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ORIGINAL PAPER

Comparative genetic mapping and genomic region collinearity analysis of the powdery mildew resistance gene *Pm41*

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Abstract

Key message **By applying comparative genomics analyses, a high-density genetic linkage map narrowed the powdery mildew resistance gene *Pm41* originating from wild emmer in a sub-centimorgan genetic interval**.

Abstract Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, results in large yield losses worldwide. A high-density genetic linkage map of the powdery mildew resistance gene *Pm41*, originating from wild emmer (*Triticum turgidum* var. *dicoccoides*) and previously mapped to the distal region of chromosome 3BL bin 0.63–1.00, was constructed using an $F_{5:6}$ recombinant inbred line population derived from a cross of durum wheat cultivar Langdon and wild emmer accession IW2. By applying comparative genomics analyses, 19 polymorphic sequence-tagged site markers were developed and integrated into the *Pm41* genetic linkage map. Ultimately, *Pm41* was mapped in a 0.6 cM genetic interval flanked by markers *XWGGC1505* and *XWGGC1507*, which correspond to 11.7, 19.2, and 24.9 kb orthologous genomic regions in *Brachypodium*, rice, and sorghum, respectively. The *XWGGC1506* marker co-segregated with *Pm41* and could be served as a starting point for chromosome landing and map-based cloning as well as marker-assisted selection of *Pm41*. Detailed comparative genomics analysis of the markers flanking the *Pm41* locus in wheat and the putative orthologous genes in *Brachypodium*, rice, and sorghum suggests that the gene order is highly conserved between rice and sorghum. However, intra-chromosome inversions and re-arrangements are evident in the wheat and *Brachypodium* genomic regions, and gene duplications are also present in the orthologous genomic regions of *Pm41* in wheat, indicating that the *Brachypodium* gene model can provide more useful information for wheat marker development.

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Introduction

Bread wheat is one of the most important food crops in the world, and powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the severest fungal wheat disease worldwide. Breeding and deployment of resistant cultivars are the most profitable and environmental friendly method to prevent disease losses. Consequently, powdery mildew resistance genes have been extensively employed in breeding program, and more than 60 powdery mildew resistance genes/alleles have currently been identified at 43 loci (McIntosh et al. 2013).

Wild emmer (*Triticum turgidum* var. *dicoccoides*, $2n = 4x = 28$, AABB), the progenitor of domesticated wheat, is a valuable resource for wheat powdery mildew disease resistance breeding (Nevo et al. 2002; Moseman et al. 1984), and several resistance genes have been identified from this source. Examples are *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), *Ml3D232* (Zhang et al. 2010), *PmG3M* (Xie et al. 2012), *PmAs846* (Xue et al. 2012), and *MlIW170* (Liu et al. 2012), some of which already provide useful resistance in wheat breeding program.

Due to rapid evolution of the pathogen population, new virulent *Bgt* races often appear that can circumvent important race-specific resistance genes. Therefore, discovering novel resistance genes and identifying the molecular markers will contribute to development of durable cultivars with broad-spectrum disease resistance and the use of marker-assisted selection to pyramid multiple resistance genes.

Many kinds of molecular markers, including restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), sequence-tagged sites (STS), and amplified fragment length polymorphisms (AFLPs), have been used to construct genetic linkage maps and to localize powdery mildew resistance genes in wheat. However, the large size of the wheat genome (17 gigabase), its hexaploid nature (AABBDD), numerous repetitive DNA sequences (90 %), and un-availability of an assembled reference genome sequence create very difficult and time-consuming obstacles to construct high-density genetic linkage maps for genes in wheat. As an alternative approach, comparative genomics analysis has been applied to construct high-resolution genetic linkage maps of interesting wheat genes by use of the available genome sequences of rice (International Rice Genome Sequencing Project 2005), sorghum (Paterson et al. 2009), *Brachypodium distachyon* (The International *Brachypodium* Initiative 2010), and wheat EST sequence (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). The recent released shotgun genome sequences of hexaploid wheat Chinese Spring (Brenchley et al. 2012), *T. urartu* (Ling et al. 2013), and *Aegilops tauschii* (Jia et al. 2013) now provide even more useful information to develop molecular markers linked to the targeted genes. Through comparative genomics analysis, high-density genetic linkage maps of vernalization (*VRN*) genes (Yan et al. 2003, 2004, 2006), pairing homologous 1 (*Ph1*) (Griffiths et al. 2006), grain protein content-B1 (*Gpc-B1*) (Uauy et al. 2006), and yellow rust resistance gene *Yr36* (Fu et al. 2009) were constructed to facilitate the map-based cloning. However, comparative analyses of the wheat leaf rust resistance gene *Lr10* (Feuillet et al. 2003) and powdery mildew resistance gene *Pm3* (Yahiaoui et al. 2004) with the rice genome sequence have revealed that genomic re-arrangements have occurred in some genomic regions containing resistance genes (Guyot et al. 2004; Yahiaoui et al. 2004; Keller et al. 2005).

The physical map of chromosome 3B had been constructed through a chromosome-based approach (Paux et al. 2008; www.iwgsc.org). Survey sequencing of chromosome 3B bacterial artificial chromosomes (BACs) and comparison with rice and *Brachypodium* genome sequences have indicated that numerous non-collinear genes are interspersed within a highly conserved ancestral grass gene backbone, which makes it more difficult to make a study of 3B chromosome through comparative genomics analysis (Choulet et al. 2010). Moreover, because of tandem or inter-chromosomal gene duplications in the telomeric regions, the gene density in the distal regions is two times higher than that in the proximal regions.

Powdery mildew resistance gene *Pm41* was previously mapped on chromosome 3B bin 0.63–1.00 (Li et al. 2009). In this paper, we have described the following: (1) a comparative genomics analysis of the genomic regions of *Pm41* with *Brachypodium*, rice, and sorghum; and (2) construction of a high-density genetic linkage map as a framework for map-based cloning and marker-assisted selection of *Pm41* in wheat breeding programs.

Materials and methods

Plant materials

A set of 175 $F_{5:6}$ recombinant inbred lines (RILs) from a cross between the powdery mildew susceptible durum wheat cultivar, Langdon, and the resistant wild emmer accession, IW2, were used to construct a high-density linkage map of the *Pm41* gene (Li et al. 2009). A highly susceptible common wheat line Xuezao was used as the susceptible control.

Powdery mildew evaluations

The prevailing *Bgt* isolate E09, kindly provided by Dr. Xiayu Duan of the Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing, China, was used to evaluate the powdery mildew disease responses. E09 was virulent on cultivars carrying the *Pm1, Pm3a, Pm3b, Pm3c, Pm3d, Pm3e, Pm3f, Pm5a, Pm6, Pm7, Pm8, Pm17,* and *Pm19* resistance genes, but avirulent on the wild emmer accession IW2 (Li et al. 2009).

The RILs were tested for powdery mildew responses under controlled greenhouse conditions with temperature of 22 °C/18 °C for the day/night cycle. At least 25 seeds were planted for each RIL, the seedlings were inoculated with *Bgt* isolate E09, and the disease phenotypes were recorded 15 days after inoculation when the susceptible control Xuezao had become severely infected. The infection types (IT) were scored on a 0–4 scale, namely with 0 representing no visible symptoms, 0; for necrotic flecks, and 1–4 for highly resistant, moderately resistant, moderately susceptible, and highly susceptible (Liu et al. 1999). Reactions were classified into two groups, resistant (R = 0–2 IT) and

susceptible (S = 3–4 IT). Only the homozygous resistant and homozygous susceptible RILs were selected for construction of the high-density genetic linkage map of the *Pm41* gene.

Polymerase chain reaction (PCR)

Genomic DNA was isolated from the seedling leaves of parental lines IW2 and Langdon as well as the homozygous resistant and homozygous susceptible RILs using the CTAB protocol (Saghai-Maroof et al. 1984). Resistant and susceptible bulks were established by mixing equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible RILs and used to screen polymorphic markers linked to *Pm41*.

The polymerase chain reaction (PCR) mixture contained 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 in a total volume of 10 µl. Amplification programs were as follows: 94 °C for 5 min, followed by 35 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. Then, 3 µl of each PCR product was 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.

Comparative genomics analysis

Since *Pm41* was previously mapped in the distal region of chromosome 3BL (Li et al. 2009), expressed sequence tags (EST) bin-mapped on chromosome 3BL deletion bin 0.63–1.00 (Munkvold et al. 2004; http://wheat.pw.usda.gov) were screened for polymorphisms between IW2 and Langdon as well as the resistant and susceptible DNA bulks. The polymorphic EST markers were tested in the RIL mapping population, and the corresponding EST sequences of the polymorphic EST markers flanking *Pm41* were used to perform a BLAST search against the genome sequence databases of *Brachypodium* (http://mips.helmholtz-muenchen.de/plant/Brachypodium/), rice (http://rice.plantbiology.msu.edu/), and sorghum (http://mips.helmholtz-muenchen.de/plant/sorghum/) to identify orthologous gene pairs. The orthologous genomic regions were identified through comparative genomics analysis of the putative highly conserved gene pairs in *Brachypodium*, rice, and sorghum. The putative gene pairs with high level of collinearity among *Brachypodium*, rice, and sorghum were preferentially used to search homologous wheat ESTs to develop new polymorphic markers linked to the *Pm41* locus.

STS marker development

After identifying the orthologous genomics regions, the coding sequences (CDS) of orthologous gene pairs among *Brachypodium*, rice, and sorghum were used as queries to search the NCBI database (http://www.ncbi.nlm.nih.gov/) for orthologous wheat ESTs, which were subsequently used to search the Chinese Spring 454 shotgun draft genome sequence database (http://www.cerealsdb.uk.net/CerealsDB/Documents/DOC_search_reads.php) and the International Wheat Genome Sequencing Consortium (IWGSC) Chinese Spring chromosome 3B survey sequences (http://www.wheatgenome.org/). The orthologous Chinese Spring contigs were used as template to design primers with DNAMAN software. The primer-designing parameters were as follows: amplification product size of 200–800 bp with the optimum 500 bp, primer length of 18–22 bp, Tm of 55–65 °C, and GC content of 40–60 %. Adjacent amplicons overlapped to ensure amplification of the entire contig. The designed primers were tested on the Chinese Spring nullisomic-tetrasomics of homoeologous group 3 and screened for polymorphisms between the Langdon and IW2 parental lines and between the resistant and susceptible DNA bulks. PCR products were mixed with 2 µl of loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, and 0.25 % xylene cyanol), separated on 8 % non-denaturing polyacrylamide gels (39 acrylamide/1 bisacrylamide), and visualized following silver staining. The chromosome 3B-specific polymorphic STS markers were used for RILs genotyping to construct the high-density genetic linkage map.

High-density genetic linkage map construction

Chi-squared (χ^2) tests for goodness-of-fit were performed to estimate deviations of observed data from theoretically expected segregation ratios. Linkages between molecular markers and the *Pm41* locus were analyzed using Mapmaker 3.0 with a LOD score threshold of 3.0 (Lincoln et al. 1992). The genetic map was constructed with the software Mapdraw V2.1 (Liu and Meng 2003).

Results

Genetic analysis of *Pm41* in the RIL population

The Langdon and IW2 parental lines as well as the set of 175 $F_{5:6}$ RILs were inoculated with *Bgt* isolate E09. Langdon was highly susceptible (IT 4), and IW2 was highly resistant (IT 0;), which is consistent with the results of Li et al. (2009) (Fig. 1). The RILs segregated as 78 resistant,

86 susceptible, and 11 segregating in response to *Bgt* isolate E09, and fit a 1:1 single Mendelian loci ratio ($\chi^2_{1:1} = 0.39$, $P > 0.05$), as expected for the previous result showing that IW2 powdery mildew resistance is controlled by a single locus *Pm41* (Li et al. 2009). Homozygous resistant and homozygous susceptible RILs were selected for genotyping to construct the high-density genetic linkage map of *Pm41*.

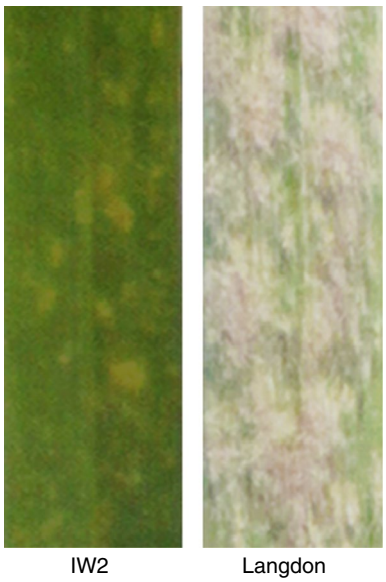

Fig. 1 Mild chlorotic lesion phenotype (0;) exhibited by the resistant IW2 parent (*left*) and the susceptible phenotype of the Langdon parent (*right*) showing extensive mildew sporulation (4) at 15 days after inoculation with *Bgt* isolate E09

Identification of EST markers linked to *Pm41*

ESTs bin-mapped on chromosome 3BL bin 0.63–1.00 were tested for polymorphisms between the Langdon and IW2 parental lines as well as the resistant and susceptible DNA bulks. Out of 150 EST primer pairs tested, two EST markers, *XWGGC1501* (developed from BM138632) and *XWGGC1519* (developed from BG263661), were polymorphic between Langdon and IW2 as well as the resistant and susceptible DNA bulks and could be linked to *Pm41* after genotyping the RILs. The *XWGGC1501* and *XWGGC1519* markers were located 2.7 cM distal and 4.6 cM proximal to the powdery mildew resistance gene *Pm41*, respectively (Fig. 2).

Comparative genomics analysis

The BM138632 and BG263661 sequences were used as queries to search orthologous genes from genome sequences of *Brachypodium*, rice, and sorghum. Both BM138632 and BG263661 revealed orthologs on *Brachypodium* chromosome 2L (Bd2g60490 and Bd2g61140), rice chromosome 1L (Os01g71670 and Os01g72520), and sorghum chromosome 3L (Sb03g045490 and Sb03g046200), respectively (Table 1). Three genomic regions span 443.5, 534.5, and 625.5 kb in *Brachypodium*, rice, and sorghum, respectively, and appear to be the orthologous genomic regions to wheat harboring the powdery mildew resistance gene *Pm41* (Figs. 2, 3). Detailed comparative analyses revealed that 54 of 73 predicted *Brachypodium* genes

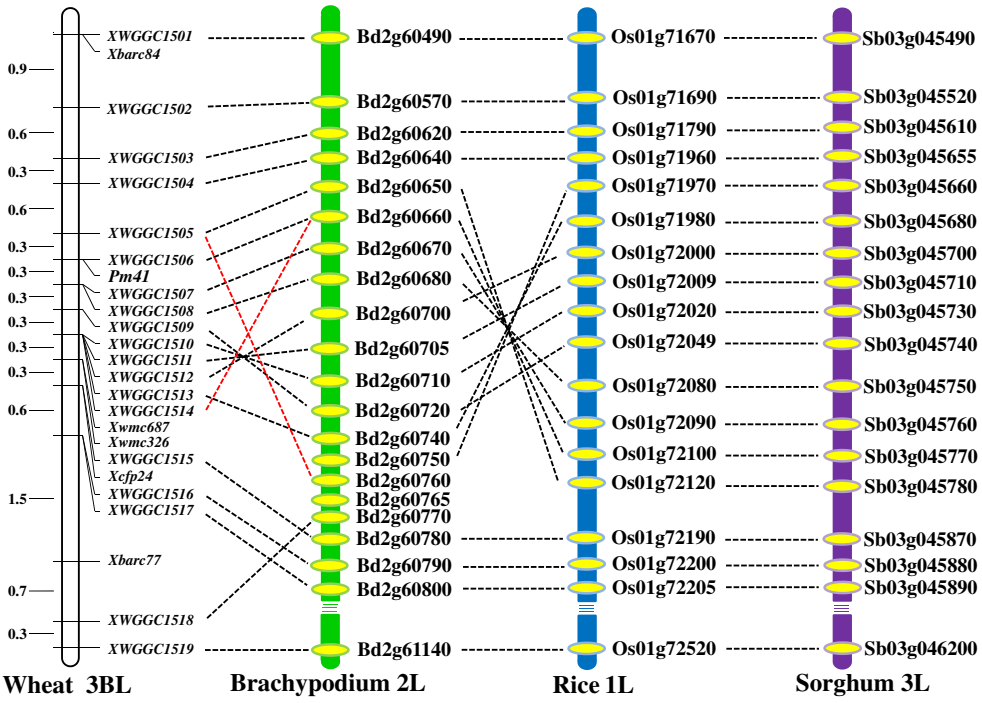

Fig. 2 Comparative genomics mapping of the *Pm41* powdery mildew resistance gene. Genetic linkage map of *Pm41*, orthologous genes on the *Brachypodium* 2L, rice 1L, and sorghum 3L chromosomes, respectively

Table 1 Orthologous gene pairs among the collinear genomic regions of wheat, *Brachypodium*, rice, and sorghum

Wheat ESTs	*Brachypodium*	Rice	Sorghum	Predicted function
BM138632	Bd2g60490	Os01g71670	Sb03g045490	Glucan endo-1,3-beta-D-glucosidase
CJ856616	Bd2g60650	Os01g72120	Sb03g045780	Glutathione S-transferase
		Os01g72110		Transposable element protein
DR741857	Bd2g60660	Os01g72100	Sb03g045770	Calcium-binding protein
CD887903	Bd2g60670	Os01g72090	Sb03g045760	Phycoerythrobilin ferredoxin oxidoreductase
HX169518	Bd2g60680	Os01g72080	Sb03g045750	Calmodulin-like protein 1
	Bd2g60690			WD-repeat protein
		Os01g72070		Retrotransposon protein
BQ620217	Bd2g60700	Os01g72000	Sb03g045700	Spotted leaf protein
HX019866	Bd2g60705	Os01g72009	Sb03g045710	Putative uncharacterized protein
			Sb03g045715	Hypothetical protein
			Sb03g045720	MKI67 FHA domain-interacting nucleolar phosphoprotein
CD490623	Bd2g60710	Os01g72020	Sb03g045730	Ankyrin-repeat protein
		Os01g72030		Retrotransposon protein
HX148298	Bd2g60720	Os01g72049	Sb03g045740	Protein OSB3, chloroplastic/mitochondrial
	Bd2g60730	Os01g71990	Sb03g045690	Pyrroline-5-carboxylate reductase
CD936937	Bd2g60740	Os01g71980	Sb03g045680 Sb03g045670	Rho-GTPase-activating protein Flavonol sulfotransferase
	Bd2g60750	Os01g71970	Sb03g045660	GRAS family transcription factor containing protein
BG263661	Bd2g61140	Os01g72520	Sb03g046200	Phosphoesterase family protein

are orthologs to 61 of 82 predicted rice genes and 61 of 77 predicted sorghum genes in the corresponding genomics regions. Out of 82 predicted rice genes, 63 are orthologs to 62 of 77 predicted sorghum genes in the corresponding genomics regions. High levels of genomic collinearity were also observed between rice and sorghum. Three genomics regions, Bd2g60490–Bd2g60640, Bd2g60760–Bd2g60820, and Bd2g60960–Bd2g61140, in *Brachypodium* showed high collinearity and share gene orders with rice and sorghum. However, gene duplications, insertions/deletions, and rearrangements are widespread among the three species. Two segmental inversions, Bd2g60650–Bd2g60750 and Bd2g60830–Bd2g60950, were found in *Brachypodium* compared to rice and sorghum gene orders in corresponding orthologous genomic regions (Fig. 3). Furthermore, another small segmental inversion from Bd2g60700 to Bd2g60720 was also observed within the segmental inversion of Bd2g60650 to Bd2g60750 in *Brachypodium* and has the same gene order from Bd2g60700 to Bd2g60720 as that of rice and sorghum (Fig. 2).

Polymorphic marker development and high-density linkage map construction

The putative orthologous genes with same order and high level of synteny among *Brachypodium*, rice, and sorghum are likely to have the same order and synteny as those of the wheat genes on chromosome 3BL, and these were preferentially used to develop molecular markers linked to *Pm41*. The putative genes between Bd2g60490 and Bd2g61140 were selected to develop markers to narrow the collinearity regions among the wheat, *Brachypodium*, rice, and sorghum. The coding sequences (CDS) of *Brachypodium* genes from Bd2g60490 to Bd2g61140 were used as queries to search orthologous wheat ESTs, the Chinese Spring IWGSC chromosome 3B survey sequences, and contigs of the Chinese Spring 454 shotgun draft genome sequences. In total, 19 polymorphic markers, from *XWGGC1501* to *XWGGC1519*, were developed and genotyped in the RILs to construct a high-density genetic linkage map of *Pm41* (Table 2; Fig. 2). These markers permitted mapping of *Pm41* within a sub-centimorgan interval that co-segregated with *XWGGC1506* and is flanked by *XWGGC1505* and *XWGGC1507* (Fig. 4) with a genetic distance of 0.3 cM on each side. This region also corresponds to an 11.7 kb genomic region (Bd2g60650–Bd2g60670) in *Brachypodium,* an 19.2 kb genomic region (Os01g72090–Os01g72120) in rice, and an 24.9 kb genomic region (Sb03g045760–Sb03g045780) in sorghum, respectively (Fig. 2).

Collinearity analysis of the *Pm41* genomic region in wheat with *Brachypodium*, rice, and sorghum

Of the 19 polymorphic markers mapped in the *Pm41* high-density genetic linkage map in wheat, 18 markers could

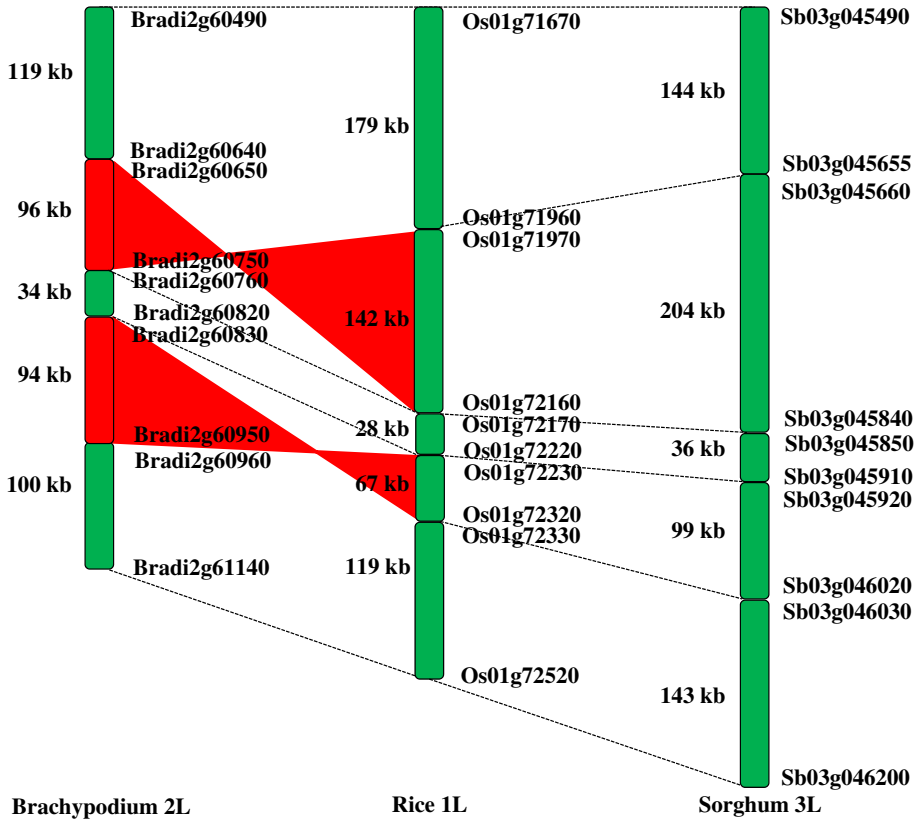

Fig. 3 Structure variations of the *Pm41* orthologous genomic regions in *Brachypodium*, rice, and sorghum. *Green regions* indicate the same gene order among *Brachypodium*, rice, and sorghum. *Red regions* represent *Brachypodium* inversions in gene order compared with rice and sorghum (color figure online)

be used to identify orthologous genes in the corresponding genomic regions of *Brachypodium*, rice, and sorghum (Fig. 2). The wheat *XWGGC1518* ortholog is Bd2g60770 in *Brachypodium*, but this ortholog is not present in the rice and sorghum genomes. The order of eight molecular markers, *XWGGC1501, XWGGC1502, XWGGC1503, XWGGC1504, XWGGC1515, XWGGC1516, XWGGC1517,* and *XWGGC1519*, in wheat chromosome 3BL is consistent with orthologs in the *Brachypodium*, rice, and sorghum genomes (Fig. 2). A segmental inversion was observed from the *XWGGC1509* to *XWGGC1512* markers in the wheat 3BL chromosome, and these markers correspond to conserved genomic blocks in *Brachypodium* (Bd2g60700–Bd2g60720), rice (Os01g72000–Os01g72049), and sorghum (Sb03g045700–Sb03g045740) (Fig. 2). The 0.6 cM genomic region harboring *Pm41* (*XWGGC1505–XWGGC1508*) is syntenic to the orthologous genomic region in *Brachypodium* (Bd2g60650–Bd2g60680) and has a conserved gene order. However, inversions in gene orders occur in the rice (Os01g72080–Os01g72120) and sorghum (Sb03g045750–Sb03g045780) syntenic genomic regions (Fig. 2).

Gene duplication analysis

Genetic linkage maps and comparative genomics analyses between the wheat 3BL, *Brachypodium*, rice, and sorghum genomes revealed complex gene duplications and rearrangements in corresponding genomic regions of *Pm41*. The two wheat *XWGGC1506* and *XWGGC1514* markers are homologous to the Bd2g60660, Os01g72100, and Sb03g045770 genes of *Brachypodium*, rice, and sorghum, respectively, suggesting that gene duplications have occurred in this region of the wheat genome. The *Pm41* flanking marker *XWGGC1505* is also homologous to 3, 6, and 9 glutathione transferases in the corresponding *Brachypodium*, rice, and sorghum genomic regions, indicating that several duplication events have occurred in the three species (Table 3; Figs. 2, 5). Sequences comparisons of the glutathione transferases in *Brachypodium*, rice, and sorghum also revealed that the breakpoint of the segmental inversion Bd2g60650–Bd2g60750 in *Brachypodium* occurred in the duplicated genes (Figs. 2, 5).

Discussion

Wild emmer is an important germplasm for resistance to biotic and abiotic stress in wheat breeding programs (Nevo et al. 2002). More than 13 alleles in 8 loci conferring resistance to powdery mildew have been identified from wild emmer. Among these, *Pm16* appeared to be composed of different alleles at same loci on the emmer 5BS

Table 2 STS markers linked to the powdery mildew resistance gene *Pm41*

Wheat ESTs	Markers	Type	Fragment size (bp)[a]		Segregation rate[b]	Forward primer (5′–3′)	Reverse primer (5′–3′)
			IW2	Langdon			
BM138632	*XWGGC1501*	D	550	–	A:B = 77:87	TACAGGTCCAATGGCATCAA	GATGTGTAGGTCAGCCCGTT
HX197528	*XWGGC1502*	C	208	342	A:B = 78:86	GCTTCCACATTTGTCGGT	GGATGAAACTTGCTCTTGA-GAC
HX129030	*XWGGC1503*	D	–	339	A:B = 80:84	CACCAGCACCGTTTATTACT	GCTCCTGTTCAATCCGAC
CO346255	*XWGGC1504*	D	–	506	A:B = 79:85	GGTTTGGACTATGGACGGAT	CGTTGGGCAGGTATGAGAAT
CJ856616	*XWGGC1505*	D	294	–	A:B = 77:87	ATCACTCTGTCTGGAGCGAA	GAAAGCGCCGACAACCCT
DR741857	*XWGGC1506*	C	/	/	A:B = 78:86	TGCCACATTGTTCTTGTCAT	TCCTGGAATCTCTCATCAGTT
CD887903	*XWGGC1507*	C	458	436	A:B = 77:87	CTTCGTTACACGCTGTCC	GGATTTGCCTGTTTCTGG
HX169518	*XWGGC1508*	C	/	/	A:B = 77:87	TCTCCCTTCCATTTGACAT	CAAGAAACTCCAAGCCAGT
HX148298	*XWGGC1509*	C	724	550	A:B = 78:86	TGGATGAAGAGGAAGGGTT	AGGCGTATGGATAAAGAGGT
CD490623	*XWGGC1510*	C	561	569	A:B = 79:85	GGCTGCTCTACTATCTCCTTG	CTCCCTCTGTTCACAAATGT
HX019866	*XWGGC1511*	C	536	544	A:B = 79:85	CACACACACTGTTGGGCA	TCTCCATCAGGTATGTCGC
BQ620217	*XWGGC1512*	D	364	–	A:B = 79:85	CGACCATTGATACATTACCTG	AACATCTTGGAAATAGCGG
CD936937	*XWGGC1513*	C	666	1,050	A:B = 79:85	GGCTTTATTCCTGGCTCG	ATCTCAACCTCGCCCTTT
DR741857	*XWGGC1514*	C	364	352	A:B = 79:85	AGATGGGAGTTGGGTTGGC	TGGGTCGGTATGAATGCG
HX142006	*XWGGC1515*	C	447	471	A:B = 77:87	GCACTACGCCACTACACTATG	GGAGGACGGTGAACAAGTT
CJ601500	*XWGGC1516*	C	480	472	A:B = 75:89	GAGTACTAGCATTCCGTGTC	AAGTTGTTATTCGCATGACC
CJ664410	*XWGGC1517*	D	287	–	A:B = 75:89	GCTGCCGTTCCAAATCTTG	GCACCATAACTTCAAAGGCT
DN829167	*XWGGC1518*	C	296	281	A:B = 74:90	AATCTAACGGTTTATGGGTC	TTTGAGAAAACAAGTTTGCG
BG263661	*XWGGC1519*	C	562	576	A:B = 75:89	TCTACCAGGGCCAGATGTTC	GGTAAGGAAATCCTGTGGCA

D dominant marker, *C* co-dominant marker

[a] Some of the polymorphic markers showed different DNA conformations, and the fragments size could not be estimated accurately

[b] A: Genotypes same as IW2. B: Genotypes same as Langdon

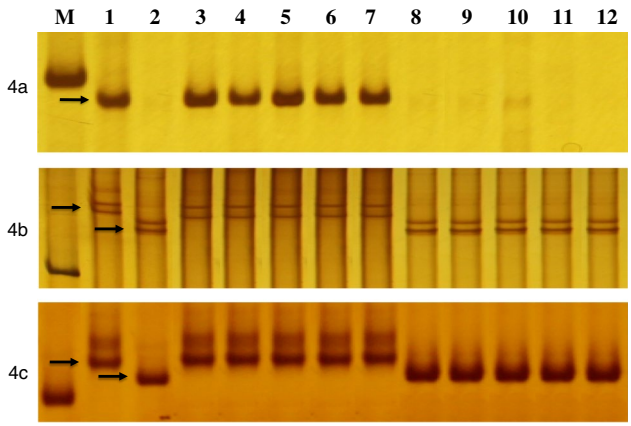

Fig. 4 PCR amplification patterns of STS markers, *XWGGC1505* (**a**), *XWGGC1506* (**b**), and *XWGGC1507* (**c**). The *black arrows* show the DNA fragments that are polymorphic between resistant and susceptible lines. Lanes 1 and 2 are IW2 and Langdon, respectively, lanes 3–7 represent homozygous resistant RILs, and lanes 8–12 represent homozygous susceptible RILs. The two bands for marker *XWGGC1506* are the results of DNA conformation difference

Table 3 Glutathione transferase duplications in the *Pm41* orthologous genomic regions of *Brachypodium*, rice, and sorghum

Brachypodium	Rice	Sorghum
Bd2g60650	Os01g72120	Sb03g045780
Bd2g60760	Os01g72130	Sb03g045790
Bd2g60765	Os01g72140	Sb03g045800
	Os01g72150	Sb03g045810
	Os01g72160	Sb03g045820
	Os01g72170	Sb03g045830
		Sb03g045840
		Sb03g045850
		Sb03g045860

chromosome because of the different reactions to multiple *Bgt* isolates (Reader and Miller 1991; Liu et al. 2002; Chen et al. 2005; Liu et al., unpublished data). Co-segregated with STS marker *Xcau516*, *Pm26* and *MlIW170* are allelic and linked to *Pm42* on 2BS (Rong et al. 2000; Hua et al.

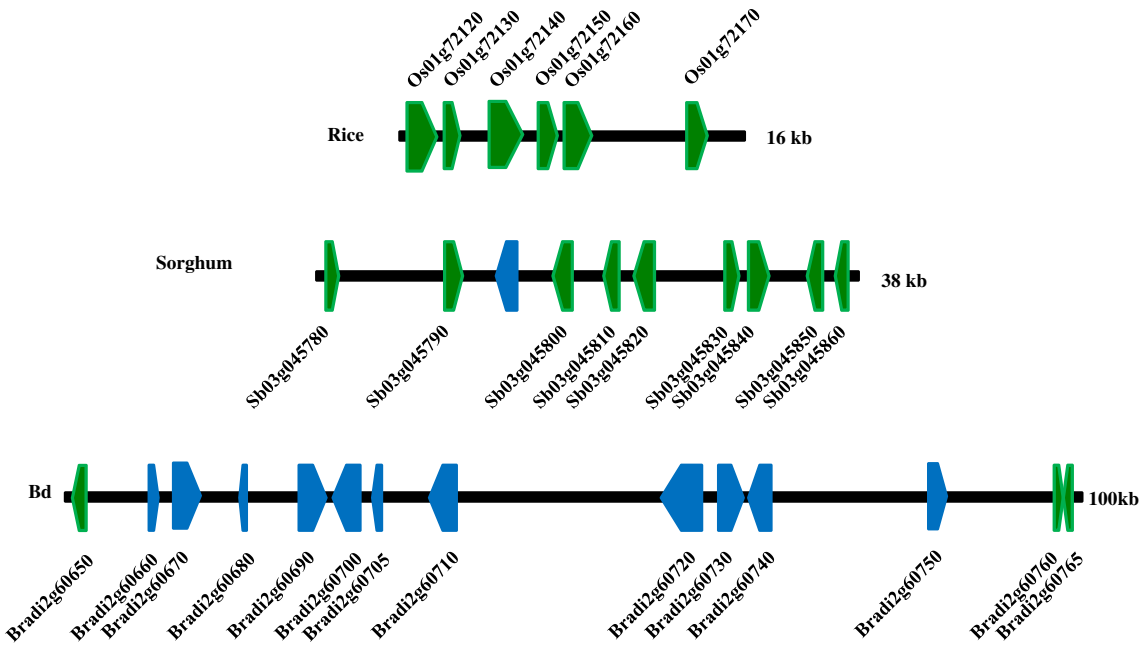

Fig. 5 Genomic organization of the duplicated genes encoding glutathione transferases in the *Pm41* orthologous genomic regions of *Brachypodium*, rice, and sorghum. The genes illustrated in *green color* are paralogs in each species (color figure online)

2009; Liu et al. 2012; unpublished data). *Pm36*, *Ml3D232*, and *PmAs846* also mapped within same genetic interval of the 5BL emmer chromosome (Blanco et al. 2008; Zhang et al. 2010; Xue et al. 2012), and these alleles provide resistance against all available *Bgt* isolates collected in China (Liu et al., unpublished data). These results indicate that these alleles are most likely the same gene. Both *MlIW72* and *PmG16* mapped to the 7AL distal end and may be new alleles of the *Pm1* loci (Ji et al. 2007; Ben-David et al. 2010). No allelic variation has been found for *MlZec1* on 2BL (Mohler et al. 2005) or *PmG3M* on 6BL (Xie et al. 2012).

The *Pm41* powdery mildew resistance gene was identified in the IW2 wild emmer accession collected from Mount Hermon, Israel, and mapped within an 2.7 cM interval between EST marker *BE489472* and SSR marker *Xwmc687* on chromosome 3BL bin 0.63–1.00 (Li et al. 2009). Since no assembled reference genome information is available for wheat genomics research, comparative genomics approaches have been applied widely to anchor high-density linkage maps of important wheat traits to *Brachypodium*, rice, and sorghum genome sequences. By applying comparative genomic analysis, the *Ml3D232* and *MlIW170* powdery mildew resistance genes and the *Iw1* wax inhibition gene have been narrowed to sub-centimorgan intervals in wheat (Zhang et al. 2010; Liu et al. 2012; Adamski et al. 2013; Wu et al. 2013). Comparative genomics analyses have revealed better collinearity between the wheat and *Brachypodium* genomes than those of rice and sorghum in the disease resistance gene region (Liu et al. 2012; Zhang et al. 2010; Xue et al. 2012). In the *Pm41* genomic region, the synteny levels between the genes identified in the 3BL wheat chromosome and their orthologs in the rice chromosome 1 and *Brachypodium* chromosome 2 were 58 and 65 %, respectively. However, the synteny conservation of the telomeric region was lower than that of the centromeric region (Rustenholz et al. 2011). The powdery mildew resistance gene *Pm41* has been mapped to the distal region of chromosome 3BL bin 0.63–1.00 (Li et al. 2009). Fortunately, three relatively high collinearity genomic regions identified in *Brachypodium*, rice, and sorghum correspond to the *Pm41* genomic region in wheat. Within the *Pm41* corresponding genomics regions, 74–80 % of the genes are orthologs between *Brachypodium*, rice, and sorghum, suggesting that a conserved structure exists within these genomic intervals among the three species.

We have used wheat ESTs, the Chinese Spring IWGSC survey sequences, and the Chinese Spring shotgun contigs (Brenchley et al. 2012) as templates for primer design. Compared with ESTs alone, the Chinese Spring contigs provide sequence information about introns and intergenic regions that are very useful for polymorphic markers development. By using this comparative data, we have developed 19 new polymorphic STS markers and have integrated these into the *Pm41* high-density genetic linkage map, and we anticipate that the recent released draft genome sequences of *T. urartu* (Ling et al. 2013) and *Ae. tauschii*

(Jia et al. 2013) will be even more helpful for wheat genomics research and marker development. We were able to map the *Pm41* within a 0.6 cM interval between *XWGGC1505* and *XWGGC1507* that corresponds to 11.7, 19.2, 24.9 kb genomic regions in the *Brachypodium*, rice, and sorghum genomes, respectively. The *XWGGC1506* marker co-segregated with *Pm41* and subsequently can be used as starting point for chromosome landing and map-based cloning of the *Pm41* gene.

Homologous genes consist of orthologs and paralogs. Orthologs are derived from a common ancestor by speciation, with or without an identical function, and paralogs have diverged by gene duplication within a single species (Abrouk et al. 2010). Gene duplication is one of the driving forces for genome evolution and serves to provide diversity for adaptation to stresses and environmental changes (Flagel and Wendel 2009; Zhang 2003). Gene duplications occurring in the targeted genomics regions can cause complexity in comparative genomics analysis and marker design. In the current study, Bd2g60760, Bd2g60765, and Bd2g60650 were paralogs. The *XWGGC1505* marker was developed from the wheat EST homologous to *Brachypodium* gene Bd2g60760. However, high-density linkage-mapping result indicated that *XWGGC1505* is orthologous to *Brachypodium* gene Bd2g60650. Therefore, putative genes without duplications in the syntenic genome regions of *Brachupodium,* rice, and sorghum should be considered first for marker development.

Genomic inversion is also a prominent feature of plant genomic architecture that like gene duplication provides a driven force in genome evolution. Thirty-one genomic inversions were identified after comparing the *Ae. tauschii* SNP genetic map with rice and sorghum genome sequences (Luo et al. 2009). In the current study, we have found two paracentric inversions (Bradi2g60650–Bradi2g60750 and Bradi2g60830–Bradi2g60950) in *Brachypodium* compared with the orthologous genomic regions of the *Pm41* locus in rice and sorghum. Moreover, the breakpoints of one segmental inversion (Bradi2g60650–Bradi2g60750) were observed in the duplicated genes (Fig. 2). Our high-density genetic linkage map of the *Pm41* reveals that the wheat genomic region (*XWGGC1505–XWGGC1513*) is syntenic to the orthologous genomic region in *Brachypodium* (Bradi2g60650–Bradi2g60750) except for a small segmental inversion (Bradi2g60700–Bradi2g60720), but complete inversion was observed in the orthologous genomic regions of rice (Os01g71970–Os01g72120) and sorghum (Sb03g045660–Sb03g045780). *Brachypodium* is even more closely related to wheat than rice and sorghum, and hence can provide more useful information for wheat marker development and functional genomics research.

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