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ORIGINAL ARTICLE

Genetic analysis of a novel broad-spectrum powdery mildew resistance gene from the wheat-*Agropyron cristatum* introgression line Pubing 74

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Abstract

Main conclusion **A novel broad-spectrum powdery mildew resistance gene *PmPB74* was identified in wheat-*Agropyron cristatum* introgression line Pubing 74.**

Development of wheat cultivars with broad-spectrum, durable resistance to powdery mildew has been restricted by lack of superior genetic resources. In this study, a wheat-*A. cristatum* introgression line Pubing 74, originally selected from a wide cross between the common wheat cultivar Fukuhokomugi (Fukuho) and *Agropyron cristatum* (L.) Gaertn ($2n = 4x = 28$; genome PPPP), displayed resistance to powdery mildew at both the seedling and adult stages. The putative alien chromosomal fragment in Pubing 74 was below the detection limit of genomic in situ hybridization (GISH), but evidence for other non-GISH-detectable introgressions was provided by the presence of three STS markers specific to *A. cristatum*. Genetic analysis indicated that Pubing 74 carried a single dominant gene for powdery mildew resistance, temporarily designated *PmPB74*. Molecular mapping showed that *PmPB74* was located on wheat chromosome arm 5DS, and flanked by markers *Xcfd81* and *HRM02* at genetic distances of 2.5 and 1.7 cM, respectively. Compared with other lines with powdery mildew resistance gene(s) on wheat chromosome arm 5DS, Pubing 74 was resistant to all 28 *Blumeria graminis* f. sp *tritici* (*Bgt*) isolates from different wheat-producing regions of northern China. Allelism tests indicated that *PmPB74* was not allelic to *PmPB3558* or *Pm2*. Our work showed that *PmPB74* is a novel gene with broad resistance to powdery mildew, and hence will be helpful in broadening the genetic basis of powdery mildew resistance in wheat.

Keywords *Blumeria graminis* f. sp. *tritici* · Disease resistance · *Triticum aestivum* · Wide cross

Abbreviations

Fukuho	cv. Fukuhokomugi
GISH	Genomic in situ hybridization
HRM	High resolution DNA melting
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat

Introduction

Powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*, *Bgt*) is a destructive wheat disease all over the world, causing not only significant yield loss but also severe quality deterioration (Bowen et al. 1991; Everts and Leath 1992). Resistant cultivars are the most economical and environmental strategy to reduce the prevalence of powdery mildew, considering that fungicide application can cause environmental problems and likely future acquisition of fungicide tolerance by the pathogen (Huang et al. 1997; Paillard et al. 2000; Huang and Roder 2004).

More than 70 powdery mildew resistance alleles have been identified, a number of which were introduced from wild relatives of common wheat (Friebe et al. 1996; Gill

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et al. 2011; Mohler et al. 2013; McIntosh et al. 2014; Petersen et al. 2015), such as *Triticum monococcum* ($2n = 2x = 14$; genome AA) (Shi et al. 1998; Yao et al. 2007; Schmolke et al. 2012), *Aegilops tauschii* ($2n = 2x = 14$; genome DD) (Miranda et al. 2006, 2007; Schneider et al. 2008), *Secale cereale* L. ($2n = 2x = 14$; genome RR) (Friebe et al. 1994; Mohler et al. 2001), *Haynaldia villosa* L. ($2n = 2x = 14$; genome VV) (Chen et al. 1995, 2013; Xie et al. 2012; Zhang et al. 2012), and *Thinopyrum intermedium* ($2n = 6x = 42$; genome JJJsJsSS) (He et al. 2009; Liu et al. 2014; Shen et al. 2015). However, some powdery mildew resistance genes, such as *Pm8* from *S. cereale* L. have become ineffective due to changes in the pathogen population (Hsam and Zeller 2002; McDonald and Linde 2002; Parks et al. 2008). Consequently, researchers are constantly seeking novel germplasms with broad-spectrum resistance to the disease.

Various molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP), have been used to map powdery mildew resistance genes in wheat. SSR markers including genomic SSR or EST-derived SSR markers have most commonly been used (Röder et al. 1998; Paillard et al. 2003; Sourdille et al. 2004; Somers et al. 2004; Yu et al. 2004). Currently, SNP markers are becoming increasingly popular (Akhunov et al. 2009; Berard et al. 2009; Chao et al. 2010; Lai et al. 2012; Allen et al. 2013), followed by the development of the wheat 9 and 90 K SNP chip platforms (Cavanagh et al. 2013; Avni et al. 2014; Wang et al. 2014). Upon SNP identification, various systems have been devised for SNP profiling, such as single strand conformation polymorphism (SSCP), kompetitive allele specific PCR (KASPar) assay, and fluorescent high resolution DNA melting (HRM) analysis. HRM analysis, a powerful tool for discrimination of a single SNP, has been successfully used in wheat (Matsuda et al. 2012; Tan et al. 2013; Terracciano et al. 2013).

Agropyron cristatum (L.) Gaertn ($2n = 4x = 28$; genomes PPPP), a perennial species of the Triticeae, harbors many favorable traits that can be exploited for wheat genetic improvement (Dewey 1984; Dong et al. 1992), such as enhanced fertile tiller number per plant (Ye et al. 2015), high grain number per spike (Wu et al. 2006; Luan et al. 2010), and high resistance to powdery mildew and other diseases (Han et al. 2014; Lu et al. 2015). Various wheat-*A. cristatum* derivative lines with elite traits including addition, translocation and introgression lines have been produced (Wu et al. 2006; Han et al. 2014; Ye et al. 2015; Lu et al. 2015; Zhang et al. 2015a). Pubing 74 is a putative wheat-*A. cristatum* introgression line, which displays a high level of resistance to powdery mildew at both the seedling and adult stages. However, the genetic basis of the resistance was still uncharacterized. We determined the chromosomal location of the resistance gene and also evaluated its effectiveness against a comprehensive set of *Bgt* isolates from a wide range of wheat-producing regions in China.

Materials and methods

Materials

Pubing 74 was originally selected from a wide cross between the common wheat cultivar (cv.) Fukuhokomugi (Fukuho) and *A. cristatum* (accession No. Z559). Common wheat cv. Mingxian 169 highly susceptible to powdery mildew was crossed with Pubing 74 to generate F_2 and F_2-derived F_3 family populations. Common wheat cv. Zhongzuo 9504 was used as the susceptible control in the powdery mildew assessment. Wheat lines with known powdery mildew resistance genes on wheat chromosome arm 5DS, including wheat landrace derivative Ulka/8*Cc (Briggle 1966; Qiu et al. 2006), German wheat cv. Tabasco (Gao et al. 2012), Chinese wheat cv. Liangxing 66 (Huang et al. 2012), wheat-*A. cristatum* introgression line PB3558 (Lu et al. 2015), common wheat line D57 (Ma et al. 2011), indigenous germplasm X3986-2 (Ma et al. 2014), and Chinese breeding line KM2939 (Ma et al. 2015), were used in this study. Tabasco was provided by Dr. Shibin Cai (Jiangsu Academy of Agricultural Sciences), D57 by Dr. Zhengqiang Ma (Nangjing Agricultural University), X3986-2 and KM2939 by Dr. Diaoguo An (Chinese Academy of Sciences, Shijiazhuang, Hebei province). The other wheat lines are maintained by our laboratory. All 28 single spore-derived *Bgt* isolates used in this study were kindly provided by Dr. Hongjie Li (Institute of Crop Science, Chinese Academy of Agricultural Sciences).

Genomic in situ hybridization and meiosis observation

Genomic in situ hybridization (GISH) was conducted according to Luan et al. (2010). *Agropyron cristatum* and Fukuho genomic DNA were used as the probe and blocker, respectively. *Agropyron cristatum* genomic DNA was labeled with Dig-Nick-Translation Mix (Roche, Mannheim, Germany). Wheat and *A. cristatum* chromosomes were pseudo-colored as blue and red, respectively. The procedure used for meiotic studies was described by Jauhar and Peterson (2006). Young spikes from Pubing 74 with pollen mother cells (PMCs) at the metaphase I (MI) stage were fixed in Carnoy solution (6-ethanol: 3-chloroform: 1-acetic acid, by vol.) for 24 h and stored at 4 °C until used. All cytological images were taken under a Nikon Eclipse E600

fluorescence microscope and captured with a CCD camera (Diagnostic Instruments, Sterling Heights, MI, USA).

Evaluation of powdery mildew response

The prevailing *Bgt* isolate E09 was used to test Pubing 74 × Mingxian 169 F_1 hybrids, an F_2 population, and F_2-derived F_3 families at the seedling stage. Seedlings at the one-leaf-stage were inoculated by dusting conidiospores of *Bgt* isolate E09 from susceptible cv. Zhongzuo 9504. Infection types (ITs) were scored on the first leaf using a 0–4 scale around 15 days after inoculation (Liu et al. 2002). Scores of 0–2 were classified as resistant and 3–4 as susceptible. Twenty seedlings of each line in the $F_{2:3}$ population were tested against *Bgt* isolate E09 to determine the genotypes of the F_2 individuals. We used 20 seedlings for each line, since this was adequate to reduce the probability of erroneously determining a heterozygous plant as homozygous resistant to 0.3 %, and to reduce the probability of determining a heterozygous plant as homozygous susceptible to $9e^{-13}$.

Twenty-eight *Bgt* isolates originating from different wheat-producing regions of Northern China (Sun et al. 2015) were used to compare the reaction patterns of Pubing 74 and lines with known alleles on chromosome arm 5DS. The reactions to 28 *Bgt* isolates were determined using detached leaf segments as described by Limpert et al. (1988). Three leaf segments from different plants of each genotype were examined and the tests were repeated three times. Besides, a mixture of *Bgt* isolates mainly composed of E09 was employed to inoculate the populations as well as two parents in the field at the adult stage. All plants were sown in 2.0 m rows, spaced 0.3 m apart. The susceptible control cv. Zhongzuo 9504 was planted in every fifth row to ensure that all plants were evenly infected. Disease reactions were scored using a 0–9 scale at the ear emergence and milky ripe stages.

DNA extraction and bulked segregant analysis

Genomic DNA was isolated from leaves of young seedlings following the CTAB method (Allen et al. 2006). To detect *A. cristatum* chromosomal fragments in Pubing 74, three sequence-tagged-site (STS) markers (*Agc2970*, *Agc6287* and *Agc21686*) designed according to the expressed sequence tags (EST) of the *A. cristatum* transcriptome (Zhang et al. 2015b). Bulked segregant analysis (BSA) was applied as described by Michelmore et al. (1991). Briefly, resistant and susceptible DNA bulks were constructed by separately mixing equal amounts of DNA from 20 homozygous resistant (IT = 0) and 20 homozygous susceptible (IT = 4) F_2 plants, homozygosity being established on the basis of progeny testing.

Genotyping with publicly available and new developed molecular markers

SSR markers evenly distributed across all the wheat chromosomes were used for a polymorphism survey on the two parents and two DNA bulks, and polymorphic markers were subsequently used for genotyping the segregating population. A series of *Xbwm* SSR markers, which were developed recently and located on wheat chromosome arm 5DS (Lu et al. 2015), were chosen for genotyping. Three HRM markers were designed based on the flanking sequences of SNPs, taking the sequences and their physical locations on wheat chromosome arm 5DS as reference (Jia et al. 2013). PCR was performed in 10 μL volumes containing 0.1 U Taq DNA polymerase, 2 μM forward and reverse primers, and approximately 30 ng of template DNA. The PCR amplification conditions were: 35 cycles at 95 °C for 30 s, 55–65 °C for 30 s and 72 °C for 30 s with an initial denaturation at 95 °C for 2 min and a final extension step at 72 °C for 5 min.

When PCR was carried out with HRM markers, 1 μL 10 x LC Green (Idaho Technology, Salt Lake City, UT, USA) was included in 10 μL PCR volumes. The resulting PCR products were analyzed using a light scanner (Idaho Technology), by ramping the temperature from 55 to 95 °C at 0.1 °C per second. Data were analyzed using the analytical light scanning software, and the amplification patterns of the HRM markers were shown in two different ways: normalized melting peaks and normalized melting curves.

Statistical analysis and linkage map construction

Chi squared (χ^2) tests were used to compare the observed and theoretically expected ratios. Polymorphic markers were used for constructing a linkage map flanking the powdery mildew resistance gene in Pubing 74, and a linkage map was established with Mapmaker 3.0 and Mapdraw (Lincoln et al. 1993; Liu and Meng 2003). The LOD score threshold was set at 3.0 and a maximum genetic distance at 50 cM. The Kosambi mapping function was used to estimate genetic distances between linked markers and the powdery mildew resistance gene based on recombination values.

Results

Pubing 74 is a novel wheat-*A. cristatum* introgression line

Pubing 74 was produced from a wide cross between *A. cristatum* and wheat cv. Fukuho, and then selected over

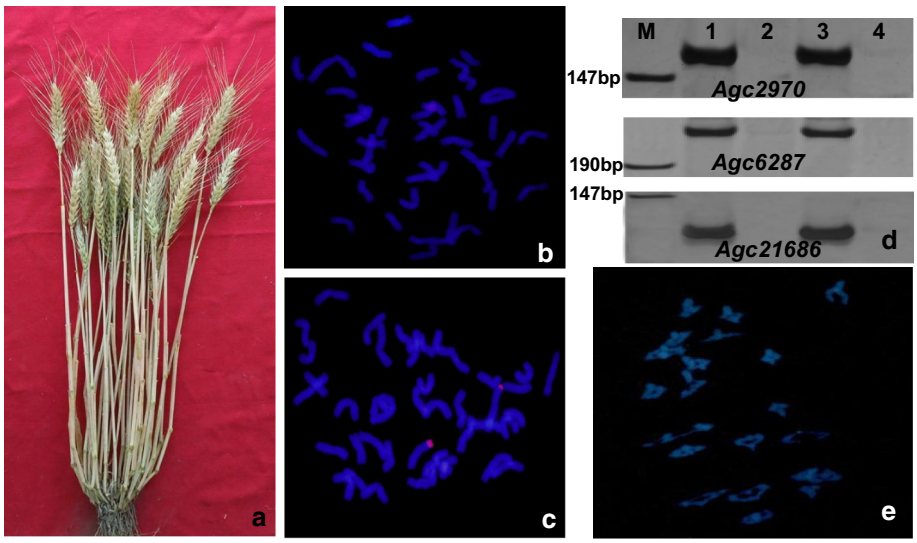

Fig. 1 Identification of wheat-*A. cristatum* introgression line Pubing 74. **a** Normal appearance of a Pubing 74 plant. **b**, **c** GISH detection of Pubing 74 (**b**) and one wheat-*A. cristatum* translocation line WAT12-9 as the positive control (**c**). **d** PCR patterns of three STS markers specific to *A. cristatum* (*Agc2970*, *Agc6287* and *Agc21686*). *Lanes M* DNA ladder; *1 A. cristatum*; *2* Fukuho; *3* Pubing 74; *4* Mingxian 169. **e** PMC from Pubing 74 with 21 bivalents at meiotic metaphase I (MI)

five selfing generations. Pubing 74 displayed full fertility and normal agronomic performance, such as 56–73 grains per spike, and 15–20 fertile tillers per plant (Fig. 1a). GISH was performed to determine whether Pubing 74 is a wheat-*A. cristatum* introgression line. No visible hybridization signal was detected in Pubing 74 (Fig. 1b), despite strong hybridization signals in the positive control (Fig. 1c). However, evidence for the presence of *A. cristatum* chromatin was provided by STS markers specific to the *A. cristatum* P genome. STS markers *Agc2970*, *Agc6287* and *Agc21686* were amplified in *A. cristatum* and Pubing 74, but not in Fukuho or Mingxian 169 (Fig. 1d). These results indicated that *A. cristatum* chromatin was present in Pubing 74, but was presumably too small to be detected by the standard cytological methods. Meiotic metaphase PMC cells showed normal 21 bivalent chromosome pairing in Pubing 74 (Fig. 1e), indicating that Pubing 74 could be a stable wheat-*A. cristatum* introgression line.

Inheritance of powdery mildew resistance in Pubing 74

Pubing 74 was highly resistant under natural field epidemic conditions over several years. It was also resistant at the seeding and adult plant stages under controlled conditions (Fig. 2). The inheritance data presented in Table 1 showed that resistance to *Bgt* race E09 was conferred by a single dominant gene, which was tentatively designated *PmPB74*. Results from seedling and adult plant tests were identical.

Chromosomal location of *PmPB74* in Pubing 74

Three hundred and seventy-eight wheat SSR markers distributed randomly throughout the wheat genome were screened for polymorphisms between the parents and DNA

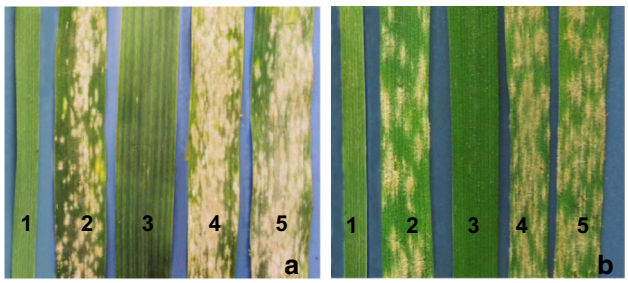

Fig. 2 Responses to powdery mildew of Pubing 74 at the adult (**a**) and seedling (**b**) stages. *1 A. cristatum*; *2* Fukuho; *3* Pubing 74; *4* Mingxian 169; *5* Zhongzuo 9504

bulks, and nine markers showed polymorphisms. Among these nine markers, only *Xcfd81* showed evidence of linkage with *PmPB74*. Since *Xcfd81* was known to be on chromosome arm 5DS, additional SSR markers on that chromosome arm were surveyed for polymorphisms. Four SSR markers (*Xgpw5201*, *Xwmc805*, *Xgpw302* and *Xcfd40*) were polymorphic and linked to *PmPB74* (Fig. 3). Four of 25 *Xbwm* SSR markers, and three of 15 HRM markers were also polymorphic and linked to *PmPB74* (Fig. 3; Table 2). The linkage map based on the powdery mildew response and marker data indicated that *PmPB74* was flanked by markers *Xcfd81* and *HRM02* at genetic distances of 2.5 and 1.7 cM, respectively (Fig. 3). *Xcfd81* was earlier reported to be located on the bin C-5DS1-0-0.63, hence *PmPB74* is also likely to be located on this bin. However, the three STS markers specific to *A. cristatum* were not linked with *PmPB74*, nor were they linked to each other, suggesting that there were multiple small chromosome segments from *A. cristatum* distributed at different chromosomal locations in Pubing 74. Amplification patterns of two SSR markers (*Xcfd81* and *Xbwm25*) and two HRM markers (*HRM01* and *HRM02*) are shown as examples in Figs. 4, 5, respectively.

Table 1 Genetic analysis of powdery mildew resistance to the *Bgt* isolate E09 in Pubing 74 × Mingxian 169 F_1, F_2 and $F_{2:3}$ populations

Parents and populations	No. of plants	No. of plants			Expected ratio	χ^2	*P* value
		H_R	H_Z	H_S			
Pubing 74	20	20		0			
Mingxian 169	20	0		20			
Pubing 74 × Mingxian 169 F_1	16		16	0			
Pubing 74 × Mingxian 169 F_2	258	196		62	3:1	0.13	0.72
Pubing 74 × Mingxian 169 $F_{2:3}$	204	54	101	49	1:2:1	0.26	0.88

H_R homozygous resistant lines, H_Z heterozygous resistant lines, H_S homozygous susceptible lines

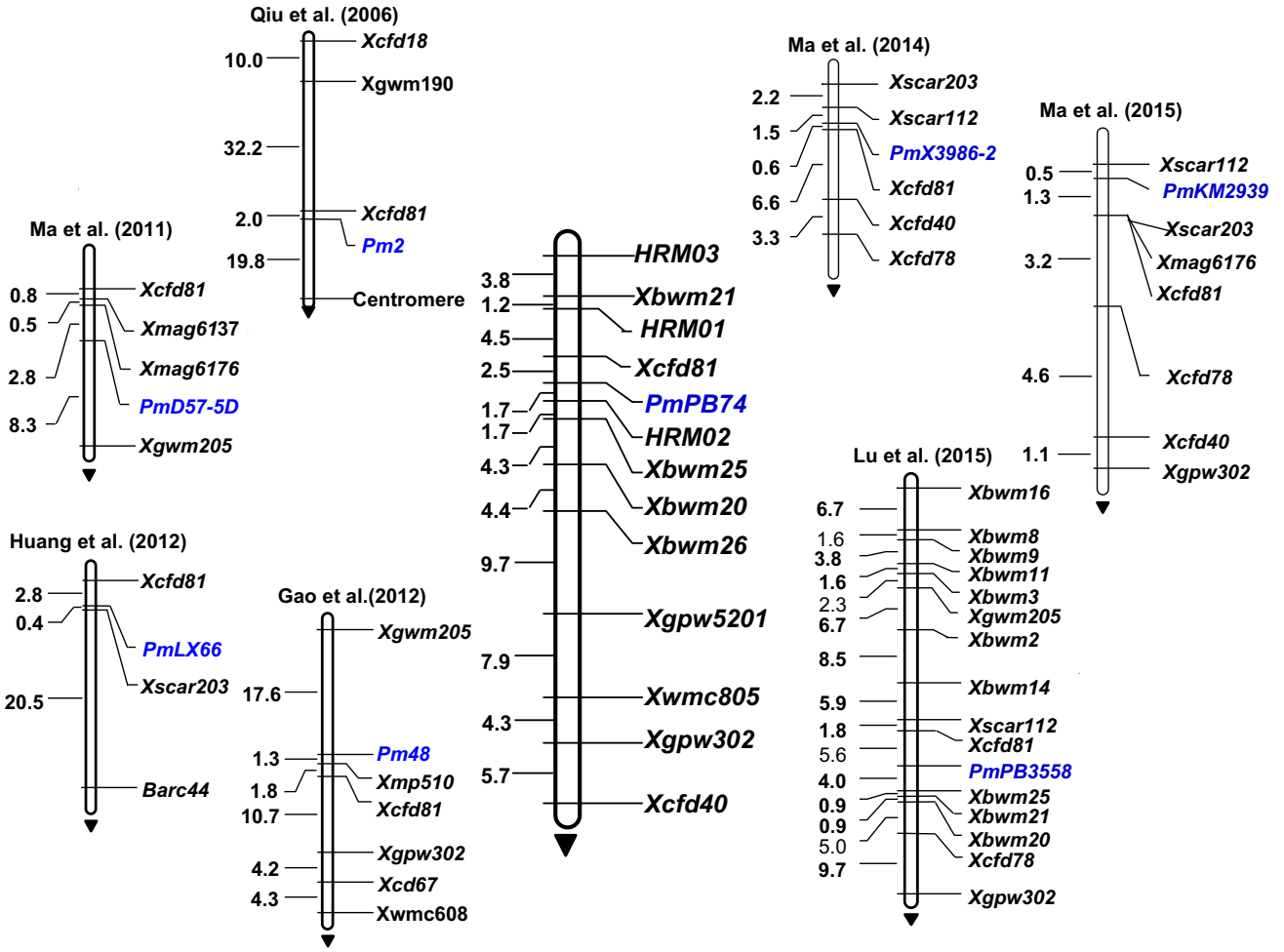

Fig. 3 Genetic mapping of *PmPB74* and comparison with the documented powdery mildew genes on wheat chromosome 5DS. Genetic distances in cM are shown on the *left*, and *black arrows* point to the centromeres

Comparative reactions of Pubing 74 and other lines with known powdery mildew resistance genes on chromosome arm 5DS to 28 *Bgt* isolates

To determine the relationships of *PmPB74* with other genes located on chromosome 5DS, 28 *Bgt* isolates including E09 from different wheat-producing regions of Northern China were used in inoculations. As shown in Table 3, Pubing 74 and *A. cristatum* were resistant to all the 28 isolates tested. However, Ulka/8*Cc (*Pm2*) was susceptible to 12 isolates, Tabasco (*Pm48*) was susceptible to eight isolates, Liangxing 66 (*PmLX66*) was susceptible to 12 isolates, and *PB3558* (*PmPB3558*) was susceptible to seven isolates. The disease reactions of seven lines to six *Bgt* isolates as examples are shown in Fig. 6. Besides, seven isolates were used to compare the reactions of

Table 2 Primer sequences of all the markers used in this study

Marker names	Forward primer (5′–3′)	Reverse primer (5′–3′)	Marker types	References
Xcfd81	tatccccaatcccctctttc	gtcaattgtggcttgtccct	SSR	Somers et al. (2004)
Xcfd40	gcgacaagtaattcagaacgg	cgcttcggtaaagtttttgc	SSR	Somers et al. (2004)
Xgpw5201	atctagccactccaccagatg	gacaaaacctccctccttcc	SSR	Somers et al. (2004)
Xgpw302	agtagtcctttccactcatcca	tagccgtgtgtccacagtcaaa	SSR	Somers et al. (2004)
Xwmc805	gatgctgctgcaccaaactc	gccttttccatgccacact	SSR	Somers et al. (2004)
Xbwm21	gtgcgtttcgttgaaggtc	aatggtcgccatgcaact	SSR	Lu et al. (2015)
Xbwm25	acgaaccccaccctcatta	atcacgccccatttttctc	SSR	Lu et al. (2015)
Xbwm20	gcttcatcctcagcttcgtc	ggaggaaacaaaggcacaga	SSR	Lu et al. (2015)
Xbwm26	ctttttgcctccatggtgat	ccgtgcgatataagaacacg	SSR	In this study
HRM01	cccaaagtggtgttaccgttatt	gattctttgtcgcactggtaa	SNP	In this study
HRM02	acggcataaatgattactcgcg	ccaccaatcttgctcaacttca	SNP	In this study
HRM03	cagaagaaagggcattcctaaca	tgggcatttaaggcatccct	SNP	In this study
Agc2970	cgattccactagggaacgaa	cacgcgttttgtgactccta	STS	Zhang et al. (2015b)
Agc6287	taggcacagccaaccagtct	tgccatcaatcatgagcctc	STS	Zhang et al. (2015b)
Agc21686	taaatgcgataatcccgctg	tgttattgctgcaagcattggt	STS	Zhang et al. (2015b)

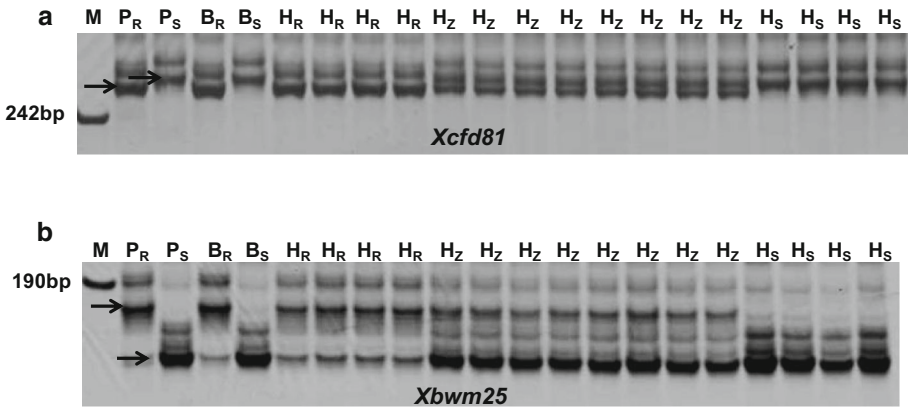

Fig. 4 Amplification profiles of SSR markers *Xcfd81* (**a**) and *Xbwm25* (**b**). *Lanes M* DNA ladder; *PR* resistant parent Pubing 74; *PS* susceptible parent Mingxian 169; *BR* resistant DNA bulk, *BS* susceptible DNA bulk; *HR* homozygous resistant F_2 individual; *HZ* heterozygous resistant F_2 individual; *HS* homozygous susceptible F_2 individual. Polymorphic PCR bands are indicated by *arrows*

Pubing 74 and three lines (D57, X3986-2 and KM2939). As shown in Table 4, both D57 and X3986-2 were each susceptible to two *Bgt* isolates, and KM2939 was susceptible to E21. Therefore, Pubing 74 displayed a broader spectrum of disease resistance than any other wheat line tested above. Finally, when 20 $F_{2:3}$ lines homozygous resistant and 20 $F_{2:3}$ lines homozygous susceptible to isolate E09 were tested with the other 27 isolates at the seedling stage, all these lines showed disease reactions identical to those inoculated with E09, thus showing that *PmPB74* conferred resistance to all the isolates.

PmPB74 and *PmPB3558* were both derived from *A. cristatum* accession Z559. To determine whether *PmPB74* was *PmPB3558*, the *Bgt* isolate Bg44-6, which was virulent to *PmPB3558* (and Mingxian 169) but avirulent to *PmPB74*, was used to inoculate the Pubing 74 × Mingxian 169 $F_{2:3}$ population at the seedling stage. A segregation pattern identical with that of E09 was obtained and *PmPB74* was assigned to the same chromosomal location, indicating that *PmPB74* was indeed not identical to *PmPB3558*. To further determine whether *PmPB74* and *PmPB3558*, and also *PmPB74* and *Pm2* were different alleles in a single locus, crosses between Pubing 74 and Ulka/8*Cc, and between Pubing 74 and PB3558, respectively, were made. When large F_2 seedling populations were tested with isolate E09, which was avirulent to all three host lines, two of 6986 F_2 plants from Pubing 74 × Ulka/8*Cc were susceptible to powdery mildew (IT = 4), and just one of 2260 F_2 plants from Pubing 74 × PB3558 displayed susceptibility to powdery mildew (IT = 4). Thus, *PmPB74* was not allelic to *Pm2* nor to *PmPB3558*. To confirm the conclusion that *PmPB74*

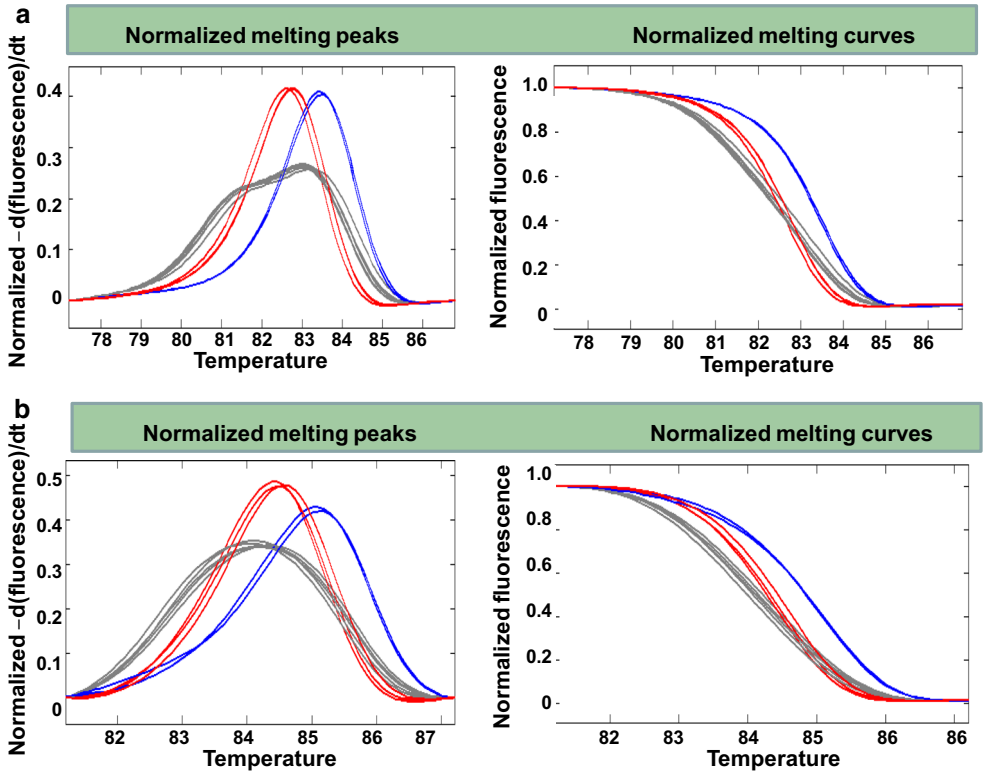

Fig. 5 Amplification profiles of HRM markers: *HRM01* (**a**) and *HRM02* (**b**). *Left* and *right panels* display normalized melting peaks and normalized melting *curves* for the same amplicons, respectively. *Lines* with *different colours* represent different genotypes: *red lines* represent Mingxian 169 and the homozygous susceptible F_2 plants; *blue lines* represent Pubing 74 and the homozygous resistant F_2 plants; *gray lines* represent heterozygous F_2 plants

represents a novel locus, we inoculated Pubing 74 × PB3558 $F_{2:3}$ lines with E09, and two lines showing 3 resistant:1 susceptible segregation were identified from 168 $F_{2:3}$ lines. Similarly, we tested the relationship between *PmPB74* and *Pm2* in Pubing 74 × Ulka/8*Cc $F_{2:3}$ lines, and two segregating lines were identified from 392 $F_{2:3}$ lines.

Discussion

Introgression lines are valuable sources of resistance genes in common wheat

The wild relatives of wheat have been used effectively as donors of desirable genes conferring superior agronomic traits. However, large alien chromosomal fragments may carry additional genes that confer undesirable traits in wheat, a phenomenon known as 'linkage drag'. Therefore, smaller alien chromosomal fragments are generally preferred. Nevertheless, small alien chromosomal fragments with desirable genes are sometimes too small to be detected by standard cytological methods. Examples include the alien introgression of small chromosomal fragments from *Dasypyrum villosum* and *Thinopyrum ponticum* to wheat (Caceres et al. 2012; Chen et al. 2012), rust resistance genes introgressed from *Ae. geniculata* and *Ae. triuncialis* to wheat (Kuraparthy et al. 2007a, b), and powdery mildew and stripe rust resistance genes putatively derived from *T. intermedium* (He et al. 2009; Liu et al. 2013; Huang et al. 2014). Pubing 74, was obtained from distant hybridization between the common wheat cv. Fukuho and *A. cristatum*, displayed high resistance to powdery mildew at both the seedling and adult stages. The putative chromosomal fragment from *A. cristatum* was below the detection limit of GISH, but evidence for other non-GISH-detectable introgressions was provided by the presence of three STS markers specific to *A. cristatum*. However, these markers were genetically independent of *PmPB74* and also independent of each other, suggesting that there were multiple small chromosome segments from *A. cristatum* distributed at different chromosomal locations in Pubing 74. Therefore, further studies are needed to develop more markers specific to *A. cristatum* and also linked to *PmPB74*. Nevertheless, the resistance in Pubing 74 was presumably derived from *A. cristatum*, considering that *A. cristatum* was the only parent highly resistant to powdery mildew.

Table 3 Comparative reactions to 28 *Bgt* isolates on Pubing 74 and other lines with known powdery mildew resistance genes on 5DS

Bgt isolates	*A. cristatum*	Pubing 74 (*PmPB74*)	Ulka/8*Cc (*Pm2*)	Tabasco (*Pm48*)	Liangxing 66 (*PmLX66*)	PB3558 (*PmPB3558*)	Mingxian 169	Zhongzuo 9504
E03	0	0	0	0	0	0	4	4
E09	0	0	0	0	1	0	4	4
E11	0	0	0	0	0	0	4	4
E16	0	0	1	0	0	0	4	4
E18	0	0	3	3	4	0	4	4
E20	0	0	4	4	4	0	4	4
E21	0	0	3	0	0	0	4	4
E22	0	0	0	0	4	1	4	4
E23	0	0	0	0	1	0	4	4
Bg44-4	0	0	0	3	3	0	4	4
Bg44-6	0	0	3	4	4	4	3	4
Bg57-5	0	0	0	3	0	0	4	4
Bg69-2	0	0	0	0	0	0	4	4
Bg70-1	0	0	4	0	0	3	4	3
Bg71-2	0	0	1	0	1	0	3	4
Bg74-2	0	0	0	0	0	0	4	4
Bg75-3	1	0	0	0	3	2	4	4
Bg76-1	0	0	4	1	3	1	4	4
Bg77-1	1	0	3	0	3	4	4	3
Bg78-1	0	0	1	1	0	1	4	4
Bg79-1	0	0	4	3	4	0	4	4
Bg80-3	0	0	0	1	0	0	4	4
Bg81-3	0	0	0	0	0	0	4	4
Bg82-1	1	1	4	3	3	3	4	3
Bg83-2	0	0	3	4	3	4	4	4
Bg84-1	1	1	3	0	1	4	3	4
Bg84-3	0	0	3	0	3	4	4	4
Bg85-2	0	0	0	0	0	0	4	4

PmPB74 is a novel resistance gene

PmPB74 occupied a different chromosomal position and conferred a different array of powdery mildew response compared with other genes at or near the *Pm2* locus. *PmPB74* and *PmPB3558* are present in wheat-*A. cristatum* introgression lines, both of which display high levels of resistance to powdery mildew at both the seedling and adult stages (Lu et al. 2015). However, *PmPB74* and *PmPB3558* conferred different response spectra, and an allelism test indicated that they were not allelic. Similar results were also obtained for *PmPB74* and *Pm2*. We also compared the response spectra of lines carrying *PmPB74* and other resistance genes located on wheat chromosome 5DS. When inoculated with 28 *Bgt* isolates, the lines with *Pm48* and *PmLX66* was susceptible to 8 and 12 *Bgt* isolates, respectively (Table 3). When inoculated with seven out of 28 *Bgt* isolates, the lines with *PmD57-5D* and *PmX3986-2* were susceptible to two isolates, and KM2939 was susceptible to one isolate (Table 4). By contrast, *PmPB74* was highly resistant to all the 28 *Bgt* isolates, showing that the resistance spectrum of *PmPB74* was broader than all of the other genes mentioned above. We concluded that *PmPB74* is a novel gene.

PmPB74 is potentially valuable for resistance breeding

Most powdery mildew resistance sources derived from wild relatives of wheat are usually not directly applicable in wheat breeding because of linkage drag. Pubing 74 not only displayed a broad-spectrum of resistance against *Bgt* isolates from northern China, but also exhibited superior agronomic performance without linkage drag. Therefore,

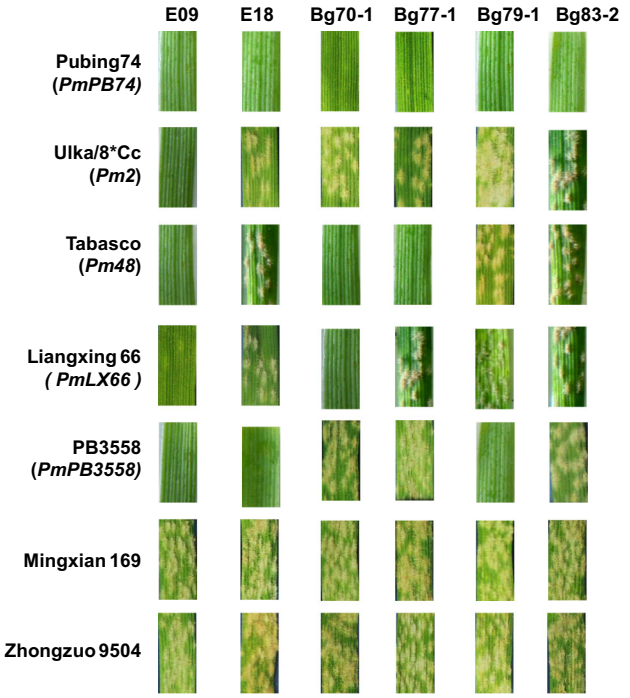

Fig. 6 Powdery mildew reactions of Pubing 74, Ulka/8*Cc, Tabasco, Liangxing 66, PB3558, Mingxian 169 and Zhongzuo 9504 to six *Bgt* isolates

Table 4 Disease reactions to seven *Bgt* isolates on Pubing 74, D57-5D, X3986 and KM2939

Bgt isolates	Pubing 74 (*PmPB74*)	D57 (*PmD57-5D*)	X3986-2 (*PmX3986-2*)	KM2939 (*PmKM2939*)
E03	0	0	0	0
E09	0	0	4	0
E11	0	0	0	0
E18	0	3	2	1
E20	0	4	3	2
E21	0	0	0	3
E23	0	0	0	0

Pubing 74 was an ideal germplasm potentially useful to enhance the powdery mildew resistance at different wheat genetic backgrounds. In this study, no virulence to *PmPB74* was found; therefore, identification of *PmPB74* and its closely linked markers will be useful for breeders to combine it with other powdery mildew resistance genes. Actually, transferring *PmPB74* into different commercial wheat varieties is currently being conducted in our group, and a range of powdery mildew resistant introgression lines have been obtained. In conclusion, the identification of *PmPB74* reported here and its corresponding closely linked molecular markers will be beneficial to increasing the diversity of the genetic sources of powdery mildew resistance.

Author contribution statement L. H. Li and Y. Q. Lu designed the research; M. M. Yao, Y. Q. Lu, J. P. Zhang, L. Q. Song, W. H. Liu, X. M. Yang and X. Q. Li performed technical work; Y. Q. Lu and M. M. Yao analyzed the data. Y. Q. Lu wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Akhunov E, Nicolet C, Dvorak J (2009) Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. Theor Appl Genet 119:507–517

Allen GC, Flores-Vergara MA, Krasynanski S, Kumar S, Thompson WF (2006) A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat Protoc 1:2320–2325

Allen AM, Barker GL, Wilkinson P, Burridge A, Winfield M, Coghill J, Uauy C, Griffiths S, Jack P, Berry S, Werner P, Melichar JP, McDougall J, Gwilliam R, Robinson P, Edwards K (2013) Discovery and development of exome-based, co-dominant single nucleotide polymorphism markers in hexaploid wheat (*Triticum aestivum* L.). Plant Biotechnol J 11:279–295

Avni R, Nave M, Eilam T, Sela H, Alekperov C, Peleg Z, Dvorak J, Korol A, Distelfeld A (2014) Ultra-dense genetic map of durum wheat × wild emmer wheat developed using the 90K iSelect SNP genotyping assay. Mol Breed 34:1–14

Berard A, Le Paslier MC, Dardevet M, Exbrayat-Vinson F, Bonnin I, Cenci A, Haudry A, Brunel D, Ravel C (2009) High-throughput single nucleotide polymorphism genotyping in wheat (*Triticum* spp.). Plant Biotechnol J 7:364–374

Bowen KL, Everts KL, Leath S (1991) Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust. Phytopathology 81:503–511

Briggle LW (1966) Three loci in wheat involving resistance to *Erysiphe graminis* f. sp. *tritici*. Crop Sci 6:461–465

Caceres ME, Pupilli F, Ceccarelli M, Vaccino P, Sarri V, Depace C, Cionini PG (2012) Cryptic introgression of *Dasypyrum villosum* parental DNA in wheat lines derived from intergeneric hybridization. Cytogenet Genome Res 136:75–81

Cavanagh CR, Chao SM, Wang SC, Huang BE, Stephen S et al (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057–8062

Chao S, Dubcovsky J, Dvorak J, Luo MC, Baenziger SP et al (2010) Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). BMC Genom 11:727

Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ (1995) Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/

6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91:1125–1128
Chen G, Zheng Q, Bao YG, Liu SB, Wang HG, Li XF (2012) Molecular cytogenetic identification of a novel dwarf wheat line with introgressed *Thinopyrum ponticum* chromatin. J Biol Sci 37:149–155
Chen PD, You CF, Hu Y, Chen SW, Zhou B, Cao AZ, Wang X (2013) Radiation-induced translocations with reduced *Haynaldia villosa* chromatin at the *Pm21* locus for powdery mildew resistance in wheat. Mol Breed 31:477–484
Dewey DR (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed) Gene manipulation in plant inprovement. Plenum Press, New York, pp 209–279
Dong YC, Zhou RH, Xu SJ, Li LH, Cauderon Y, Wang RRC (1992) Desirable characteristics in perennial Triticeae collected in China for wheat improvement. Hereditas 116:175–178
Everts KL, Leath S (1992) Effect of early season powdery mildew on development, survival, and yield contribution of tillers of winter wheat. Phytopathology 82:1273–1278
Friebe B, Heun M, Tuleen N, Zeller FJ, Gill BS (1994) Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci 34:621–625
Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87
Gao HD, Zhu FF, Jiang YJ, Wu JZ, Yan W, Zhang QF, Jacobi A, Cai SB (2012) Genetic analysis and molecular mapping of a new powdery mildew resistant gene *Pm46* in common wheat. Theor Appl Genet 125:967–973
Gill BS, Friebe BR, White FF (2011) Alien introgressions represent a rich source of genes for crop improvement. Proc Natl Acad Sci USA 108:7657–7658
Han HM, Bai L, Su JJ, Zhang JP, Song LQ, Gao AN, Yang XM, Li XQ, Liu WH, Li LH (2014) Genetic rearrangements of six wheat-*Agropyron cristatum* 6P addition lines revealed by molecular markers. PLoS One 9:e91066
He RL, Chang ZJ, Yang ZJ, Yuan ZY, Zhan HX, Zhang XJ, Liu JX (2009) Inheritance and mapping of powdery mildew resistance gene *Pm43* introgressed from *Thinopyrum intermedium* into wheat. Theor Appl Genet 118:1173–1180
Hsam SLK, Zeller FJ (2002) Breeding for powdery mildew resistance in common wheat (*T. aestivum* L.). In: Belanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) The powdery mildews, a comprehensive treatise. APS Press, St Paul, pp 219–238
Huang XQ, Roder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica 137:203–223
Huang XQ, Hsam SLK, Zeller FJ (1997) Identification of powdery mildew resistance genes in common wheat (*Triticum aestivum* L. em Thell).9. Cultivars, land races and breeding lines grown in China. Plant Breed 116:233–238
Huang J, Zhao ZH, Song FJ, Wang XM, Xu HX, Huang Y, An DG, Li HJ (2012) Molecular detection of a gene effective against powdery mildew in the wheat cultivar Liangxing 66. Mol Breed 30:1737–1745
Huang Q, Li X, Chen WQ, Xiang ZP, Zhong SF, Chang ZJ, Zhang M, Zhang HY, Tan FQ, Ren ZL, Luo PG (2014) Genetic mapping of a putative *Thinopyrum intermedium*-derived stripe rust resistance gene on wheat chromosome 1B. Theor Appl Genet 127:843–853
Jauhar PP, Peterson TS (2006) Cytological analyses of hybrids and derivatives of hybrids between durum wheat and *Thinopyrum bessarabicum*, using multi-colour fluorescent GISH. Plant Breed 125:19–26
Jia JZ, Zhao SC, Kong XY, Li YR, Zhao GY et al (2013) Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496:91–95
Kuraparthy V, Chhuneja P, Dhaliwal HS, Kaur S, Bowden RL, Gill BS (2007a) Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. Theor Appl Genet 114:1379–1389
Kuraparthy V, Sood S, Chhuneja P, Dhaliwal HS, Kaur S, Bowden RL, Gill BS (2007b) A cryptic wheat-*Aegilops triuncialis* translocation with leaf rust resistance gene *Lr58*. Crop Sci 47:1995–2003
Lai KT, Duran C, Berkman PJ, Lorenc MT, Stiller J, Manoli S, Hayden MJ, Forrest KL, Fleury D, Baumann U, Zander M, Mason AS, Batley J, Edwards D (2012) Single nucleotide polymorphism discovery from wheat next-generation sequence data. Plant Biotechnol J 10:743–749
Limpert E, Andrivon D, Felsenstein FG (1988) Influence of different benzimidazole concentrations in agar medium on senescence of wheat leaf segments and on growth and sporulation of the wheat powdery mildew pathogen. J Plant Dis Protect 95:301–306
Lincoln SE, Daly MJ, Lander ES (1993) Constructing linkage maps with MAPMAKER/Exp version 3. 0: a tutorial reference manual, 3rd edn. Whitehead Institute for Medical Res, Cambridge
Liu RH, Meng JL (2003) MapDraw: a microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas 25:317–321
Liu ZY, Sun QX, Ni ZF, Nevo E, Yang TM (2002) Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. Euphytica 123:21–29
Liu J, Chang ZJ, Zhang XJ, Yang ZJ, Li X, Jia JQ, Zhan HX, Guo HJ, Wang JM (2013) Putative *Thinopyrum intermedium*-derived stripe rust resistance gene *Yr50* maps on wheat chromosome arm 4BL. Theor Appl Genet 126:265–274
Liu ZH, Xu M, Xiang ZP, Li X, Chen WQ, Luo PG (2014) Registration of the novel wheat lines L658, L693, L696, and L699, which are resistant to *Fusarium* head blight, stripe rust, and powdery mildew. J Plant Regist 9:121–124
Lu YQ, Wu XY, Yao MM, Zhang JP, Liu WH, Yang XM, Li XQ, Du J, Gao AN, Li LH (2015) Genetic mapping of a putative *Agropyron cristatum*-derived powdery mildew resistance gene by a combination of bulked segregant analysis and single nucleotide polymorphism array. Mol Breed 35:1–13
Luan Y, Wang XG, Liu WH, Li CY, Zhang JP, Gao AN, Wang YD, Yang XM, Li LH (2010) Production and identification of wheat-*Agropyron cristatum* 6P translocation lines. Planta 232:501–510
Ma HQ, Kong ZX, Fu BS, Li N, Zhang LX, Jia HY, Ma ZQ (2011) Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat. Theor Appl Genet 123:1099–1106
Ma PT, Xu HX, Luo QL, Qie YM, Zhou YL, Xu YF, Han HM, Li LH, An DG (2014) Inheritance and genetic mapping of a gene for seedling resistance to powdery mildew in wheat line X3986-2. Euphytica 200:149–157
Ma PT, Xu HX, Xu YF, Li LL, Qie YM, Luo QL, Zhang XT, Li XQ, Zhou YL, An DG (2015) Molecular mapping of a new powdery mildew resistance gene *Pm2b* in Chinese breeding line KM2939. Theor Appl Genet 128:613–622
Matsuda R, Iehisa JC, Takumi S (2012) Application of real-time PCR-based SNP detection for mapping of *Net2*, a causal D-genome gene for hybrid necrosis in interspecific crosses between tetraploid wheat and *Aegilops tauschii*. Genes Genet Syst 87:137–143
McDonald BA, Linde C (2002) The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124:163–180
McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2014) Catalogue of gene symbols for wheat: 2013–2014

supplement. Komugi-wheat genetic resources database. http://www.shigen.nig.ac.jp/wheat/komugi/

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Miranda LM, Murphy JP, Marshall D, Leath S (2006) *Pm34*: a new powdery mildew resistance gene transferred from *Aegilops tauschii* Coss. to common wheat (*Triticum aestivum* L.). Theor Appl Genet 113:1497–1504

Miranda LM, Murphy JP, Marshall D, Cowger C, Leath S (2007) Chromosomal location of *Pm35*, a novel *Aegilops tauschii* derived powdery mildew resistance gene introgressed into common wheat (*Triticum aestivum* L.). Theor Appl Genet 114:1451–1456

Mohler V, Hsam SLK, Zeller FJ, Wenzel G (2001) An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8*/*Pm17* locus of common wheat. Plant Breed 120:448–450

Mohler V, Bauer C, Schweizer G, Kempf H, Hartl L (2013) *Pm50*: a new powdery mildew resistance gene in common wheat derived from cultivated emmer. J Appl Genet 54:259–263

Paillard S, Goldringer I, Enjalbert J, Doussinault G, de Vallavieille-Pope C, Brabant P (2000) Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. I–Is specific seedling resistance selected? Theor Appl Genet 101:449–456

Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of winter wheat (*Triticum aestivum* L.). Theor Appl Genet 107:1235–1242

Parks R, Carbone I, Murphy JP, Marshall D, Cowger C (2008) Virulence structure of the Eastern US wheat powdery mildew population. Plant Dis 92:1074–1082

Petersen S, Lyerly JH, Worthington ML, Parks WR, Cowger C, Marshall DS, Brown-Guedira G, Murphy PJ (2015) Mapping of powdery mildew resistance gene *Pm53* introgressed from *Aegilops speltoides* into soft red winter wheat. Theor Appl Genet 128:303–312

Qiu YC, Sun XL, Zhou RH, Kong XY, Zhang SS, Jia JZ (2006) Identification of microsatellite markers linked to powdery mildew resistance gene *Pm2* in wheat. Cereal Res Commun 34:1267–1273

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schmolke M, Mohler V, Hartl L, Zeller FJ, Hsam SLK (2012) A new powdery mildew resistance allele at the *Pm4* wheat locus transferred from einkorn (*Triticum monococcum*). Mol Breed 29:449–456

Schneider A, Molnar I, Molnar-Lang M (2008) Utilisation of *Aegilops* (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica 163:1–19

Shen XK, Ma LX, Zhong SF, Liu N, Zhang M, Chen WQ, Zhou YL, Li HJ, Chang ZJ, Li X, Bai GH, Zhang HY, Tan FQ, Ren ZL, Luo PG (2015) Identification and genetic mapping of the putative *Thinopyrum intermedium*-derived dominant powdery mildew resistance gene *PmL962* on wheat chromosome arm 2BS. Theor Appl Genet 128:517–528

Shi AN, Leath S, Murphy JP (1998) A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology 88:144–147

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi LL, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genom 4:12–25

Sun YL, Zou JW, Sun HG, Song W, Wang XM, Li HJ (2015) *PmLX66* and *PmW14*: new alleles of *Pm2* for resistance to powdery mildew in the Chinese winter wheat cultivars Liangxing 66 and Wennong 14. Plant Dis 99:1118–1124

Tan YY, Fu HW, Zhao HR, Lu S, Fu JJ, Li YF, Cui HR, Shu QY (2013) Functional molecular markers and high-resolution melting curve analysis of low phytic acid mutations for marker-assisted selection in rice. Mol Breed 31:517–528

Terracciano I, Maccaferri M, Bassi F, Mantovani P, Sanguineti MC, Salvi S, Simkova H, Dolezel J, Massi A, Ammar K, Kolmer J, Tuberosa R (2013) Development of COS-SNP and HRM markers for high-throughput and reliable haplotype-based detection of *Lr14a* in durum wheat (*Triticum durum* Desf.). Theor Appl Genet 126:1077–1101

Wang SC, Wong D, Forrest K, Allen A, Chao SM et al (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. Plant Biotechnol J 12:787–796

Wu J, Yang XM, Wang H, Li HJ, Li LH, Li XQ, Liu WH (2006) The introgression of chromosome 6P specifying for increased numbers of florets and kernels from Agropyron cristatum into wheat. Theor Appl Genet 114:13–20

Xie WL, Ben-David R, Zeng B, Dinoor A, Xie CJ, Sun QX, Röder MS, Fahoum A, Fahima T (2012) Suppressed recombination rate in 6VS/6AL translocation region carrying the *Pm21* locus introgressed from *Haynaldia villosa* into hexaploid wheat. Mol Breeding 29:399–412

Yao GQ, Zhang JL, Yang LL, Xu HX, Jiang YM, Xiong L, Zhang CQ, Zhang ZZ, Ma ZQ, Sorrells ME (2007) Genetic mapping of two powdery mildew resistance genes in einkorn (*Triticum monococcum* L.) accessions. Theor Appl Genet 114:351–358

Ye XL, Lu YQ, Liu WH, Chen GY, Han HM, Zhang JP, Yang XM, Li XQ, Gao AN, Li LH (2015) The effects of chromosome 6P on fertile tiller number of wheat as revealed in wheat-*Agropyron cristatum* chromosome 5A/6P translocation lines. Theor Appl Genet 128:797–811

Yu JK, Dake TM, Singh S, Benscher D, Li W, Gill B, Sorrells ME (2004) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. Genome 47: 805–818

Zhang RQ, Wang X, Chen PD (2012) Molecular and cytogenetic characterization of a small alien-segment translocation line carrying the softness genes of *Haynaldia villosa*. Genome 55:639–646

Zhang J, Zhang JP, Liu WH, Han HM, Lu YQ, Yang XM, Li XQ, Li LH (2015a) Introgression of *Agropyron cristatum* 6P chromosome segment into common wheat for enhanced thousand-grain weight and spike length. Theor Appl Genet 128:1827–1837

Zhang JP, Liu WH, Han HM, Song LQ, Bai L, Gao ZH, Zhang Y, Yang XM, Li XQ, Gao AN, Li LH (2015b) De novo transcriptome sequencing of *Agropyron cristatum* to identify available gene resources for the enhancement of wheat. Genomics 106:129–136