Mapping Powdery Mildew Resistance Gene *pmYBL* on Chromosome 7B of Chinese Wheat (*Triticum aestivum* L.) Landrace Youbailan

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Abstract

Chinese wheat landrace Youbailan has excellent resistance to powdery mildew caused by *Blumeria graminis* f. sp. *tritici*. In the present study, genetic analysis indicated that a recessive gene, tentatively designated *pmYBL*, was responsible for the powdery mildew resistance of Youbailan. *pmYBL* was located in the 695-to-715-Mb genomic region of chromosome 7BL, with 19 gene-linked single-nucleotide polymorphism (SNP) markers. It was flanked by *SNP1-12* and *SNP1-2* with genetic distances of 0.6 and 1.8 centimorgans, respectively. The disease reaction

patterns of Youbailan and four cultivars (lines) carrying the powdery mildew (*Pm*) genes located on chromosome arm 7BL indicated that *pmYBL* may be allelic or closely linked to these genes. All of the SNP markers linked to *pmYBL* were diagnostic, indicating that these markers will be useful for pyramiding *pmYBL* using marker-assisted selection.

Keywords: genetic mapping, powdery mildew, RNA-seq, wheat

Wheat is an important food crop worldwide. Powdery mildew, caused by the fungal pathogen *Blumeria graminis* (DC.) Speer f. sp. *tritici* emend. É. J. Marchal, is a serious disease that reduces wheat production. Fungicides are proven to be effective to control wheat powdery mildew but usually with additional cost and deleterious impact on the environment. In contrast, host resistance has been proposed as an effective and environmentally friendly measure to control the disease. Therefore, it is necessary to explore wheat germplasm resources to identify resistance genes against powdery mildew. At the same time, identifying the resistance genes and their tightly linked molecular markers is necessary for breeding disease-resistant wheat cultivars using marker-assisted selection (MAS). To date, 92 formally designated powdery mildew (*Pm*) resistance genes or alleles at 61 loci (*Pm1* to *Pm65*, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, and *Pm31* = *Pm21*) have been documented (Hao et al. 2008; Li et al. 2019; Singrün et al. 2003; Xie et al. 2012; Zhang et al. 2004). Among them, 5, 3, 17, 5, 5, and 2 alleles have been characterized at six loci; namely, *Pm1*, *Pm2*, *Pm3*, *Pm4*, *Pm5*, and *Pm24*, respectively. Due to the high selection pressure of single disease resistance gene or cultivar deployment in monocultures over a wide area, fast evolution of the *B. graminis* f. sp. *tritici* population often led to the emergence of new virulent *B. graminis* f. sp. *tritici* isolates that could overcome host resistance. Therefore, identifying new disease-resistant germplasm and mapping *Pm* resistance genes are of ongoing interest.

Wheat landraces represent valuable germplasm resources for powdery mildew resistance, from which diverse *Pm* genes or alleles have been identified. Among the formally designated *Pm* genes, *Pm5d*, *Pm5e*, *Pm24*, *Pm24b*, *Pm45*, *Pm47*, and *Pm61* were identified from Chinese wheat landraces IGV1-455, Fuzhuang30, Chiyacao, Baihulu, D57, Hongyanglazi, and Xuxusanyuehuang, respectively (Huang et al. 1997, 2003; Ma et al. 2011; Nematollahi et al. 2008; Sun et al. 2018; Xiao et al. 2013; Xue et al. 2012). *Pm59* and *Pm63* were found from wheat landraces collected from Afghanistan and Iran, respectively (Tan et al. 2018a,b). Provisionally designed powdery mildew resistance genes or alleles from wheat landraces were also reported, such as *MLxbd*, *pmX*, *MIHLT*, *PmSGD*, and *PmBYTT* (Fu et al. 2013; Wang et al. 2015; Xu et al. 2018a,b; Xue et al. 2009).

With the development of molecular biology techniques, molecular markers have been routinely applied in mapping powdery mildew resistance genes in wheat. Among the available molecular marker techniques, single-nucleotide polymorphism (SNP) is preferred for gene mapping due to the larger numbers and high polymorphisms for genotyping (Ball et al. 2010; Yu et al. 2011). In addition, the next-generation sequencing technology with bulked segregant analysis (BSA) is an efficient method to rapidly and efficiently map genes responsible for mutant phenotypes and, ultimately, develop a precise genetic linkage map. Many disease resistance genes from wheat have been mapped using this method, including stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) resistance genes *Yr15* (Ramirez-Gonzalez et al. 2015), *YrZH22* (Wang et al. 2017), *Yr26* (J. Wu et al. 2018), *YrMM58* and *YrHY1* (Wang et al. 2018), and *YrZM103* (Zhang et al. 2017), and powdery mildew resistance genes *Pm4b* (P. Wu et al. 2018) and *PmSGD* (Xu et al. 2018b).

Chinese wheat landrace Youbailan is highly resistant to powdery mildew. The present article reports the identification and molecular mapping of a powdery mildew resistance gene from Youbailan using RNA sequencing (RNA-seq) with BSA. A high-density molecular map of the powdery mildew resistance gene in Youbailan will be of great value in wheat molecular breeding and gene cloning.

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Materials and Methods

Plant material and *B. graminis* f. sp. *tritici* isolates. F₁ hybrids and BC₁, F₂, and F_{2:3} segregating generations were developed from

the cross between Youbailan and highly susceptible cultivar Chancellor. A segregating F₂ population consisting of 231 individuals and their derivative F_{2:3} progenies were used to evaluate the powdery mildew response. Four cultivars—namely, Fuzhuang 30 (*Pm5e*), Hongyoumai (*PmHYM*), Xiaobaidongmai (*Mlxbd*), and Tangmai 4 (*PmTm4*)—which carry known *Pm* genes or alleles mapped in the same chromosome region on 7BL were selected for powdery mildew reaction comparison with Youbailan using 28 *B. graminis* f. sp. *tritici* isolates. In all, 39 wheat cultivars (lines) with known *Pm* genes were used to test the genetic diversity of the 7BL chromosome region using a set of markers linked to the newly identified *Pm* gene in Youbailan. Wheat cultivar Jingshuang 16 was used to increase the *B. graminis* f. sp. *tritici* isolates. All germplasm and *B. graminis* f.

sp. *tritici* isolates used in this study were maintained at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.

Evaluation of powdery mildew resistance. Seed of the F₁, BC₁, and F₂ populations and the F₂-derived F₃ lines (15 individuals of each line) were planted in plastic trays. Youbailan and Chancellor, as well as wheat landraces Fuzhuang 30, Hongyoumai, and Xiaobaidongmai and cultivar Tangmai 4, were planted in pots (10 cm in diameter, 6 to 8 seeds/cultivar). When the first leaves were fully expanded, inoculation was performed by dusting conidia from the sporulating susceptible seedlings of Jingshuang 16 onto the tested seedlings. After inoculation, the seedlings were maintained in an MLR-352H-PC Panasonic Incubator (PHC Corporation of North America, Wood

Fig. 1. Density distribution of single-nucleotide polymorphism loci on 21 wheat chromosomes.

Table 1. Primers of 31 single-nucleotide polymorphism (SNP) markers for mapping powdery mildew resistance gene *pmYBL*^a

SNP ID	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	Extended primer sequence (5'–3')
SNP1-2	ACGTTGGATGTACCTGAGCAACAAGAAGGC	ACGTTGGATGTGACCCATCATCTTTGCTG	TGACTAGACCTTCTGG
SNP1-3	ACGTTGGATGATAAGCAAGAATGCACCC	ACGTTGGATGTCCATAGTCAAACCATCTC	CACCCCCCAAGTTA
SNP1-4	ACGTTGGATGAAGTTGGAGTGGTAGCATC	ACGTTGGATGAAGATCACATCAGCCATCCC	CACATATATTGGCGCTTTTITAG
SNP1-10	ACGTTGGATGCAAACTTCTCACTGCAACC	ACGTTGGATGCCTCAACTATAAACCGCATC	CAGTCAACCATTTCC
SNP1-12	ACGTTGGATGTACGTATGCAGGTTTCTCGG	ACGTTGGATGTTGACTGTTTCTGCGAGTCC	CCTCCTCCTCTAGCGCTAGGGA
SNP1-13	ACGTTGGATGACGACACGTCTGTGACTTTG	ACGTTGGATGATAGTACTCTGCACCAAGC	CCTGACTTTACAATGAAATTAACAACC
SNP1-16	ACGTTGGATGTGTTTGGTGTGTTGATCCCC	ACGTTGGATGTCATCGCTGTCAAGAAGCTG	TGTTTGATCCCCATAAGATAAC
SNP1-18	ACGTTGGATGTAAATGGGCGGAGGACATTG	ACGTTGGATGATATCAGGAATGGTGGAGGG	GGGTGAGGACATTGTGCAACC
SNP1-19	ACGTTGGATGAACAGAGGTTTCTTGACGCG	ACGTTGGATGATAGCAGAAGTGCTGGGAAC	CTCTAGGCTCTGAAACTATTTCTAG
SNP1-20	ACGTTGGATGTGTTCTCTCAGCTACTTCC	ACGTTGGATGATTGCAGGAGGATGTAGCAG	AGCAGAACCTTCGCA
SNP1-21	ACGTTGGATGGTACCATCTTAGTCAACGAC	ACGTTGGATGGCGTTGTCTCTGCTTATGTC	ATTTTGAAATGAGAATCTGTTTCTCT
SNP1-23	ACGTTGGATGGCTAAACTACTGTTGCATGA	ACGTTGGATGAGAAGACCCCTTTGTTGCCAT	CACAAAATTACACTTCAATATTTCC
SNP1-24	ACGTTGGATGGCTAATTGACTTCTTGGTAG	ACGTTGGATGCTGGATGAGCATATTTGGAC	TGGTAGTTGTAGACGGA
SNP1-26	ACGTTGGATGAAGCGGCAGAAGGCATATAC	ACGTTGGATGCGGATCATAGTAAGCTCACC	CCAAGGAACAACACTACTGAT
SNP1-27	ACGTTGGATGAGCTTCTTACCGCAATCAC	ACGTTGGATGGTCTAATACAGAGCACACG	GGCGTTTAAACAGGGGATTGTGCC
SNP1-31	ACGTTGGATGTACTCTACGTTCTAGGTTTC	ACGTTGGATGTAGCAGCAGGACCTACAAG	GGAGTTTCTTATGAAATACAATAAA
SNP1-32	ACGTTGGATGTGATCCCCATGATCATGGAC	ACGTTGGATGTGCGACACCACTTCTGTAG	CGTAGATGCCGGATGTGG
SNP1-34	ACGTTGGATGGCTCCACATGAATCCATTCC	ACGTTGGATGGCATGATTGTGAGTGTGG	TTAGTGGTACCTGAGCATTA
SNP1-35	ACGTTGGATGATGGTCATGGAGGACTCCGA	ACGTTGGATGAAAGAGCAGGGGCTGCTTGT	CAGAACAGGAGGAGGTGGTG
SNP1-36	ACGTTGGATGCCCTGAACCTGCAGCTTTG	ACGTTGGATGCTCAACCTCAACCTTTTCC	GTCTGGTGCAATTTTGGTATT
SNP1-41	ACGTTGGATGAGAAGGGTACATGACAGTGG	ACGTTGGATGCACAAAGAAGAGGAGCAAG	CCCCGCCAAATGTCGGATTCC
SNP1-42	ACGTTGGATGCCATTTTGCTTTAGGAACC	ACGTTGGATGCAAAGCTGTGACCAGTTACC	AGGCCGAAGTGCAGGATTGTCTAA
SNP1-43	ACGTTGGATGCAGATGACAAAGTCAAGTCTC	ACGTTGGATGGAATCAGCACAAGCATGGTC	GGAGCAGAATGGCGAGGGC
SNP1-44	ACGTTGGATGAACGAGGGAAGTCTAGCTAC	ACGTTGGATGGATCATTTTTAGTTCGGAGC	CCAGTTCAAATAAATGCTATTTCAATGA
SNP1-48	ACGTTGGATGTATCTTAACCTAGTCCCCC	ACGTTGGATGCTCTGTTACAGTCTCTCAGG	GCAAGTTCATCGTATCTAAATTATGGCG
SNP1-50	ACGTTGGATGGGTATGCAGGATCGAAATG	ACGTTGGATGAACCATATAAAGGGCCACG	GGTGTGACAGTACCTGAATCTGA
SNP1-51	ACGTTGGATGTTTGTAGTGGAAGCAAGGAC	ACGTTGGATGCATGAGAGGTTTATCACTC	ATGACGGGATCACAAA
SNP1-53	ACGTTGGATGACGAGTCATCTTGACCAAAC	ACGTTGGATGTGTCGAAGTTGAAGACTGGG	TGGTAAGAATCTGTGAATGATGAGAC
SNP1-54	ACGTTGGATGTGTTGGTATGCGGACAATG	ACGTTGGATGCACAATGCTAGGTTGCTTGG	GGTGGCTTGGGAGTGTCCAAGG
SNP1-56	ACGTTGGATGGATCAGCCAAGATGGTGAAG	ACGTTGGATGTCTATCTTGACAGTAGAGA	CTCTCAACTTGAAAGATTATCTGGC
SNP1-60	ACGTTGGATGGTGCAACACAGGGTTTGCTT	ACGTTGGATGGCTTCTAAGCATGTTCTGG	CCAGCGTCACAGAATGCC

^a Annealing temperature of the first-round PCR was 56°C and the second-round PCR was 52°C.

Dale, IL, U.S.A.) at a temperature of 18°C under a daily cycle of 12 h of light and 12 h of darkness.

Infection types (ITs) were scored at 10 days postinoculation (dpi) based on six degrees of infection (0, 0; 1, 2, 3, and 4) (Sheng 1988; Xu et al. 2018a). Plants with ITs 0 to 2 were classified as resistant and those with ITs 3 to 4 as susceptible. According to the reactions of the $F_{2:3}$ lines, the corresponding individual lines were divided into three groups: homozygous resistant, heterozygous, and homozygous susceptible. The χ^2 tests for goodness-of-fit were performed to evaluate deviations of the observed phenotypic data from the expected segregation ratios for the F_2 population and $F_{2:3}$ lines.

RNA sequencing. Segments of inoculated leaf tissues (approximately 3 cm) from the parental cultivars Youbailan and Chancellor and the resistant and susceptible bulks (40 homozygous resistant and 40 homozygous susceptible lines) were sampled at 10 dpi for RNA isolation. Sequencing was performed using an Illumina HiSeq X Ten platform (Shanghai OE Biotechnology Co., Ltd., Shanghai, China).

SNP analysis. SNPs were extracted using software SAM tools (Li et al. 2009). SNP loci with a depth (DP) value ≥ 4 were retained from the original SNP calling data. To obtain homozygous traits associated with markers from different parents, the putative SNP loci were trimmed based on an SNP index ≥ 0.8 (Takagi et al. 2013). To classify and prioritize the SNP loci, the bulk frequency ratio (BFR) was calculated in the corresponding bulk as previously described (Ramirez-Gonzalez et al. 2015; Trick et al. 2012). Putative SNP loci with a value over the BFR threshold of 4 were used to draw a high-density diagram across the whole genome with intervals of 10 Mb. *Pm* gene locations were determined according to the density peak of the SNPs.

Mapping the powdery mildew resistance gene of Youbailan using SNP markers. Genomic DNA was extracted as previously described (Rogers and Bendich 1985). The genotypes obtained from screening the DNA of each F_2 plant derived from the cross of Youbailan $\times$ Chancellor for 31 polymorphic SNP markers, which were located in the mapped *Pm* gene positional interval, were examined for genetic mapping. Genotyping was performed using Sequenom iPLEX Gold (Sequenom, San Diego, CA, U.S.A.) assays. A linkage map of the chromosome region carrying the target gene was constructed using JoinMap 4.0 software (<https://joinmap.software.informer.com/4.0/>). The linkage group was identified based on a logarithm of odds threshold of 3.0. Recombination fractions were converted into centimorgan distances, which were estimated using the Kosambi map function. The genetic map was generated with MapDraw V2.1 (Liu and Meng 2003).

Results

Phenotype and inheritance of powdery mildew resistance in Youbailan. At the seedling stage, 28 *B. graminis* f. sp. *tritici* isolates were used to inoculate Youbailan to evaluate its powdery mildew resistance. Youbailan was resistant to all tested isolates with ITs of 0; (E07, E09, E11, E15, E16, E18, E23-1, E23-2, E24, E26, E30-1, E31, E32, E49, E50, E60, E67, E69, E70, E71, Bgt-1, Bgt-2, and Bgt-3) or 1 (E01, E05, E06, E13, and E68), indicating excellent powdery mildew resistance.

Youbailan, Chancellor, and their F_1 , BC_1 , F_2 , and $F_{2:3}$ populations were inoculated with *B. graminis* f. sp. *tritici* isolate E09. Youbailan was highly resistant with an IT 0; and Chancellor was highly susceptible with an IT 4. In all, 11 F_1 (Youbailan/Chancellor) and 19 hybrid BC_1 (Youbailan/Chancellor/Chancellor) individuals were susceptible, with IT of 4. The 231 F_2 plants segregated for 54 resistant and 177 susceptible plants, fitting a 1:3 segregation ratio ($\chi^2 = 0.33$, $P = 0.57$). The 231 F_2 -derived $F_{2:3}$ lines segregated for 54 homozygous resistant, 120 segregating, and 57 homozygous susceptible lines, fitting a 1:2:1 segregation ratio ($\chi^2 = 0.43$, $P = 0.81$). These results indicated that the resistance of Youbailan to *B. graminis* f. sp. *tritici* isolate E09 was controlled by a recessive gene, tentatively designated *pmYBL*.

SNP calling and identification of *pmYBL* genomic interval. The parents Youbailan and Chancellor, as well as the homozygous resistant (R-pool) and homozygous susceptible (S-pool) bulks, were subjected to

RNA-seq analysis using an Illumina HiSeq X Ten platform, and 197.7, 206.9, 206.8, and 191.4 million clean reads, respectively, were obtained.

Using a DP threshold of 4 and selecting SNP loci of the two parents based on an SNP index ≥ 0.8 , 126,026 SNP loci were obtained. To further identify SNP loci associated with the powdery mildew resistance, the SNP loci for the resistant and susceptible bulks were filtered based on a BFR ≥ 4 to classify and prioritize the SNP loci. In total, 9,380 SNPs were retained. The density distribution of SNP loci

Fig. 2. Genetic linkage map for the powdery mildew resistance gene *pmYBL* on chromosome arm 7BL, with genetic distances in centimorgans shown on the left and locus names on the right. SNP = single-nucleotide polymorphism.

showed a strong peak in the 695-to-715-Mb region on chromosome 7B, indicating a high probability of linkage with the resistance gene in Youbailan (Fig. 1).

Mapping of the *pmYBL* gene. Thirty-one SNPs (Table 1) on chromosome arm 7BL in the 695-to-715-Mb region were selected for designing and validating PCR primers. Nineteen polymorphic SNP markers were identified and used for genotyping the F_2 segregation population. *pmYBL* was mapped within a 42.8-centimorgan (cM) genetic interval, as defined by markers *SNP1-18* to *SNP1-44*. The average genetic distance between the two neighboring SNP markers in this region was 2.25 cM. The nearest SNP markers to *pmYBL* were *SNP1-12* and *SNP1-2*, with genetic distances of 0.6 and 1.8 cM, respectively, from the target gene (Fig. 2).

Comparison of the reactions to *B. graminis* f. sp. *tritici* and integration mapping of *Pm* genes mapped on chromosome 7BL. In addition to *pmYBL* from Youbailan, several powdery mildew resistance genes or alleles such as *Pm5e*, *PmHYM*, *Mlxbd*, and *PmTm4*

were identified from Chinese wheat landraces Fuzhuang 30, Hongyoumai, and Xiaobaidongmai and cultivar Tangmai 4, respectively, and previously mapped on chromosome arm 7BL. The disease reactions of Youbailan, Fuzhuang 30, Hongyoumai, Xiaobaidongmai, and Tangmai 4 to the 28 *B. graminis* f. sp. *tritici* isolates were evaluated. Youbailan was resistant to all tested isolates, whereas Fuzhuang 30 was susceptible to isolates E06, E07, E11, E13, and E23-1; Hongyoumai was susceptible to E01, E06, E13, and E23-1; and Xiaobaidongmai and Tangmai 4 were susceptible to isolate E68 (Table 2).

Based on the sequences of the tightly linked molecular markers to the *Pm* genes identified on 7BL, including *Pm5d*, *PmTm4*, *Mlxbd*, *PmHYM*, *PmBYYT*, *PmSGD*, and *pmYBL*, and their physical positions in the Chinese Spring 7B reference genome, an integrated linkage map was constructed and those seven genes could be placed on a 56.6-Mb physical interval (661.9 to 718.5 Mb) (Fig. 3).

Validation of the SNP markers linked to *pmYBL* in *Pm*-resistant germplasm. To evaluate the efficiency of SNP markers

Table 2. Reactions of wheat cultivars Youbailan, Fuzhuang 30, Hongyoumai, Xiaobaidongmai, and Tangmai 4 and the susceptible control Chancellor to 28 *Blumeria graminis* f. sp. *tritici* isolates

Cultivars (lines)	<i>B. graminis</i> f. sp. <i>tritici</i> isolates ^a								
	E01	E05	E06	E07	E09	E11	E13	E15	E16
Youbailan	1	1	1	0;	0;	0;	1	0;	0;
Fuzhuang 30	1	1	3	3	1	3	3	0;	0
Hongyoumai	3	1	3	ND	1	ND	4	ND	0
Xiaobaidongmai	1	2	1	1	0;	1	1	0;	0;
Tangmai 4	1	2	1	1	0;	1	1	0;	0;
Chancellor	4	4	4	4	4	4	4	4	4

(Continued on next page)

^a ND = no data.

Fig. 3. Powdery mildew (*Pm*) genes mapped on chromosome 7BL were compared using tightly linked markers with those genes to blast against the Chinese Spring 7B genomic sequences (physical positions in megabases shown on the left and locus names or gene names shown on the right). **A**, Physical interval Xgwm611 to W7BL-15 of section **B**, expanded version.

linked to *pmYBL* in detecting the target powdery mildew resistance genes in wheat breeding programs, we tested the gene-linked SNP markers in a set of 39 wheat cultivars (lines) with known *Pm* genes (Fig. 4). *SNP1-26* was the most diagnostic SNP marker, showing 97% agreement with the presence of *pmYBL*, followed by *SNP1-3* and *SNP1-2*, which were both present with 92% accuracy. The nearest marker, *SNP1-12*, has a diagnostic ability of 72%, and *SNP1-2* has a diagnostic ability of 67%. In total, 9 (*SNP1-3*, *SNP1-4*, *SNP1-16*, *SNP1-18*, *SNP1-19*, *SNP1-21*, *SNP1-23*, *SNP1-26*, and *SNP1-44*) and 14 (*SNP1-2*, *SNP1-3*, *SNP1-4*, *SNP1-21*, *SNP1-23*, *SNP1-26*, *SNP1-34*, *SNP1-43*, *SNP1-44*, *SNP1-48*, *SNP1-51*, *SNP1-53*, *SNP1-56*, and *SNP1-60*) SNP markers could be used as diagnostic markers to distinguish Fuzhuang 30 and Chiyachao, respectively, from Youbailan. All 19 SNPs could be used as diagnostic markers to differentiate Xiaobaidongmai from Youbailan.

Candidate genes. The physical positions of *SNP1-2* and *SNP1-12* were found on chromosome 7B:704271943 and 7B:709198270, respectively. Therefore, *pmYBL* is located in this genomic interval. According to information from the Chinese Spring genome, 70

genes were annotated between markers *SNP1-2* and *SNP1-12*. Among those genes, 11 and 15 differential expression genes were identified between the parents and the bulks, respectively. Nine genes that differentially expressed between both parents and bulks were considered as candidate genes of *pmYBL*. Among them, six genes were upregulated in expression and three genes were down-regulated in expression (Table 3).

Discussion

Many *Pm* genes have been identified and localized on different wheat chromosomes, providing a good number of powdery mildew resistance genes for wheat breeding. However, due to monoculture of wheat cultivars carrying a single resistance gene in a wide area and the coevolution of pathogen virulence genes, some resistance genes have become ineffective within a short period of agricultural use (Hsam et al. 2001). Hence, for the sustainable protection of wheat from powdery mildew, continuous efforts are necessary to search for new resistance genes (Xiao et al. 2013). In the present study, Chinese wheat landrace Youbailan showed excellent resistance to 28 tested

Table 2. (Continued from previous page)

<i>B. graminis</i> f. sp. <i>tritici</i> isolates ^a																		
E18	E23-1	E23-2	E24	E26	E30-1	E31	E32	E49	E50	E60	E67	E68	E69	E70	E71	Bgt-1	Bgt-2	Bgt-3
0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1	0;	0;	0;	0;	0;	0;
0;	3	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	1	0;	0	0	0;	0;	0;
0;	3	0;	0;	ND	0;	ND	0;	0	0;	0;	0;	1	0	0	0	0;	0;	0
0;	1	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	3	0;	0;	0;	0;	0;	0;
0;	1	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	3	0;	0;	0;	0;	0;	0;
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Fig. 4. Single-nucleotide polymorphism loci linked to *pmYBL* were tested across 39 varieties with known powdery mildew (*Pm*) genes.

Table 3. Differentially expressed genes in powdery-mildew-resistant parent Youbailan versus susceptible parent Chancellor or in the resistant bulk versus the susceptible bulk in the genomic region between markers *SNP1-2* and *SNP1-12*

Gene ID	Location	Up or down expression ^a		Description
		P _R versus P _S	R versus S bulk	
<i>TraesCS7B02G438200</i>	7B:704279683–704282776	–	Up	Hypothetical protein TRIUR3_03383
<i>TraesCS7B02G438300</i>	7B:704286867–704289083	–	Up	Predicted protein
<i>TraesCS7B02G438500</i>	7B:704316702–704317852	–	Up	Premnaspirodiene oxygenase
<i>TraesCS7B02G438800</i>	7B:704625191–704628034	–	Up	Predicted protein
<i>TraesCS7B02G439100</i>	7B:704667966–704672613	Up	Up	Predicted: protein MAK16 homolog
<i>TraesCS7B02G439900</i>	7B:705251306–705254066	Up	Up	Predicted protein
<i>TraesCS7B02G440000</i>	7B:705254105–705257483	Up	Up	E3 ubiquitin-protein ligase KEG
<i>TraesCS7B02G440100</i>	7B:705733614–705736627	Up	Up	Endo-1,3;1,4-β-D-glucanase
<i>TraesCS7B02G440900</i>	7B:706107402–706109634	Up	Up	Secologanin synthase
<i>TraesCS7B02G441100</i>	7B:706159712–706162097	Up	Up	Predicted protein
<i>TraesCS7B02G441200</i>	7B:706383400–706387417	–	Down	Predicted protein
<i>TraesCS7B02G442200</i>	7B:706977079–706980031	Down	Down	Hypothetical protein F775_05928
<i>TraesCS7B02G443000</i>	7B:707912516–707913888	–	Up	Hypothetical protein TRIUR3_32371
<i>TraesCS7B02G443100</i>	7B:707946356–707946526	Up	–	Hypothetical protein TRIUR3_01690
<i>TraesCS7B02G444800</i>	7B:709047924–709049408	Down	Down	Predicted protein, partial
<i>TraesCS7B02G444900</i>	7B:709081284–709082771	Down	–	1-Aminocyclopropane-1-carboxylate oxidase
<i>TraesCS7B02G445000</i>	7B:709093597–709095609	Down	Down	Predicted protein, partial

^a P_R and P_S represent Youbailan and Chancellor, respectively; R and S represent resistant and susceptible, respectively; and – indicates no difference.

isolates, indicating its broad-spectrum resistance against *B. graminis* f. sp. *tritici*. Genetic analysis revealed that a recessive gene, named *pmYBL*, confers resistance to powdery mildew in Youbailan. *pmYBL* was assigned to a 695-to-715-Mb physical interval on chromosome arm 7BL and was flanked by SNP markers *SNP1-12* and *SNP1-2* with genetic distances of 0.6 and 1.8 cM, respectively. A saturated genetic map with cosegregated or tightly linked markers is necessary for map-based cloning and MAS of the target gene. In the present study, 19 SNP markers were found to be linked to the *pmYBL* gene. After testing 39 cultivars (lines) with different known *Pm* genes, *SNP1-26* was the most diagnostic marker, followed by the *SNP1-3* and *SNP1-2*, which could be used for MAS of *pmYBL* in wheat breeding programs.

To date, 13 powdery mildew resistance genes or alleles, including *Pm5a-e*, *PmH*, *PmTm4*, *Mlxbd*, *PmHYM*, *PmBYYT*, *PmSGD*, *PmDHT*, and *pmYBL*, have been mapped to the distal region of chromosome 7BL (Hsam et al. 2001; Hu et al. 2008; Huang et al. 2003; Qie et al. 2019; Wang et al. 2009; Xu et al. 2018a,b; Xue et al. 2009). Due to limited markers and sequence information of *Pm5a*, *Pm5b*, *Pm5c*, *Pm5e*, *PmH*, and *PmDHT*, these genes were not placed on the integrated map. The other seven genes could be placed on a 56.6-Mb physical interval (661.9 to 718.5 Mb), and only *PmBYYT* was at a different position. Overlapping of *Pm5d*, *Mlxbd*, and *PmHYM* was observed and more markers are needed to distinguish them. *PmSGD* and *PmTm4* were found at different positions. *pmYBL* was placed at different position from *PmSGD* but shared an overlap with *PmTm4*. Meanwhile, 28 different *B. graminis* f. sp. *tritici* isolates were able to distinguish *Pm5e*, *PmHYM*, *Mlxbd*, *PmTm4*, and *pmYBL*. The integrated linkage map and disease response patterns of these genes indicated that a cluster of *Pm* genes or different alleles may exist on the distal region of chromosome 7BL.

Resistance gene *pmYBL* was located on the 7B:704271943-7B:709198270 physical interval of Chinese Spring chromosome 7B, with 17 differentially expressed genes annotated. Although 9 of these genes were shared by the comparisons of both parents and bulks, 4 may be associated with disease resistance. Gene *TraesCS7B02G439100* is a MAK16 homolog protein with two nuclear localization signals (Milhon et al. 2000). Gene *TraesCS7B02G440000* is an E3 ubiquitin-protein ligase that specialized in recognizing the target proteins and played an important role in the ubiquitin pathway (Lyzenga et al. 2013). Gene *TraesCS7B02G440100* is an endo-1,3;1,4-β-D-glucanase that functions on decomposing fungal cell walls and could be related to disease resistance. Singh et al. (2018) found that transgenic tea overexpressing the *Solanum tuberosum* endo-1,3-β-D-glucanase gene conferred resistance to blister blight disease. Gene *TraesCS7B02G444900* is a 1-aminocyclopropane-1-carboxylate (ACC) oxidase. ACC oxidase is a

key enzyme in ethylene synthesis, and ethylene plays an important role in plant disease resistance. However, the expression of the ACC oxidase gene was downregulated in Youbailan and, therefore, the ethylene pathway may not be related to the *Pm* resistance. Further studies are needed to determine the functions of these candidate genes and their relationship to powdery mildew resistance.

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