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Identification and genetic mapping of a powdery mildew resistance gene in wild emmer (*Triticum dicoccoides*) accession IW72 from Israel

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Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (Bgt), is a devastating disease of wheat (*Triticum aestivum*) in China and worldwide, causing severe yield losses annually. Wild emmer (*T. dicoccoides*) accession IW72 collected from Israel is resistant to powdery mildew at the seedling and adult stages. Genetic analysis indicated that the resistance was controlled by a single dominant gene, temporarily designated *MlIW72*. The F_2 population and F_3 families derived from a hybrid between IW72 and susceptible durum wheat line Mo75 were used for molecular mapping of the resistance gene. *MlIW72* was linked with SSR loci *Xgwm344*, *Xcfa2040*, *Xcfa2240*, *Xcfa2257* and *Xwmc525* on the long arm of chromosome 7A. In addition, two STS markers, MAG2185 (derived from RFLP marker PSR680) and MAG1759 (developed from EST CD452874), were mapped close to *MlIW72*. All these markers were physically located in the terminal bin 0.86–1.00 of 7AL. The chromosome location and genetic mapping results suggested that the powdery mildew resistance gene identified in wild emmer accession IW72 might be a new allele at the *Pm1* locus or a new locus closely linked to *Pm1*.

Keywords Genetic mapping · Powdery mildew · Resistance gene · *Triticum dicoccoides* · Wild emmer

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Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (Bgt), is one of the most devastating diseases of wheat (*Triticum aestivum*, AABBDD; 2n = 42) in China and worldwide and severe epidemics often occur in areas with cool and humid climates, causing significant yield losses. Compared with fungicides, resistant cultivars are cost-effective and environmentally safe approach to control the disease. However, the race-specific resistance genes commonly used are often overcome by new races possessing corresponding virulence genes in relatively short time periods. Thus, it is necessary to discover more diverse resistance genes to enrich those available for resistance breeding. About 50 formally designated *Pm* resistance alleles occur at 32 loci (Huang and Röder 2004; McIntosh et al. 2003, 2004, 2005; Miranda et al. 2006, 2007). In addition, there are several temporarily designated resistance genes (Qiu et al. 2005; Yao et al.

2007; Zhu et al. 2005; Zhou et al. 2005; Mohler et al. 2005). Many of the resistance genes were introduced from, or found in, wheat relatives.

Wild emmer, *Triticum turgidum* var. *dicoccoides* [*T. dicoccoides*, (AABB; 2n = 28)], the ancestor of cultivated wheat, has great potential for wheat improvement (Nevo et al. 2002), being a genetic resource of genes for quality and grain protein content (Nevo et al. 2002; Xu et al. 2004; Uauy et al. 2006), as well as pathogen resistance (Gerechter-Amitai and Stubbs 1970; Moseman et al. 1984, 1985; Nevo et al. 1991; Buerstmayr et al. 2003). Wild emmer genotypes originating from Israel were highly resistant to wheat powdery mildew in China (Xie et al. 2003). Several resistance genes were identified in, and transferred from, wild emmer to common wheat (Reader and Miller 1991; Rong et al. 2000; Liu et al. 2002; Mohler et al. 2005).

Molecular markers, such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNAs (RAPDs) and microsatellites or simple sequence repeats (SSRs), have been widely used and have greatly accelerated progress in wheat genetic resources and breeding. Molecular markers for more than 30 powdery mildew resistance genes/alleles in wheat have been reported (Huang and Röder 2004; McIntosh et al. 2003, 2004, 2005; Zhu et al. 2005; Miranda et al. 2006, 2007; Yao et al. 2007). *Pm3b* was successfully isolated by map-based cloning with the aid of closely linked molecular markers (Yahiaoui et al. 2004).

The present paper reports the genetic analysis and molecular mapping of a powdery mildew resistance gene in one of these Israeli wild emmer genotypes highly resistant to powdery mildew in China.

Materials and methods

Plant materials

Wild emmer accession IW72 (collected in Kokhav-Hashahar, Israel, the original accession number was TZ74) was highly resistant to powdery mildew (no visible disease symptoms). A susceptible durum wheat line Mo75 was kindly provided by Prof. Xiao Chen (Institute of Crops, China Academy of Agricultural Science). An F_2 population and derived F_3 families from Mo75/IW72 were used for genetic analysis and mapping.

Evaluation of powdery mildew resistance

Bgt isolate E09, virulent to *Pm1a*, *Pm3a*, *Pm5a*, *Pm7* and *Pm8*, but avirulent to IW72, was used for disease response tests. Two hundred and five F_2 plants were evaluated at the adult stage (from heading to grain filling) in the inoculated nursery for response to powdery mildew. Plants with no visible disease symptoms were classified resistant, whereas those with freely sporulating lesions were regarded as susceptible. The derived F_3 progenies were tested at the seedling stage in a greenhouse to confirm the phenotypes of the F_2 plants and to establish the genotypes. Fifteen to 20 seedlings of each F_3 family were inoculated at the one-leaf stage by brushing conidia from neighboring sporulating susceptible seedlings of cv. Xuezao on to fully expanded first leaves. The results were recorded two weeks after inoculation when the Xuezao control and susceptible test seedlings showed distinct mildew symptoms. Reactions were scored on a 0, 0, and 1 to 4 infection type (IT) scale, with 0 representing no visible symptoms, 0; representing necrotic flecks, and 1, 2, 3, 4 for highly resistant (necrosis with low sporulation), resistant (necrosis with medium sporulation), susceptible (no necrosis with medium to high sporulation), and highly susceptible (no necrosis with full sporulation) reactions, respectively.

Molecular marker analysis

Total DNA was extracted from leaf tissue samples of each F_2 adult plant following the method of Song and Henry (1995). Two DNA pools were created by combining equal amounts of DNA from 8 resistant or 8 susceptible F_2 plants for bulked segregant analysis (BSA) (Michelmore et al. 1991).

Wheat microsatellite markers (gwm, wmc, cfa, and cfd series) mapped to the A and B genomes were chosen for marker analysis. Relevant information for these markers is published on the GrainGenes website (http://www.wheat.pw.usda.gov). In addition, STS markers (Yao et al. 2007), that were closely linked to *Pm1a* and other powdery mildew resistance genes located on chromosome 7AL in einkorn wheat, were also tested.

PCR was performed in a volume of 10 µl containing 50 ng of genomic DNA, 10 mM Tris-Hcl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of dNTPs, 20 ng of each primer pair, and 0.75 U Taq DNA

polymerase. The amplification programs were as follows: 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 50–60°C (depending on the specific primers) for 1 min, 72°C for 2 min and a final extension at 72°C for 10 min. The PCR products were separated in 8% non-denaturing polyacrylamide gels (39 acrylamide : 1 bisacrylamide). Gels were then silver-stained (Xu et al. 2002) and photographed.

Chromosomal arm and physical bin assignments of markers

The markers linked to the powdery mildew resistance were tested on DNA samples of Chinese Spring homoeologous group 7 nullisomic-tetrasomics and ditelosomics to assign chromosome and chromosome arm locations. Four Chinese Spring 7AL deletion lines (d7AL-1 FL0.39, d7AL-16 FL0.86, d7AL-17 FL0.71, d7AL-21 FL0.74) were used to physically locate the markers. Each marker was mapped to a chromosome bin flanked by breakpoints of the largest deletion possessing the fragment and the smallest deletion lacking it after comparing the amplification patterns (Qi et al. 2003; Sourdille et al. 2004).

Data analysis

Chi-squared tests were used to determine the goodness-of-fit of observed data with expected segregation ratios. Linkage between molecular markers and the resistance gene was analyzed using Mapmaker 3.0, with a LOD score of 3.0 as threshold.

Results

Genetic analysis of the powdery mildew resistance in wild emmer accession IW72

IW72 was highly resistant to Bgt isolate E09 (IT 0 or 0;). Of 205 F_2 plants from Mo75/IW72, 154 were resistant and 51 were susceptible at the adult stage, indicating single locus segregation ($\chi^2_{3:1} = 0.0016$, df = 1, $P > 0.95$). This segregation was further confirmed by progeny seedling tests. Among 154 F_3 lines derived from resistant F_2 plants, 47 were homozygous resistant, 107 segregated, and 51 susceptible F_2 plants were homozygous susceptible ($\chi^2_{1:2:1} = 0.583$, df = 2, $P > 0.75$). The resistance gene was temporarily designated *MlIW72*.

Mapping of *MlIW72*

Initially, 28 wheat SSR markers mapping to the A and B genomes were screened for polymorphism between the resistant and susceptible DNA pools. One marker, GWM344, was polymorphic between the pools. *Xgwm344* was found co-segregating with *MlIW72* (Fig. 1). On the wheat microsatellite maps constructed by Röder et al. (1998) and Peng et al. (2002), *Xgwm344* appears on the long arms of both chromosomes 7B and 7A, then other SSR markers located on 7AL and 7BL were tested for polymorphism, and four of them (CFA2040, CFA2240, CFA2257 and WMC525) detected polymorphisms between the resistant and susceptible pools. All four co-dominant loci, *Xcfa2040*, *Xcfa2240*, *Xcfa2257* and *Xwmc525*, were also co-segregated with *MlIW72*.

Five STS primer pairs of markers linked to powdery mildew resistance genes located on chromosome 7AL in einkorn wheat reported by Yao et al. (2007) were screened for polymorphism between IW72 and Mo75, and the resistant and the susceptible DNA pools. Two, MAG1759 and MAG2185, were co-segregated with *MlIW72* (Fig. 2). MAG1759 showed a co-dominant inheritance pattern whereas MAG2185, amplifying a 240 bp polymorphic fragment, was a coupling-phase dominant marker closely associated with the resistance allele.

Linkage analysis showed that *MlIW72* was flanked by *Xmag2185* and *Xmag1759* with genetic distances of 3.3 cm and 8.2 cm, respectively (Fig. 3a). The

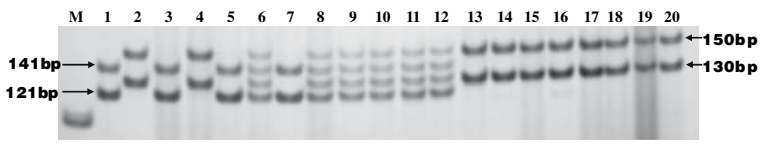

Fig. 1 PCR product of GWM344 in resistant parent IW72 (Lane 1), susceptible parent Mo75 (Lane 2), resistance pool (Lane 3), susceptibile pool (Lane 4), resistant plants (Lanes 5–12) and susceptible plants (Lanes 13–20). F_2 plants 6, and 8–12 were heterozygous. M, 1 kb DNA ladder

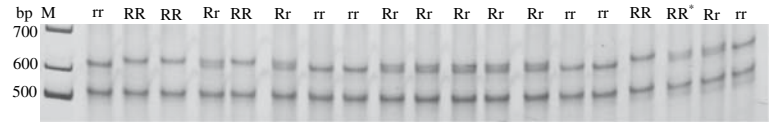

Fig. 2 Amplification patterns of MAG1759 in homozygous resistant plants (RR), heterozygous resistant plants (Rr) and homozygous susceptible plants (rr). * indicates recombinant. M, 1 kb DNA ladder

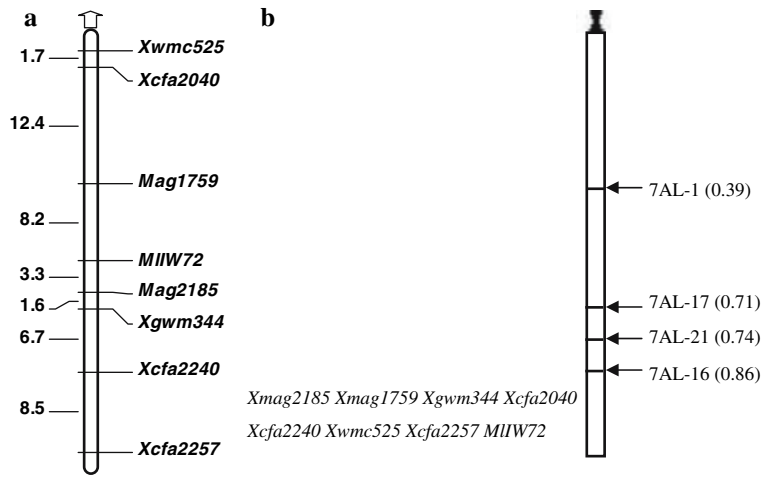

Fig. 3 Molecular mapping of *MlIW72*. (**a**) Linkage map of *MlIW72* and its linked markers. The numbers on the left indicated the genetic distances (cM) between adjacent loci. Arrow indicates the centromere direction. (**b**) Physical map of chromosome 7AL

linked microsatellite loci *Xgwm344*, *Xcfa2240*, *Xcfa2257*, *Xwmc525* and *Xcfa2040* were at greater distances from *MlIW72*.

A set of Chinese Spring homoeologous group 7 nullisomic-tetrasomics and ditelosomics were used to assign the chromosomal locations of the molecular marks. All except *Xmag2185*, which had no corresponding PCR band in Chinese Spring, were absent in N7AT7B, N7AT7D, and Dt7AL, indicating they were located on the long arm of chromosome 7A. These results were further confirmed when four Chinese Spring 7AL deletion lines (d7AL-1 FL0.39, d7AL-16 FL0.86, d7AL-17 FL0.71, d7AL-21 FL0.74) were used to determine the sub-chromosomal region harbouring the markers. No amplification products of the markers were observed in the deletion lines indicating their physical locations in chromosome 7AL distal bin 0.86–1.00 (Fig. 4). We concluded that *MlIW72* was distally located in chromosome 7AL (Fig. 3b).

Discussion

A dominant powdery mildew resistance gene effective at both the seedling and adult stages was identified in wild emmer accession IW72. Molecular mapping indicated that *MlIW72* was physically located in terminal bin 0.86–1.00 of chromosome 7AL, close to several markers including *Xgwm344*, *Xmag2185* and *Xmag1759* (Fig. 3).

Several different powdery mildew resistance genes have been located on chromosome 7AL; these include *Pm1a*, *Pm1e*, *Pm9* and *mlRD30* originating from common wheat (Hsam et al. 1998; Singrün et al. 2003,

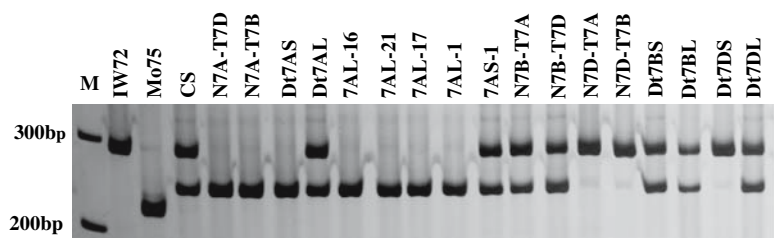

Fig. 4 Amplification of CFA2240 in IW72, Mo75, Chinese Spring homoeologous group 7 nulli-tetrasomics, ditelosomics and chromosome 7AL deletion lines

2004; Schneider et al. 1991), *Pm1b* and likely *Pm1c* transferred from *T. monococcum* (Hsam et al. 1998), *Pm1d* derived from *T. spelta* (Hsam et al. 1998), *PmU* introduced from *T. urartu* (Qiu et al. 2005), and *Mlm2033* and *Mlm80* identified in *T. monococcum* (Yao et al. 2007). Srnic et al. (2005) also located two partially dominant major genes transferred to winter wheat from *T. monococcum* and *T. timopheevii* in chromosome 7AL. *MlIW72* is the first reported *Pm* gene in *T. dicoccoides* to be located on chromosome 7AL. Thus chromosome 7AL in common wheat and its relatives appears to be an important region for powdery mildew resistance genes.

Singrün et al. (2003) found that *Pm22* and *Pm1c* were both closely linked to *Xgwm344-7A* and demonstrated that the earlier named *Pm22* was an allele (*Pm1e*) of the complex *Pm1* locus. Yao et al. (2007) mapped two powdery mildew resistance genes, *Mlm2033* and *Mlm80*, on the long arm of chromosome 7A in einkorn. These genes were closely linked to *Xgwm344*, *Xmag2185* and *Xmag1759*, and suggesting a likely association with the *Pm1* locus. In the present study, *MlIW72* was located to a similar chromosome region providing additional evidence for a complex *Pm1* site.

The present work adds to the potential of wild emmer for wheat improvement (Nevo et al. 2002). Potentially useful genes found in wild emmer can be readily transferred to common wheat by direct hybridization, backcrossing and selection.

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