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ORIGINAL PAPER

Identification and comparative mapping of a powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*) on chromosome 2BS

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Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is an important foliar disease of wheat worldwide. Wild emmer (*Triticum turgidum* var. *dicoccoides*) is a valuable genetic resource for improving disease resistance in common wheat. A powdery mildew resistance gene conferring resistance to *B. graminis* f. sp. *tritici* isolate E09 at the seedling and adult stages was identified in wild emmer accession IW170 introduced from Israel. An incomplete dominant gene, temporarily designated *MlIW170,* was responsible for the resistance. Through molecular marker and bulked segregant analyses of an F_2 population and F_3 families derived from a cross between susceptible durum wheat line 81086A and IW170, *MlIW170* was located in the distal chromosome bin 2BS3-0.84-1.00 and flanked by SSR markers *Xcfd238* and *Xwmc243*. *MlIW170* co-segregated with *Xcau516*, an STS marker developed from RFLP marker *Xwg516* that co-segregated with powdery mildew resistance gene *Pm26* on 2BS. Four EST–STS markers, *BE498358*, *BF201235*, *BQ160080*, and *BF146221,* were integrated into the genetic linkage map of *MlIW170*. Three AFLP markers, *XPaacMcac, XPagcMcta*, *XPaacMcag*, and seven AFLP-derived SCAR markers, *XcauG2*, *XcauG3*, *XcauG6*, *XcauG8*, *XcauG10*, *XcauG20*, and *XcauG25*, were linked to *MlIW170*. *XcauG3*, a resistance gene analog (RGA)-like sequence, co-segregated with *MlIW170*. The non-glaucousness locus *Iw1* was 18.77 cM distal to *MlIW170*. By comparative genomics of wheat–*Brachypodium*–rice genomic co-linearity, four EST–STS markers, *CJ658408*, *CJ945509*, *BQ169830*, *CJ945085*, and one STS marker *XP2430*, were developed and *MlIW170* was mapped in an 2.69 cM interval that is co-linear with a 131 kb genomic region in *Brachypodium* and a 105 kb genomic region in rice. Four RGA-like sequences annotated in the orthologous *Brachypodium* genomic region could serve as chromosome landing target regions for map-based cloning of *MlIW170*.

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The seed stock of IW170 and its derivatives are available upon request to Zhiyong Liu, China Agricultural University at zhiyongliu@cau.edu.cn.

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Introduction

In cool and humid areas all over the world, powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a serious fungal disease of wheat affecting grain yield and end-use quality. In recent years, certain agronomic practices, such as popularization of semi-dwarf cultivars, improvement of irrigation conditions, and increasing use of nitrogenous fertilizers have increased crop yield, but have also had the paradoxical side effect of aggravating powdery

mildew. Although chemical agents are universally used to control this disease at present, resistant wheat cultivars are the most effective, economical, and environmentally safe means of prevention. However, race-specific resistance genes tend to lose effectiveness within short period due to the selective increase of virulent races. Deployment of diversified resistance genes has been suggested as a remedy to this dilemma, necessitating a search for new powdery mildew resistance genes. To date, more than 40 wheat powdery mildew resistance loci have been identified, some of them derived from diploid or tetraploid wild relatives of common wheat (McIntosh et al. 2008).

Wild emmer, *Triticum turgidum* var. *dicoccoides* ($2n = 4x = 28$; genome AABB), the progenitor of cultivated tetraploid and hexaploid wheats, is rich in genetic diversity for resistances to powdery mildew (Moseman et al. 1984). Several powdery mildew resistance genes, such as *Pm16* (Reader and Miller 1991), *Pm26* (Rong et al. 2000), *Pm30* (Liu et al. 2002), *MlZec1* (Mohler et al. 2005), *MlIW72* (Ji et al. 2007), *Pm36* (Blanco et al. 2008), *Pm41* (Li et al. 2009), *Pm42* (Hua et al. 2009), *PmG16* (Ben-David et al. 2010), and *Ml3D232* (Zhang et al. 2010) were identified in wild emmer, or transferred to cultivated wheat or cultivated wheat derivatives.

Molecular markers accelerate the identification and cloning of disease resistance genes in wheat. Various markers, including restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs), have been used to map more than 30 powdery mildew resistance genes. Currently, SSRs are the markers of choice for mapping in wheat. They are evenly distributed along the chromosomes and are powerful tools for genetic mapping and marker-assisted selection of disease resistance genes. Thousands of publically available wheat SSR markers have been developed (Röder et al. 1998; Somers et al. 2004; http://wheat.pw.usda.gov).

AFLP technology has the capability of detecting polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. AFLP has become widely used for generating high-density linkage maps for major genes (Büschges et al. 1997; Hartl et al. 1999) and quantitative trait loci (QTLs) (Liu and Bai 2010). Since AFLP is time-consuming and costly, polymorphic AFLP fragments can be converted into sequence tagged site (STS) and sequence-characterized amplified region (SCAR) markers.

Expressed sequence tags (ESTs) are conserved portions of expressed genes and therefore can be used to conduct comparative genomics analyses. Thousands of wheat ESTs have been physically located to specific chromosome bins by applying Southern hybridization to a set of Chinese Spring deletion lines (Qi et al. 2004). These physically mapped ESTs can be used to develop polymorphic EST–STS markers to construct high-density linkage maps provided that the bin location of the target gene has been determined.

Comparative genomics provides a powerful tool for research on the large genomes of cereals, such as wheat and barley. The positional cloning of *VRN* genes (Yan et al. 2003, 2004, 2006), *Ph1* (Griffiths et al. 2006) and *Gpc-B1* (Uauy et al. 2006) in wheat benefited greatly from the available rice genome sequence. However, comparative analyses of the wheat *Lr10* (Feuillet et al. 2003) and *Pm3* (Yahiaoui et al. 2004) genes with the rice genome sequence showed that the rice genome contains genes homologous to *Lr10* and *Pm3*, but at non-orthologous positions, indicating that genomic rearrangements interrupt colinearity of wheat and rice in some genomic regions containing resistance genes (Guyot et al. 2004; Yahiaoui et al. 2004). Recently, *Brachypodium* was proposed as new model organism for functional genomics in grasses (Draper et al. 2001). High levels of genomic colinearity between wheat and *Brachypodium* were demonstrated at the *Q* (Faris et al. 2008) and *Lr34* loci (Bossolini et al. 2007). The available genomic sequence of *Brachypodium* is useful for developing molecular markers in targeted genomic regions of wheat. Using a comparative genomics approach, Zhang et al. (2010) developed high-density EST–STS marker linkage map of powdery mildew resistance gene *Ml3D232* derived from wild emmer.

In the present study, we report genetic analysis and comparative genomic mapping results of a powdery mildew resistance gene on chromosome 2BS derived from wild emmer accession IW170.

Materials and methods

Plant materials

Wild emmer accession IW170, kindly provided by Drs. T. Fahima and E. Nevo, University of Haifa, Israel, is immune to local prevailing *Bgt* isolate E09, and was crossed with the highly susceptible durum line 81086A. F_1 plants, an F_2 segregating population and F_3 families were tested for powdery mildew response and used for genetic mapping of the resistance gene as described by Liu et al. (2002).

Chromosomal arm assignment and bin mapping of markers linked to the powdery mildew resistance gene were conducted using Chinese Spring (CS) and selected CS nullisomic-tetrasomics, ditelosomics and deletion lines of homoeologous group 2 (kindly provided by Drs. WJ Raupp and BS Gill, Wheat Genetics Resource Centre, Kansas State University, USA).

Powdery mildew tests

Local isolate E09 of *B. graminis* f. sp. *tritici*, provided by Dr. Xiayu Duan, Institute of Plant Protection, Chinese Academy of Agricultural Science, was used for evaluation of powdery mildew responses under controlled greenhouse conditions. Isolate E09 is virulent to *Pm1*, *Pm3a*, *Pm3c*, *Pm5*, *Pm7*, *Pm8*, *Pm17,* and *Pm19* (Zhou et al. 2005) and avirulent to wild emmer accession IW170 and its derivatives. A highly susceptible common wheat cv. Xuezao was planted as control. Inoculation was performed as described by Li et al. (2009), and the response of each plant was scored on a 0–4 infection type (IT) scale 15 days after inoculation when the control was sporulating profusely. Reactions were classified into two groups, resistant (R, IT 0–2) and susceptible (S, IT 3–4). Twenty-five $F_{2:3}$ seedlings per F_2 individual as well as 25 F_1 seedlings were tested to confirm response phenotypes. Genotypes of F_2 individuals in the mapping population were deduced from the responses of the F_3 lines.

DNA isolation

Genomic DNA was extracted from the uninfected seedling leaves of parental wild emmer IW170, durum wheat 81086A and F_2 plants by the cetyltrimethylammonium bromide (CTAB) method (Allen et al. 2006).

Molecular marker analysis

For bulked segregant analysis, two DNA bulks were separately constituted by using equal amounts of DNA from ten homozygous-resistant and ten homozygous-susceptible F_2 plants from the segregating population (81086A/IW170). Wheat microsatellite markers (*Xgwm*, *Xwmc*, *Xbarc*, and *Xcfd* series; GrainGenes website http://wheat.pw.usda.gov) mapped to A and B genomes were chosen for testing. STS marker *Xcau516* was developed by end-sequencing RFLP probe WG516 (kindly provided by Drs. David Benscher & Mark Sorrells, Cornell University, USA). EST–SSR markers (*Xcau*) were developed according to flanking sequences of microsatellite motifs in wheat ESTs deposited in public EST databases. In order to saturate the interval containing the powdery mildew resistance gene with molecular markers, 47 EST–STS primers developed from wheat ESTs mapped to chromosome 2BS were also evaluated (http://wheat.pw.usda.gov/SNP/primers/contig_primer_list.xls). In addition, 86 AFLP P*st*I/M*se*I primer combinations were screened and some of the polymorphic AFLP markers were converted into SCAR markers (*XcauG*) after sequencing the polymorphic DNA fragments.

PCR were conducted in total volumes of 10 μl containing 10 mM Tris–HCl, PH 7.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 25 ng of each primer, 50–100 ng genomic DNA, and 0.75 U Taq DNA polymerase. Amplifications were performed at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 50–60°C (depending on the specific primers) for 45 s, and 72°C for 90 s, with a final extension at 72°C for 10 min. Five microlitre PCR products mixed with 2 μl loading buffer were separated on 5% denaturing polyacrylamide gels with a 19:1 of acrylamide:bisacrylamide ratio or 8% non-denaturing polyacrylamide gels with a 39:1 of acrylamide:bisacrylamide. Gels were silver stained and photographed.

Chromosome arm assignment and physical bin mapping of polymorphic markers

Polymorphic markers linked to the resistance gene were detected in a set of Chinese Spring nullisomic–tetrasomics, ditelosomics, and deletion lines of homoeologous group 2. Polymorphic markers were mapped to chromosome bins flanked by breakpoints of the largest deletion possessing the fragment and the smallest deletion lacking it after comparing the amplification patterns.

Comparative genomics analysis

The sequences of STS, EST–SSR, and EST–STS markers flanking the target resistance gene were used as queries to search rice and *Brachypodium* genome sequences to identify orthologous loci in rice and *Brachypodium* genomes. Then, orthologous gene pairs between rice and *Brachypodium* were compared within the homologous genomic regions. Wheat EST sequences homologous to putative *Brachypodium* and rice genes within the orthologous genomic regions were used to design PCR primers to develop markers polymorphic between the resistant and susceptible parental lines. Polymorphic EST markers were tested in the F_2 population for linkage map construction.

Data analysis

Chi-squared (χ^2) tests for goodness-of-fit were used to compare observed and from theoretically expected segregation ratios. Linkage analysis of polymorphic molecular markers and the resistance gene was conducted using Mapmaker 3.0 software (Lincoln et al. 1992) with a LOD score threshold of 3.0. Genetic maps were constructed using MapDraw V2.1 (Liu and Meng 2003).

Results

Genetic analysis of the powdery mildew resistance in IW170

When wild emmer accession IW170, durum line 81086A, the F_1 hybrid, and F_3 families were challenged with *Bgt*

isolate E09, IW170 was highly resistant (IT 0) and 81086A was highly susceptible (IT 4). All hybrid F_1 seedlings had an intermediate resistant (IT 2) phenotype, indicating that the powdery mildew resistance in IW170 was incompletely dominant. The F_3 families segregated 24 homozygous resistant: 62 segregating: 27 homozygous susceptible, as expected for variation at a single locus ($\chi^2_{1:2:1} = 1.23$, $P_{df2} = 0.54$). The incompletely dominant gene for resistance in IW170 was temporarily designated as *MlIW170*.

Identification and physical bin mapping of polymorphic markers linked to *MlIW170*

First, SSR primers were surveyed for polymorphisms between the parental lines and between resistant and susceptible DNA bulks. Nine SSR markers, *Xbarc297*, *Xbarc318*, *Xcfd238*, *Xgwm614*, *Xgwm210*, *Xwmc243*, *Xwmc257*, *Xwmc154*, and *Xwmc25* detected such polymorphisms and linked to *MlIW170*.

Chinese Spring homoeologous group 2 nullisomic–tetrasomics, ditelosomics, and deletion lines were used to assign the chromosomal and physical bin locations of the powdery mildew resistance gene *MlIW170* and its linked SSR markers. All 9 SSR markers listed above mapped to distal bin 2BS3-0.84-1.00 indicating that *MlIW170* was also in that bin (Fig. 1a).

Four EST-derived STS markers, *BF201235*, *BQ160080*, *BE498358*, and *BF146221* (Table 1), were linked to the resistance gene after screening 47 EST–STS primer pairs mapped to 2BS bin 0.84-1.00. *BF201235* was closest to *MlIW170* with a proximal genetic distance of 2.15 cM (Figs. 1b, 2a). EST–SSR marker *Xcau357* (Table 1) was 7.53 cM distal to the resistance gene (Fig. 1b).

AFLP were employed to saturate the powdery mildew resistance gene region with molecular markers. Ten AFLP

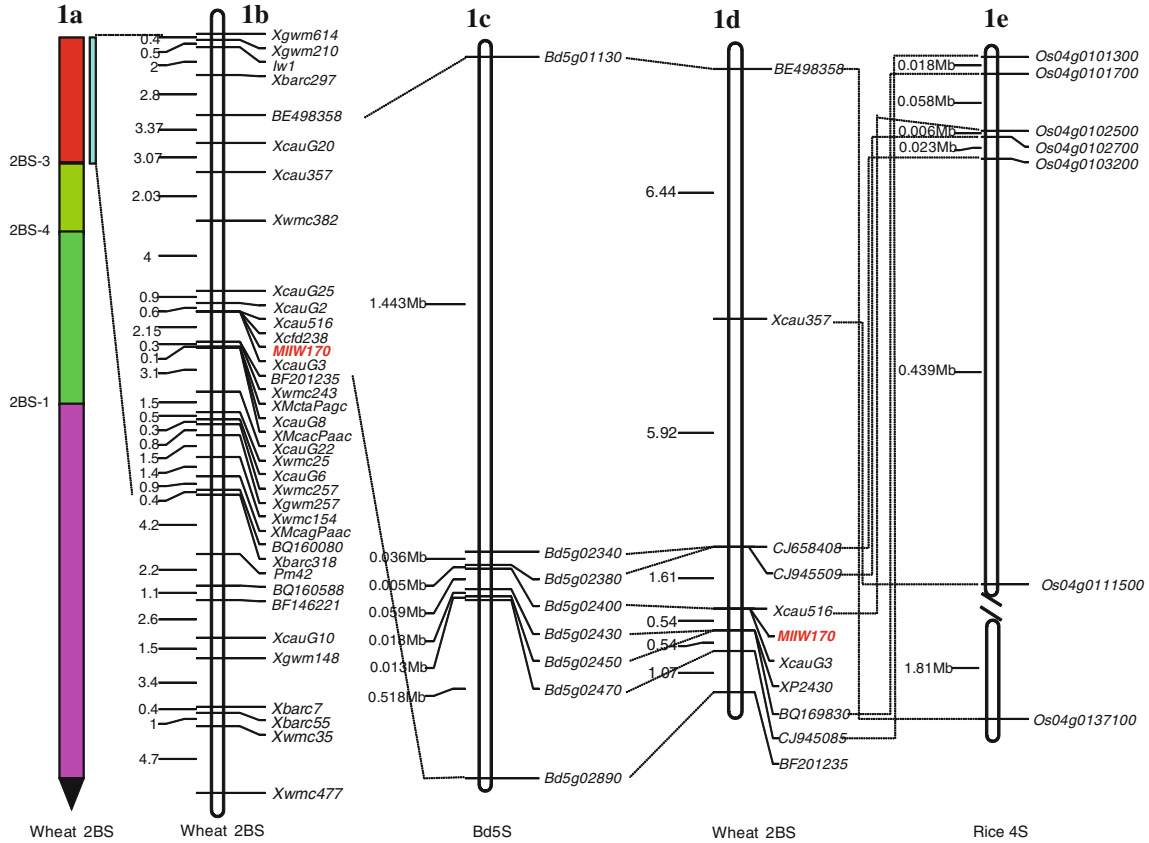

Fig. 1 Genetic and comparative genomics linkage map of powdery mildew resistance gene *MlIW170* derived from wild emmer. **a** Physical bin map of *MlIW170*. *MlIW170* was mapped to distal bin 2BS3-0.84-1.00. **b** Genetic map of the *MlIW170* gene region on wheat chromosome 2BS with genetic distances in cM shown on the *left*, Markers shown on the *right*. **c** The orthologous region of *MlIW170* on *Brachypodium* Bd5 with physical distances in Mb on the *left*, orthologous genes shown on the *right*. **d** EST–STS marker linkage map of powdery mildew resistance gene *MlIW170* with genetic distances in cM shown on the *left*, EST–STS Markers shown on the *right*. **e** The orthologous region of *MlIW170* on rice chromosome 4 with physical distances in Mb on the *left*, orthologous genes shown on the *right*

markers linked to *MlIW170* were identified after screening 86 AFLP P*st*I/M*se*I primer combinations. Seven of the ten markers were converted into SCAR markers, viz. *XcauG2*, *XcauG3*, *XcauG6*, *XcauG8*, *XcauG10*, *XcauG20*, and *XcauG25* (Table 2), which can be used more conveniently in fine mapping. The positions of these markers relative to *MlIW170* are shown in Fig. 1b. *XcauG3* co-segregated with *MlIW170* (Figs. 1b, 2b). Sequence analysis of the polymorphic DNA fragment revealed that *XcauG3* is part of a resistance gene analog (RGA).

The linkage between *MlIW170* and STS marker *Xcau516*

RFLP marker *Xwg516* co-segregated with powdery mildew resistance gene *Pm26* also originating from wild emmer

Table 1 STS, EST–STS, and EST–SSR markers linked to powdery mildew resistance gene *MlIW170*

STS marker	Forward primer (5′–3′)	Reverse primer (5′–3′)
Xcau516	AAGAAGAAGCAAAGCGT	AGAGAAGAGAAAGAGATAGC
XP2430	ACCAGTATTATTGCCAGTTAGC	TGACTCCCTCCACATTCAA
BF201235	GGAGTTTGAGAACGCCAGAG	AAAGCTTGGCAATCCTCTCA
BQ160080	GCCTGGCTCAACCGTAATAA	AGGGCTTAGAGAGGCCAAAG
BE498358	TAATAGGACACCGAGCGACC	GCGTCAGCCAGCTACTCG
BF146221	CTTGGAGGTGTCGTCCTTGT	CGAGTTCCAGATGCAGTACG
CJ658408	CCCACCTTCAAGAACATCG	GCAGCAGCTTCTTCCTTT
CJ945509	TGAACGAGGGGTTGTGA	GGCTTCTTGTTGTTCCACTC
BQ169830	ATGGACGCTCAGTGGCT	GTCGCTTCCAGCACATC
CJ945085	TCAGCACAGCATAACCCA	GACCCTTGGTGTCCTCTTT
Xcau357	TTAGAAACGACAGTGCAGGG	GGTGCAAGTACAGAGGAGCC

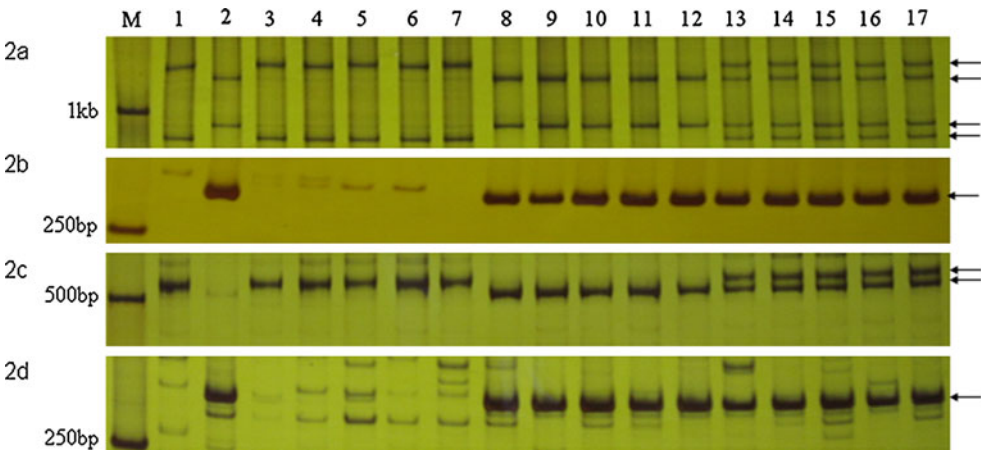

Fig. 2 PCR amplification patterns of markers *BF201235* (**a**), *XcauG3* (**b**), *Xcau516* (**c**), and *XP2430* (**d**) in 8% non-denatured polyacrylamide gels. The *arrows* on the right side indicate the DNA fragments polymorphic between resistant and susceptible plants. *M* 2 kb DNA ladder. *Lanes 1* and *2* are IW170 and 81086A, respectively, *lanes 3–7* represent homozygous resistant plants, *lanes 8–12* represent homozygous susceptible plants, and *lanes 13–17* represent heterozygous resistant plants

Table 2 AFLP and AFLP-derived SCAR markers linked to powdery mildew resistance gene *MlIW170*

SCAR marker	AFLP primer pairs	Forward primer (5′–3′)	Reverse primer (5′–3′)
	XmcacPaac	GATGAGTCCTGAGTAACAC	GACTGCGTACATGCAGAAC
	XmctaPagc	GATGAGTCCTGAGTAACTA	GACTGCGTACATGCAGAGC
	XmcagPaac	GATGAGTCCTGAGTAACAG	GACTGCGTACATGCAGAAC
XcauG2	*XmcaaPgac*	CAACAGCACAGCTATAACAT	AGGACGCGAGACCACAG
XcauG3	*XmcaaPgag*	GCAGGAGGCGAAACAACT	TAACAACCATTTGTGCCAATG
XcauG6	*XmcacPaat*	CTATGGTATTGTCCGCTAG	GCAGAATTTTCACAGTTGC
XcauG8	*XmcacPagc*	TTAACACGAACCCGTCG	GCTTTCTTTGCCATCCTT
XcauG10	*XmcacPaag*	CTGCAGAAGAAGTAGGCT	CACTACTATGCTTTTCTCCT
XcauG20	*XmcatPgat*	TTAACATGCCACGTTGAC	TTAGCTGATGGTTGTTGC
XcauG25	*XmctaPagc*	CTGCAGAGCACATATCAAAG	ACTAAGAACTGCATGCAGG

(Rong et al. 2000). To clarify the relationship between *MlIW170* and *Pm26*, the RFLP probe WG516 was end-sequenced and STS marker *Xcau516* was developed (Table 1). *Xcau516* co-segregated with *MlIW170* when genotyped on the 81086A/IW170 F_2 population (Figs. 1b, 2c). Thus *Pm26* and *MlIW170* appear to be located on same genomic region.

MlIW170 is linked to morphological marker *Iw1* for non-glaucousness

IW170 is non-glaucous whereas the durum line 81086A is glaucous. The F_2 population of 81086A/IW170 segregated 80 non-glaucous:33 glaucous, fitting to an expected 3:1 ratio ($\chi^2_{3:1} = 1.07$, $P_{df1} = 0.3$) with dominance of non-glaucousness. Linkage analysis showed that *MlIW170* was 18.77 cM proximal to *Iw1* (Fig. 1b).

Comparative genomics analysis

The sequences of 4 STS and EST–STS flanking markers, *BE498358*, *Xcau357* (*CA695634*), *Xcau516*, and *BF201235* were used as queries to search for orthologous genes in the rice and *Brachypodium* genomic sequences. Both *BE498358* and *Xcau516* detected orthologs on the short arm terminal regions of rice chromosome 4 (*Os04g0137100* and *Os04g0102500*) and *Brachypodium* chromosome 5 (*Bd5g01130* and *Bd5g02400*) (Fig. 1c, e). An ortholog of *Xcau357* was found in rice (*Os04g0111500*), but not in *Brachypodium*. *BF201235* was homologous to *Brachypodium* gene *Bd5g02890*. However, the homolog of *BF201235* was on rice chromosome 12 (*Os12g0277500*) rather than chromosome 4. Compared with the short arm terminal regions of wheat 2BS and the *Brachypodium* 5S, the rice 4S region showed an inversion in gene order. Thus, a 2.1 Mb genomic region (*Bd5g01130–Bd5g02890*) homologous to the wheat genomic region harboring the powdery mildew resistance gene *MlIW170* was detected in the *Brachypodium* genome. The orthologous genomic region of *MlIW170* in rice genome could not be identified from the available information. Since the genomic region between *BE498358* and *Xcau516* in wheat is orthologous to the rice genomic region between *Os04g0137100* and *Os04g0102500*, the orthologous genomic region of *MlIW170* was most likely distal to *Os04g0102500* in the rice genome.

Developing a high-density EST–STS marker linkage map of *MlIW170*

The genomic sequences of putative *Brachypodium* genes between *Bd5g01130* and *Bd5g02890* were used as queries to search homologous wheat ESTs and to develop polymorphic EST–STS markers. Five EST–STS markers, *CJ658408*, *CJ945509*, *XP2430*, *BQ169830*, and *CJ945085*, were polymorphic between the parental lines as well as the resistant and susceptible bulks, and were subsequently used to construct a high-density linkage map of *MlIW170* after testing the segregating population (Fig. 1d; Table 1). *MlIW170* mapped between *CJ658408* and *CJ945085* in an interval of 2.69 cM, that is colinear with a 131 kb genomic region (*Bd5g02340–Bd5g02470*) in *Brachypodium* and a 105 kb genomic region (*Os04g0101300–Os04g0103200*) in rice (Fig. 1c, e). Detailed annotation and comparative analysis revealed high colinearity between the rice and *Brachypodium* genomic regions. Eight of 11 predicted rice genes are orthologous to eight of 14 predicted *Brachypodium* genes (Table 3). Among the 14 *Brachypodium* genes, four of them, *Bd5g02360*, *Bd5g02370*, *Bd5g02410*, and *Bd5g02430*, were resistance gene analogs (RGAs). However, putative RGA sequences were not found in the corresponding rice orthologous genomic positions. Molecular mapping results showed that a RGA-like sequence *XP2430*, homologous to the *Brachypodium* RGA *Bd5g02430*, was 0.54 cM proximal to *MlIW170* (Figs. 1d, 2d). Overall, *MlIW170* was located in a 2.1 cM interval that is collinear with a genomic region containing RGA-like sequences in *Brachypodium*.

Discussion

Wild emmer harbors considerable genetic diversity for powdery mildew resistance (Moseman et al. 1984; Xie and Nevo 2008). Among 380 wild emmer accessions from Israel, 94% had intermediate to high levels of resistance to *Bgt* isolate E09 (Xie et al. 2003; our unpublished data). Ten powdery mildew resistance genes/alleles, including *Pm16* (Reader and Miller 1991; Chen et al. 2005), *Pm26* (Rong et al. 2000), *Pm30* (Liu et al. 2002), *MlZec1* (Mohler et al. 2005), *MlIW72* (Ji et al. 2007), *Pm36* (Blanco et al. 2008), *Pm41* (Li et al. 2009), *Pm42* (Hua et al. 2009), *PmG16* (Ben-David et al. 2010) and *Ml3D232* (Zhang et al. 2010) from wild emmer have been identified and mapped to chromosomes 5BS, 2BS, 5BS, 2BL, 7AL, 5BL, 3BL, 2BS, 7AL, and 5BL, respectively.

In the present study, an incompletely dominant resistance gene *MlIW170* in wild emmer accession IW170 was identified and mapped to the distal region of chromosome 2BS (bin 0.84-1.00) by molecular marker analysis and Chinese Spring deletion bin mapping. Five powdery mildew resistance genes, *Pm6*, *Pm26*, *MlZec1*, *Pm33*, and *Pm42*, were previously located on chromosome 2B. *Pm6* originated from the G genome of *T. timopheevii* (Jorgensen and Jensen 1973) and *Pm33* was identified in *T. carthlicum* (Zhu et al. 2005). Both *Pm6* and *Pm33* were located on chromosome 2BL. *MlZec1*, a dominant resistance gene derived from wild emmer, was mapped distally to SSR

Table 3 Ortholgous gene pairs between *Brachypodium*, rice, and mapped wheat STS, EST–STS markers

Wheat marker	*Brachypodium* gene	Rice gene	Predicted function
CJ658408	*Bd5g02340*	*Os04g0103200*	Proteasome family protein
	Bd5g02350	*Os04g0103100*	Beta3-glucuronyltransferase
	Bd5g02360		Putative bacterial blight-resistance protein *Xa1*
	Bd5g02370		Putative bacterial blight-resistance protein *Xa1*
CJ945509	*Bd5g02380*	*Os04g0102700*	*N*-acylethanolamine amidohydrolase
	Bd5g02390		Serine-type peptidase
		Os04g0102600	Splicing factor 3B subunit 2
Xcau516	*Bd5g02400*	*Os04g0102500*	Phosphoglycerate mutase-like protein
	Bd5g02410		NBS-LRR resistance-like protein
		Os04g0102200	5S ribosomal RNA
		Os04g0102000	5S ribosomal RNA
	Bd5g02420		F-box domain containing protein
XP2430	*Bd5g02430*		NBS-LRR resistance-like protein
	Bd5g02440	*Os04g0101800*	C3HC4-type RING finger
BQ169830	*Bd5g02450*	*Os04g0101700*	Phagocytosis and cell motility protein
	Bd5g02460	*Os04g0101400*	Cytochrome P450 family protein
CJ945085	*Bd5g02470*	*Os04g0101300*	MRG family protein

marker *Xwmc356* in terminal bin 2BL 0.89–1.00 (Mohler et al. 2005). Two recessive powdery mildew resistance genes, *Pm26* and *Pm42* from wild emmer were located on chromosome 2BS (Rong et al. 2000; Hua et al. 2009). Our integrated mapping results indicate that *MlIW170* is 17.15 cM distal to *Pm42* (Fig. 1b), indicating *MlIW170* and *Pm42* are unlikely to be the same gene. *Pm26* was mapped on 2BS, co-segregating with RFLP marker *Xwg516* (Rong et al. 2000). STS marker *Xcau516*, developed from *Xwg516* by end-sequencing, co-segregated with *MlIW170* in our F_2 population of 113 plants. The allelic relationship between *MlIW170* and *Pm26* could not be determined because a genetic stock containing *Pm26* was not publically available (M Feldman, personal communication). However, the co-segregating of RFLP marker *Xwg516* and its derived STS marker *Xcau516* with *Pm26* and *MlIW170* indicated that *Pm26* and *MlIW170* are likely to be identical, allelic or tightly linked.

In order to saturate the genetic map of *MlIW170*, additional markers were produced by the AFLP method. AFLP-derived SCAR marker *XcauG3* co-segregated with *MlIW170*. A NCBI Blast result indicated that *XcauG3* was part of a resistance gene analog (RGA), providing very useful information for fine mapping of *MlIW170*. Plant disease resistance genes tend to be clustered and co-segregation of *MlIW170* and *XcauG3* may imply that both are located in the same or adjacent gene clusters.

Large genome sizes, abundant repetitive elements, and polyploidy hamper wheat genomics research. Comparative genomics analysis among wheat and other grasses with smaller sequenced genomes is a useful tool to facilitate fine mapping and map-based cloning in wheat. Several studies showed close colinearity between wheat and rice genomes (Peng et al. 2004; Conley et al. 2004), but there are also limitations to wheat–rice comparative analyses (Paterson et al. 2004). Comparative analyses of *Lr21* (Huang et al. 2003), *Lr10* (Feuillet et al. 2003), and *Pm3b* (Yahiaoui et al. 2004) with rice showed that wheat and rice have very limited colinearity in these regions (Keller et al. 2005). Recently, *Brachypodium distachyon*, with its small genome and simple growth requirements, was proposed as new model organism for functional genomics in grasses (Draper et al. 2001). Studies indicated that phylogenetic relationships between *Brachypodium* and wheat were closer than between rice and wheat (Catalan et al. 1995; Vogel et al. 2006). *Brachypodium* proved useful in mapping and cloning the wheat *Ph1* gene (Griffiths et al. 2006). In addition, very close genomic colinearities between wheat and *Brachypodium* were demonstrated for the *Q* (Faris et al. 2008), *Lr34* (Bossolini et al. 2007), and *Ml3D232* loci (Zhang et al. 2010).

In the present study, genes in wheat chromosome 2BS distal bin 2BS3-0.84-1.00 showed high levels of colinearity with the short arms of chromosome 5 of *Brachypodium* and chromosome 4 of rice (Table 3). Eight of 11 putative rice genes had orthologs in the *Brachypodium* genomic region. By comparative genomic analysis, five polymorphic EST–STS markers, *CJ658408*, *CJ945509*, *BQ169830*, *CJ945085*, and *XP2430*, were developed and *MlIW170* was placed in a 2.69 cM interval that is colinear with 131 and 105 kb orthologous genomic regions in *Brachypodium* and rice, respectively (Fig. 1c, e). The gene order was conserved among wheat, rice, and *Brachypodium* in the corresponding genomic regions. However, microcolinearity

differences caused by gene amplification and insertion/deletion were observed between wheat, *Brachypodium,* and rice. Three of 11 predicted rice genes were not found in the corresponding *Brachypodium* genomic region. Among 14 predicted *Brachypodium* genes in this region, six were absent from the rice orthologous position and four of them were RGAs. NBS-LRR genes, as the largest class of disease resistance genes, play important roles in defending plants from infection of pathogens. The number of NBSs varies from 129, 245, and 239 to 508 in maize, sorghum, *brachypodium,* and rice, respectively, suggesting rapid evolution of these genes in grass species (Li et al. 2010). Disease resistance genes often cluster together in plant genomes due to genetic duplication and rapid evolution and therefore more frequently interrupting the microcolinearity between genomes. Comparative genomics analyses of the rice genome with the wheat and *Brachypodium* resistance gene analogies have shown that the rice genome contains genes homologous to these RGAs. Nevertheless, these genes are located at non-homologous positions, suggesting that wheat and rice have limited collinearity in genomic regions harboring resistance genes and wheat and *Brachypodium* NBS-LRR gene families may have a syntenic order. The four RGAs, *Bd5g02360*, *Bd5g02370*, *Bd5g02410*, and *Bd5g02430*, present in the *MlIW170* orthologous genomic region of *Brachypodium*, provided crucial information for fine mapping and map-based isolation of *MlIW170* from wild emmer. Two RGA markers, *XcauG3* and *XP2430*, may serve as starting points for wheat BAC library screening and chromosome landing for cloning *MlIW170*. Our finding provides further evidences that *Brachypodium* is more closely related to wheat than that of rice and could serve as powerful tool for fine mapping and cloning disease resistance genes in wheat via comparative genomics analyses.

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