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ORIGINAL PAPER

Molecular identification of a new powdery mildew resistance gene *Pm41* on chromosome 3BL derived from wild emmer (*Triticum turgidum* var. *dicoccoides*)

Genqiao Li · Tilin Fang · Hongtao Zhang · Chaojie Xie · Hongjie Li · Tsomin Yang · Eviatar Nevo · Tzion Fahima · Qixin Sun · Zhiyong Liu

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Abstract Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is an important wheat disease in China and other parts of the world. Wild emmer (*Triticum turgidum* var. *dicoccoides*) is the immediate progenitor of cultivated tetraploid and hexaploid wheats and thus an important resource for wheat improvement. Wild emmer accession IW2 collected from Mount Hermon, Israel, is highly resistant to powdery mildew at the seedling and adult plant stages. Genetic analysis using an F_2 segregating population and $F_{2:3}$ families, derived from a cross between susceptible durum cultivar Langdon and wild emmer accession IW2, indicated that a single dominant gene was responsible for the resistance of IW2. Bulked segregant and molecular marker analyses detected that six polymorphic SSR, one ISBP, and three EST-STS markers on chromosome 3BL bin 0.63–1.00 were linked to the resistance gene. Allelic variations of resistance-linked EST-STS marker *BE489472* revealed that the allele was present only in wild emmer but absent in common wheat. Segregation distortion was observed for the powdery mildew resistance allele and its linked SSR markers with preferential transmission of Langdon alleles over IW2 alleles. The resistance gene was introgressed into common wheat by backcrossing and marker-assisted selection. Since no designated powdery mildew resistance gene has been found on chromosome 3BL, the resistance gene derived from wild emmer accession IW2 appears to be new one and was consequently designated *Pm41*.

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G. Li · T. Fang · H. Zhang · C. Xie · T. Yang · Q. Sun (✉) · Z. Liu (✉)
State Key Laboratory for Agrobiotechnology/Key Laboratory of Crop Genomics and Genetic Improvement, Ministry of Agriculture/Beijing Key Laboratory of Crop Genetic Improvement/Key Laboratory of Crop Heterosis Research & Utilization, Ministry of Education, China Agricultural University, Beijing 100193, People's Republic of China
e-mail: qxsun@cau.edu.cn

Z. Liu
e-mail: zhiyongliu@cau.edu.cn

H. Li
National Key Facility for Crop Gene Resources and Genetic Improvement/Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

E. Nevo · T. Fahima
Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31905, Israel

Present Address:
T. Fang
Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078, USA

Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most important diseases of common wheat (*Triticum aestivum* L.) worldwide. Severe epidemics of this disease often occur in areas with cool and humid climates, causing significant yield losses (Bennett 1984). In 1990, the grain yield reduction of 1.4 billion kg of wheat due to powdery mildew epidemics were recorded in China (Zhuang 2003), and about 6 million ha of wheat production is affected annually (http://www.agri.gov.cn/). Breeding resistant cultivars are the most economical and

environmentally safe method to decrease fungicide application and to reduce yield reduction due to the disease. To date, 40 loci for resistance to powdery mildew (*Pm1–Pm43*. *Pm18*, *Pm22*, and *Pm23* were deleted) have been identified (McIntosh et al. 2008; Hao et al. 2008; R. McIntosh, pers. commun.) and only powdery mildew resistance gene *Pm3* has been cloned so far (Yahiaoui et al. 2004). Five of these loci (*Pm1*, *Pm3*, *Pm4*, *Pm5*, and *Pm8*) have more than one allele conferring resistance. However, many of the resistance loci have been ineffective and only a few, such as *Pm2*, *Pm4*, *Pm21*, and *Pm30* confer resistance against the currently prevailing pathogen isolates in released cultivars in China (Hua et al. 2009). Other effective powdery mildew resistance genes, such as *Pm1c*, *Pm12*, *Pm13*, *Pm16*, and *Mlxbd*, continue to be resistant but have not been exploited due to poor agronomic traits associated with either alien chromosome segments or un-adapted genetic backgrounds in Chinese breeding programs (Duan et al. 1998; Qiu and Zhang 2004). An increased effort is required to explore new powdery mildew resistance genes and to improve the agronomic traits of lines with currently designated genes.

Wild emmer (*T. turgidum* var. *dicoccoides*) (AABB, $2n = 4X = 28$) is the immediate progenitor of cultivated tetraploid and hexaploid wheats. The wild emmer gene pool contains many economically important genes for resistance to diseases and pests, and tolerance to a range of ecological stresses that can be used in wheat improvement (Nevo and Beiles 1989; Nevo 1995; Nevo et al. 2002; Peng et al. 2000). Wild emmer is highly resistant to powdery mildew (Gerechter-Amitai and van Silfhout 1984; Moseman et al. 1984; Xie et al. 2003). Several loci conferring resistance to powdery mildew have been transferred from wild emmer into tetraploid and hexaploid wheats, e.g., *Pm16* (Chen et al. 2005), *Pm26* (Rong et al. 2000), *Pm30* (Liu et al. 2002), *MlZec1* (Mohler et al. 2005), *Pm36* (Blanco et al. 2008), and *Pm42* (Hua et al. 2009). Among them, *Pm16* was initially located on chromosome 4A (Reader and Miller 1991) and later assigned to 5BS by molecular markers, suggesting that it may be allelic or identical to *Pm30* (Liu et al. 2002; Chen et al. 2005). However, results of recent inoculations using multiple *Bgt* isolates revealed differential reactions between *Pm16* and *Pm30* in 4 *Bgt* isolates, which suggests that either *Pm16* and *Pm30* are different alleles or that Brigand (*Pm16*) may carry an additional mildew resistance gene (Hua et al. 2009). *Pm26*, a recessive gene located on chromosome 2BS, co-segregated with RFLP marker *Xwg516* (Rong et al. 2000). Another recessive gene, *Pm42*, was recently characterized on 2BS, 36.8 cM proximal to *Pm26* (Hua et al. 2009). In tetraploid wheat, *Pm36* was located on chromosome 5BL (Blanco et al. 2008). Two temporarily designated loci, *MlZec1* (Mohler et al. 2005) and *MlIW72* (Ji et al. 2007), were located on chromosomes 2BL and 7AL, respectively. This list suggests that wild emmer is a valuable resource of powdery mildew resistance genes, which can be mapped in the future and introgressed into durum and common wheats in an attempt to genetically increase their resistance to the pathogen.

PCR-based microsatellites, or simple sequence repeats (SSR), have the advantages of abundance, high efficiency, and co-dominance and are widely used in linkage map construction, gene tagging, and gene cloning. Several thousand wheat SSR markers have been deposited in the GrainGenes database (http://wheat.pw.usda.gov). SSR markers linked to powdery mildew resistance genes *Pm1*, *Pm2*, *Pm3*, *Pm4*, *Pm5*, *Pm12*, *Pm16*, *Pm27*, *Pm30*, *Pm33*, *Pm35*, *Pm36*, and *Pm37* have been reported (McIntosh et al. 2008). Using chromosome 3B BAC-end sequences (BES), Paux et al. (2006, 2008) developed insertion site-based polymorphism (ISBP) markers based on the presence of junctions between transposable element (TE) and nearby DNA sequences. These ISBP markers from 3B physical map provide a valuable source of chromosome-specific markers for gene mapping in wheat.

We reported here the molecular identification of a new powdery mildew resistance gene, which was derived from wild emmer accession IW2 collected at Mount Hermon, Israel, and its marker-assisted introgression into common wheat genetic background.

Materials and methods

Plant materials

Wild emmer accession IW2 was highly resistant to *Bgt* isolate E09, a prevailing pathotype in the Beijing area, with infection type (IT) 0, in both the seedling and adult plant stages. Durum wheat cultivar Langdon was highly susceptible to E09 with IT 3–4. The F_1 hybrid between Langdon and IW2 was self-pollinated to generate the F_2 population and corresponding F_3 families.

Chinese Spring (CS) wheat and selected nullisomic–tetrasomic lines (N3AT3B, N3AT3D, N3BT3A, and N3BT3D), ditelosomic lines (Dt3AL and Dt3BL), and deletion lines (d3BL-2:0.22 and d3BL-7:0.63) of homoeologous group 3 were used for chromosomal arm assignment and bin mapping of molecular markers.

Evaluation for powdery mildew resistance

Bgt E09 was used to inoculate IW2, Langdon, and Langdon/IW2 hybrid plants at the seedling stage under

controlled greenhouse conditions. The F_2-derived F_3 families, 20 seedlings each, were tested to confirm the phenotypes and to establish the resistance genotype of each F_2 plant. Seeds were planted in pots (10 cm in diameter), 20 plants in each pot, and the common wheat line Xuezao was used as the susceptible control. Ten Chinese *Bgt* isolates were used to compare the seedling reactions of IW2 and 25 accessions with known powdery mildew resistance genes. Cultivar Chancellor was used as the susceptible control. Seedlings were inoculated with the isolate when the first leaf was fully expanded. Infection types were scored on a 0–4 scale (Liu et al. 1999) 15 days after inoculation when the susceptible controls showed obvious disease symptoms (IT 4). Reactions were classified into two groups, resistant (R, IT 0–2) and susceptible (S, IT 3–4).

PCR amplification and electrophoretic analysis

Genomic DNA was extracted from parental wild emmer IW2, durum wheat Langdon, and F_2 population plants using the CTAB protocol (Sharp et al. 1988). Resistant and susceptible bulks, which were composed of equal amounts of DNA from 10 homozygous-resistant and 10 homozygous-susceptible F_2 plants, respectively, were used for bulked segregant analysis (BSA) (Michelmore et al. 1991).

Wheat SSR, EST-STS (http://wheat.pw.usda.gov/SNP/primers/contig_primer_list.xls), and ISBP markers (Paux et al. 2006, Paux et al. 2008) mapped to the A and B genomes were used to screen the parents, resistant, and susceptible bulks. The resulting polymorphic markers were used to genotype the F_2 population. PCR was performed in 10 μl volume of reaction mixture containing 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 20 ng of each primer, 50 ng genomic DNA, and 0.75 U Taq DNA polymerase. PCR conditions were an initial denaturation at 94°C for 5 min followed by 38 cycles of 94°C for 45 s, 50–60°C (depending on the specific SSR primers) for 45 s, and 72°C for 90 s, and a final extension at 72°C for 10 min. PCR products were mixed with 2 μl loading buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, and 0.25% xylene cyanol) and separated in 8% non-denaturing polyacrylamide gels (39 acrylamide : 1 bisacrylamide) as described by Liu et al. (2002). Gels were then silver stained and photographed.

Introgression of the powdery mildew resistance gene into common wheat

In order to transfer the putative single gene in IW2 to hexaploid wheat, resistant F_2 plants of Langdon/IW2 were backcrossed to the susceptible common wheat line 87-1. The progenies were inoculated with *Bgt* isolate E09. The resistant BC_3F_1 plants were selfed to produce families from which homozygous-resistant BC_3F_2 plants were selected.

Chromosomal arm and physical bin assignments of polymorphic markers

Chromosomal and bin locations of the disease resistance gene-linked polymorphic markers were determined using CS nullisomic–tetrasomics, ditelosomics, and deletion lines of homoeologous group 3. The use of deletion lines enables markers to be localized to a chromosome bin flanked by breakpoints of the largest deletion possessing the fragment and the smallest deletion lacking it after comparing the amplification patterns.

Data analysis and genetic mapping

Chi-squared test (χ^2) was used to determine the suitability of observed data with expected segregation ratios. Linkage between molecular markers and the resistance gene was analyzed using Mapmaker 3.0b (Lincoln et al. 1992) with an LOD score of 3.0 as the threshold. The genetic map was drawn with the software Mapdraw V2.1 (Liu and Meng 2003).

Results

Inheritance of powdery mildew resistance in wild emmer IW2

When inoculated with the isolate E09, IW2 was highly resistant (IT 0;) whereas durum wheat Langdon was highly susceptible (IT 3–4) at both seedling and adult growth stages. Langdon/IW2 F_1 hybrid plants were resistant (IT $1–1^+$), indicating incompletely dominant resistance. Since it was not possible to reliably score heterozygous F_2 plants, the F_2-derived F_3 families were tested to confirm the phenotypes and to establish the resistance genotype of each F_2 plant. The F_2 population segregated 186 seedlings resistant: 177 susceptible, suggesting two complementary genes ratio rather than a single gene ($\chi^2_{3:1} = 115.2, P < 0.01$). The F_3 families segregated 58 homozygous resistant:128 segregating:177 homozygous susceptible, a distribution that confirmed neither hypothesis ($\chi^2_{1:2:1} = 109.6$, $P < 0.01$, $\chi^2_{1:8:7} = 72.8$, $P < 0.01$, Table 1). All of the susceptible F_2 plants generated homozygous susceptible F_3 progenies, whereas the progenies of the resistant plants were either homozygous resistant or segregated in ratios similar to the F_2 population. These results suggest that the powdery mildew resistance in IW2 is controlled by a single allele that is not normally transmitted.

Table 1 Genetic analysis of *Pm41* and its linked molecular markers in an F_2 population of Langdon/IW2

Loci	A(D)[a]	H[a]	B[a]	Expected ratio	χ^2 [b]	Frequency of A[c]	Frequency of B[c]	Direction of skewness
Xbarc84	62	125	176	A:H:B = 1:2:1	106.8**	0.17	0.48	Langdon
BE489472	59	128	176	A:H:B = 1:2:1	106.9**	0.16	0.48	Langdon
Pm41	58	128	177	A:H:B = 1:2:1	109.6**	0.16	0.49	Langdon
Xwmc687	58	127	178	A:H:B = 1:2:1	112.1**	0.16	0.49	Langdon
Xwmc326	56	128	179	A:H:B = 1:2:1	114.9**	0.15	0.49	Langdon
BE637789	58	128	177	A:H:B = 1:2:1	109.5**	0.16	0.49	Langdon
Xbarc77	61	125	177	A:H:B = 1:2:1	109.3**	0.17	0.49	Langdon
Xcfp26	182		181	D:B = 3:1	119.7**	–	–	
BE517780	53	131	180	A:H:B = 1:2:1	115.6**	0.15	0.50	Langdon
Xwmc236	186		177	D:B = 3:1	109.3**	–	–	
Xgwm114	185		178	D:B = 3:1	111.8**	–	–	

** Significant at $P < 0.01$

[a] A: homozygous for the allele from IW2. B: homozygous for the allele from Langdon. H: heterozygous. D: dominant allele of IW2

[b] Values for significance at $P = 0.05$ are 3.88 (3:1) and 5.99 (1:2:1)

[c] The frequencies were calculated based on the homozygous marker genotypes in the F_2 generation

Molecular mapping of the powdery mildew resistance gene in IW2

Initially, 126 SSR markers mapped to the A and B genomes of wheat were screened for their polymorphism between the parental lines and the resistant and susceptible DNA bulks. Two SSR markers, *Xwmc326* and *Xbarc77*, were polymorphic between the parents, as well as the bulks. These markers proved to be linked to the resistance locus by testing on the individuals of F_2 generation. Both *Xwmc326* and *Xbarc77* were located on the long arm of chromosome 3B (Röder et al. 1998; Somers et al. 2004). Further SSR markers located on 3BL were screened. Four (*Xbarc84*, *Xwmc687*, *Xgwm114*, and *Xwmc236*) were polymorphic between the resistant and susceptible bulks, and were closely linked to the resistance gene. Of the six polymorphic markers, four (*Xbarc84*, *Xwmc687*, *Xwmc326*, and *Xbarc77*) were co-dominant, and two (*Xwmc236* and *Xgwm114*) were dominant. A linkage map including the resistance locus and its closely linked markers was constructed (Fig. 1). Since no powdery mildew resistance gene was previously located on chromosome 3BL, this gene was thus designated *Pm41*.

ISBP markers and wheat ESTs physically mapped to 3BL were surveyed to identify polymorphic markers linked to *Pm41*. Of 58 ISBP and 85 EST primer pairs screened, one ISBP marker (*Xcfp26*) and three EST-STS markers (*BE489472*, *BE637789*, and *BE517780*) were polymorphic between the resistant and susceptible bulks, as well as the parents, and were closely linked with *Pm41* (Table 2, Fig. 1). *BE489472* was found to be tightly linked to *Pm41* with a genetic distance of 0.8 cM.

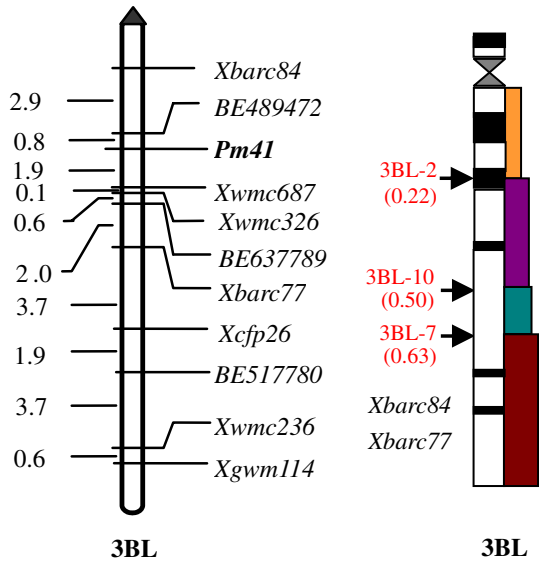

Fig. 1 Linkage map and chromosome bin physical map of powdery mildew resistance gene *Pm41* on chromosome 3BL

Physical bin mapping of the powdery mildew resistance gene *Pm41*

Chinese Spring homoeologous group 3 nullisomic-tetrasomics, ditelosomics, and deletion lines were used to assign the chromosomal and physical bin locations of *Pm41*-linked SSR markers. Both SSR markers *Xbarc84* and *Xbarc77* were absent in 3BL-2 and 3BL-7 (Fig. 2), which is consistent with the reports of Sourdille et al. (2004) and Paux et al. (2008). Since *Pm41* was flanked by these markers, *Pm41* was thus located on the distal bin 3BL-7 (0.63–1.00, Fig. 1).

Table 2 Details of EST-STS markers close to the powdery mildew resistance locus *Pm41*

EST accession	Forward primer (5′–3′)	Reverse primer (5′–3′)
BE489472	GAATTGGGGCAGATTTCTTG	GAAGAGCGATCATGGAGAGG
BE637789	CAAGGACGACTGCTGGCTA	ATCTTGATGACGAACTCGGG
BE517780	GCATCCTAGGGAGGTCATCA	ATCTCCGGGATAGAAAGCGT

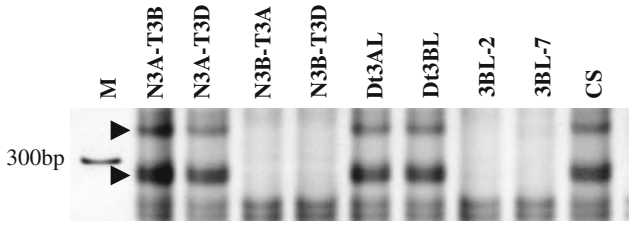

Fig. 2 Amplification pattern of SSR marker *Xbarc84* in Chinese Spring homoeologous group 3 nulli-tetrasomics, ditelosomics, and 3BL deletion lines. *Arrows* indicates the specific bands

Segregation distortion of the 3BL chromosome

The segregation of powdery mildew resistance gene *Pm41* and its linked SSR markers in the F_2 population significantly deviated from the expected 3:1 or 1:2:1 ratios (Table 1). Excesses of Langdon alleles were observed for *Xbarc84*, *Xwmc687*, *Xwmc326*, *Xbarc77*, *BE489472*, *BE637789*, *BE517780*, and *Pm41*. The distortion of the three dominant markers *Xcfp26*, *Xwmc236*, and *Xgwm114* could not be tested due to indistinguishable of IW2 alleles in the homozygous and heterozygous resistant plants. When segregating F_3 progenies derived from heterozygous F_2 plants were analyzed, similar bias toward Langdon alleles were found for the *Pm41* locus (Table 3).

Introgression of *Pm41* into a common wheat background

In order to introduce *Pm41* into common wheat, resistant Langdon/IW2 F_2 plants were pollinated with the susceptible common wheat line 87-1. Three resistant F_1 plants were backcrossed to wheat line 87-1, and all of the nine resistant BC_1F_1 plants were heterozygous at the four (*Xbarc84*, *Xwmc687*, *Xwmc326*, and *Xbarc77*) co-dominant SSR loci. Resistant progenies were further backcrossed twice with 87-1, and homozygous-resistant BC_3F_2 plants were selected. Line 8K118 (87-1*4//Langdon/IW2) provides an example of a free threshing *Pm41* containing hexaploid wheat line for future reference.

Table 3 Genetic analysis of *Pm41* locus in heterozygous F_2-derived F_3 families

F_3 family	F_2-2	F_2-3	F_2-6	F_2-8	F_2-29	F_2-36	Total
Resistant	29	31	18	26	22	17	143
Susceptible	47	34	11	31	26	18	167
$\chi^2_{3:1}$	55.0**	25.8**	2.6	26.3**	21.8**	13.0**	137.8**

The six F_2-derived F_3 families shown here represent a random selection of the 128 F_3 families that segregated

** Significant at $P < 0.01$

Differential reactions of IW2 and wheat accessions with known genes for *Bgt* resistance

Differential reactions of IW2 and 25 wheat cultivars/lines possessing known powdery mildew resistance genes to 10 *Bgt* isolates are listed in Table 4. IW2 was highly resistant to all the isolates tested and gave the same response patterns as Brigand (*Pm16*), Yangmai 5/Sub 6 V (*Pm21*), and Xiaobaidong (*Mlxbd*).

Molecular detection of powdery mildew resistance gene *Pm41* in wild emmer and common wheat germplasm by tightly linked EST-STS marker *BE489472*

Allelic variations of EST-STS marker *BE489472* were evaluated on wild emmer and common wheat cultivars/lines to test the frequency of *Pm41* in the gene pool (Fig. 3, Supplement Table 1). Out of the 78 wild emmer accessions collected from 12 sites in Israel, 13 detected the same amplification patterns as that of IW2 (Fig. 3, Supplement Table 1). No *BE489472* amplification pattern as that of IW2 could be found in 60 wheat cultivars/lines, as well as 25 entries with known powdery mildew resistance genes, indicating *Pm41* may be present only in the wild emmer populations (Fig. 3, Supplement Table 1).

Discussion

Powdery mildew resistance gene *Pm41* is a new locus from wild emmer

Wild emmer is a promising genetic resource for improvement of resistance to powdery mildew in both durum and common wheat (Gerechter-Amitai and van Silfhout 1984; Moseman et al. 1984; Nevo et al. 2002). Potentially useful genes in wild emmer can readily be transferred to common wheat by direct hybridization, backcrossing, and selection.

Table 4 Infection types of 27 wheat cultivars/lines to 10 isolates of *Blumeria graminis* f. sp. *tritici*

Cultivar/Line	*Pm* gene	*Bgt* isolate									
		E03	E05	E09	E15	E20	E23	B01	B02	B04	B05
Chancellor	–	S	S	S	S	S	S	S	S	S	S
Axminister/8*cc	*Pm1*	S	S	S	S	S	S	S	S	R	S
Ulka/8*cc	*Pm2*	R	R	R	R	S	R	R	R	R	S
Asosan/8*cc	*Pm3a*	R	S	R	R	S	S	S	S	S	S
Chul/8*cc	*Pm3b*	S	S	S	R	S	S	S	S	S	S
Sonora/8*cc	*Pm3c*	S	S	S	R	S	S	S	S	S	S
Kolibri	*Pm3d*	R	S	S	R	S	S	S	R	S	S
W150	*Pm3e*	R	S	S	S	S	S	S	S	S	S
Mich.amber/8*cc	*Pm3f*	S	S	S	S	S	R	S	R	S	S
Khapli/8*cc	*Pm4a*	R	R	R	S	S	R	R	R	R	S
Armada	*Pm4b*	R	R	R	S	S	R	R	R	R	S
81-7241	*Pm4c* (*Pm23*)	R	R	R	R	S	R	R	R	R	R
Hope/8*cc	*Pm5*	S	S	S	S	S	S	S	S	S	S
Coker747	*Pm6*	S	S	S	S	S	S	R	R	S	S
CI14189	*Pm7*	S	S	S	S	S	S	S	S	S	S
Kavkaz	*Pm8*	S	S	S	S	S	S	S	R	S	S
Coker 983	*Pm5* + *6*	R	S	S	R	S	S	R	R	R	S
Wembley	*Pm12*	R	R	R	R	R	R	R	R	R	S
R4A	*Pm13*	R	R	R	S	R	R	R	R	R	R
Brigand	*Pm16*	R	R	R	R	R	R	R	R	R	R
Amigo	*Pm17*	R	S	S	R	S	R	S	S	R	S
Yangmai 5/Sub 6V	*Pm21*	R	R	R	R	R	R	R	R	R	R
Chiyacao	*Pm24*	R	S	R	R	R	R	R	R	R	S
5P27	*Pm30*	R	R	R	R	S	R	R	R	R	R
Xiaobaidong	*Mlxbd*	R	R	R	R	R	R	R	R	R	R
IW2	*Pm41*	R	R	R	R	R	R	R	R	R	R
P63	*Pm42*	R	R	R	R	S	R	R	R	R	R

R Resistant, *S* susceptible

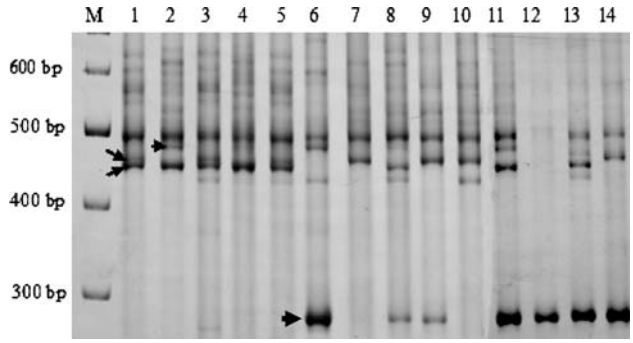

Fig. 3 Allelic variations of EST-STS marker *BE489472* in tetraploid and common wheat germplasms. M: 100 bp ladder, 1: IW2, 2: Langdon, 3: IW3, 4: IW4, 5: IW10, 6: IW101, 7: Heng 7228, 8: Liangxing 99, 9: Jimai 20, 10: Han 00-7050, 11: Axminster/8Cc (*Pm2*), 12: R4A (*Pm13*), 13: Brigand (*Pm16*), 14: Yangmai 5/Sub6 V (*Pm21*). *Arrows* indicates the specific bands

Ten wild emmer accessions collected from Mount Hermon in northern Israel showed resistance to Chinese *Bgt* isolate E09 at both seedling and adult stages (Xie et al. 2003). In the present study, an incompletely dominant resistance gene, designated *Pm41*, in wild emmer accession IW2 was located to the distal bin (3BL7-0.63-1.00) of chromosome 3BL. Ceoloni et al. (1988) transferred the powdery mildew resistance gene *Pm13* from *Ae. longissima* into wheat by *ph1*-induced homoeologous recombination. Cenci et al. (1999) mapped *Pm13* to a translocated $3S^{l}S$ segment (T3BL.3BS-$3S^{l}$) linked to RFLP marker *Xcdo-460-3BS*. Seedling tests showed that *Pm13* was susceptible to one (E15) of 10 *Bgt* isolates, while *Pm41* was resistant to all of them (Table 4). In addition to *Pm41*, *Pm16*, *Pm21*, and *Mlxbd* were all resistant to the 10 isolates. However, *Pm16* was mapped to 5BS (Chen et al. 2005). *Haynaldia*

villosa-derived *Pm21* was located on the translocated chromosome 6AL/6VS (Chen et al. 1995). *Mlxbd* was identified in Chinese wheat landrace Xiaobaidong and located on chromosome 7BL (Huang et al. 2000). As no powdery mildew resistance gene has been identified on wheat chromosome 3BL, *Pm41* proved to be a new resistance locus.

Allelic variations of EST-STS marker *BE489472* revealed that *Pm41* was present only in wild emmer germplasm

Tightly linked molecular markers can be used as a diagnostic tool for identification of the resistance genes. A diagnostic marker, *csLV34*, has been reported for detection of leaf rust resistance gene *Lr34* (Lagudah et al. 2006). Analyzing allelic variations of *BE489472* on a set of wild emmer and cultivated wheat lines indicated that *Pm41*-linked allele was present in wild emmer but absent in common wheat germplasm (Fig. 3, Supplement Table 1). Among the 78 tested wild emmer accessions collected at 12 sites in Israel, *Pm41*-linked *BE489742* allele was detected in 13 accessions from five sites. *Pm41*-linked *BE489742* allele was found in five out of 11 wild emmer accessions collected from Mount Hermon, suggesting the presence of *Pm41*. Genetic mapping results of powdery mildew resistance genes in IW3 and IW10, collected from Mount Hermon, conformed the presence of *Pm41* (Li et al. 2009). However, allelic variations of EST-STS marker *BE489742* and diversified reaction to *Bgt* E09 indicated that uncatalogued powdery mildew resistance genes may available in the wild emmer gene pool and need to be further investigated. Recent cloning of *GPC-1B* (Uauy et al. 2006) and *Yr36* (Fu et al. 2009) genes originating from wild emmer suggested that the high grain protein content and broad-race resistance to stripe rust (*Puccinia striiformis* Westend.) in high temperature loci were not incorporated into the domestication of cultivated tetraploid and hexaploid wheat. Exploiting exotic genes from wild emmer population and transferring them into cultivated wheat lines will contribute to wheat resistance to biotic and abiotic stresses, production and end-product nutrition. *Pm41* provides an additional source of disease resistance to *Bgt* isolates, which can be used for gene pyramiding in wheat breeding programs.

Distorted segregation of the 3BL region in Langdon/IW2

Distorted segregation ratios have been reported in many crop species including barley (*Horderum vulgare* L.) (Graner et al. 1991; Cistué et al. 2005), rice (*Oryza sativa* L.) (Harushima et al. 1996; Xu et al. 1997), and maize (*Zea mays* L.) (Wendel et al. 1987; Lu et al. 2002). This phenomenon has also been reported in wheat near *Sr11* on chromosome 6B (Sears and Loegering 1961; Luig 1964) and *Sr36* on chromosome 2B (Nyquist 1962). Zhang and Dvorák (1990) mapped a segregation distortion factor, *Sd1*, proximal to the *Lr19* locus in recombinants of *Lophopyrum ponticum* chromosome 7Ag and wheat chromosome 7D. Prins and Marais (1999) found a second segregation distortion factor, *Sd2*, on 7BL translocation derivatives from *L. ponticum*. Segregation distortion also occurred for markers on chromosomes 1DL, 3DS, 4DS, 5DL, and 7DS in an *Aegilops tauschii* F_2 population (Faris et al. 1998). In a cross between durum wheat Langdon and wild emmer H52, Peng et al. (2000) reported that gametes carrying Langdon alleles had stronger vigor and higher competition ability than those with H52 alleles, on chromosomes 5A and 5B in the segregating population. Kumar et al. (2007) located genes *SDR1*, *SDR2*, and *SDR3* for segregation distortions on chromosomes 5BL, 5BS, and 5BL, respectively. Likewise, in the present study, powdery mildew resistance gene *Pm41* and its linked molecular markers covering a genetic distance of 18 cM exhibited similar distortion on 3BL (Table 1).

A strategy for fine genetic mapping and positional cloning of *Pm41*

Positional cloning strategies require a high-resolution linkage map of the region containing the target gene (Tanksley et al. 1995). Powdery mildew resistance gene *Pm3* was isolated by a map-based cloning approach (Yahiaoui et al. 2004). Within hexaploid wheat, the largest chromosome, 3B, exclusively accounts for approximately one gigabase (Lee et al. 2004). Recently, a one-gigabase physical map of chromosome 3B was constructed using a Chinese Spring 3B-specific BAC library (Paux et al. 2008) and 67,968 BAC-ends were sequenced to develop SSR and ISBP (insertion site-based polymorphism) markers (Paux et al. 2006, 2008). One ISBP marker (*Xcfp26*) was assigned in the present linkage map (Fig. 2). A high-density linkage map in the *Pm41* gene region could be developed using the physical map information.

Comparative genomics analysis has also been proposed as a powerful tool for fine mapping of important genes in wheat and barley (Yan et al. 2003; Turner et al. 2005). Using wheat ESTs and the rice genetic map, comparative genomics analysis indicated a high level of conservation in gene order and content between wheat homoeologous group 3 and rice chromosome 1 despite more than 50 million years of independent evolution (Sorrells et al. 2003; Paux et al. 2006). Munkvold et al. (2004) mapped 703 ESTs to chromosome 3B, including 206 ESTs in the distal bin 3BL-7-0.63-1.00. These ESTs can be used to

develop EST-STS markers to construct a high-density linkage map of the *Pm41* region. Three EST-STS markers developed from BE489472, BE637789, and BE517780 were polymorphic and located on the linkage map. In addition to the rice map, the most recently available *Brachypodium distachyon* draft genome sequence (www.brachypodium.org) may provide a 'bridge' species to perform comparative genomic analysis for fine genetic mapping and cloning of *Pm41* (Bossolini et al. 2007).

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