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ORIGINAL PAPER

Genetic and comparative genomics mapping reveals that a powdery mildew resistance gene *Ml3D232* originating from wild emmer co-segregates with an NBS-LRR analog in common wheat (*Triticum aestivum* L.)

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Abstract Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most important wheat diseases worldwide and breeding for resistance using diversified disease resistance genes is the most promising approach to prevent outbreaks of powdery mildew. A powdery mildew resistance gene, originating from wild emmer wheat (*Triticum turgidum* var. *dicoccoides*) accessions collected from Israel, has been transferred into the hexaploid wheat line 3D232 through crossing and backcrossing. Inoculation results with 21 *B. graminis* f. sp. *tritici* races indicated that 3D232 is resistant to all of the powdery mildew isolates tested. Genetic analyses of 3D232 using an F_2 segregating population and F_3 families indicated that a single dominant gene, *Ml3D232*, confers resistance in the host seedling stage. By applying molecular markers and bulked segregant analysis (BSA), we have identified polymorphic simple sequence repeats (SSR), expressed sequence tags (EST) and derived sequence tagged site (STS) markers to determine that the *Ml3D232* is located on chromosome 5BL bin 0.59–0.76. Comparative genetic analyses using mapped EST markers and genome sequences of rice and Brachypodium established co-linearity of the *Ml3D232* genomic region with a 1.4 Mb genomic region on

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The seed stock of 3D232 has been deposited to gene bank of CIMMYT, Beijing, China. Request seeds of 3D232 should address to Dr. Zhonghu He, CIMMYT Beijing at zhhecaas@gmail.com or directly to Dr. Zhiyong Liu, China Agricultural University at zhiyongliu@cau.edu.cn.

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Brachypodium distachyon chromosome 4, and a 1.2 Mb contig located on the *Oryza sativa* chromosome 9. Our comparative approach enabled us to develop new EST–STS markers and to delimit the genomic region carrying *Ml3D232* to a 0.8 cM segment that is collinear with a 558 kb region on *B. distachyon*. Eight EST markers, including an NBS-LRR analog, co-segregated with *Ml3D232* to provide a target site for fine genetic mapping, chromosome landing and map-based cloning of the powdery mildew resistance gene. This newly developed common wheat germplasm provides broad-spectrum resistance to powdery mildew and a valuable resource for wheat breeding programs.

Introduction

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most important wheat diseases worldwide (Bennett 1984). Disease resistance has proved to be an effective and environmentally safe method to control wheat pathogens. However, resistance genes tend to be short-lived due to changes in pathogen virulence, which can lead to a single resistance genes becoming ineffective over wide production area. Deployment of diversified disease resistance genes and gene pyramiding have been suggested as a remedy for this dilemma, provided that a sufficient number of distinct resistance genes are available for introgression into a desirable cultivar. Currently, 43 loci (*Pm1–Pm43*) with 59 resistance genes/alleles to powdery mildew have been reported in wheat (McIntosh et al. 2008; Luo et al. 2009; Li et al. 2008; Hua et al. 2009; He et al. 2009).

Wild emmer (*T. turgidum* var. *dicoccoides*) is considered to be the progenitor of cultivated tetraploid and hexaploid wheats, and has been reported to be polymorphic for resistance to several pathogens, including stripe rust (*Puccinina striiformis* f. sp. *tritici*), leaf rust (*Puccinia recondita* f. sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*), and powdery mildew (Nevo et al. 2002; Moseman et al. 1984). Disease resistance genes introduced into cultivated tetraploid and hexaploid wheats from wild emmer have been described for stripe rust, leaf rust and powdery mildew (McIntosh et al. 2008). To date, eight powdery mildew resistance loci have been transferred into durum and common wheats from wild emmer and assigned to specific chromosomes or chromosome arms. Among these, *Pm16* was originally placed on chromosome 4A (Reader and Miller 1991), but subsequently has been reassigned to 5BS via SSR marker mapping and possibly is allelic to *Pm30* (Liu et al. 2002; Chen et al. 2005). The *Pm26* and *Pm42* recessive resistance genes are 36.8 cM apart on chromosome arm 2BS (Rong et al. 2000; Hua et al. 2009). *Pm41* was reported on 3BL (Li et al. 2008) and *MlZec1* was physically located distal to chromosome 2BL (Mohler et al. 2005). Two loci, *Pm36* and *MlIW72*, have also been introduced into tetraploid durum wheat. *Pm36* was mapped to chromosome 5BL and linked to the EST BJ261635 marker (Blanco et al. 2008), whereas *MlIW72* localized at the terminus of chromosome 7AL and is most likely allelic to *Pm1* (Ji et al. 2007).

Molecular markers tightly linked to disease resistance genes can be used in breeding programs to facilitate marker assisted selection (MAS), gene pyramiding, and as a starting point for map-based cloning. Microsatellites, or simple sequence repeats (SSR), have the advantages of being abundant, codominant, stable, and randomly distributed throughout the genome. These markers have a high level of polymorphism, are easy to detect and can be transferred between research labs, hence they are widely used in constructing linkage maps, and for gene tagging and cloning. Thousands of wheat SSR markers have been developed and deposited in the GrainGene database (Röder et al. 1998; Somers et al. 2004; http://wheat.pw.usda.gov). Among these, SSR markers linked to several powdery mildew resistance genes/alleles have been identified (Huang and Röder 2004; McIntosh et al. 2008).

Expressed sequence tags (ESTs) are derived from transcribed regions of the genome. By applying Southern hybridization to a set of Chinese Spring deletion lines, thousands of wheat ESTs have been located on specific chromosome bins (Qi et al. 2004). These physically mapped ESTs can be used to design PCR primers to develop polymorphic sequence tagged site (STS) markers provided that the target gene's chromosomal bin position has been determined. EST sequences are known to be conserved between grass species and therefore provide useful information to conduct comparative genomics analysis (Sorrells et al. 2003).

A perception is that fine genetic mapping and map-based cloning in hexaploid wheat is too complex for traditional mapping strategies and even some of the more modern molecular markers because of the genome size (17 Gb), polyploid nature and highly repetitive sequences (>80%) within the wheat genome. However, comparative genomics has revealed a high level of co-linearity within genomes of the grass family, and this synteny, combined with the available genome sequences of rice and Brachypodium (Devos 2005; Yan et al. 2003, 2004, 2006; The International Brachypodium Initiative 2010), provides useful information for characterizing wheat genome structure, developing closely linked molecular markers and cloning wheat target genes. Detailed comparative genomics analyses of the rice genome with the wheat leaf rust *Lr10* and the powdery mildew *Pm3* resistance genes have shown that the rice genome contains genes homologous to *Lr10* and *Pm3*. Nevertheless, these genes are located at

non-homologous positions, suggesting that wheat and rice have limited collinearity in some genomic regions harboring resistance genes (Guyot et al. 2004; Yahiaoui et al. 2004; Wicker et al. 2007). As an alternative, Draper et al. (2001) advocated that *Brachypodium distachyon* be developed as a model for genomics analysis of cereals and temperate grasses because of its small genome size and close relatedness to members of the Pooideae subfamily in the grass family. The recent sequence of Brachypodium and other resources developed for genomics analyses (The International Brachypodium Initiative 2010) now provides useful information for comparative analyses of barley (Turner et al. 2005) and wheat (Bossolini et al. 2007) traits.

We report here (1) the identification and molecular mapping of a powdery mildew resistance gene *Ml3D232* transferred into common wheat from wild emmer, and (2) the development of high-density genetic linkage map of *Ml3D232* through comparative genomic analysis using the rice and Brachypodium sequences.

Materials and methods

Plant materials

Wild emmer accessions (*T. turgidum* var. *dicoccoides*, $2n = 4x = 28$, AABB) I222 (kindly provided by Dr. Z. K. Gerechter-Amitai, Agricultural Research Organization, The Volcani Center, Israel) were used as a powdery mildew resistant donor to cross with the susceptible 87-1 Chinese elite common wheat line. Then, several backcrosses were carried out to introduce the resistance gene into hexaploid wheat. The common wheat line 3D232 containing homozygous powdery mildew resistance (87-1*6/I222, BC_5F_6) was subsequently selected for crosses with the highly susceptible common wheat line Xuezao to produce F_1 hybrids, F_2 segregating populations and F_3 families. Cultivars/lines with known powdery mildew resistance genes also were used to compare resistance reactions of line 3D232 to 21 different Chinese *B. graminis* f. sp. *tritici* isolates (Table 1). Chinese Spring (CS) and its nullisomic-tetrasomics, ditelosomics and deletion lines of homoeologous group 5 (kindly provided by Drs. W. J. Raupp and B. S. Gill, Wheat Genetics Resource Centre, Kansas State University, USA) were used for chromosomal arm assignment and bin mapping of the molecular markers.

Powdery mildew evaluations

Twenty-one Chinese *B. graminis* f. sp. *tritici* isolates were used to compare the reactions of line 3D232 and 30 wheat accessions with known powdery mildew resistance genes (Table 1). Seedlings were inoculated when the first leaf of seedlings was fully expanded by brushing conidia from neighboring sporulating susceptible seedlings of Xuezao onto the test seedlings. The powdery mildew isolate E09, which is virulent on plants harboring *Pm1*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3e*, *Pm3f*, *Pm5*, *Pm6*, *Pm7*, *Pm8*, *Pm17* and *Pm19* resistance alleles, avirulent on line 3D232 and fully virulent to Xuezao, was used to evaluate the disease phenotypes of F_1 hybrids, F_2 population and $F_{2:3}$ progenies under controlled greenhouse conditions. Infection types (IT) were scored 15 days after inoculation when Xuezao became heavily infected, using a scale of 0, 0; and 1, 2, 3, 4. A "0" reaction indicated no visible symptoms, "0;" reactions produced necrotic flecks, and 1, 2, 3 and 4 phenotypes were 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. Phenotypes were pooled into two groups, resistant (R, IT = 0 to 2) and susceptible (S, IT = 3, 4). F_2 genotypes were based on the responses of their F_3 families and classified as homozygous resistant, segregating and homozygous susceptible.

Genomic DNA isolation and marker analysis

Genomic DNA was extracted from the uninfected seedling leaves of each F_2 plant by the CTAB method (Saghai-Maroof et al. 1984). For bulked segregant analysis (Michelmore et al. 1991), separate DNA bulks were assembled by using equal amounts of DNA from 10 homozygous resistant and 10 homozygous susceptible F_2 plants, respectively. Wheat genomic SSRs (*Xgwm, Xwmc, Xbarc, Xcfa*, and *Xcfd* series) and EST markers were chosen for polymorphism analyses. Primer sequences information of these genomic SSR and EST markers is available online at GrainGenes website (http://wheat.pw.usda.gov). The resulting polymorphic markers were used to genotype the F_2 populations. PCR amplifications were conducted in 10 µl reactions containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 25 ng of each primer, 50 ng genomic DNA and 0.75U Taq DNA polymerase. Amplifications were performed at 94°C for 5 min, followed by 40 cycles at 94°C for 45 s, 50–60°C (depending on specific primers) for 45 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. 3 µl of each PCR product were mixed with 2 µl of loading buffer and separated on 8% non-denaturing polyacrylamide gels (39 acrylamide: 1 bisacrylamide). After electrophoresis, the gels were silver stained and photographed.

Table 1 Differential reactions of 31 wheat cultivars/lines possessing known powdery mildew resistance genes after inoculation with 21 isolates of *Blumeria graminis* f. sp. *tritici*

Cultivar/line	*Blumeria graminis tritici* isolate																					Pm
	E01	E02	E03	E05	E06	E07	E09	E11	E13	E15	E16	E17	E18	E20	E21	E23	E25	E26	E30	E31	E32	
Chancellor	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	–
Axminister/8*Cc[a]	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	*1*
Ulka/8*Cc	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	R	S	*2*
Asosan/8*Cc	S	S	R	S	S	S	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	*3a*
Chul/8*Cc	S	S	S	S	S	R	S	R	R	R	S	R	S	S	S	S	S	R	S	S	S	*3b*
Sonora/8*Cc	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	*3c*
Kolibri	S	S	R	S	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	*3d*
W150	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*3e*
Michigen Amber/8*Cc	R	S	R	R	S	R	R	R	R	S	S	S	S	S	S	R	R	R	R	R	R	*3f*
Khapli/8*Cc	R	S	R	R	R	R	R	R	R	S	S	R	S	S	S	R	R	R	R	R	R	*4a*
Armada	R	S	R	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	*4b*
Hope/8*Cc	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*5a*
Aquila	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	R	S	*5b*
Coker747	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*6*
CI14189	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*7*
Kavkaz	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	*8*
Wembley	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*12*
R4A	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	*13*
Brigand	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*16*
Amigo	S	S	R	S	S	S	S	R	R	R	S	S	S	S	S	R	S	R	R	R	R	*17*
XX186	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*19*
Yangmai5/Sub.6v	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*21*
81-7241	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	*23*
Chiyacao	R	R	R	S	S	R	R	R	S	R	R	R	S	R	R	R	R	R	R	R	S	*24*
5P27	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	*30*
Normandie	R	R	R	R	S	S	R	S	S	R	R	R	R	R	S	S	R	R	R	R	R	*1 + 2 +9*
Maris Huntsman	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	R	S	*2 + 6*
Coker 983	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S	*5 + 6*
Mission	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	R	R	R	R	R	R	*4b + 5b*
P63	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	*Pm42*
3D232	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	*Ml3D232*

R resistant, *S* susceptible

[a] Seven times backcrossed to cv. Chancell

Chromosome arm assignment and physical bin mapping

Polymorphic markers linked to the resistance gene were located using a set of Chinese Spring homoeologous group 5 nullisomic-tetrasomics, ditelosomics and deletion lines (Table 2). Markers were located to chromosome bins by determining the smallest deletion bin possessing them.

Comparative genomics analysis and EST marker development

To locate genomic regions homologous to the wheat powdery mildew resistance locus *Ml3D232* in rice and Brachypodium genomes, EST sequences flanking the *Ml3D232* locus were used to search for orthologous loci in the rice and Brachypodium genome sequences. Then,

Table 2 Chinese Spring 5BL deletion lines used for chromosomal bin mapping

Deletion line	Description	Fraction length
5BL-12	19″ + 1″[d5BL-12]	0.08
5BL-6	19″ + 1′[del5BL-6] + 1‴[5A]	0.29
5BL-11	20″ + 1″[d5BL-11]	0.59
5BL-14	18″ + 1″[d5BL-14]	0.75
5BL-9	18″ + 1″[del5BL-9] + 1'[del7AL-11] + 1′[7A] + t′[del5BS-9L]	0.76
5BL-16	19″ + 1″[del5BL-16] + 1′[del2DL-12] + 1′[2D]	0.79
5BL-13	17″ + 1″[d5BL-13]	0.82

orthologous gene pairs between rice and Brachypodium were compared within the homologous genomics regions. Wheat EST sequences homologous to Brachypodium genes within the genomic region were used to design PCR primers to evaluate polymorphisms between the resistant and susceptible parental lines. Polymorphic EST markers were mapped in the F_2 population for linkage map construction.

Data analysis

Chi-squared (χ^2) tests for goodness-of-fit were used to evaluate deviations of observed data from theoretically expected segregation ratios. Linkages between markers and the resistance gene were determined using Mapmaker 3.0, with a LOD score threshold of 3.0 (Lincoln et al. 1992).

Results

Reactions of 3D232 to 21 isolates of *B. graminis* f. sp. *tritici*

Reactions of 3D232 and 30 wheat accessions possessing known powdery mildew resistance genes to 21 *Bgt* isolates are listed in Table 1. The accession 3D232 was highly resistant to all 21 isolates and had similar resistance reaction patterns as plants with *Pm12*, *Pm16* and *Pm21* resistance genes, suggesting that 3D232 harbors broad-spectrum powdery mildew resistance.

Genetic analysis of powdery mildew resistance genes in 3D232

3D232 was highly resistant to isolate E09 (IT 0;), whereas Xuezao was highly susceptible (IT 4). All hybrid F_1 seedlings had a 0;-1 IT phenotype, indicating that the powdery mildew resistance in 3D232 is dominant. The F_2 population segregated as 117 resistant and 40 susceptible, which fits a 3:1 single Mendelian loci ratio ($\chi^2_{3:1} = 0.019$, $P > 0.01$). The $F_{2:3}$ progenies segregated as 39 homozygous resistant:78 segregating:40 homozygous susceptible, as expected of a 1:2:1 ratio ($\chi^2_{1:2:1} = 0.025$, $P > 0.01$). These results suggest that a single dominant powdery mildew resistant locus, which we provisionally designate *Ml3D232*, has been transferred into the common wheat line 3D232 from the wild emmer accession I222.

Identification of microsatellite markers linked to *Ml3D232*

Initially, GWM and WMC microsatellite primers were used to screen polymorphisms between the parental lines as well as the resistant and susceptible DNA bulk segregant lines. The SSR markers *Xwmc75*, *Xwmc289*, *Xwmc415*, *Xgwm335, Xgwm499* and *Xgwm639* revealed polymorphisms between the resistant and susceptible parents as well as the bulk segregants. After testing the F_2 segregating population, a linkage map for powdery mildew disease resistance gene *Ml3D232* was constructed (Fig. 1). Among the six polymorphic SSR markers, *Xwmc75*, *Xwmc289*, *Xgwm335* and *Xgwm499* were co-dominant, while *Xwmc415* and *Xgwm639* were dominant.

Chromosome arm assignment and physical bin mapping

Chinese Spring homoeologous group 5 nullisomic-tetrasomics, ditelosomics and deletion lines were used to assign the chromosomal and physical bin locations of the powdery mildew resistance gene *Ml3D232* and its linked SSR markers. *Xwmc415* and *Xgwm75* were not detected in 5BL-6, 5BL-11 and 5BL-12, and *Xgwm75* was also absent in 5BL-14 (Fig. 2a, b). The results indicated that *Xwmc415* is located at 5BL bin 0.59–0.75 and that *Xgwm75* is located at 5BL bin 0.75–0.76. Therefore, the powdery mildew resistant gene *Ml3D232* could be assigned to 5BL bin 0.59–0.76 (Fig. 1).

Identification of EST markers linked to *Ml3D232*

To saturate the genomic region harboring the powdery mildew resistance gene *Ml3D232*, 19 ESTs that physically mapped to the 0.59–0.76 interval within chromosome 5BL were selected for polymorphism screening. Six EST-derived STS markers, BE494426, BE442763, BE445282, BE407068, CA635388 and CJ832481, were polymorphic between the parental lines as well as the resistant and susceptible bulks, and were shown to be linked to the resistance gene *Ml3D232* after testing the segregating

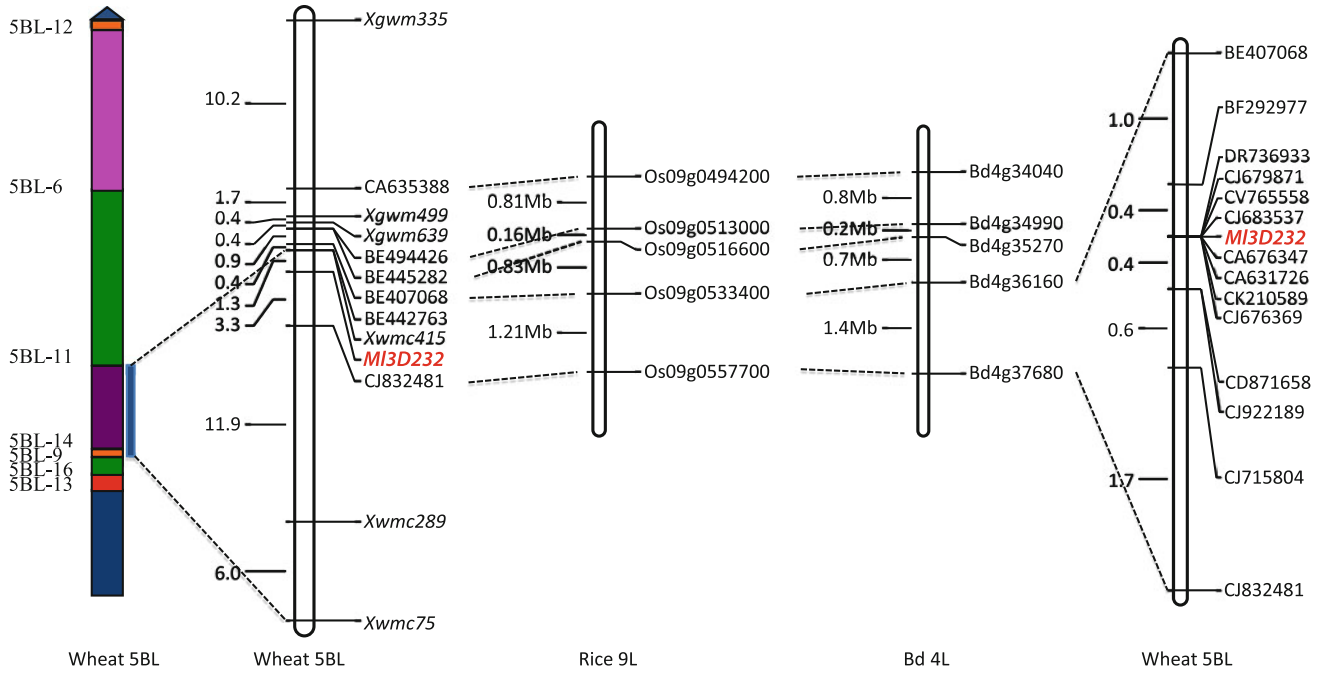

Fig. 1 Genetic, physical bin and comparative mapping of powdery mildew resistance gene *Ml3D232* derived from wild emmer

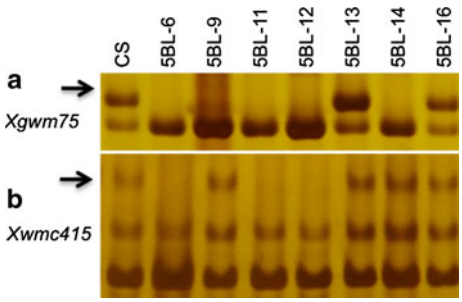

Fig. 2 Amplification patterns of SSR markers *Xgwm75* and *Xwmc415* in Chinese Spring 5BL deletion lines

population (Fig. 1). No polymorphisms between the resistant and susceptible lines were detected for BJ261635, an EST-STS marker linked to powdery mildew resistance gene *Pm36* (Blanco et al. 2007), therefore, this marker could not be mapped in our study.

Comparative genetic mapping *Ml3D232* using rice and *Brachypodium* genome sequences

The sequences of the *Ml3D232*-linked ESTs CA635388, BE494426, BE445282, BE407068, BE442763 and CJ832481 were used to blast the rice and Brachypodium genome sequences. Except for BE442763, all of the wheat ESTs were detected on the long arms of rice chromosome 9 and Brachypodium chromosome 4, respectively (Fig. 1). A 1.2 Mb genomic region (Os09g0533400–Os09g0557700) in the rice 9L and a 1.4 Mb genomic region (Bd4g36160–Bd4g37680) in Brachypodium 4L were conserved in the *Ml3D232* genomic region in wheat covered by the EST markers BE407068 and CJ832481 (Fig. 1). After searching homologous wheat ESTs using the Brachypodium genes residing between Bd4g36160–Bd4g37680, 12 new polymorphic EST markers homologous to 12 Brachypodium genes were constructed and mapped in the *Ml3D232* linkage map covering 4.1 cM between BE407068 and CJ832481 (Fig. 1). The two flanking EST markers BF292977 and CD871658 narrowed the genomic region carrying *Ml3D232* to a 0.8 cM region that is co-linear with a 558 kb genomic region in *B. distachyon*. Eight wheat EST markers co-segregated with *Ml3D232* in the mapping population, and their corresponding homologous region in Brachypodium (Bd4g36840–Bd4g37120) spans 314 kb and contains 29 putative genes. Among the 29 Brachypodium genes, only Bd4g36980 is homologous to a known NBS-LRR resistance gene analogy (RGA). However, putative RGA sequences were not found in the corresponding rice genomic region.

Discussion

Wild emmer provides diverse resistance to wheat pathogens, including stripe rust (Grama and Gerechter-Amitai 1974), stem rust (Nevo et al. 1991), leaf rust (Moseman et al. 1985) and powdery mildew (Moseman et al. 1984). In this study, a new broad-spectrum powdery mildew resistance common wheat germplasm 3D232 has been

developed by introgressing powdery mildew resistance gene from wild emmer originated from Israel into Chinese common wheat elite line. To investigate the inheritance of the powdery mildew resistance derived from wild emmer, a segregating population was developed by crossing the resistant backcrossed inbred line 3D232 with a susceptible line Xuezao. The segregation pattern supported the hypothesis that the broad-spectrum resistance is controlled by a single, dominant gene *Ml3D232*. A SSR and EST linkage map of *Ml3D232* was then constructed using BSA approach (Michelmore et al. 1991). Six SSR markers, *Xwmc75*, *Xwmc289*, *Xwmc415*, *Xgwm335, Xgwm499* and *Xgwm639*, were found to be linked to Ml3D232.

The SSR markers *Xgwm639, Xwmc415* and *Xwmc289* have multiple loci in the common wheat genome. *Xgwm639* was detected at three loci on chromosome arms 5AL, 5BL and 5DL (Röder et al. 1998; Sourdille et al. 2004), *Xwmc415* was found at two loci on chromosome arms 5AL and 5BL (Somers et al. 2004), and *Xwmc289* was assigned to chromosome arms 5BL and 5DL (Somers et al. 2004). However, *Xgwm335, Xgwm499* and *Xwmc75* were single-locus markers that only mapped to chromosome 5BL. These results and the physical mapping results with Chinese Spring nullisomic-tetrasomics, ditelosomics and deletion lines enabled us to locate the powdery mildew resistance gene *Ml3D232* on chromosome 5BL bin 0.59–0.76.

To date, eight powdery mildew resistance genes have been identified from wild emmer and introgressed into common and durum wheats (Reader and Miller 1991; Rong et al. 2000; Liu et al. 2002; Mohler et al. 2005; Blanco et al. 2007; Ji et al. 2007; Li et al. 2008; Hua et al. 2009). Among these, only *Pm30* (Liu et al. 2002) and *Pm36* (Blanco et al. 2007) have been assigned to chromosome 5B. *Pm30* was derived from the wild emmer accession C20 originating from the Rosh Pinna population in Israel and mapped to 5BS bin 0.56–0.71 (Liu et al. 2002). Chen et al. (2005) recently reported that *Pm16*, originally located on chromosome 4A, is linked to the SSR marker *Xgwm159* and might be identical to *Pm30*. However, the differential reactions we have found with multiple *B. graminis* f. sp. *tritici* isolates indicate that *Pm16* and *Pm30* are not identical, but most likely are alleles (Table 1).

Pm36 was transferred into durum wheat from wild emmer and mapped on 5BL closely linked to EST marker BJ261635 (Blanco et al. 2008). To characterize the relationship between *Ml3D232* and *Pm36*, EST marker BJ261635 was tested in our segregation population. Unfortunately, BJ261635 failed to reveal polymorphisms between the resistant and susceptible plants used in our study and hence could not be used to assess the relationships between *Ml3D232* and *Pm36*. However, when compared to the consensus microsatellite map developed by Somers et al. (2004), *Ml3D232*, flanked by SSR markers *Xwmc415* and *Xwmc75*, was located on the same genomic region of *Pm36*, flanked by SSR markers *Xcfd7* and *Xwmc75* in 5BL. Postulation and allelism tests need to be conducted to clarify if *Ml3D232* and *Pm36* are allelic or closely linked powdery mildew resistance genes in disease resistance gene cluster.

The physical bin mapping of wheat ESTs in a series of Chinese deletion lines (Qi et al. 2004) provides useful information to develop closely linked EST-based polymorphic markers when the physical bin position of the target gene is known. The positional cloning of vernalization genes (*Vrn1*, *Vrn2* and *Vrn3*) (Yan et al. 2003, 2004, 2006) and high grain protein content gene (*GPC-1B*) (Distelfeld et al. 2004; Uauy et al. 2006) in wheat have benefited greatly from the comparative genomics analyses of the rice genome sequence. However, the isolation of the wheat disease resistance genes, *Lr21* (Huang et al. 2003), *Lr10* (Feuillet et al. 2003) and *Pm3b* (Yahiaoui et al. 2004) have shown that wheat and rice have very limited co-linearity in these regions of the genome due to massive genomic rearrangements (Keller et al. 2005). We were able to generate additional EST markers for comparative genomic analysis against the rice genome sequence to locate rice homologous genomic regions. Our comparative genomic analyses between the *Ml3D232* genetic regions in wheat with the homologous genomic regions in rice revealed no RGA-like disease resistance genes in the rice sequences. In this regard, our results are similar to those observed during cloning of the *Lr10* (Feuillet et al. 2003) and *Pm3b* (Yahiaoui et al. 2004) resistance genes. These studies all point to the limited value of the rice genome sequence for cloning disease resistance genes in wheat.

However, the recent sequencing of the Brachypodium genome (The International Brachypodium Initiative 2010) provides a new comparative genomics approach for fine mapping and cloning wheat genes. Brachypodium is more closely related to temperate cereals like wheat than to tropical rice, both in whole genome comparisons (The International Brachypodium Initiative 2010), and detailed analyses of particular gene regions (Bossolini et al. 2007). Our comparative genomics analyses of the rice and Brachypodium genomic regions sharing homology to the region encompassing the wheat powdery mildew resistance gene *Ml3D232* have revealed the presence of an NBS-LRR disease resistance gene Bd4g36980 in Brachypodium, but not in rice. The wheat EST CJ683537, a putative NBS-LRR sequence that is orthologous to Bd4g36980, was found to co-segregate with *Ml3D232* and may be useful as a chromosome-landing probe for future screening of BAC libraries (Tanksley et al. 1995).

Disease resistance genes appear to have evolved very rapidly in cereals due to gene duplications and massive

chromosomal rearrangements. In this regard, the NBS-LRR gene family has rarely been found in a syntenic order in rice and sorghum comparisons (The International Brachypodium Initiative 2010). Same situation was observed between rice and Brachypodium comparisons in this study. The homology of Brachypodium NBS-LRR gene Bd4g36980 could not be found at orthologous region of chromosome 9, but at a non-orthologous position of chromosome 11 (Os11g0686500) in rice genome. However, the fact that the wheat NBS-LRR-like EST CJ683537 is orthologous to Bd4g36980 and co-segregated with the powdery mildew resistance gene *Ml3D232* provides additional evidence that the wheat and Brachypodium NBS-LRR gene families have a syntenic order. Thus, the NBS-LRR like wheat EST CJ683537 may serve as a starting point for fine genetic mapping, wheat BAC library screening, chromosome landing and map-based cloning of *Ml3D232*.

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