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ORIGINAL ARTICLE

Fine mapping of powdery mildew resistance gene *Pm4e* in bread wheat (*Triticum aestivum* L.)

Khan Nasr Ullah[1] · Na Li[1] · Tao Shen[1] · Peisi Wang[1] · Wenbin Tang[1] · Shengwei Ma[1] · Zhimeng Zhang[1] · Haiyan Jia[1] · Zhongxin Kong[1] · Zhengqiang Ma[1]

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Abstract

Main conclusion **Fine mapping of wheat powdery mildew-resistance gene *Pm4e* to a 0.19 cM interval with sequence-based markers provides the foundation for map-based cloning and marker-assisted selection with breeder-friendly markers.**

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is a wheat foliar disease that poses a serious threat to global wheat production. *Pm4* is a resistance gene locus that has played a key role in controlling this disease in wheat production and a few resistance alleles of this locus have been identified. We have previously mapped the *Pm4e* allele to a 6.7 cM interval on chromosome 2AL. In this study, *Pm4e* was delimited to a 0.19 cM interval flanked by *Xwgrc763* and *Xwgrc865*, through employment of a larger segregating population, derived from the cross of resistant parent D29 with susceptible parent Yangmai 158 (Y158), and enrichment of the genetic interval with markers developed on Chinese Spring (C.S.) survey sequence. In this interval, *Pm4e* co-segregated with a few markers, some of which were either D29-dominant or Y158-dominant, implying great sequence variation in the interval between D29 and Y158. Most of these co-segregation markers could not differentiate the *Pm4* alleles from each other. Survey of 55 wheat cultivars with four co-dominant markers showed that the *Pm4e*-co-segregating loci always co-exist. Annotation of the *Pm4e* interval-corresponding C.S. sequence revealed more than a dozen resistance gene analogs clustered in a 2.4 Mb region, although C.S. is susceptible to the *Pm4e*-avirulent isolate Bgt2. This study has established the foundation for map-based cloning of *Pm4e*. Moreover, some of the co-dominant markers developed in this study could help in marker-assisted transfer of *Pm4*e into elite cultivars.

Keywords Wheat · Powdery mildew · Chinese Spring · *Pm4* · Fine mapping · Marker-assisted selection

Introduction

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (Bgt) is a devastating wheat foliar disease, predominant in regions with cool and maritime climate, and causes severe yield losses once epidemics occur (Everts and Leath 1992). To control this disease through breeding, a number of resistance genes have been identified. With the recent characterization of *Pm55*, *Pm57, Pm58*, *Pm59, Pm60,* and *Pm61*, 88 formally designated powdery mildew-resistance genes or alleles mapping to 57 different loci (*Pm1–Pm61, Pm18=Pm1c, Pm22=Pm1e, Pm23=Pm4c, Pm31=Pm21*) have been documented so far (Zhang et al. 2016; Liu et al. 2017; McIntosh et al. 2017; Wiersma et al. 2017; Zou et al. 2017; Tan et al. 2018; Sun et al. 2018), and some of them have played important roles in stabilizing wheat yield.

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Khan Nasr Ullah and Na Li contributed equally.

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✉ Zhengqiang Ma
zqm2@njau.edu.cn

[1] The Applied Plant Genomics Laboratory of Crop Genomics and Bioinformatics Centre, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China

More and more studies demonstrated that the resistance gene locus could evolve into multiple alleles or carry tightly arranged resistance genes, for example, *Pm1* (Liang et al. 2016; Yao et al. 2007), *Pm2* (Ma et al. 2015; Sánchez et al. 2016), *Pm3* (Yahiaoui et al. 2004; Bhullar et al. 2010; Zhao et al. 2016), *Pm4 (*Briggle 1966; Hao et al. 2008; Schmolke et al. 2012; Li et al. 2017)*, Pm5* (Huang et al. 2003), and *Pm24* (Huang et al. 2000; Xue et al. 2012), for wheat powdery mildew resistance. Five alleles at the *Pm4* locus have been reported. *Pm4a* was introduced from *T. dicoccum* accession Khapli and *T. durum* accession Yuma (Briggle 1966; Ma et al. 2004). *Pm4b* was a *T. carthlicum* gene (The et al. 1979). Both *Pm4c* and *Pm4e* were identified in common wheat cultivars (Hao et al. 2008; Li et al. 2017). A resistance gene originated from *T. monococcum* was assumed to be *Pm4d* (Schmolke et al. 2012). Moreover, a few other powdery mildew-resistance genes mapped on chromosome 2AL could be either allelic or closely linked to the *Pm4*, such as *pmX* (Fu et al. 2013), *PmLK906* (Niu et al. 2008) and *PmPS5A* (Zhu et al. 2005).

Pm4 is one of the most widely used powdery mildew-resistance locus and can be found alone or in combination with other powdery mildew-resistance genes in many resistance cultivars in China (Huang et al. 1997). *Pm4a* has been overcome due to the emergence of new virulent strains and its wide utilization in breeding programs globally (Duan et al. 2002) and in China (Li et al. 2017); however, the allelic expansion of the *Pm4* locus has increased the resistance duration of this locus. Investigation of the allelic variations in detail is, therefore, important, not only for increase of the allelic diversity, but also for understanding the host–pathogen interaction and resistance gene pyramiding (Srichumpa et al. 2005; Koller et al. 2018).

This study was aimed at fine mapping of *Pm4e*, with an ultimate goal of resolving the complexity of *Pm4* locus and providing breeders with friendly markers that can be efficiently used in marker-assisted breeding.

Materials and methods

Plant materials

D29 containing *Pm4e* is a common wheat landrace introduced from Sichuan province of China, without known pedigree. CI14123 (Khapli/8*CC), Armada and 81-7241 are three common wheat lines containing *Pm4a*, *Pm4b,* and *Pm4c*, respectively. Yangmai158 (Y158) is an elite Chinese common wheat cultivar, developed by the Yangzhou Lixiahe Regional Institute of Agricultural Sciences, China. Chinese Spring is a common wheat landrace. Sumai No.3, a common wheat cultivar, was used as the susceptible control in resistance evaluation. The segregation population was derived from selfing of BC_6F_1 plants with heterozygous *Pm4e*. In addition, 55 Chinese wheat cultivars or lines from different ecological areas were used in survey with *Pm4e*-linked markers.

Resistance evaluation

According to the method described by Xu et al. (2008), all the seedlings grown in rectangular trays were inoculated at one leaf stage by dusting the conidiospores of *Pm4e*-avirulent isolate Bgt2 freshly increased on susceptible cultivar Sumai No. 3. Sumai No. 3 and Y158 were used as susceptible controls. After inoculation, the trays were placed in a growth chamber set with over 80% humidity, 14 h light, and a 22 °C/18 °C temperature day/night cycle. Disease symptoms were scored 7 day post-inoculation when the control plants of Sumai No.3 and Y158 were heavily diseased, according to 0–5 scale as mentioned by Yao et al. (2007). The 0, 1, 2, 3, 4, and 5 in this scale represent no visible symptoms, visible necrosis without sporulation, sparse sporulation, moderate sporulation, abundant sporulation, and abundant sporulation with mycelia covering more than 80% of the leaf area, respectively. A score of 0–2 was considered as resistant and 3–5 as susceptible.

DNA extraction and PCR amplification

DNA was extracted from young seedling tissue using the procedure of Ma et al. (1994). For bulked segregant analysis (BSA) (Michelmore et al. 1991), resistant and susceptible bulks were made by combination of equal amount of DNA from ten homozygous resistant and ten homozygous susceptible F_2 plants, respectively, following progeny testing.

Polymerase chain reaction of markers was performed in PE9600 thermal cycler (Perkin Elmer), in a volume of 10 µl containing 10–20 ng of template, 2 pmol of each of the primers, 2 nmol of each of the deoxynucleoside triphosphates, 15 nmol of $MgCl_2$, 0.1 U Taq DNA polymerase, and 1 × PCR buffer. The PCR profile was set as one cycle of 94 °C for 3 min, followed by 36 cycles of 94 °C for 30 s, 50–60 °C (depending on the specific primers) for 40 s, and 72 °C for 50 s, and a final extension at 72 °C for 5 min. The PCR products were separated on 8% non-denaturing polyacrylamide gels with a 19:1, 29:1, or 39:1 acrylamide/bisacrylamide ratio, and then silver-stained as described by Santos et al. (1993). PCR products from amplification with the *Pm4a* co-segregating marker STS-BCD1231 (Ma et al.

2004) were separated on a 1% agarose gel, and viewed under UV light after staining with ethidium bromide.

Recombinant screening, marker development, and map construction

Recombinant screening was performed with the published *Pm4e*-flanking markers with *Xhbg327* at one side and *Xgdm93* at the other side (Li et al. 2017). Marker *Xsts-bscd1231*, which co-segregated with *Pm4e*, was also used in genotyping. The obtained recombinants were transferred to greenhouse to obtain the corresponding $F_{2:3}$ progenies. To narrow down the *Pm4e* interval, the genomic information from the corresponding Chinese Spring (C.S.) chromosome 2AL sequence available via the IWGSC wheat sequence repository at URGI-INRA-Versailles, France (http://wheat-urgi.versailles.inra.fr/) was used to develop additional PCR-based markers for mapping and genotyping by taking advantage of simple sequence repeats (SSR), cleaved amplified polymorphism (CAP), single nucleotide polymorphism (SNP), and repeats junctions. In addition, the corresponding *T. urartu* 2AL scaffolds and *T. durum* 2AL contigs were also used to develop markers. All primer sequences were designed using MacVector (Accelrys, UK). If the PCR products of a marker were monomorphic sizewise between the parents, they were digested with restriction enzymes *Eco*RI, *Hha*I, *Rsa*I, *Msp*I, *Hin*dIII, *Mbo*I, and *Alu*I to reveal potential sequence composition variations. Each digestion was performed in a 5 µl reaction mix with 0.05 µg DNA, according to supplier's manual.

Physical mapping and gene annotation

The sequences associated with the two flanking markers most closely linked to *Pm4e* were used as queries in search against the C.S. chromosome 2A sequence available via the IWGSC to determine the boundaries of the interval. The gene annotation information of the interval was then retrieved from IWGSC RefSeq v1.0 annotation (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/).

Linkage analysis

Linkage analysis was conducted with Mapmaker 3.0 (Lincoln et al. 1993). An LOD score of 3.0 was used as the threshold for linkage. Genetic distances were estimated using Kosambi function (Kosambi 1944). Chi-squared (χ^2) tests for goodness-of-fit were used to evaluate deviations of the observed data from the expected segregation ratios.

Results

Phenotyping and genotyping of the segregation population

Li et al. (2017) found, using an F_2 population derived from the cross of D29 with Y158, that *Pm4e* is a dominant-resistance gene and was 4.9 cM from *Xgdm93* at one side and 1.8 cM from *Xhbg327* at the other side (Li et al. 2017). To fine map this gene, we surveyed a population of 772 plants derived from selfing of BC_6F_1 plants with *Pm4e*, which were obtained in development of the *Pm4e* near isogenic lines using Y158 as the recurrent parent. Inoculation with Bgt2 of the population seedlings yielded 575 resistant plants and 197 susceptible plants, which fit well the expected 3:1 segregation ratio ($\chi^2_{3:1} = 0.0017$, $P = 0.90–0.95$) and was in consistent with the conclusion by Li et al. (2017). Genotyping these plants with *Xsts_bcd1231*, *Xgdm93,* and *Xhbg327* showed that *Xsts_bcd1231* co-segregated with the phenotype and was encompassed in the 3.51 cM *Xgdm93–Xhbg327* interval (Fig. 1a). Fifty-three plants with recombination occurring between *Xgdm93* and *Xhbg327* were identified and phenotypically and genotypically verified using the progenies derived from selfing.

High-density mapping

To enrich the *Pm4e* interval with more markers, a total of 176 markers, majority of which are SSR-based, were designed based on the C.S. genomic DNA sequence corresponding to the interval and homologous scaffolds or contigs from *T. urartu* and *T. durum*. However, only 14 markers detected length or presence/absence polymorphism between D29 and Y158 as well as between the resistant and susceptible pools (Table 1). Of these polymorphic markers, except for WGRC349 derived from the *T. urartu* scaffold50306, all others were developed based on the Chinese Spring 2A survey sequence. Digestion of the monomorphic PCR products with restriction enzymes revealed that WGRC421 and WGRC427 detected polymorphism between the two parents when digested with restriction enzymes *Hha*I and *Eco*RI, respectively. All these polymorphic markers were mapped to the *Pm4e* interval using the data from recombinant genotyping, resulting in a reduction of the average adjoining marker interval to 0.38 cM (Fig. 1b). We were, therefore, able to delimit *Pm4e* to a 0.19 cM genetic interval flanked by *Xwgrc763* and *Xwgrc865* (Fig. 1b).

Besides *Xsts-bcd1231, Xwgrc872, Xwgrc869, Xwgrc883, Xwgrc929,* and *Xwgrc1096* showed co-segregation with *Pm4e* (Fig. 1b). These markers mapped to an approximately

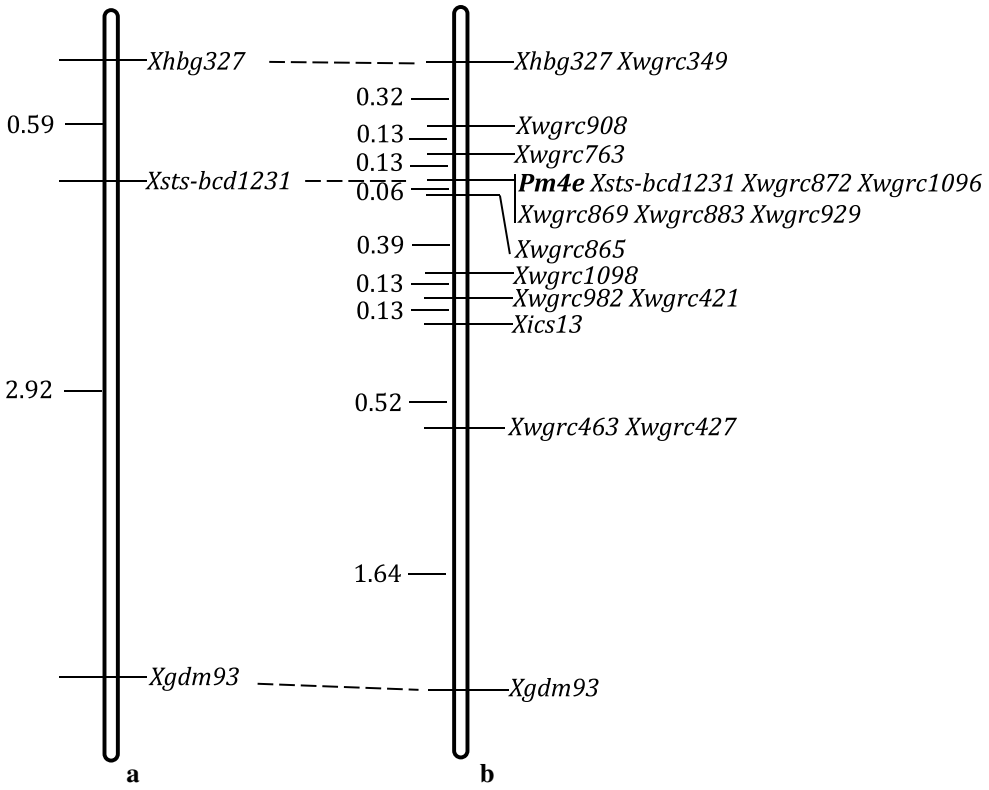

Fig. 1 Map of *Pm4e* before (**a**) and after (**b**) marker enrichment. Genetic distance is shown to the left in cM

Table 1 Information of markers developed in this study that detected polymorphism

Marker name	Forward primer	Reverse primer
WGRC763	AATCATCACTATCTTTGACAAGTT	GCCAGTTGAAGCCTTTTAGA
WGRC872	TCATCATCACCACCAGCACC	TGGACACGGTTCGGTTTACC
WGRC865	TGAACTGTGAGGTATCCCACCC	GCTTCATCTTTCCCCCCTTC
WGRC869	GCCATCCCCTTGACTTACAC	GAGGAGGAGAATCTTTCCACG
WGRC982	TTTTCCTTGGCACTGGTTCG	GGATGGGTGACTTGTAACTGTTTGC
WGRC908	CAAGGTTGATAAGTGTCTGTGGGC	GCGAAGTTCATTGGTCCGTTG
WGRC929	TCCAAACTTGACACGCTTG	TGGGATAGCAGTGAAGGAGG
WGRC883	AATCCACCTCTCCGCTTTC	AATCTCGCATCAACAGCAAG
WGRC349	CCAACCAAAGCCCTGTTTTC	GCACACTATGTCGTAATCCGTG
WGRC463	GAGATGGACGAGTAGAATGCAAG	CAGTGTGGTGGACGGTGGA
WGRC421	GTTGCCAAGATCGGTAACAA	ACTTGCCGAGACAACAATAAAT
WGRC427	GTGTGGGTGCGACGATATTG	TGCGGTCAACTATAATACAAGAAT
WGRC1096	CATTTCCTTCTGCTCCTCATTGG	TTCCTAACCCCTCCTCCTTCAC
WGRC1098	ACTTTGTGTGTTGGGTGGGC	CTAGAGACATTGTTGTTGCTGCTAC
WGRC3242	TGCTTTTGACAGTGAGCC	CGTCATTACAGTGTGTTCTG

360-kb block of C.S. 2AL. In the *Xwgrc763* and *Xwgrc865* interval, except for *Xwgrc763*, *Xwgrc872,* and *Xwgrc869*, the other four marker loci were either D29-dominant (*Xwgrc929*) or Y158-dominant (*Xwgrc883, Xwgrc865, Xwgrc1096*) (Fig. 2), implying great sequence variation in the *Pm4e* block.

Comparative mapping of *Pm4e* and *Pm4b*

Wu et al. (2018) recently published a marker map of *Pm4b*. We surveyed all the *Pm4b*-linked markers in this map but the SRAP marker Me8/Em7$_{-220}$ and found that only one marker ICS13 detected polymorphism between D29 and Y158. *Xics13* was mapped 0.71 cM distal to the *Pm4e*

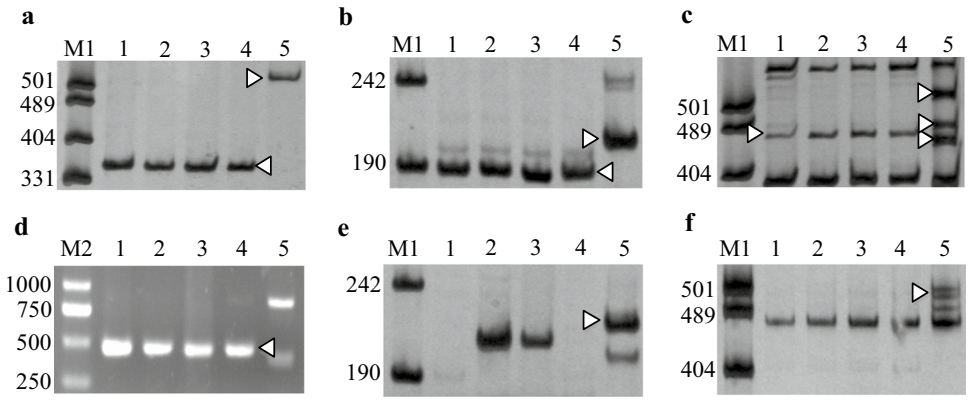

Fig. 2 Polymorphic pattern of markers WGRC763 (**a**), WGRC872 (**b**), WGRC869 (**c**), BCD1231 (**d**), WGRC883 (**e**) and WGRC865 (**f**) in CI14123 (*Pm4a*, 1), Armada (*Pm4b*, 2), 81-7241 (*Pm4c*, 3), D29 (*Pm4e*, 4) and Y158 (5). **a**, **c** On 8% 39:1 acrylamide/bisacrylamide gels; **b**, **e**, **f** on 8% 29:1 acrylamide/bisacrylamide gels; **d** on a 1% agarose gel. Arrows indicate the polymorphic bands mapping to chromosome 2A. M1 and M2, DNA markers pUC19/*Msp*I and D2000, respectively. Numbers to the left indicate molecular size in bp

interval (Fig. 1b) and 1.3 cM proximal to *Pm4b* (Wu et al. 2018). Applying the markers' mapping to the *Pm4e* interval to lines contains *Pm4a*, *Pm4b,* and *Pm4c*, we showed that *Xwgrc883* differentiated the *Pm4e* line from the *Pm4b* and *Pm4c* lines (Fig. 2). WGRC883 could be a co-dominant marker for *Pm4b* and *Pm4c* (Fig. 2e).

Gene annotation of the *Pm4e* interval-corresponding C.S. sequence

The flanking marker loci *Xwgrc908* (the closer locus *Xwgrc763* was not used due to its involvement in a potential inversion, see Fig. 4) and *Xwgrc865* defined a 6.1 Mb sequence region of C.S. chromosome 2A (IWGSC, v1.0). According to the gene annotations of IWGSC RefSeq v1.0, this region contains 122 high confidence genes (Electronic Supplementary Table 1), some of which are homologs of known disease resistance or defense-associated proteins, such as NBS–LRR-resistance proteins, receptor-like protein kinases, harpin-induced-like protein, and thaumatin-like protein. The NBS–LRR-resistance gene analogs (RGAs) are mainly clustered into two regions, defined by TraesCS2A01G560600.1-TraesCS2A01G560900.1 and TraesCS2A01G564200.1-TraesCS2A01G564900.1. To verify the linkage of these RGAs with *Pm4e*, markers were designed and used in survey of D29 and Y158. Five markers produced the expected PCR products in at least one of the lines, but WGRC3242, designed based on the NBS–LRR analog TraesCS2A01G560700.1, was the only one detected polymorphism (Fig. 3a). As expected, *Xwgrc3242* co-segregated with *Pm4e*. Although *Xwgrc3242* was monomorphic between D29 and C.S. (Fig. 3a), C.S. was susceptible to the isolate Bgt2, implying that C.S. does not possess the *Pm4e* allele. However, it is possible that C.S. carries a gene allelic to *Pm4e*.

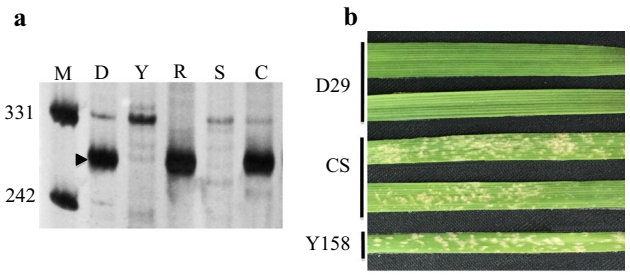

Fig. 3 Polymorphic pattern of TraesCS2A01G560700.1-derived marker WGRC3242, resolved in the 8% 29:1 acrylamide/bisacrylamide gel (**a**) and comparison of resistance response of D29 and C.S. to Bgt2 at seedling stage (**b**). Y158 was the susceptible control. In **a**, D (D29), Y (Y158), R (resistant pool), S (susceptible pool), C (C.S.), M, DNA marker pUC19/*Msp*I. Arrow indicates the mapped polymorphic band. Numbers to the left indicate molecular size in bp

Pm4e haplotype in cultivars

Fifty-five lines from different ecological regions of China were surveyed with co-dominant markers WGRC763, WGRC872, WGRC869, and WGRC982. The results showed that *Xwgrc872* and *Xwgrc869*, which co-segregated with *Pm4e*, always co-existed in eight of the lines (Table 2). Different from this, recombination of *Xwgrc982* with *Xwgrc872* and *Xwgrc869* was observed in 11 of the lines that produced a product same as in D29. However, no recombination was observed between *Xwgrc872/Xwgrc869* and *Xwgrc763* (Fig. 1b). *Xwgrc763* was the other flanking marker locus and was closer to the *Pm4e* than *Xwgrc982*. Since WGRC763, WGRC872, and WGRC869 could not differentiate among the four *Pm4* alleles, it appeared that a *Pm4* haplotype was detected in these lines. Examining these lines with the commonly used *Pm4a*-co-segregating dominant marker *STS-BCD1231* yielded supportive results (Table 2, Fig. 1b).

Table 2 Survey of 55 Chinese wheat cultivars with *Xwgrc872*, *Xwgrc763*, *Xwgrc869*, *Xsts_bcd1231,* and *Xwgrc982*

Cultivars	*Xwgrc763*	*Xwgrc872*	*Xwgrc869*	*Xsts_bcd1231*	*Xwgrc982*
Zhoumai 26	−	−	−	−	−
Zhoumai27	+	+	+	+	+
Jimai 23	−	−	−	−	−
Jimai 31	−	−	−	−	−
Shannong 0905	−	−	−	−	−
Bainong 419	+	+	+	+	+
4610	−	−	−	−	−
Yanhang 2	−	−	−	−	−
Yannong 5158	−	−	/	−	+
Lumai 21	−	−	−	−	+
Long 35	−	−	−	−	+
Baofeng2018	−	−	−	−	−
Cunmai 11	−	−	−	−	−
Fengdecunmai 12	+	+	+	+	+
Huaichuan P(9)19	−	−	−	−-	−
Junjunmai K8	−	−	−	−	−
Puxing 5	−	−	−	−	−
Quanmai 890	+	+	+	+	+
Ruihua 055	−	−	−	−	−
Wanke 06725	−	−	−	−	−
Xigao 2	−	−	−	−	−
Xinmai296	−	−	−	−	+
Xumai 0054	−	−	−	−	−
Xuke 718	+	+	+	+	+
Yaomai18	−	−	−	−	−
Yimai 6	−	−	−	−	−
Yikemai 5	−	−	−	−	−
Yuliang 1688	−	−	−	−	−
Zhongyu 1152	−	−	−	−	−
D28	−	−	−	−	−
PF5	−	−	−	−	+
PF6	−	−	−	−	+
Shannong 612	−	−	−	−	−
Shannong 981	−	−	−	−	+
Shumai 969	−	−	−	−	−
Shumai 580	−	−	−	−	+
Shumai 1675	−	−	−	−	−
Shumai 1701	−	−	−	−	−
Aikang58	+	+	+	+	+
Zhoumai 22	−	−	−	−	−
Zhoumai 10170	+	+	+	+	+
BFB10	−	−	−	−	+
GY12029	−	−	−v	−	−
Shengxuan 6	−	−	−	−	−
Tianming 108	−	−	−	−	−
Huihong 225	−	−	−	−	−
Suyanmai 017	−	−	−	−	−
Guohong 9	−	−	−	−	−
Xinmai 116	−	−	−	−	+
Yangmai20	+	+	+	+	+
Shannong 269	−	−	−	−	−
Shannong 861	−	−	−	−	+
LS6109	−	−	−	−	−
Shannong 999	−`	−	−	−	−
Shannong 2015–9	−	−	−	−	−

+ Amplified band pattern the same as D29 (*Pm4e*); − amplified band pattern the same as Y158; /missing data

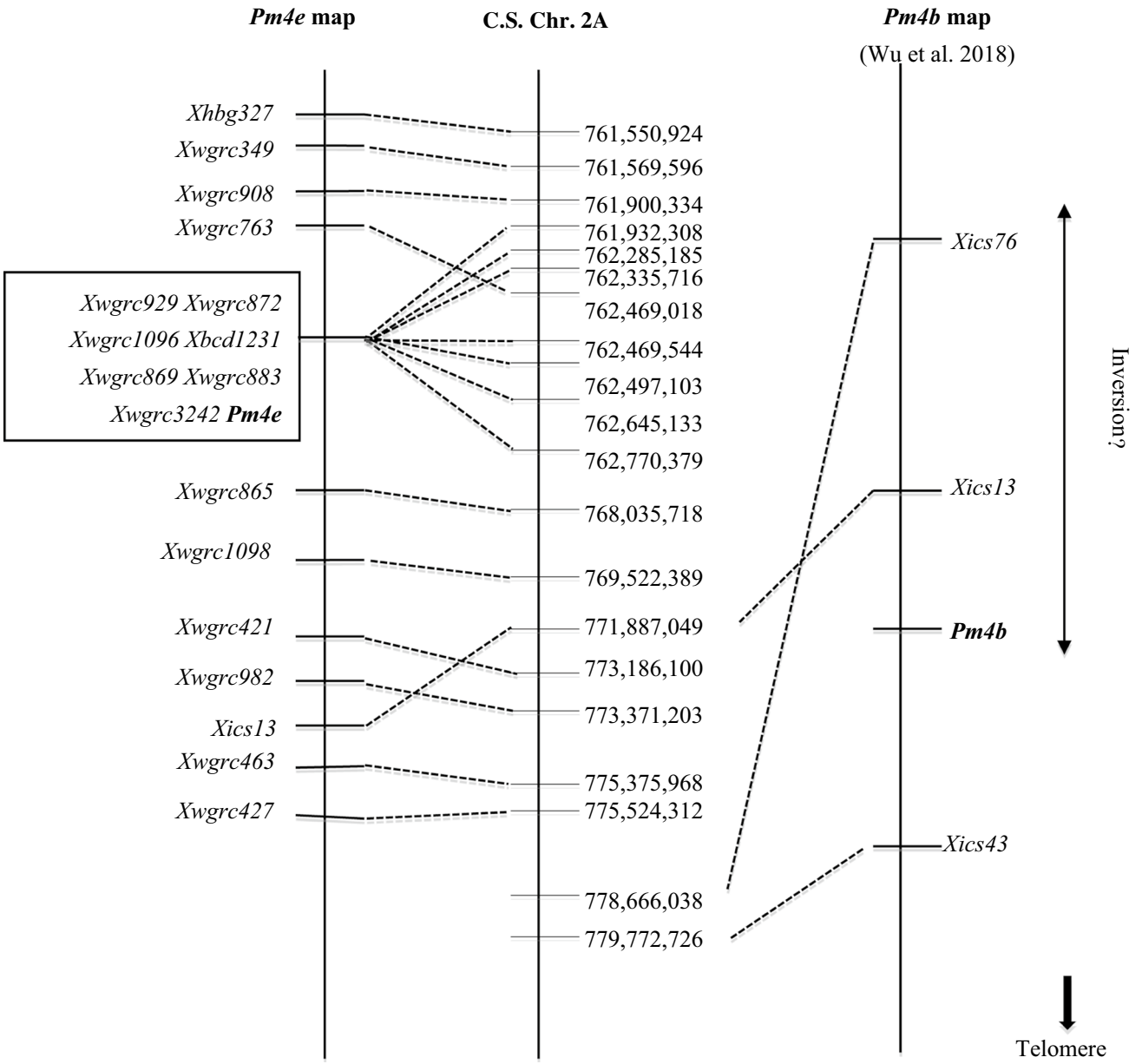

Fig. 4 Alignment of the markers on the *Pm4e* and *Pm4b* maps (Wu et al. 2018) to the C.S. 2A genomic DNA sequence. The marker order in the *Pm4e* map was not drawn in proportional to the genetic distance and the physical positions in bp of the C.S. map was not drawn in proportional to the physical distance

Discussion

Multiple genes for powdery mildew resistance on the long arm of wheat chromosome 2A have been reported. The existence of five formally designated *Pm4* genes and at least five temporarily designated genes closely linked to *Pm4* locus makes it one of the complex regions as previously known for *Pm1*, *Pm2*, *Pm3,* and *Pm5* loci (Li et al. 2017). Unlike the well-understood true allelic series of *Pm3* locus, no candidate gene has been cloned for the *Pm4* locus so far. Moreover, none of the genes or alleles at or near the *Pm4* locus has been fine mapped except *Pm4b* (Wu et al. 2018). The fine mapping of *Pm4e* and the development of new markers linked to the *Pm4* locus are hoped to lay the foundation for the map-based cloning of *Pm4* and its utilization in breeding programs. It is still necessary to determine whether or not the *Pm4* locus represents a true allelic series or paralogous copies of one gene. It is noted that multiple copies of RGAs exist in the *Pm4e*-interval-corresponding C.S. sequence.

Among the five so-called *Pm4* alleles, *Pm4d* was reasoned to be a gene linked, instead of allelic, to *Pm4a* and *Pm4e* (Li et al. 2017). Using resistance spectrum test (Li et al. 2017) and marker WGRC883, we were able to distinguish *Pm4e* from the other *Pm4* alleles. In consistence with their allelic relationship, all markers co-segregated with *Pm4e,* but the Y158-dominant *Xwgrc883* identified a common haplotype in the *Pm4*-carrying lines (Fig. 2). Among these markers, BCD1231 and the BCD1231-derived STS markers have been shown to co-segregate with *Pm4* alleles in a few studies (Ma et al. 1994, 2004; Niu et al. 2010; Fu et al. 2013; Li et al. 2017). In a fine mapping study, *Pm4a* was indeed mapped to the same position as *Pm4e* (data not shown). The *Pm4e* interval also overlaps with *Pm4b* interval reported by Hao et al. (2008). However, this is not in line with the mapping position of *Pm4b* reported by Wu et al. (2018) (Fig. 4). Alignment of the markers on the *Pm4e* map to the C.S. 2A sequence revealed a near-perfect collinearity of the *Pm4e* interval apart from an inversion involving *Xwgrc763* and *Xwgrc929*/*Xwgrc872*/*Xwgrc1096* (Fig. 4). Comparing to the C.S. sequence, the *Pm4b* interval appears to be involved in a large inversion event that causes the relative position change of *Pm4b* (Fig. 4). However, this still requires verification due to the inconsistency of *Pm4b* map in Hao et al. (2008) and Wu et al. (2018). A comparative high-resolution mapping and allelism test of all these genes might be able to clarify some of the issues.

Although *Pm4e* has been mapped to an interval as small as 0.19 cM, the corresponding physical distance is approximately 6.1 Mb in C.S. This is equal to a physical/genetic distance ratio of 32.3 Mb/cM, far greater than the 1.7 Mb/cM for flowering repressor gene VRN2 (Yan et al. 2004) and the 1 Mb/cM in the case of *Mla* (Wei et al. 1999), thus makes *Pm4e* cloning more difficult. In the marker block co-segregating with *Pm4e*, four markers were either D29-dominant or Y158-dominant, suggesting that great sequence variations are present between D29 and Y158, which could inhibit recombination occurrence, as in the case of the *Pm1* locus (Liang et al. 2016). Nevertheless, the presence of co-dominant markers (*Xwgrc872* and *Xwgrc869*) in the block implies that it is still possible to reach higher mapping resolution through using larger mapping population or altering the susceptible mapping parents.

Currently, most of the cloned race-specific disease-resistance genes in cereal crops encode CC–NBS–LRR-type proteins, for example, *Pm3b* (Yahiaoui et al. 2004), *Pm2* (Sánchez et al. 2016), *Pm60* (Zou et al. 2017), *Pm21* (He et al. 2018), *Lr1* (Cloutier et al. 2007), and *Sr33* (Periyannan et al. 2013) in wheat. In the 6.1 Mb C.S. sequence corresponding to *Pm4e*, more than a dozen RGAs are distributed over a 2.4 Mb region, but they do not provide resistance to the *Pm4e*-avirulent isolate Bgt2. It is worth examining if *Pm4e* is evolved from one of these RGAs, which is now under way in our lab.

The diagnostic markers previously developed for the *Pm4* locus were either dominant or mapped away from the gene. Thus, for selection of the *Pm4* genes, more makers are often needed, and sometimes, an additional time-consuming step of progeny testing is required to confirm the heterozygosity. In this study, we identified co-dominant marker loci *Xwgrc763* and *Xwgrc982* that were tightly linked to *Pm4e*, and more importantly, co-dominant marker loci *Xwgrc872* and *Xwgrc869* that co-segregated with *Pm4e*. They could be markers of choice in marker-assisted selection (MAS) of *Pm4e* and pyramiding the *Pm4* genes with other genes to obtain more stable and durable resistance against powdery mildew.

Author contribution statement Khan NU conducted genotyping, phenotyping, data analysis, and prepared the draft; N Li contributed to experiment design and data analysis and preparation of the draft; T Shen, P Wang, WB Tang, and Z Zhang participated in genotyping and phenotyping; S Ma contributed to sequence analysis; HY Jia and ZX Kong contributed to implementation of the project, Z Ma conceived the project, designed the experiments, and reviewed the article.

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Compliance with ethical standards

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

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