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ORIGINAL PAPER

Identification and genetic mapping of the putative *Thinopyrum intermedium*-derived dominant powdery mildew resistance gene *PmL962* on wheat chromosome arm 2BS

X. K. Shen · L. X. Ma · S. F. Zhong · N. Liu · M. Zhang · W. Q. Chen · Y. L. Zhou · H. J. Li · Z. J. Chang · X. Li · G. H. Bai · H. Y. Zhang · F. Q. Tan · Z. L. Ren · P. G. Luo

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Abstract

Key message **Powdery resistance putatively derived from *Thinopyrum intermedium* in the wheat line L962 is controlled by a single dominant gene designated *PmL962* and mapped to chromosome arm 2BS.**

Abstract Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a destructive disease affecting the production of wheat (*Triticum aestivum*). Powdery mildew resistance was putatively transferred from *Thinopyrum intermedium* to the common wheat line L962, which conferred resistance to multiple Chinese *Bgt* isolates. Genetic analysis of the powdery mildew response was conducted by crossing the resistant line L962 with the susceptible line L983. Disease assessments of the F_1, F_2, and $F_{2:3}$ populations from the cross L983/L962 indicated that resistance was controlled by a single dominant gene. A total of 373 $F_{2:3}$ lines and 781 pairs of genomic simple sequence repeat (SSR) primers were employed to determine the chromosomal location of the resistance gene. The gene was linked to four publicly available and recently developed wheat genomic SSR markers and seven EST-STS markers. The resistance gene was mapped to chromosome arm 2BS based on the locations of the linked markers. Pedigree, molecular marker and resistance response data indicated that the powdery mildew resistance gene in L962 is novel. It was temporarily designated *PmL962*. It is flanked by *Xwmc314* and *BE443737*at genetic distances of 2.09 and 3.74 cM, respectively, and located in a 20.77 cM interval that is co-linear with a 269.4 kb genomic region on chromosome 5 in *Brachypodium distachyon* and a 223.5 kb genomic region on rice (*Oryza sativa*) chromosome 4. The markers that are closely linked to this gene have potential applications in marker-assisted breeding.

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X. K. Shen and L. X. Ma contributed equally to this work.

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X. K. Shen · W. Q. Chen · Y. L. Zhou · P. G. Luo (✉)
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100193, China
e-mail: lpglab@sicau.edu.cn

X. K. Shen · L. X. Ma · S. F. Zhong · N. Liu · M. Zhang · X. Li · H. Y. Zhang · F. Q. Tan · Z. L. Ren · P. G. Luo
Provincial Key Laboratory of Plant Breeding and Genetics, Sichuan Agricultural University, Chengdu 611130, Sichuan, China

H. J. Li
The National Key Facility for Crop Gene Resources and Genetic Improvement (NFCRI), Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Z. J. Chang
Institute of Crop Genetics, Shanxi Academy of Agricultural Science, Taiyuan 030031, Shanxi, China

G. H. Bai · P. G. Luo
Hard Winter Wheat Genetics Research Unit, USDA-ARS, 4008, Throckmorton Hall, Manhattan KS66506, USA

Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, is a major wheat fungal disease that affects grain yield and end-use quality (Zhuang and Li 1993). Since the 1970s, this disease has become widespread in most winter wheat regions in China, and currently affects 6–8 million hectares (ha) of wheat annually (Zhao et al. 2013). Although powdery mildew has become widespread in southwest China relatively recently compared to other diseases such as stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*), it currently exceeds stripe rust as the most damaging disease in the region due to popularization of semi-dwarf cultivars grown under high inputs of irrigation and nitrogenous fertilizer (Luo et al. 2009c). Although chemical methods are widely used for control, resistant cultivars are more effective, economical, and environmentally friendly means of controlling the disease. However, race-specific resistance, which is widely used in wheat cultivars, is usually short-lived, especially when a single resistance gene is deployed over a wide area. Therefore, ongoing efforts are required to identify new sources of resistance for use in breeding programs. To date, more than 50 wheat powdery mildew resistance loci have been identified, some of which are derived from wild relatives of common wheat (http://wheat.pw.usda.gov/; Mohler et al. 2013).

Alien gene transfer is an important means of increasing the genetic diversity of disease resistance in wheat. Of the permanently designated *Pm* loci, 28 genes were transferred from wild relatives and sparsely cultivated subspecies, including *Aegilops squarrosa*, *Ae. speltoides*, *Ae. longissima*, *Ae. ovata*, *Dasypyrum villosum*, *T. turgidum* var. *dicoccoides* and var. *dicoccum*, *T. timopheevii*, *T. monococcum*, *Thinopyrum intermedium*, and cereal rye (*Secale cereale* L.) (http://wheat.pw.usda.gov/; Mohler et al. 2013). *Thinopyrum intermedium* (Host) (Barkworth and D.R. Dewey) ($2n = 6x = 42$; JJJ^{s}J^{s}SS) [syn. *Elytrigia intermedia* (Host) Nevski] has been hybridized extensively with wheat and has also proven to be a useful source of resistance to various diseases of hexaploid wheat (*Triticum aestivum* L.) ($2n = 42$; AABBDD) (Li and Wang 2009; Luo et al. 2009c; Huang et al. 2014; Liu et al. 2013, 2014).

Thinopyrum intermedium provides a valuable gene pool for wheat disease resistance because of its resistance to wheat streak mosaic virus (Friebe et al. 1996), Fusarium head blight (FHB) (Fedak and Han 2005; Huang et al. 2014; Liu et al. 2014), leaf rust (Autrique et al. 1995), stem rust (Fedak 1999), and powdery mildew (Liu and Wang 2005; Liu et al. 2014). Two powdery mildew resistance genes, *Pm40* (Luo et al. 2009c) and *Pm43* (He et al. 2009), and two stripe rust resistance genes, *Yr50* (Huang et al. 2014) and *YrL693* (Huang et al. 2014), were reportedly transferred from *Th. intermedium* to common wheat. The wheat line YU25, derived from a single plant from an interspecific cross of wheat and *Th. intermedium* (Zhang et al. 2011), is resistant to stripe rust, powdery mildew, and FHB (Liu et al. 2014). Previous genetic analysis of its resistance to powdery mildew using segregating populations from a cross and backcross of MY11 and YU25 demonstrated that resistance in YU25 is controlled by two dominant genes that were temporarily designated *PmE* and *PmYU25* and mapped to chromosomes 7BS and 2DL based on linkages of 13.0 cM to simple sequence repeat (SSR) marker *Xgwm297-7B* and 16.6 cM to SSR marker *Xgwm210-2D*, respectively (Ma et al. 2007). Subsequently, the line GRY19 was selected from an F_4 population of MY11/YU25, and genetic analysis confirmed that GRY19 carried only *PmE*, which was mapped to chromosome arm 7BS with five closely linked SSR markers and permanently designated *Pm40* (Luo et al. 2009c). However, the precise chromosomal location of *PmYU25* was still uncertain due to homology of SSR markers on homoeologous chromosomes. The same marker could match more than one locus in different genomes. The powdery mildew resistant line L962 was developed from the progeny of the cross MY11/YU25.The resistance phenotypes observed in the field implied that it carries *PmYU25* and lacks *Pm40*.

SSRs serve as a valid marker system because of their abundance in plant genomes and ease of detection via PCR in combination with polyacrylamide or high-resolution agarose gel electrophoresis. SSRs are widely applied in the construction of high-density genetic maps in plants and for identifying specific genes (Gupta et al. 1999). Until recently, SSRs were the markers of choice and served as powerful tools for genetic mapping and marker-assisted selection of disease resistance genes in hexaploid wheat, because of their high level of polymorphism and wide distribution across all chromosomes. Thousands of publically available wheat SSR markers have been developed (Röder et al. 1998; Gupta et al. 2002; http://wheat.pw.usda.gov).

In contrast to SSRs, expressed sequence tags (ESTs) are useful for conducting comparative genomic analyses because they are usually located in conserved regions of expressed genes. There are enormous numbers of published ESTs, and many have been physically localized to specific chromosome bins using Southern hybridization and a set of Chinese Spring deletion lines (Qi et al. 2004). These ESTs can be converted to EST-STS markers that can in turn be used to construct high-density maps.

Other studies have documented the high collinearity of chromosome regions between wheat and model species, including rice and *B. distachyon* (Sorrells et al. 2003; The International Brachypodium Initiative 2010). Therefore, the combination of available ESTs and comparative genomics is a powerful tool for research on cereals with large genomes (Liu et al. 2012). This approach has been used in

map-based cloning of disease resistance genes in wheat, including *Lr34/Yr18/Pm38* (Krattinger et al. 2009) and the stripe rust resistance gene *Yr36* (Fu et al. 2009).

Toward the eventual objective of marker-assisted selection, fine mapping, and map-based cloning, the objectives of this study were to study the inheritance of powdery mildew resistance in L962, to confirm the chromosomal location of the gene, and to map the gene with SSR and EST-STS markers in conjunction with comparative genomics methods.

Materials and methods

Plant materials

The wheat powdery mildew resistant line L962 and susceptible line L983 were selected from the F_7 population of the MY11/YU25 cross. The powdery mildew resistance in YU25 was putatively derived from *Th. intermedium* (Ma et al. 2007; Luo et al. 2009c; Zhang et al. 2011; Liu et al. 2014). The sister lines L962 and L983 were employed as parents to study the inheritance of powdery mildew resistance. YU25 and the cultivar MY11 were used as the resistant and susceptible controls, respectively. An accession of *Th. intermedium* and the common wheat cultivar, Chinese Spring, were used as controls for the detection of alien chromatin in YU25 and L962. The Chinese Spring nulli-tetrasomic (NT) lines were used to confirm the chromosomal locations of the powdery mildew resistance gene and linked markers. The F_1, F_2, and $F_{2:3}$ populations from the L983/L962 cross were used for genetic analysis of response to powdery mildew. A total of 373 F_2 plants and the derived $F_{2:3}$ lines were used in genetic mapping.

Evaluation of powdery mildew reactions

The parental lines L962 and L983 along with L658, which carries *Pm40*, were derived from the same F_7 population of MY11/YU25. Liangxing 99 carrying *Pm52* and Coker 747 carrying *Pm6* were employed as resistant controls. Twenty-eight *Bgt* single-spore isolates, collected from Beijing, Shandong, Hebei, Henan, Jiangsu, and Sichuan provinces, were used to assess the reactions of different wheat lines to powdery mildew (Table 1). Isolate *Bgt28*, collected from Yaan City, Sichuan province, was employed to screen the parents and the genetic populations that were used to map the powdery mildew resistance gene in L962. Evaluation of reactions of seedlings to the other 27 isolates was conducted in a greenhouse at the Institute of Crop Science, Chinese Academy of Agricultural Science, Beijing. For these evaluations, the experimental design, testing methods, and resistance classification based on the infection types (ITs) were consistent with those described in previously published reports (Zhao et al. 2013).

To determine the inheritance of resistance in L962, isolate *Bgt28*, which was collected from Sichuan province and is avirulent on L962 and virulent on L983, was employed to test the F_1, F_2, and $F_{2:3}$ populations of L983/L962. The seeds were planted in a greenhouse; 20–30 plants of each parental line in addition to 20–30 plants each of the F_1, F_2 and $F_{2:3}$ lines (Table 2) were planted in a randomized design in 2.5 m rows with 25 cm spacing. Susceptible control plants (Chancellor) used to spread the pathogen were planted in every third row to ensure that all plants had the same opportunity for infection. The reactions of adult plants were scored at ear emergence using a previously described rating scale (He et al. 2009).

DNA extraction and bulked segregant analysis

Total DNA was extracted from 5-week-old seedling leaves using a previously described method (Tai and Tanksley 1990). Equal amounts of DNA at a concentration of 60 ng/μl from 10 homozygous resistant and 10 homozygous susceptible F_2 individuals (genotypes based on the reactions of $F_{2:3}$ lines) were pooled to form the resistant (B_R) and susceptible (Bs) DNA bulks, respectively, for bulked segregant analysis (BSA) (Michelmore et al. 1991). Markers that were polymorphic between the resistant and susceptible parents and the DNA bulks were used to genotype the $F_{2:3}$ lines for linkage analysis.

Microsatellite marker analysis

Genomic DNA from the parents and individual F_2 plants derived from L983/L962 were used for molecular analyses. For the initial polymorphic marker survey, *gwm* (Röder et al. 1998) and *wmc* (Gupta et al. 2002) SSR markers spaced at intervals of 3–4 cM along the chromosomes according to the consensus map of Somers et al. (2004) were selected and used in BSA to screen for markers linked to the resistance gene. PCRs (25-μl volumes) were performed in a PTC-200 thermocycler (MJ Research, Watertown, MA, USA). SSR analysis was performed following a previously described procedure (Röder et al. 1998) with minor modifications. Each PCR mixture contained each SSR primer at a concentration of 200 nmol/L, 0.2 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 1 unit of Taq polymerase, and 60 ng of template DNA. PCR was performed following a previously described program (Luo et al. 2008). Each PCR product was mixed with 3 μl of loading buffer [98 % formamide, 10 mM EDTA (pH 8.0), 0.25 % bromophenol blue, and 0.25 mg/ml xylene cyanol], denatured at 95 °C for 5 min, and chilled on ice. Subsequently, a 6 μl aliquot of each sample was loaded onto a 6 % polyacrylamide gel

Table 1 Infection types on wheat seedlings inoculated with *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates from different areas

Race	Source resistance gene	Coker 747 *Pm6*	Liangxing 99 *Pm52*	L658 *Pm40*	L962 *PmL962*	L983 Unknown
Bgt68-2	Beijing	0	0	0	3	3
Bgt74-1	Hebei	3	0	0	3	3
Bgt87	Beijing	3	0	0	3	3
Bgt74-3	Hebei	3	0	0	3	4
Bgt86-3	Jiangsu	2	0	0	3	4
Bgt75-1	Henan	2	0	0	3	4
Bgt75-2	Henan	3	0	0	3	3
Bgt82-3	Shandong	0	0	0	3	3
Bgt88-3	Shandong	3	0	0	3	3
Bgt77-1	Henan	3	0	0	3	3
Bgt83-1	Shandong	0	0	0	3	3
Bgt81-2	Shandong	4	0	0	3	3
Bgt68-1	Beijing	1	0	0	3	3
Bgt69-1	Hebei	3	0	0	3	3
Bgt82-2	Shandong	0	0	0	3	3
Bgt78-3	Henan	2	0	0	0	3
Bgt79-2	Shandong	3	3	0	0	3
Bgt44-6	Shandong	3	3	0	0	3
Bgt76-3	Henan	3	0	0	3	3
Bgt78-2	Henan	3	0	0	3	3
Bgt68-3	Beijing	1	0	0	3	3
Bgt73-3	Hebei	1	0	0	0	1
Bgt72	Hebei	2	0	0	0	3
Bgt71-2	Hebei	2	0	0	3	3
Bgt44-4	Shandong	0	3	0	3	3
Bgt79-3	Shandong	2	3	0	0	3
Bgt75-3	Henan	3	2	0	0	3
Bgt28	Sichuan	–	–	0	0	3

Tests were conducted twice

'–' no data

0 no visible symptoms, *1* hypersensitive necrotic flecks, small conidia with few conidiospores, *2* colonies with moderately developed conidia, *3* colonies with well-developed hyphae and abundant disconnected conidia, *4* well-developed hyphae and joined conidia

Table 2 Phenotypes of F_1, F_2, and $F_{2:3}$ populations obtained from L983/L962when inoculated with *Bgt* isolate *Bgt28*

Generation	Observed numbers of F_1 and F_2 individuals or $F_{2:3}$ lines			Expected ratio	χ^2
	Homozygous resistant/F_2 resistant	Segregating	Homozygous susceptible/F_2 susceptible		
F_1	–	24			
F_2	281	–	92	3:1	0.022^{NS}
$F_{2:3}$	105	176	92	1:2:1	2.089^{NS}

(19:1 acrylamide:bis-acrylamide, 8 M urea and 1 × TBE [90 mM tris–borate (pH 8.3), 2 mM EDTA]) prior to separation at 80 W for approximately 1.5 h and visualization by silver staining (Bassam et al. 1991).

Development of novel SSR and EST-STS markers

To increase the marker density of the map, we chose various *Xgwm*, *Xwmc*, *Xmag*, and *Xgpw* SSR markers situated close to two markers that co-segregated with the resistance locus in BSA. The contig sequences carrying the markers that mapped to chromosome 2B can be found in the draft wheat genome sequence (Brenchley et al. 2012; Jia et al. 2013; Ling et al. 2013) using BLAST. To reduce the interval containing the powdery mildew resistance gene via the use of molecular markers, we searched this contig for additional SSR loci. Using Primer3 (Rozen and Skaletsky 2000) we developed 11 novel genomic SSR markers based

Table 3 SSR markers used for linkage mapping or for developing new polymorphic SSR markers

Marker	Forward primer 5′–3′	Reverse primer 5′–3′	Contig no.[c]	Polymorphic bands[d] (Y/N)	Linkage (Y/N)	Type
Xwmc154[a]	ATGCTCGTCAGTGTCATGTTTG	AAACGGAACCTACCTCACTCTT	5170471	Y	Y	Codominant
Xgwm210[a]	TGCATCAAGAATAGTGTGGAAG	TGAGAGGAAGGCTCACACCT	5159569	Y	Y	Dominant
Xgwm148[a]	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	5210895	Y	Y	Codominant
Xwmc314[a]	ACACGGGGTCTGATTGCTTTAC	ATCGCTTTTTGACAAGTGAGGC	–	Y	Y	Dominant
Xwmc661[a]	CCACCATGGTGCTAATAGTGTC	AGCTCGTAACGTAATGCAACTG	5247173	Y	N	–
Xwmc661-1[b]	TGCCTCTGCCACATGTGTAT	GAGGGGGTGTTGTGTCACTT	5247173	Y	N	–
Xwmc661-2[b]	GAGAGGAGGCCTCACACTTTT	CATGTGGCATCGTGATGAAT	5247173	Y	Y	Codominant
Xgwm410[a]	GCTTGAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	5234233	Y	N	–
Xgwm410-1[b]	CCGAGTGATGAAGATAATCAGG	CACCAAGGCAATATTCACACA	5234233	Y	Y	Codominant
Xgwm410-2[b]	TGTTTTGTGCCATTGTGTGT	TACAAACGCACAGCCACACT	5234233	Y	N	–
Xgwm410-3[b]	ACCGACAGAGGGAAGGTTCT	AAGGTTGGAAGGCCCACTAT	5234233	Y	N	–
Xmag3930[a]	CCTCCAAAGAGAAGCCATGA	ATGCCCTTGAGGACGAACT	5243153	Y	N	–
Xmag3930-1[b]	CTGTCGTGGAGGAGATTGGT	CGGTGCAGTTTCACACATTT	5243153	Y	N	–
Xmag3930-2[b]	TTGCATTTGCTTGATGATGG	CTGATCCCATGAAACCAAGG	5243153	Y	Y	Codominant
Xgpw1148[a]	GGTAGCCCGAACAGCTTGAG	GGAACTGTCCGAAGGTGTGT	5245833	Y	N	–
Xgpw1148-1[b]*Xgpw1148-2*[b]*Xgpw1148-3*[b]*Xgpw1148-4*[b]	CTCCTCTTCGAGGCCGAGTA	GCGGAAGTAGGGCTCTCAG	5245833	Y	N	–
	GCTCCCCCGACCTTATCAC	GCTTGACGAGGACGAACC	5245833	Y	N	–
	GCATGCATAACACCAACGTC	GCTCGGGTACATTTTGTGCT	5245833	Y	N	–
	TTCATCCTGTCATCATTTGTTGT	TGCAACTTGGTAAGCCAAAA	5245833	Y	Y	Dominant

Y yes, *N* no

[a] Published SSR markers on chromosome 2B

[b] Newly developed SSR markers on chromosome 2B

[c] Contig from which the marker was derived

on 4 contigs carrying 4 different public SSR loci (Table 3) that did not exhibit polymorphism between the parents. The novel markers were named after previously identified public SSR markers by adding a second number. Because the public markers and newly developed markers are located within the same contig, they are physically close and therefore useful for fine genetic mapping. Thus, these markers were employed to screen additional polymorphic markers and subsequently used to construct a high-density genetic map. In addition, 105 EST-STS markers developed according to the wheat ESTs mapped on chromosome 2BS were also evaluated (http://wheat.pw.usda.gov).

Chromosomal localization

To further ensure that the chromosomal locations of the linked microsatellite markers used in this study were accurate, the following lines were used: Chinese Spring nullisomic 2A tetrasomic 2B (N2AT2B), nullisomic 2A tetrasomic 2D (N2AT2D), nullisomic 2B tetrasomic 2A (N2BT2A), nullisomic 2B tetrasomic 2D (N2BT2D), nullisomic 2D tetrasomic 2A (N2DT2A), and nullisomic 2D tetrasomic 2B (N2DT2B). All lines were kindly provided by Prof. D. C. Liu, Triticeae Research Institute, Sichuan Agricultural University, Chengdu, Sichuan.

Statistical analysis and linkage mapping

Chi squared tests were performed to determine the goodness-of-fit of segregation data with hypothesized 1:2:1 ratios for $F_{2:3}$ lines using Sigmaplot 2001 software (SPSS Inc., Chicago, IL, USA). Recombination fractions were converted to map distances (cM) using the Kosambi mapping function (Kosambi 1944). Loci showing no significant deviations ($P > 0.05$) were used in the linkage analysis. The order of the linked SSR and EST-STS markers and the resistance gene was determined using JoinMap 4 (Wageningen, The Netherlands). A total of 373 $F_{2:3}$ lines derived from the L983/L962 cross (Table 1) were used to construct two different genetic maps using JoinMap4 with a LOD threshold of 3.0.

Results

Response to powdery mildew

Wheat seedlings at the one-leaf-stage were tested with 27 *Bgt* isolates in a greenhouse at the Institute of Crop Science, CAAS, Beijing. Seedlings were also tested with isolate *Bgt28* in a growth chamber (Microclima MC1750E, Snijders Scientific, Tilburg, Holland) at Sichuan Agricultural University. Only one (*Bgt73-3*) of the 28 isolates was avirulent on all five wheat genotypes examined (Table 1); this isolate was collected from Hebei. All isolates were avirulent on the line expressing *Pm40* (Table 1; Luo et al. 2009c). Four isolates were virulent on the line expressing *Pm52* (Table 1; Zhao et al. 2013). L962 was resistant to 8 of the 28 isolates (Table 1). However, all isolates except Bg73-3 were virulent on the susceptible line L983. This result indicates that L962 carries one or more resistance genes that are absent in L983.

Fig. 1 Powdery mildew responses on flag leaves of parental lines L983 (**a**) and L962 (**b**)

Inheritance of resistance to powdery mildew in L962

F_1 plants, F_2 populations and $F_{2:3}$ lines from L983/L962 crosses (Table 2) were infected via the susceptible spreader line Chancellor inoculated with culture *Bgt28*. The results confirmed that L962 was resistant and L983 was susceptible to powdery mildew (Fig. 1). The F_1 plants were resistant, with responses similar to L962, indicating that resistance was dominant. The F_2 and $F_{2:3}$ lines segregated for a single dominant gene (Table 2), which was provisionally designated *PmL962*.

Identification of microsatellite markers linked to *PmL962*

Thirty-seven (4.5 %) of the 781 microsatellites (from the *gwm* and *wmc* series) were polymorphic between L962 and

Table 4 Polymorphic EST-STS markers used in map construction

EST marker	Forward primer 5′–3′	Reverse primer 5′–3′	Contig[b]	Linkage (Y/N)	Type
BE517877[a]	GATCGATCCTTCCCCGAACC	CACGCCCAGGAGTTAACACT	5382295	Y	Dominant
BE606912	ACCTGCAAAGACACCCAACA	GCATCGTTGCTAGGAGGGAG	5232840	Y	Dominant
BF202540	CCACAAGGATCATAGCGCCT	CAGTACAGCTCTCACCACCG	5198028	Y	Dominant
BE443737	GACCCGAATGCTAGTACCGG	ATGACACCGTTTGCCATTGC	5235863	Y	Codominant
BE444541	ACTCGTCTTTCTTGCAGGGG	CCGCCAAGCTTTTCAGACAC	5201747	Y	Dominant
BG263207	CTTCTTCGGCCAGACCTCTC	AGGTCCGGATTGTTCAGCAG	5247043	Y	Codominant
BF478477	AGCTACTCCACGCCTCAGTA	AGCCCAGAGTAAGCAGCATC	5278486	Y	Dominant
BF478445	CTTCATTATGGTGCACGCCG	ATACGAGCTCCAGACCCACT	1900033	N	
BE443747	CTCTATGAGAACCCGTCGGC	CCTTAGGCAATCCACCGTGT	4411536	N	
BF292851	TCGTCAGCCAGTTCATGACC	GCAGCAGCCAACAATCAACA	3870744	N	
BE404432	ATCCCGCACCAACTGTTCAT	TCCCCGAGTTTCTTCACAGC	5212423	N	
BE426229	TGCTGGCATCCTACAAGGTG	TGGAAGCCCGTGTACAAGTC	5240081	N	
BE443085	AGATGGGAGAGGTGTGGTCA	CAGCAGGAGACCAGTGGAAG	7969569	N	
BE498683	TTCAAGGTGATGCGGTCGAA	AGGGTGCTTTTCCTGCTCTC	144932	N	
BE518440	GGTGCAGTTGTAGTTTGCCG	AGCCTGGCTGGAAAAATCGA	2777	N	
BF484232	CAAGCACTCTAACCGGGCG	CCTGTCGCACGATGTTCAAC	5299839	N	
BF482714	CGGCTGAAGATGATAGGCGT	AGTTAACCAGCGACAGGGTG	3338572	N	
BE444264	CCAAGGCCAAGGAGATCGTC	CAGTGACGGTGGTCTTCGAA	6438930	N	
BE446068	TGGAATTAACCGAGCTGCGT	TCAAGCTCGCTCGGCTAATT	5358861	N	
BE500081	TTTGCAGCCTCCATTTTGCC	CCTGGGTGCCTTTTCATTGC	9909412	N	
BE500347	GGAAGGAGAAAAGGCCCAGT	ATTGCCCCTGGGTTTGTCAA	5385940	N	
BE591604	ACGAGGGCGATTTGAGAGTG	CGAGTCCAGTGTTCCCTTCC	5260464	N	

[a] ST accession in NCBI

[b] Contig from which the marker was derived

L983. These microsatellites were used to perform BSA on the mapping population. The markers *Xgwm210-2B*, *Xgwm148-2B*, *Xwmc154-2B*, and *Xwmc314-2B*, which are located on chromosome 2BS, were polymorphic between the two parents and the contrasting DNA bulks, indicating potential linkage with *PmL962*. Subsequently, polymorphism of an additional 11 new SSR markers developed from contig sequences in the corresponding region of chromosome 2BS were tested by BSA. The results indicated that an additional four markers, *Xwmc661-2-2B*, *Xgwm410-1-2B*, *Xmag3930-2-2B*, and *Xgpw1148-4-2B*, were linked with *PmL962*.

Chromosomal assignments and genetic map of *PmL962*

Based on the published chromosomal locations of the four linked microsatellite markers (Röder et al. 1998; Song et al. 2002; Somers et al. 2004) and the reported chromosomal locations of the contigs containing the other four linked microsatellite markers (http://www.wheatgenome.org/), *PmL962* was localized to the distal end of wheat chromosome arm 2BS, and the order of SSR loci agreed well with the established SSR maps on chromosome 2B (http://wheat.pw.usda.gov/cgi-bin/graingenes). However, microsatellite markers are not always chromosome-specific because of shared partial homology (Plaschke et al. 1996). The locations of all eight linked SSR markers on chromosome 2B were verified using Chinese Spring nulli-tetrasomic lines (Table 3).

Of the 105 EST-STS markers developed from sequences mapped on chromosome 2B, 22were polymorphic (Table 4 and Supplemental Table 1) and seven were linked with *PmL962* in BSA. These linked markers were used to genotype the $F_{2:3}$ population (Supplemental Table 2). Each marker locus segregated in 1:2:1 or 3:1 ratios except for *Xgpw1148-4-2B* and *Xgwm410-1-2B*, which showed significant deviations. A linkage map spanning chromosome arm 2BS (100.2 cM in length) was constructed (Fig. 2). Because four of the nine linked SSR markers were previously shown to be located on wheat chromosome 2BS and given that the contigs containing the other four linked markers were also located on this chromosome arm, we hypothesized that *PmL962* was also located on this chromosome. Marker *Xgwm210-2B*, which showed linkage of 3.51 cM with *PmL962*, was mapped to deletion bin 2BS4-0.84-1.00. The seven EST-STS markers were newly

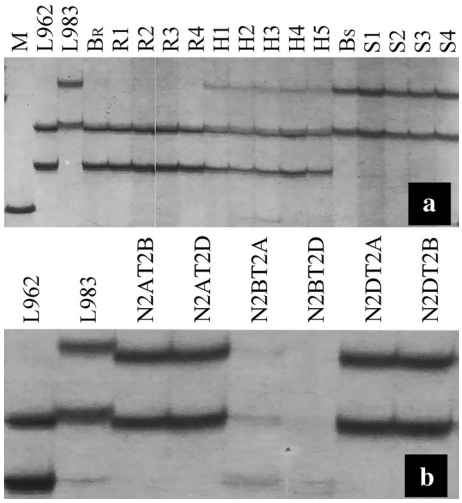

Fig. 2 Silver-stained polyacrylamide gels showing amplification of *Xwmc154*in the parents, bulked genomic DNA (**a**), and homoeologous group 2 nulli-tetrasomic lines providing evidence that*Xwmc154*is located on wheat chromosome 2B (**b**). *L962* resistant parent, *L983* susceptible parent, *R* homozygous resistant F_2 individuals, B_R resistant F_2 DNA pool, *H* heterozygous F_2 individuals, *S* homozygous susceptible F_2 individuals, B_S susceptible F_2 DNA pool, *marker* 50-bp DNA ladder

developed according to the ESTs previously mapped in this chromosome region (*BE517877*, *BE606912*, *BF202540*, *BE443737*, *BE444541*, *BG263207*, and *BF478477*). The closest marker, *BE443737*, was proximal to *PmL962* and also located in bin 2BS3-0.84-1.00 (Fig. 2a, b).

Comparative genomic analysis

The sequences of EST-STS markers, *BF202540*, *BE443737*, and *BE444541* flanking *PmL962*, were used as queries to search for orthologs in rice and *Brachypodium* genomic sequences. Both *BE202540* and *BE444541* detected orthologs on rice chromosome 4S (*Os04g0102500* and *Os04g0106300*) (Fig. 2c) and *Brachypodium* chromosome 5 (*LOC100824766* and *LOC100821269*) (Fig. 2d). Thus, a 269.4 kb genomic region (*LOC100824766–LOC100821269*) on chromosome 5 in *Brachypodium* and a 223.5 kb genomic region (*Os04g0102500* and *Os04g0106300*) on chromosome 4 in rice are homologous to the wheat genomic region harboring the powdery mildew resistance gene *PmL962*.

Discussion

Origin and mode of inheritance of *PmL962*

The discovery of novel powdery mildew resistance genes and the development of resistant cultivars constitute the most effective method of controlling powdery mildew in wheat. *Th. intermedium* is an important perennial Triticeae species and is a valuable source of resistance to powdery mildew (He et al. 2009; Luo et al. 2009c). The wheat line L962 and its resistant parent YU25 are derivatives of *Th. intermedium*. Because all common wheat parents in the pedigree of these lines are highly susceptible, the resistance trait in L962 was presumably derived from *Th. intermedium*. Genetic data indicated the presence of a single dominant resistance gene (Table 2) that was previously named *PmYU25* and located on chromosome 2DL based on a linkage of 16.6 cM with the SSR marker *Xgwm210-2D* (Ma et al. 2007). Of the formally named powdery mildew resistance genes, *Pm40* on 7BS, *Pm43* on 2DL, and *Pm52*on 2BL were derived from this donor (He et al. 2009; Luo et al. 2009c; Chang ZJ, personal communication, 2014). Because of its different chromosomal location, the resistance gene in L962 is likely novel. In addition, the resistance responses of *PmL962* to various isolates were different from those of plants harboring *Pm40*and *Pm52* (Table 1). Therefore, compared with Lianxing 99 (*Pm52*) and L658 (*Pm40*), L962 must have a different resistance gene or allele.

Resistance source and chromosomal location of *PmL962*

The microsatellite markers *Xgwm210-2B*, *Xwmc314-2B*, and *Xwmc154-2B*, which were previously assigned to chromosome 2B (Röder et al. 1998; Gupta et al. 2002; Song et al. 2002; Somers et al. 2004), were closely linked with the powdery mildew resistance gene in L962 (Fig. 3). In addition, contigs containing four other linked microsatellite markers were also on chromosome 2B (http://www.wheatgenome.org/). The localizations of 15 linked markers (except for *Xwmc626-1B-1*)on chromosome 2B were verified using Chinese Spring nulli-tetrasomic lines (Table 3). The order of these SSR loci agreed well with the established SSR maps of chromosome 2B (http://wheat.pw.usda.gov/cgi-bin/graingenes). These data provide solid evidence that *PmL962* is also located on chromosome 2B in the chromosomal region flanked by *Xwmc314* and *BE443737* at distances of 2.09 and 3.74 cM, respectively. Although *Xwmc314-2B* which is distal to *PmL962* could not be assigned to a bin based on existing data, *Xgwm210-2B*, which is distal to *Xwmc314-2B*, is located in bin 2BS3-0.84-1.00 (http://www.wheatgenome.org/; Liu et al. 2012). The proximal flanking markers *BE443737* and *Xwmc154-2B* are also located in bin 2BS3-0.84-1.00 (http://jcvi.org/cgi-bin/wheat/wheatmarker.pl; Liu et al. 2012). Thus, *PmL962* must be located in deletion bin 2BS3-0.84-1.00.

Four powdery mildew resistance genes, *Pm26*, *Pm42*, *MlIW170*, and *Ml5323*, were previously localized on chromosome 2BS. The recessive *Pm26* and *Pm42*, derived from

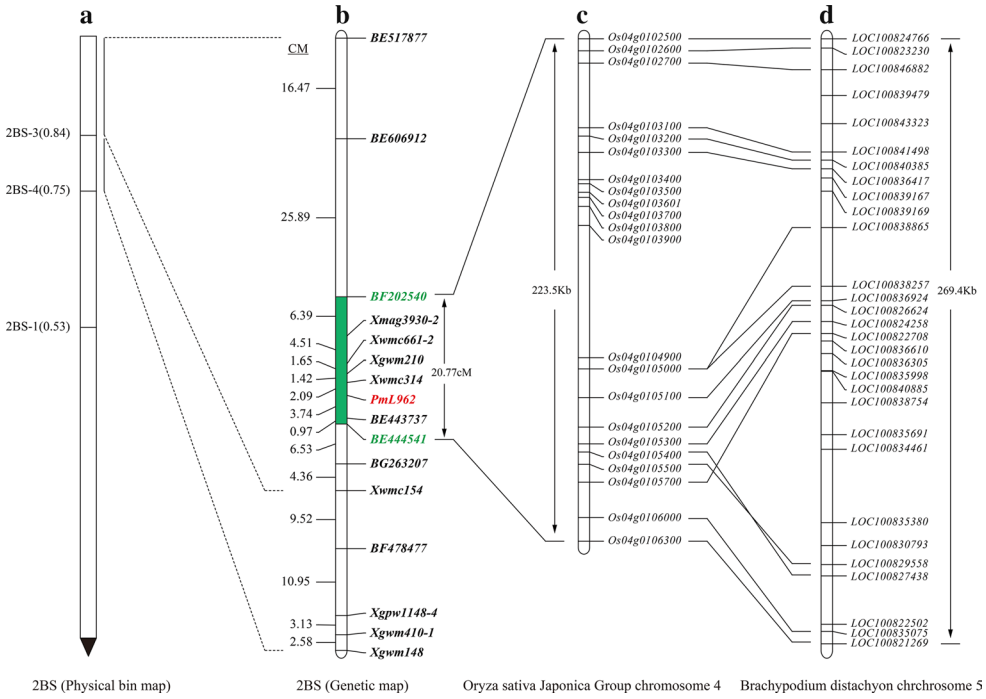

Fig. 3 Genetic and comparative genomic linkage map of the powdery mildew resistance gene *PmL962*. **a** Physical bin map of *PmL962*; *PmL962* was mapped to distal bin 2BS3-0.84-1.00; **b** genetic map of *PmL962* on wheat chromosome 2BS with genetic distances (in cM) shown at the *left*. Genetic markers are shown at the *right*; **c** orthologous region of *PmL962*in *Oryza sativa* japonica chromosome 4 with physical distances in kb shown at the *left*; orthologous genes are shown at the *right*; **d** orthologous region of *PmL962* on *Brachypodium distachyon* chromosome 4

T. turgidum var. *dicoccoides*, were localized on chromosome 2BS by Rong et al. (2000) and Hua et al. (2009), respectively. The dominant powdery mildew resistance gene *Ml5323* and the incompletely dominant powdery mildew resistance gene*MlIW170*, also from *T. turgidum* var. *dicoccoides*, were also localized on chromosome 2BS by Piarulli et al. (2012) and Liu et al. (2012). L962, which was used in the present study, was selected from the progeny of MY11/YU25. Line YU25, which is resistant to stripe rust, powdery mildew, and FHB, was derived from *Th. intermedium* (Zhang et al. 2011; Liu et al. 2014), and its resistance traits are assumed to be derived from *Th. intermedium*. However, the above powdery mildew resistance genes on chromosome 2BS are derived from *Triticum turgidum* var. *dicoccoides*. Therefore, *PmL962* is likely to be different from *Pm26*, *Pm42*, *Ml5323*, and *MlIW170*.

Pm42 was physically mapped to chromosome bin 2BS4-0.75-0.84 (Hua et al. 2009). Hence, it is likely that *PmL962* is different from *Pm42* because *PmL962* is located in bin 2BS3-0.84-1.00 (Fig. 3a, b). However, genes *Pm26*, *MlIW170,* and *Ml5323* are also located in bin 2BS3-0.84-1.00. A previous study suggested that *Pm26* and *MlIW170* are identical (Liu et al. 2012). Further comparative genomics analysis suggested that the orthologous genomic region containing MlIW170 was distal to *Os04g0102500* in the rice genome (Liu et al. 2012). Conversely, comparative genomics analysis showed that the orthologous genomic region of *PmL962* is proximal to *Os04g0102500* in the rice genome (Fig. 3c). Hence, the location of *PmL962* is also different from the locations of *Pm26* and *MlIW170*. The distance between the SSR marker *Xgwm210-2B* and *PmL962* is only 3.51 cM (Fig. 3b), whereas that between *Xgwm210-2B* and *Ml5323* is 30.5 cM (Piarulli et al. 2012). It is nearly impossible that this difference could be attributed only to random variation. Moreover, the distance between *Xgwm210-2B* and *Xwmc661-1-2B,* which are in the same contig as *Xgwm210-2B* in the linkage map of *PmL962* (Fig. 3b), is similar to the distance between *Xgwm210-2B* and *Xwmc661-2B* in the linkage map of *Ml5323* (Piarulli et al. 2012). This finding suggests that the mapping could be comparable to some degree. Thus, *PmL962* is most likely different from (and distal to) Ml5323.

Based on the pedigree, inheritance, molecular marker experiments, and genetic location, we concluded that *PmL962*differs from *Pm26*, *Pm42*, *MlIW170,* and *Ml5323* and is a new gene.

Transfer of resistance by cryptic translocation is possible

Alien chromosomal translocation is a classic and useful method for transferring genes from wild relatives to common wheat. Despite the potential to carry valuable genes, many alien translocations have questionable value in wheat breeding because large transferred chromosome segments often carry additional genes for undesirable traits or do not adequately compensate for the wheat genes they replace, resulting in ‘linkage drag’ (Young and Tanksley 1989). However, traits of interest have occasionally been transferred to recipient genotypes by cryptic translocation

without detectable cytological or genetic changes (Kuraparthy et al. 2007). Previous studies have indicated that the wheat genotype YU25, the resistant parent of L962, does not have a cytologically detectable alien chromosome segment and therefore may contain a cryptic translocation (Luo et al. 2009a, c; Huang et al. 2014). Alien chromosomal segments resulting from such small translocations are not easily detected by standard cytogenetic methods other than high-resolution FISH (Danilova et al. 2014).

In the present study, nearly all of the 781 SSR primer pairs that amplified wheat-specific products were evenly distributed over all chromosome arms with 3–4 cM spacing in L962 and L983;however, only 37 (4.5 %) of the 781 SSR primer pairs were polymorphic. These polymorphic SSRs were located on all chromosomes. This result not unexpected because L962 and L983 are sister lines. It also indicates that a large foreign chromosomal segment is not present in L983 and is similar to what was observed in YU25 (Ma et al. 2007; Luo et al. 2009a, c). Additional evidence for the absence of a large alien chromosomal segment in L962 includes the following: first, wheat primers linked with the resistance gene produced wheat-specific PCR products in genotype L962, and closely linked SSR loci flanking *Pm54* did not show significant alterations in either gene order or their between-marker distances compared with the consensus genetic map (Fig. 3b); second, L962 is genetically and agronomically uniform based on several years of observations; and third, the resistance gene in L962 behaved as a discrete Mendelian unit (Table 2). Moreover, we could not detect in situ hybridization signals in the resistant parent YU25 using *Th. intermedium* genomic DNA as a probe (data not shown, personal communication with Prof. Z. X. Tang, Institute of Agronomy, Sichuan Agricultural University, Chengdu China). Taken together, these data indicate that L962 does not possess a large alien chromosomal segment but instead contains a cryptic translocation. The pedigree provides the only evidence that L962 carries a powdery mildew resistance gene from *Th. intermedium*. Hence, one of the goals for further study is to obtain new information concerning the source of powdery mildew resistance at the DNA level.

Potential role of *PmL962* in the improvement of wheat powdery mildew resistance

Although many wheat powdery mildew resistance genes have been identified and incorporated into commercial cultivars, the resistances conferred by almost all of the genes have been overcome by the pathogen (Yang et al. 2013). Although the widely used *Pm21* still confers resistance as a major resistance gene resource in Chinese breeding programs (Cao et al. 2011), there are reports of new races with virulence on varieties possessing this gene (Ji et al. 2007; Shi et al. 2009; Yang et al. 2009). This implies a potential threat to wheat yields in China due to the loss of *Pm21*-mediated resistance. Hence, the identification of new powdery mildew resistance genes is an important long-term task to achieving lasting, broad-spectrum resistance in wheat breeding.

In this study, we identified a single dominant gene putatively derived from *Th. intermedium*, and responsible for resistance to powdery mildew in wheat L962. This gene was earlier reported on chromosome 2D based on linkage to a single linked SSR marker (Ma et al. 2007). However, the present linkage and comparative genomics analyses showed that *PmL962* is located on chromosome 2BS rather than chromosome 2D (Fig. 3b). Although the resistance spectrum of *PmL962* is not wide, it can be utilized in combination with the other *Pm* genes. We also determined the resistance spectrum of *Pm6* and *Pm52* on chromosome 2BL. Interestingly, the resistance spectrum of *PmL962* is more complementary to that of *Pm52* than to that of *Pm6*, as all isolates that were virulent to plants expressing *Pm52* were avirulent to plants expressing *PmL962* (Table 1). This indicated that pyramiding *PmL962* with *Pm52* has a greater potential advantage than pyramiding *PmL962* with *Pm6*. Moreover, Liangxing 99, which carries *Pm52,* is one of the most widely grown commercial cultivars in the winter wheat regions in northern China; therefore, introgressing *PmL962* into Liangxing 99 would be useful for enhancing and broadening the resistance spectrum (Luo et al. 2009b). More importantly, the utilization of molecular markers that are closely linked with *PmL962* and *Pm52* has led to the pyramiding of *PmL962* and *Pm52* (unpublished results). The identification of *PmL962* may aid further studies aimed at fine mapping and determining the relationship between the resistance conferred by *PmL962* and that conferred by other *Pm* genes, especially the *Pm* genes on chromosome 2BS, including *Pm26*, *Pm42*, *MlIW170,* and *Ml5323*.

Author contribution statement Z. J. Chang, Z. L. Ren, H. Y. Zhang, W. Q. Chen, and P. G. Luo designed the research; X. K. Shen, L. X. Ma, S. F. Zhong, X. Li, Z. P. Xiang, and S. F. Zhong performed the research; N. Liu, M. Zhang, Y. L. Zhou, H. Y. Zhang, F. Q. Tan, H. J. Li, and X. Li analyzed the data; and G. H. Bai, W. Q. Chen and P. G. Luo wrote the paper.

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Conflict of interest The authors declare no conflicts of interest.

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