Chromosomal Location and Comparative Genomics Analysis of Powdery Mildew Resistance Gene Pm51 in a Putative Wheat-Thinopyrum ponticum Introgression Line

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Abstract

Powdery mildew (PM) is a very destructive disease of wheat (Triticum aestivum L.). Wheat-Thinopyrum ponticum introgression line CH7086 was shown to possess powdery mildew resistance possibly originating from Th. ponticum. Genomic in situ hybridization and molecular characterization of the alien introgression failed to identify alien chromatin. To study the genetics of resistance, CH7086 was crossed with susceptible genotypes. Segregation in F2 populations and F2:3 lines tested with Chinese Bgt race E09 under controlled conditions indicated that CH7086 carries a single dominant gene for powdery mildew resistance. Fourteen SSR and EST-PCR markers linked with the locus were identified. The genetic distances between the locus and the two flanking markers were 1.5 and 3.2 cM, respectively. Based on the locations of the markers by nullisomic-tetrasomic and deletion lines of ‘Chinese Spring’, the resistance gene was located in deletion bin 2BL-0.89-1.00. Conserved orthologous marker analysis indicated that the genomic region flanking the resistance gene has a high level of collinearity to that of rice chromosome 4 and Brachypodium chromosome 5. Both resistance specificities and tests of allelism suggested the resistance gene in CH7086 was different from previously reported powdery mildew resistance genes on 2BL, and the gene was provisionally designated PmCH86. Molecular analysis of PmCH86 compared with other genes...
for resistance to Bgt in the 2BL-0.89-1.00 region suggested that PmCH86 may be a new PM resistance gene, and it was therefore designated as Pm51. The closely linked flanking markers could be useful in exploiting this putative wheat-Thinopyrum translocation line for rapid transfer of Pm51 to wheat breeding programs.

Introduction

Powdery mildew (PM), caused by Blumeria graminis f. sp. tritici (Bgt), is a globally important disease of wheat (Triticum aestivum L.). Resistant varieties are the most feasible means of controlling the disease and reducing yield losses. To date, 54 formally designated Pm resistance genes have been identified. They have been mapped to 46 loci and assigned to specific chromosomes or chromosome arms [1]. Of these loci, 29 genes were transferred from relatives, including T. turgidum var. dicoccoides, T. timopheevii, T. monococcum, Aegilops tauschii, Ae. speltoides, Ae. longissima, Ae. ovata, and from more distantly related species, including Secale cereale, Dasypyrum villosum, and Thinopyrum intermedium [2]. However, many resistance genes become ineffective because of frequent changes in pathogen populations, especially when a single resistance gene is deployed over a wide area. Therefore, new sources of effective and durable resistance from both common wheat and wild relatives are required for resistance breeding.

Tall wheatgrass, Thinopyrum ponticum (Podp.) Z.-W. Liu & R.-C. Wang [syn. Agropyron elongatum (Host) Beauv., Lophopyrum ponticum (Podp.) A. Löve, and Elytrigia elongata (Host) Nevski], has been one of the most beneficial perennial species that conferred valuable genetic variability for wheat improvement. In addition to wheat rust resistances, Th. ponticum displays resistance to powdery mildew, eyespot, wheat streak mosaic virus (WSMV), wheat curl mite (WCM), Cephalosporium stripe, and Fusarium head blight - [3]. As for rust resistance transferred from Th. ponticum, three genes for resistance to leaf rust viz. Lr19, Lr24, and Lr29, and three genes for resistance to stem rust, viz. Sr24, Sr25, and Sr26, were reported in wheat-Th. ponticum translocation derivatives [4, 5]. However, there are few reports on the transfer of powdery mildew resistance from Th. ponticum to wheat [3].

So called cryptic translocations between wheat and alien chromatin have been reported on a number of occasions. These are alien transfers that cannot be visualized by cytological means, and are also usually not detectable with markers. Recent examples were the transfers of rust resistances from Ae. geniculata and Ae. triuncialis to wheat [6, 7]. Genomic rearrangements in wheat hybrids due to cryptic introgressions of small chromosome segments from Dasypyrum villosum and Th. ponticum to wheat were also reported recently [8, 9]. However, with materials having putative cryptic translocations that cannot be detected cytologically or with markers there is always the question of alien identity.
CH7086, a _Th. ponticum_-derived wheat introgression line, was resistant to powdery mildew under greenhouse conditions in Taiyuan, Shanxi province. The resistance gene was preliminarily assigned to chromosome arm 2BL [10]. The objectives of the present study were to characterize this potential new cryptic wheat- _Th. ponticum_ translocation, and to determine its location using microsatellite and comparative genomic molecular marker analyses.

**Materials and Methods**

**Plant materials and populations**

The materials used in this study were _Th. ponticum_ (accession R431) with the genomic formula JJJ JJ [11]; partial amphiploid, Xiaoyan 7430, derived from accession R431 and provided by the Crop Science Institute, Shanxi Academy of Agricultural Sciences, Taiyuan; wheat genotypes ‘CH7086’, ‘CH5241’, ‘Zhong 8701’, ‘Jimai 26’, ‘Xiangyang 4’, ‘Misuizao’, and ‘Chinese Spring (CS)’; and various ‘CS’ nullisomic-tetrasomic (NT) stocks and deletion lines, obtained from Dr. B. Friebe, Wheat Genetic and Genomic Resources Center, Kansas State University, USA. CH7086 and CH5241 are homogeneous BC2F5-derived wheat lines obtained from the cross Zhong 8701/Xiaoyan 7430//2*Jimai 26. CH7086 is resistant to powdery mildew whereas CH5241 is susceptible. Xiaoyan 7430, the resistance donor of CH7086, was derived from the cross Misuizao/R431//Xiangyang 4 [12].

To investigate the inheritance of powdery mildew resistance introgressed from _Th. ponticum_, CH7086 was crossed to susceptible cultivars CH5241, Taichung 29, SY95-71, and Jintai 170 to generate segregating populations. The F2 and F3 were tested for segregation of powdery mildew response. An F2 population of 154 plants and 148 derived F3 lines from CH7086/CH5241 were used for microsatellite screening and gene mapping. CH7086, Xiaoyan 7430, the original mildew resistant donor accession (R431) of _Th. ponticum_, and CS were also used for genomic in situ hybridization and C-banding analyses to determine the chromosomal composition in Xiaoyan 7430 and the size of any alien introgressions in CH7086.

**Genomic in situ hybridization**

Seedling root tips were collected, pretreated in ice water for 24 h and fixed in ethanol-acetic acid (3:1) for one week. Root-tip squashes and the conventional Giemsa-C banding methods were performed according to Gill et al. [13]. For GISH analysis, total genomic DNA from _Th. ponticum_ was labeled with fluorescein-12-dUTP by nick translation following the manufacturer’s instructions (Roche Diagnostics, Indianapolis, IN). Sheared genomic DNA of Chinese Spring wheat (AABBDD, 2n=6x=42) was used as blocking DNA, and the probe-to-blocker ratio was approximately 1:120. The detection and visualization of GISH signals were performed as described by Han et al. [14]. Images of GISH and C-banded
chromosomes were taken with an Olympus BX-51 microscope using a DP70 CCD camera (Olympus, Japan).

**Testing for powdery mildew response**

The seedling reactions of line CH7086 and the parental lines inoculated with four Bgt isolates, provided by the Plant Protection Institute, Chinese Academy of Agricultural Sciences, are shown in Table 1. Among the Bgt isolates tested, E09, a prevalent pathotype in the Beijing area, is virulent to Pm1, Pm3a, Pm3c, Pm3e, Pm5a, Pm6, Pm7, Pm8, Pm17, and Pm19 [15]; E20 and E21 are the most widely virulent pathotypes in China and are virulent to most of the Pm genes including Pm4a, Pm4b, PmPs5A, and Pm33. E26 is virulent to Pm4b and Pm33, but avirulent to Pm4a and PmPs5A [16, 17]; E15, avirulent to Pm6, was used for the test of allelism between Pm6 and the resistance gene in CH7086 [16]. Procedures used in powdery mildew inoculation, incubation of inoculated plants, and reaction scoring were as described in He et al. [18]. All F2 plants and their parents were inoculated with isolate E09 at the one-leaf seedling stage to screen for powdery mildew reaction. For progeny testing, 15 to 20 F3 seedlings from each F2 plant were grown and tested with the same race. The infection types (ITs) were rated on a 0–4 scale, 7–10 days after inoculation when conidia were fully developed [18].

**Molecular marker analysis**

Wheat chromosome 2BL has good synteny with rice chromosome 4 [19, 20]. Thus, wheat ESTs that mapped to bin 2BL-0.50–1.00 were aligned to the rice genome sequences using BLASTN. ESTs with orthologous genes in the syntenic region of rice chromosome 4 were used to develop STS (sequence tagged site) markers. PCR products from STS primers were separated in 1% agarose gels, whereas PCR products from EST and SSR primers were separated in 8% non-denaturing polyacrylamide gels. In order to produce SCAR markers, amplified polymorphic bands between CH7086 and Taichung 29 were extracted from gels and re-amplified. The method of cloning and sequencing PCR products was as described by Liu et al. [21].

**Chromosome assignment and linkage analysis**

Chi-squared ($\chi^2$) tests for goodness-of-fit were used to test for deviations of observed data from theoretically expected segregations. Linkages between DNA markers and the resistance gene were established with JoinMap version 4.0 software (Wageningen, Netherlands) with a LOD threshold of 3.0. Map distances were determined using the Kosambi mapping function.
Results

Likely origin of the powdery mildew resistance

Seedling reactions of *Th. ponticum*, the partial amphiploid donor and nine wheat cultivars/lines to four *Bgt* isolates are summarized in Table 1. CH7086 and the *Th. ponticum* parent R431 were resistant to isolates E09, E20, E21, and E26 (IT 0-0;), whereas the wheat parents or lines, Xiangyang 4, Misuizao, Zhong 8701, and Jimai 26, were susceptible (IT 3-4). These results demonstrated that CH7086 was resistant to powdery mildew, with the ITs being similar to the donor Xiaoyan 7430 (IT 0-0;) as well as the donor *Th. ponticum* accession R431 (IT 0).

Attempted characterization of an alien introgression in CH7086

When GISH using *Th. ponticum* genomic DNA as probe was performed on the wheat-*Th. ponticum* partial amphiploid Xiaoyan 7430 (2n=56) and line CH7086, 14 *Th. ponticum* chromosomes were clearly distinguishable in the mitotic metaphases of Xiaoyan 7034 (results not shown). However, no GISH signals were observed in CH7086 (Figure 1A). Giemsa-C banding (Figure 1B) indicated that CH7086 contained typical wheat chromosomes without visible bands indicative of *Thinopyrum* chromatin.

Genetic analysis of powdery mildew resistance in CH7086

Wheat-*Th. ponticum* introgression line CH7086, the F<sub>1</sub> hybrid, and F<sub>2:3</sub> families from CH7086 crossed respectively with wheat lines Taichung 29, CH5241, SY95-71, and Jintai 170 were inoculated with *Bgt* isolate E09. CH7086 was highly

### Table 1. Reactions of selected donor materials, parents and controls after with four *Bgt* isolates.

| Line            | Chromosome | Genomic formula | *Bgt* isolate |
|-----------------|------------|-----------------|---------------|
|                 | number     |                | E09 | E20 | E21 | E26 |
| *Th. ponticum* R431 | 70         | JJJ*J*          | 0   | 0   | 0   | 0   |
| Xiaoyan (XY) 7430   | 56         | ABD +J*         | 0   | 0;  | 0;  | 0;  |
| Xiangyang 4        | 42         | ABD             | 4   | 3   | 3   | 3   |
| Misuizao           | 42         | ABD             | 4   | 3   | 4   | 3   |
| CH7086            | 42         | ABD             | 0   | 0;  | 0;  | 0;  |
| CH5241            | 42         | ABD             | 4   | 4   | 4   | 3   |
| Zhong 8701         | 42         | ABD             | 4   | 3   | 4   | 4   |
| Jimai 26           | 42         | ABD             | 4   | 4   | 4   | 4   |
| SY95-71            | 42         | ABD             | 4   | 4   | 4   | 4   |
| Jintai 170         | 42         | ABD             | 4   | 4   | 4   | 3   |
| Taichung 29        | 42         | ABD             | 4   | 4   | 4   | 4   |

Infection types were based on a 0–4 scale, where 0= no visible symptoms, 0; = necrotic flecks, 1= necrosis with low sporulation, 2= necrosis with moderate sporulation, 3= no necrosis with moderate to high sporulation, and 4= no necrosis with full sporulation. Scores of 0–2 were classified as resistant and 3–4 as susceptible.

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resistant (IT 0) and the wheat lines were all highly susceptible (IT 4) (Table 1). All F₁ hybrid seedlings and adult plants were highly resistant, implying that the resistance in CH7086 was dominant. F₂ populations from four crosses segregated 3 resistant: 1 susceptible and the pooled data of F₂ lines from CH5241/CH7086 and CH7086/Taichung 29 segregated 63 homozygous resistant: 128 segregating: 61 homozygous susceptible, as expected for single gene segregation ($\chi^2 = 0.10$, $P = 0.95$) (Table 2). The dominant gene for $Pm$ resistance in CH7086 was temporarily designated as $PmCH86$.

Identification and physical bin mapping of polymorphic markers linked to $PmCH86$

The 148 plants of the F₂ population segregated 1:2:1 for all fourteen markers (Table 3, Table 4). Of the microsatellite markers tested, Xgwm47, Xwmc332, Xwmc317, Xwmc817, and Xbarc159 showed linkage with powdery mildew resistance in CH7086. As these markers all map to chromosome arm 2BL, $PmCH86$ must also be located in this arm. Based on the high-density microsatellite consensus map of common wheat [22], two microsatellite markers (Xwmc332 and Xbarc159) linked with the resistance in this study were mapped at a distance of 45.7 cM on chromosome arm 2BL. As shown in Figure 2, we bin mapped the five SSR markers by CS deletion lines. Xgwm47 was the only marker located in bin 0.59–0.89 and the others, Xwmc332, Xwmc317, Xwmc817, and Xbarc159, mapped to the 2BL-6 deletion bin FL 0.89–1.00. $PmCH86$ was placed in
the interval between Xwmc332 and Xwmc317, thus the physical location of
PmCH86 was in the 2BL-6 deletion bin, which is the most distal bin of the long
arm accounting for approximately 11% of the physical length of this chromosome
arm. Evaluation of the five linked microsatellite markers, including four
codominant and one dominant (Table 3, Table 4, Figure 2), along the physical
and genetic maps of chromosome 2B allowed us to estimate the genomic location
of PmCH86.

Comparative genomics analysis

Powdery mildew resistance genes Pm6, Pm33, and PmJM22 are also located in the
distal region of chromosome 2BL [23, 24, 25]. To clarify the relationship of
PmCH86 with these known genes, we designed the primers for molecular markers
based on wheat EST and syntenic regions of the rice. An STS marker Xbcd135
which co-segregated with Pm6 was also developed (Table S1). Comparative
mapping was reported between wheat chromosome arm 2BL, rice chromosome 4
and Brachypodium chromosome 5 [19, 25]. Eleven STS and EST-STS flanking
markers, BF478581, DN949092, BM138525, CINAU140, BI479701, BQ169948,
BQ169948, BQ246670, BE500840, BE444894, and BE405017, were used as queries
to search for orthologous genes in rice and Brachypodium genomic sequences.
Both Cos66 and BE405017 detected orthologs on the long arm terminal regions of

| Parent or cross | No. of plants or lines | Expected | $\chi^2$ | $P$ |
|----------------|------------------------|----------|---------|-----|
|                |                        | Resistant| Segregating | Susceptible |
| CH7086         | P1                     | 19       |          |     |
| SY95-71        | P2                     | 15       |          |     |
| CH5241         | P3                     | 13       |          |     |
| Taichung 29    | P4                     | 13       |          |     |
| Jintai 170     | P5                     | 17       |          |     |
| CH7086-Taichung 29 | F1             | 11       |          |     |
|                  | F2                     | 88       | 26       | 3:1 | 0.292| 0.589|
|                  | F2,3                   | 25       | 52       | 27  | 1:2:1| 0.077| 0.962|
| CH7086/SY95-71 | F1                     | 7        | 42       | 3:1 | 0.133| 0.715|
|                  | F2                     | 118      |          |     |
| CH5241/CH7086  | F1                     | 21       |          |     |
|                  | F2                     | 111      | 43       | 3:1 | 0.701| 0.402|
|                  | F2,3                   | 38       | 76       | 34  | 1:2:1| 0.324| 0.850|
| CH7086/Jintai 170 | F1               | 9        |          |     |
|                  | F2                     | 149      | 40       | 3:1 | 1.483| 0.223|
| Pooled F2 data |                        | 466      | 151      | 3:1 | 0.091| 0.763|
| Pooled F2,3 data |                      | 63       | 128      | 61  | 1:2:1| 0.095| 0.953|

Values for significance of $\chi^2$ at $P=0.05$, and 3.83 for 1 df and 5.99 for 2 df, respectively.

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rice chromosome 4 (LOC_Os04g54870 and LOC_Os04g57560) and Brachypodium chromosome 5 (Bradi5g23570 and Bradi5g25740). The closest EST markers flanking \( PmCH86 \) were BI479701 and BQ246670 at distances of 4.9 cM and 1.5 cM, respectively; BI479701 was ortholog of rice gene LOC_Os04g55480 and Brachypodium gene Bradi5g23970. The ortholog of BQ246670 was found in rice (LOC_Os04g57140) and Brachypodium (Bradi5g25390). The terminal region of wheat 2BL had a similar gene order to rice 4L and Brachypodium 5L. Thus, the collinear region of the powdery mildew resistance gene \( PmCH86 \) covered 214 kb.
genomic region (LOC_Os04g56740 to LOC_Os04g57140) of chromosome 4 L in rice and 198 kb genomic region (Bradi5g25090 to Bradi5g25390) of chromosome 5 L in Brachypodium (Table S1, Figure 3). The orthologous genomic regions of PmCH86 in the rice and Brachypodium genomes could be candidate regions for fine mapping of PmCH86.

Table 4. Segregations of powdery mildew resistance and markers linked to PmCH86 in F2 population or F2:3 lines from CH5241/CH7086.

| Marker | Resistance | Codominant marker | Dominant marker | Total | $\chi^2$ (1:2:1 or 3:1) | $P$ |
|--------|------------|-------------------|-----------------|-------|------------------------|-----|
|        | genotype   | AA    | Aa   | aa  | AA | Not AA | Not aa | aa |
| Xwmc332| PmPm       | 34    | 3    | 1   | 38 |         |        |    |
|        | Pmpm       | 1     | 73   | 2   | 76 |         |        |    |
|        | pmpm       | 1     | 2    | 31  | 34 |         |        |    |
|        | Total      | 36    | 78   | 34  | 148| 0.486   | 0.784 |   |
| BQ246670| PmPm      | 37    | 1    | 0   | 38 |         |        |    |
|        | Pmpm       | 0     | 75   | 1   | 76 |         |        |    |
|        | pmpm       | 1     | 0    | 33  | 34 |         |        |    |
|        | Total      | 38    | 76   | 34  | 148| 0.324   | 0.850 |   |
| BE444894| PmPm      | 21    | 17   | 3   | 38 |         |        |    |
|        | Pmpm       | 8     | 68   | 76  |   |         |        |    |
|        | pmpm       | 3     | 31   | 34  |   |         |        |    |
|        | Total      | 32    | 116  | 148 |   | 0.901   | 0.343 |   |
| Xwmc317| PmPm       | 30    | 8    | 8   | 38 |         |        |    |
|        | Pmpm       | 65    | 11   | 76  |   |         |        |    |
|        | pmpm       | 12    | 22   | 34  |   |         |        |    |
|        | PmPm       | 107   | 41   | 148 |   | 0.577   | 0.448 |   |
| Xwmc817| PmPm       | 19    | 12   | 7   | 38 |         |        |    |
|        | Pmpm       | 19    | 46   | 11  | 76 |         |        |    |
|        | pmpm       | 6     | 5    | 23  | 34 |         |        |    |
|        | Total      | 44    | 63   | 41  | 148| 3.392   | 0.183 |   |
| BE405017| PmPm      | 16    | 22   | 38  |   |         |        |    |
|        | Pmpm       | 20    | 56   | 76  |   |         |        |    |
|        | pmpm       | 8     | 26   | 34  |   |         |        |    |
|        | Total      | 44    | 104  | 148 |   | 1.766   | 0.184 |   |
| Xbarc159| PmPm      | 10    | 17   | 11  | 38 |         |        |    |
|        | Pmpm       | 19    | 42   | 15  | 76 |         |        |    |
|        | pmpm       | 8     | 8    | 18  | 34 |         |        |    |
|        | Total      | 37    | 67   | 44  | 148| 1.986   | 0.370 |   |

AA homozygous for the CH7086 allele; aa homozygous for the CH5241 allele; Aa heterozygous.

*The data of F2:3 lines from CH5241/CH7086;
*Values for significance of $\chi^2$ at $P=0.05$ is 3.84 for 1 df and 5.99 for 2 df, respectively.

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Validation of the flanking markers in marker-assisted selection

The closest markers *Xwmc332* and BQ246670 were linked to *PmCH86* with genetic distances of 3.2 and 1.5 cM, respectively (Figure 3). The genomic DNAs of resistant and susceptible F2 plants from crosses of CH7086 with susceptible lines CH5241 and Taichung 29, as well as the parents and resistant (BR) and susceptible (BS) bulks were tested for the presence of markers linked to *PmCH86*. The specific *PmCH86*-associated 500-bp band amplified by BQ246670 was inherited as a dominant marker (Table 4, Figure 4). Marker BQ246670 may be useful for marker assisted selection (MAS) and for pyramiding *PmCH86* with other powdery mildew resistance genes in wheat depending on marker polymorphisms.

Comparison of *PmCH86* and other *Pm* genes on 2BL

*Pm6* was resistant only to race E15, and susceptible to races E1-3, E5-7, E10, E13, E16-18, E20-21, E23, E26, E30-32, and E42 at the seedling stage [16]. Timgalen carrying *Pm6* was highly susceptible (IT 3-4) to E21 and E26, whereas CH7086 was highly resistant (IT 0-0) (Table 1). This indicated that *PmCH86* differs in specificity from *Pm6*. 
In order to further clarify the genetic relationship of *PmCH86* and *Pm6*, 236 F$_2$ plants from CH7086/Timgalen were inoculated with E15, an isolate avirulent to both parents. Two susceptible plants were found, confirming that *PmCH86* and *Pm6* were not allelic, but were also not genetically independent ($\chi^2_{15:1} = 11.76$, $P_{df0.01} = 0.001 < 0.01$).

Figure 3. Genetic and comparative mapping of the *PmCH86* gene. A) Chromosome physical map of 2BL (http://www.k-state.edu/wgrc/Germplasm/Deletions/grp2L.html). B) Genetic map of wheat chromosome 2BL, only the region relatively close to *PmCH86* was shown. Genetic distances are shown to the left in cM. C) The homologous region of *PmCH86* with rice chromosome 4 (http://rice.plantbiology.msu.edu/). D) The homologous region of *PmCH86* with Brachypodium chromosome 5 (http://www.brachypodium.org/database).

Figure 4. A profile of amplification with marker BQ246670 in F$_2$ population from cross of CH7086/Taichung 29. M: DNA ladder; P$_R$: CH7086; P$_S$: Taichung 29; B$_R$: resistant bulk; B$_S$: susceptible bulk; R: homozygous resistant F$_2$ plants, S: homozygous susceptible F$_2$ plants. Asterisk indicates the critical band linked with *PmCH86*.

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Discussion

Alien gene transfer has an important role in increasing the genetic diversity available for wheat improvement [4]. *Th. ponticum* is immune to wheat powdery mildew and certain wheat- *Th. ponticum* derivatives are highly resistant to Chinese Bgt isolates. A resistance gene was recently found in two *Th. ponticum*-derived partial amphiploids [26]. However, there is no published report of transfer of powdery mildew resistance from this species to a wheat chromosome. In this study, CH7086 was produced by crossing and backcrossing Xiaoyan 7430 with susceptible wheat cultivars and selecting for powdery mildew resistance. A novel powdery mildew resistance gene, presumably transferred from *Th. ponticum* into common wheat, was mapped on chromosome arm 2BL and closely linked SSR markers were identified. However, based on GISH and Giemsa-C banding analyses of CH7086, no cytological evidence was found for an alien translocation. The gene PmCH86 must be either present in a cryptic translocation involving a small chromosome segment from *Th. ponticum*, or a wheat gene derived from an unknown source. Cryptic alien transfers have been reported in other studies [6, 7]. Further studies are needed to determine the source of PmCH86.

Five powdery mildew resistance genes, Pm6, Pm26, MIZec1, Pm33, and MILX9, were previously located on chromosome arm 2BL. Pm6 originated from the G genome of *T. timopheevii* and Pm33 was identified in *T. carthlicum* [27, 23]. Pm6 and its linked RFLP marker Xbcd135 were physically located at the region of deletion bin 2BL-6 (FL 0.89–1.00) [28], and this marker was subsequently converted into two STS markers, STSBCD135-1 and STSBCD135-2, which are closely linked to Pm6 with a genetic distance of 0.8 cM [27]. Recently, Qin et al. developed a high-density genetic linkage map of the Pm6 locus through a comparative genomics analysis using the genome sequences of rice and *Brachypodium* together with Triticaceae ESTs, and localized Pm6 at 0.2 to 1.2 cM proximal to the Xbcd135 locus. However, in the present study, PmCH86 was 8.2 cM distal to Xbcd135 (Figure 3). The low infection type conferred by PmCH86 to all races tested (Table 1) was also different from that of Timgalen [16].

Based on a microsatellite map, *T. carthlicum*-derived powdery mildew resistance gene Pm33 was placed in the region between 18.1 cM distal to Xgwm526 and 1.1 cM proximal to Xwmc317 on chromosome 2BL, and the genetic distance between Pm33 and Pm6 was estimated to be 61.7 cM [23]. In our study, PmCH86 was placed between Xwmc332 and Xwmc317 with an estimated genetic distance being about 30 cM proximal to Xwmc317 (Figure 3). This indicates that PmCH86 should not be allelic to Pm33. Yin et al. [24] located PmJM22 on 2BL in wheat cultivar Jimai 22 using microsatellite markers. Since PmJM22 was close to the region of Pm33, they may be allelic. MIZec1, a dominant resistance gene derived from wild emmer, was mapped distally to SSR marker Xwmc356 on the terminal bin 2BL 0.89–1.00 [29]. Xwmc356 was distal to Xwmc317 [22], thus being at a different position from PmCH86. The recently identified gene MILX99 was derived from commercial winter wheat cultivars Liangxing 99. MILX99 was located on chromosome 2BL in the deletion bin 2BL2-0.36-0.50 and was linked to SSR
marker Xgwm120 [30]. Based on these results and our own data, it appears that
PmCH86 is different from other known powdery mildew resistance genes on
chromosome 2BL and represents a new powdery mildew resistance locus, and was
therefore designated as Pm51. The genetic map of 2BL presented here is in minor
conflict with PmJM22 at map distance of BE405017 and BE444894 on the
previously published map by Yin et al. [24]. This is likely due to our map being
significantly longer. Similar phenomena were also hypothesised for small putative
segmental introgressions carrying stem rust resistance from Ae. speltoides into
wheat [31].

The wheat chromosome arm 2BL appears to be a hotspot region in disease
resistance genes. Rust resistance genes, Yr5, Yr7, Yr43, Yr44 [32, 33], Lr48, and
Sr28 [34, 35], have been mapped by molecular markers. Genes Sr9 (several alleles)
and Sr16 were also placed in this arm [36]. The accumulation of functional
markers in 2BL can be used to target resistance genes in the 2BL terminal regions.
Comparative maps will be useful for isolating Pm51 from a gene-rich region in the
terminal region of wheat chromosome 2BL by using the rice and Brachypodium
genomes as references. Saturation mapping of Pm51 with more functional
markers is underway to better understand the allelic relationships, gene structure
and function in this gene-rich region.

We found that the new powdery mildew resistance gene Pm51 mapped distally
on chromosome 2BL was close to the EST-PCR marker BQ246670 (1.5 cM distal).
Tightly linked markers for the Pm51 locus characterized in this study could be
used for MAS of Pm51 in wheat breeding programs or to pyramid multiple
resistance genes in a single genotype in order to achieve more durable resistance.

Supporting Information
Table S1. Molecular markers mapped to the PmCH86 region based on wheat,
rice, and Brachypodium synteny.
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Author Contributions
Conceived and designed the experiments: ZY ZC HZ. Performed the experiments:
HZ GL XZ XL HG WG. Analyzed the data: ZY HZ GL HG JJ WG LQ. Contributed
reagents/materials/analysis tools: ZC HZ GL WG YR LQ. Wrote the paper: ZY ZC
HZ LQ.
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