes of WE74, WE74 × XZ F_1 and XZ at two weeks post-inoculation with *Bgt* isolate E09.

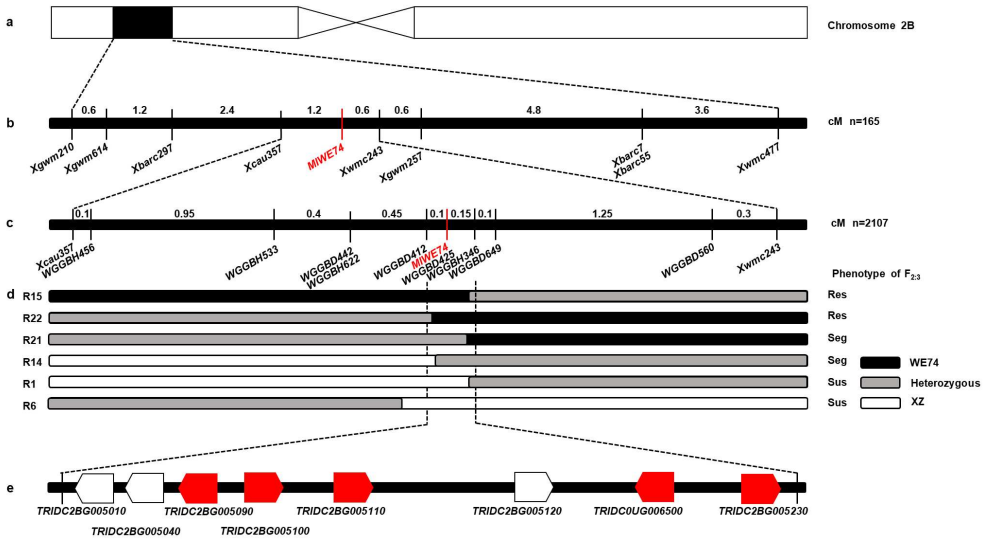
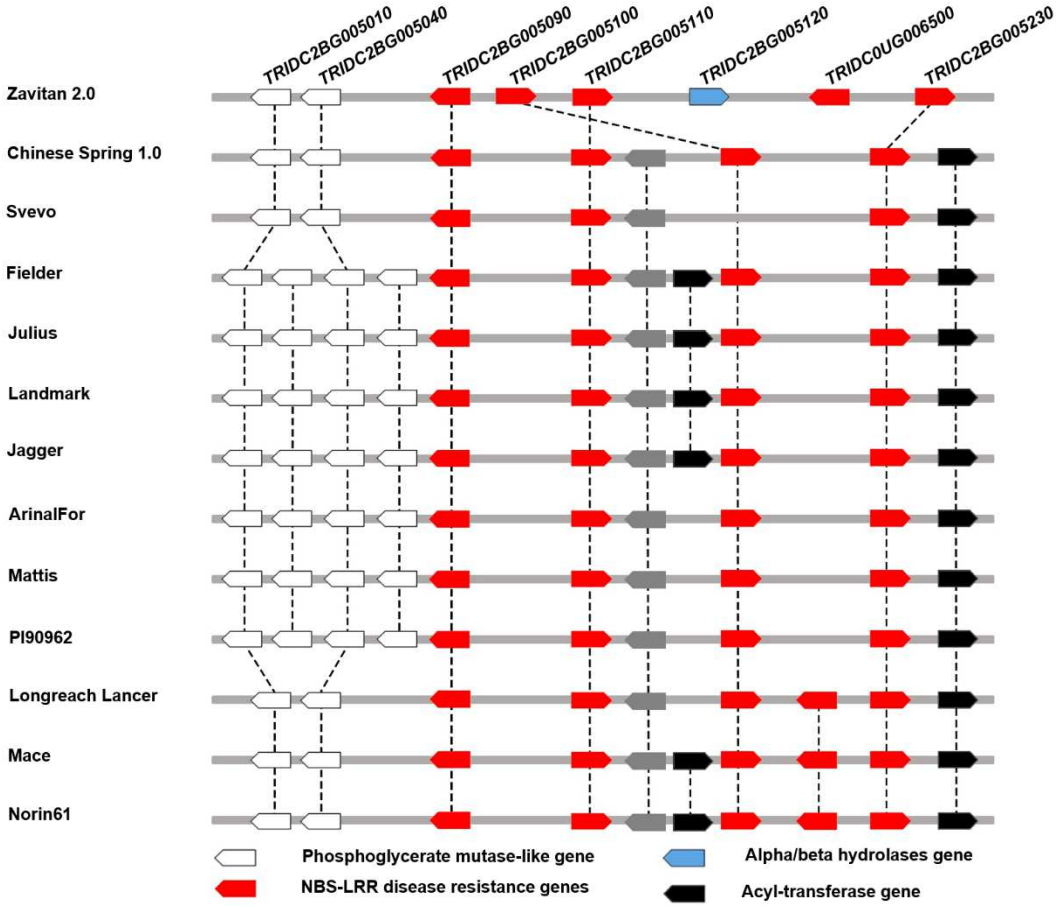


Figure 2 Fine mapping of the powdery mildew resistance gene *MlWE74*. **a** Physical location of *MlWE74* in chromosome 2B. **b** Genetic linkage map of *MlWE74*. Numbers above the line are genetic distances between adjacent markers in cM. **c** High-resolution genetic linkage map of *MlWE74*. **d** Genotypes and phenotypes of the six relevant recombinant events. Recombinant events and phenotypes are indicated at the left and right, respectively. Res, Seg and Sus represent resistant, segregation and susceptible, separately. White, black and gray blocks indicate homozygous segments from WE74, homozygous segments from XZ and heterozygous segments, respectively. **e** Annotated genes in the physical mapping interval of the *MlWE74* locus. Red pentagons represent NBS-LRR proteins related to disease resistance.



516

517 **Figure 3** Micro-collinearity of the genomic region of *MlWE74* between wild emmer, durum and
518 hexaploid wheat. Orthologous genes are linked by lines.
519

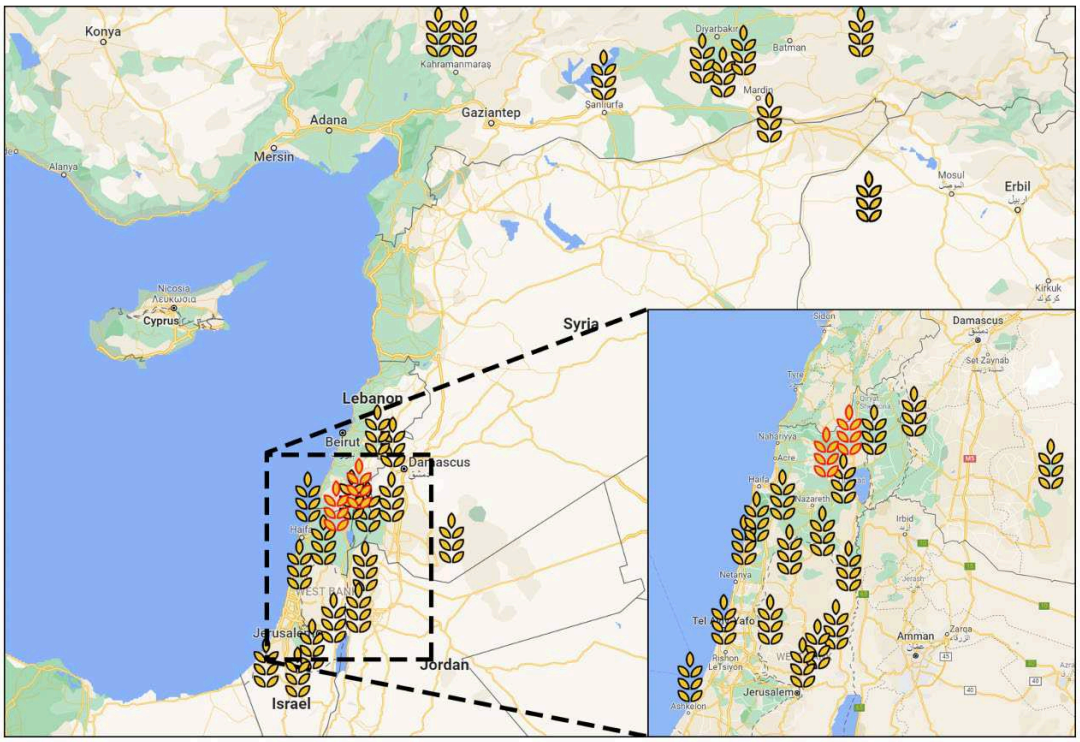


Figure 4 Geographic distribution of *MlWE74* in WEW populations from the Fertile Crescent region. Black spikes indicate absence of *MlWE74*; red spikes represent the presence of *MlWE74*, distributed only in the regions of Rosh Pinna and Amirim in Israel.

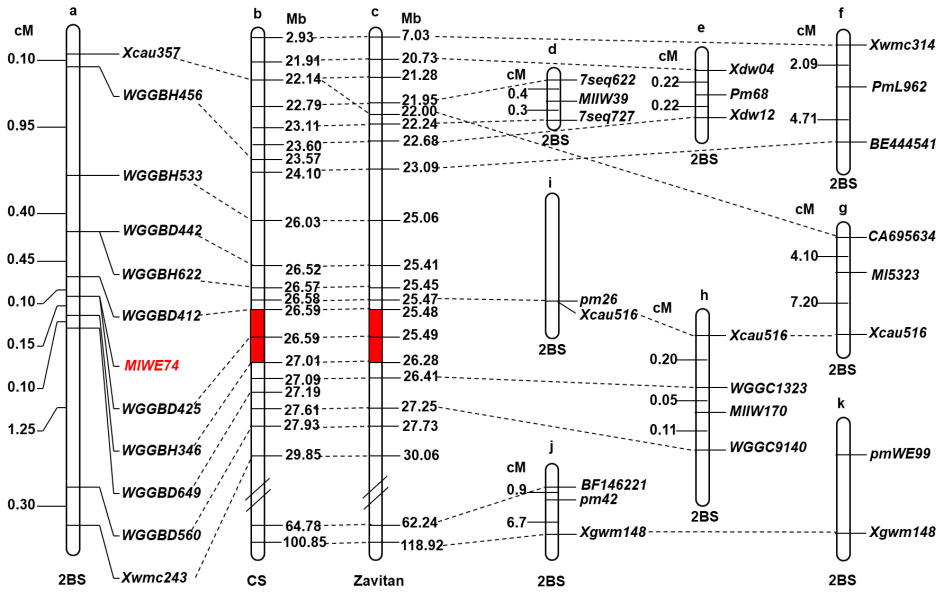


Figure 5 Comparison of genetics linkage map of *MlWE74* (a) with those reported powdery mildew resistance genes *MlIW39* (d), *Pm68* (e), *PmL962* (f), *Ml5323* (g), *MlIW170* (h), *pm26* (i), *pm42* (j) and *pmWE99* (k) using the linked markers *Xcau516* and *Xgwm148* as anchors. Physical map of the *MlWE74* region based on the Chinese Spring reference genome sequence (IWGSC RefSeq v1.0) (b) and WEW accession Zavitan reference genome sequence (v2.0) (c), physical locations in Mb are shown at the right.

Supplementary Figures and Tables

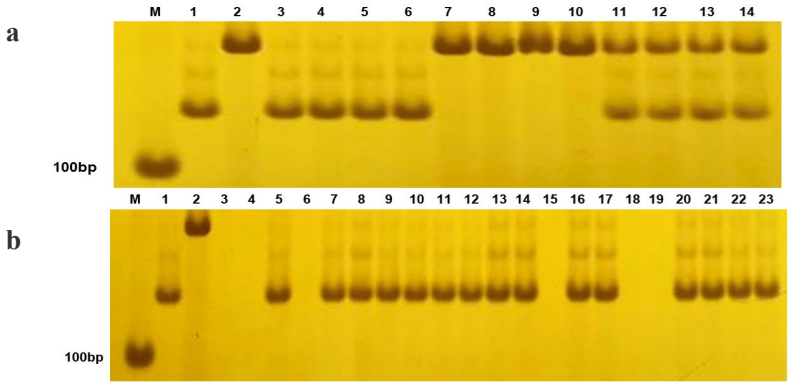


Figure S1 PCR amplification patterns of co-segregated marker *WGGBD425*. **a** Lanes 1 and 2, WE74 and XZ; lanes 3-6, homozygous resistant lines; lanes 7-10, homozygous susceptible lines; lanes 11-14, heterozygous resistant lines in WE74/XZ population. b Lanes 1-4, WE74, XZ, IW170 and Pm26; lanes 5-14, IW11-IW20; lanes 15-23, IW61-IW69.

Table S1 Primers used in mapping of *MlWE74*.

Table S2 Genotypes of markers for the 76 recombinant $F_{2:3}$ families derived from WE74/XZ cross.

Table S3 The interval between flanking markers *WGGBD412* and *WGGBH346* in the genomes of different wheat varieties.

Table S4 Annotated genes in the physical interval of different wheat genomes (- means no matched gene).

Table S5 Haplotype variation of the *MlWE74* co-segregated marker *WGGBD425* in wild emmer wheat.

Table S6 Powdery mildew response of 15 wild emmer wheat accessions carrying *MlWE74* to *Bgt* E09.

Table S7 Infection types of WE74, Pm26 and IW170 to 10 *Bgt* isolates.

Supplementary Files

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- Tables.xlsx