



# Chromosomal mapping of a locus associated with adult-stage resistance to powdery mildew from *Agropyron cristatum* chromosome 6PL in wheat

Yida Lin<sup>1,2</sup> · Shenghui Zhou<sup>2</sup> · Xuezhong Liang<sup>2</sup> · Baojin Guo<sup>2</sup> · Bing Han<sup>2</sup> · Haiming Han<sup>2</sup> · Jingpeng Zhang<sup>2</sup> · Yuqing Lu<sup>2</sup> · Zhi Zhang<sup>2</sup> · Xinming Yang<sup>2</sup> · Xiuquan Li<sup>2</sup> · Weihua Liu<sup>2</sup> · Lihui Li<sup>1,2</sup>

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## Abstract

**Key message** The powdery mildew resistance locus was mapped to *A. cristatum* chromosome 6PL bin (0.27–0.51) and agronomic traits evaluation indicated that this locus has potential breeding application value.

**Abstract** *Agropyron cristatum* ( $2n = 4x = 28$ , PPPP) is a wild relative of wheat with an abundance of biotic and abiotic stress resistance genes and is considered one of the best exogenous donor relatives for wheat breeding. A number of wheat-*A. cristatum* derived lines have been generated, including addition lines, translocation lines and deletion lines. In this study, the 6P disomic addition line 4844-12 ( $2n = 2x = 44$ ) was confirmed to have genetic effects on powdery mildew resistance. Four 6P deletion lines (del16a, del19b, del21 and del27) and two translocation lines (WAT638a and WAT638b), derived from radiation treatment of 4844-12, were used to further assess the 6P powdery mildew resistance locus by powdery mildew resistance assessment, genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH) and 6P specific sequence-tagged-site (STS) markers. Collectively, the locus harboring the powdery mildew resistance gene was genetically mapped to a 6PL bin (0.27–0.51). The genetic effects of this chromosome segment on resistance to powdery mildew were further confirmed by del16a and del27 BC<sub>3</sub>F<sub>2</sub> lines. Comprehensive evaluation of agronomic traits revealed that the powdery mildew resistance locus of 6PL (0.27–0.51) has potential application value in wheat breeding. A total of 22 resistant genes were annotated and 3 specific gene markers were developed for detecting chromatin of the resistant region based on genome re-sequencing. In summary, this study could broaden the powdery mildew resistance gene pool for wheat genetic improvements.

## Introduction

Wheat (*Triticum aestivum* L.) is one of the staple crop species worldwide, but wheat diseases have long been threatening yield security (Dinu et al. 2018; Shewry 2009; Singh et al. 2016). Powdery mildew, caused by *Blumeria graminis*

(DC.) Speer f. sp. *tritici* Em. Marchal (*Bgt*) (Jin et al. 2020), can infect various tissues of wheat, including stems, leaves and ears, affects yields by reducing the photosynthesis of leaves, and is a major destructive wheat disease worldwide. In addition, a large quantities of pesticides used to treat powdery mildew increased production costs and pollute the environment. (An et al. 2019; Wang et al. 2020b). Although 68 resistance genes of powdery mildew were identified from wheat and its relatives (He et al. 2021), most of these genes do not have broad-spectrum resistance; moreover, an increasing number of genes have lost their resistance to new races, and only a few of resistance genes are used in wheat breeding (Li et al. 2020; Zhang et al. 2021). Therefore, it is extremely important to explore new powdery mildew resistance genes.

Wild relatives of wheat provide a rich gene pool of resistance to powdery mildew, and approximately forty powdery mildew resistance genes have been reported. For instance, *Pm7*, *Pm8* and *Pm56* are derived from

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Yida Lin and Shenghui Zhou have contributed equally to this work.

✉ Lihui Li  
lilihui@caas.cn

<sup>1</sup> State Key Laboratory of Crop Stress Biology for Arid Areas and College of Agronomy, Northwest A&F University, Yangling 712100, China

<sup>2</sup> National Key Facility for Crop Gene Resources and Genetic Improvement/Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

*Secale cereale* (Hao et al. 2018; McIntosh et al. 2011; Singh et al. 2018), *Pm21*, *Pm55* and *Pm62* are derived from *Dasyphyrum villosum* (He et al. 2020; Zhang et al. 2018, 2016b). Similarly, *Pm34*, *Pm35* and *Pm58* are derived from *Aegilops tauschii* (Miranda et al. 2007, 2006; Wiersma et al. 2017), and *Pm40* and *Pm43* are derived from *Thinopyrum intermedium* (He et al. 2009; Luo et al. 2009). Notably, *Pm21* is a cluster of genes that are widely used in wheat improvement and breeding, therefore, obtaining powdery mildew resistance genes especially broad-spectrum resistance genes from wheat relatives by distant hybridization is an effective method for wheat breeding (Cao et al. 2011; He et al. 2018; Wu et al. 2021).

*Agropyron cristatum* ( $2n=4x=28$ , PPPP) evolved under unfavorable environmental conditions and has strong tolerance to biotic and abiotic stresses (Dong et al. 1992; Wu et al. 2006). As expected, it carries many genes with excellent agronomic traits, such as many yield related genes and disease resistance genes, and is considered one of the best exogenous donor relatives for wheat breeding (Jiang et al. 2018; Li et al. 2017; Pan et al. 2017; Zhang et al. 2016a). In recent years, resistance genes on chromosome segments of 2P and 6P have been preliminarily mapped by deletion and translocation lines. For instance, stripe rust and leaf rust resistance genes have been mapped to 6PS (0.81–1.00) (Zhang et al. 2017b), and leaf rust and powdery mildew resistance genes have been mapped to 2PL (0.66–0.86) (Jiang et al. 2018; Li et al. 2017). Moreover, a novel broad-spectrum powdery mildew resistance gene from wheat-*A. cristatum* introgression line Pubing 74 was created, and could be detected by the 6PL specific sequence-tagged-site (STS) molecular marker *Agc2970* (Lu et al. 2016). Copete and Cabrera (2017) used wheat conserved orthologous set (COS) markers to verify that *A. cristatum* exhibits resistance to powdery mildew via loci on chromosome arms 2PL and 6PL. Thus, genomic mapping of powdery mildew resistance loci from *A. cristatum* and the study of their association with crop yields are necessary and relevant tasks.

In this study, a novel powdery mildew resistance locus was identified on the *A. cristatum* 6P chromosome. Various methods including disease evaluation, genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH), molecular markers analysis and genetic analysis, were employed to map the 6P chromosome segment where the resistance locus is located and to evaluate the application potential of this locus in wheat breeding. In addition, a total of 22 resistance genes have been annotated by re-sequencing, and 3 gene markers have been developed that could be used to detect chromatin fragments of resistance locus. This study provides an important insight into discovering disease resistance genes of *A. cristatum* in wheat and has important value for wheat breeding.

## Materials and methods

### Plant materials

*A. cristatum* (Z559;  $2n=4x=28$ , PPPP) was collected from Xinjiang, China. The common wheat cultivar Fuku-hokomugi (Fukuho;  $2n=6x=42$ , AABBDD) from Japan was used as the current parent. The wheat-*A. cristatum* 6P disomic addition line 4844–12 ( $2n=2x=44$ ) was generated by distant hybridization of Z559 and Fukuho (Wu et al. 2006). WAT638a, WAT638b del16a, del19b, del21 and del27 were created by irradiating 4844–12 with  $^{60}\text{Co}$ - $\gamma$  rays, followed by pollination with Gaocheng8901 (Song et al. 2016). The resulting  $F_1$  progeny were backcrossed with Fukuho, obtained from the generation  $BC_5F_6$ . Zhongzuo9504 and Fukuho were used as the susceptible controls for powdery mildew resistance evaluation. The 6PL translocation line WAT638b was used to evaluate breeding potential. All the plant materials were preserved at the Center of Crop Germplasm Resources Research at the Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China.

### Assessment of powdery mildew resistance and agronomic traits

*Bgt* isolates E20 was provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The powdery mildew resistance of the following lines was assessed during the adult stage in greenhouse in Beijing: homozygous 4844–12, del16a, del19b, del21, del27, WAT638a and WAT638b; the control parents Fukuho and Zhongzuo9504, 20 plants per material. In addition, 150 del16a and del27 plants from  $BC_3F_2$  populations were planted in pots, with five plants per pot. The chi-square ( $\chi^2$ ) test was used to test the population segregation ratios. Adult plant resistance was investigated three times after flowering and then scored when symptoms were severe on the susceptible line Zhongzuo9504 (Li et al. 2017). Powdery mildew resistance was evaluated by immunity type (IT) using a 0–9 scale. An IT of 0–4 indicated resistance, and at IT of 5–9 indicated susceptibility; specifically, an IT was 0 indicated immune; 0, nearly immune; 1–2, highly resistant; 3–4, moderately resistant; 5–6, moderately susceptible; 7–8, highly susceptible; and 9, extremely susceptible (Sheng and Duan 1991).

Agronomic traits of del16a, del19b, del21, del27 and the translocation line WAT638b were assessed in the field (2020–2021) in Henan via assessment of powdery mildew resistance (natural infection) and investigation of yield related traits including spike length, spikelet number per spike, kernel number per spikelet, grain number per spike, grain length and

thousand grain weight. These traits were also used to evaluate their breeding potential.

### Cytological identification

GISH was carried out to determine the karyotype of del16a, del19b, del21, del27, WAT638a, WAT638b and 4844–12 by genomic probe of Z559 to distinguish between Fukuho and *A. cristatum* chromosomes. FISH was used with oligonucleotide probes, including Oligo-pSc119.2–1 (6-FAM-5') and Oligo-pTa535-1 (Tamra-5') to detect chromosome variation in Fukuho, the probes were synthesized by Sangon Biotech (Shanghai) (Tang et al. 2014). Nitrous oxide gas and 90% acetic acid were used to treat the root tips of all the materials, similar processing procedures were performed as described by Wang et al. (2020b), in which a mixture of cellulase (R-10, Yakult Japan) and pectinase (Y-23, Yakult Japan) was used for cellenzymatic hydrolysis (Zhu et al. 2017). Root tip cells were exposed to ultraviolet light for 80 s at 125,000  $\mu\text{J}/\text{cm}^2$  and counterstained with 4',6-Diamidino-2-phenylindole (DAPI). Fluorescent signals were observed via an Olympus AX80 fluorescence microscope (Olympus Corporation, Tokyo, Japan), imaged with a charge-coupled device (CCD) camera (Diagnostic Institute, Inc. Sterling Height, MI, USA) and modified with Photoshop CS6.

### Molecular marker analysis

DNA of 4844–12, del16a, del19b, del21, del27, WAT638a and WAT638b was obtained by the modified cetyltrimethylammonium bromide (CTAB) method (Allen et al. 2006). Molecular markers were derived from the specific expressed-sequence-tags (ESTs) developed by the transcriptome sequencing of Z559 (Zhang et al. 2017a), and several EST markers were mapped to the 31 bins of chromosome 6P (Song et al. 2016). These 31 markers were used to determine the genotypes of the 4844–12 and their derived offspring. The genotypes of del27 and del16a BC<sub>3</sub>F<sub>2</sub> population were determined via the Z559-specific repeat marker *AcPR3* (Han et al. 2017). All the primers used were synthesized by Sangon Biotech (Shanghai). The PCR amplification system used consisted of 5.0  $\mu\text{l}$  of 2 $\times$  Green Master Mix, 1.0  $\mu\text{l}$  of DNA (50–100 ng/ $\mu\text{l}$ ), 2.0  $\mu\text{l}$  of a mixture of forward and reverse primers (2  $\mu\text{M}$ ) and up to 10.0  $\mu\text{l}$  of ddH<sub>2</sub>O. The PCR products were detected on a 1% agarose gels and 8% nondenaturing polyacrylamide gels.

### The disease resistance gene prediction and markers development

The del16a and del27 were re-sequenced to determine the physical interval of 6PL bin (0.27–0.51). The fresh leaves of seedlings were collected, mixed, and immediately frozen in liquid nitrogen for DNA extraction. The

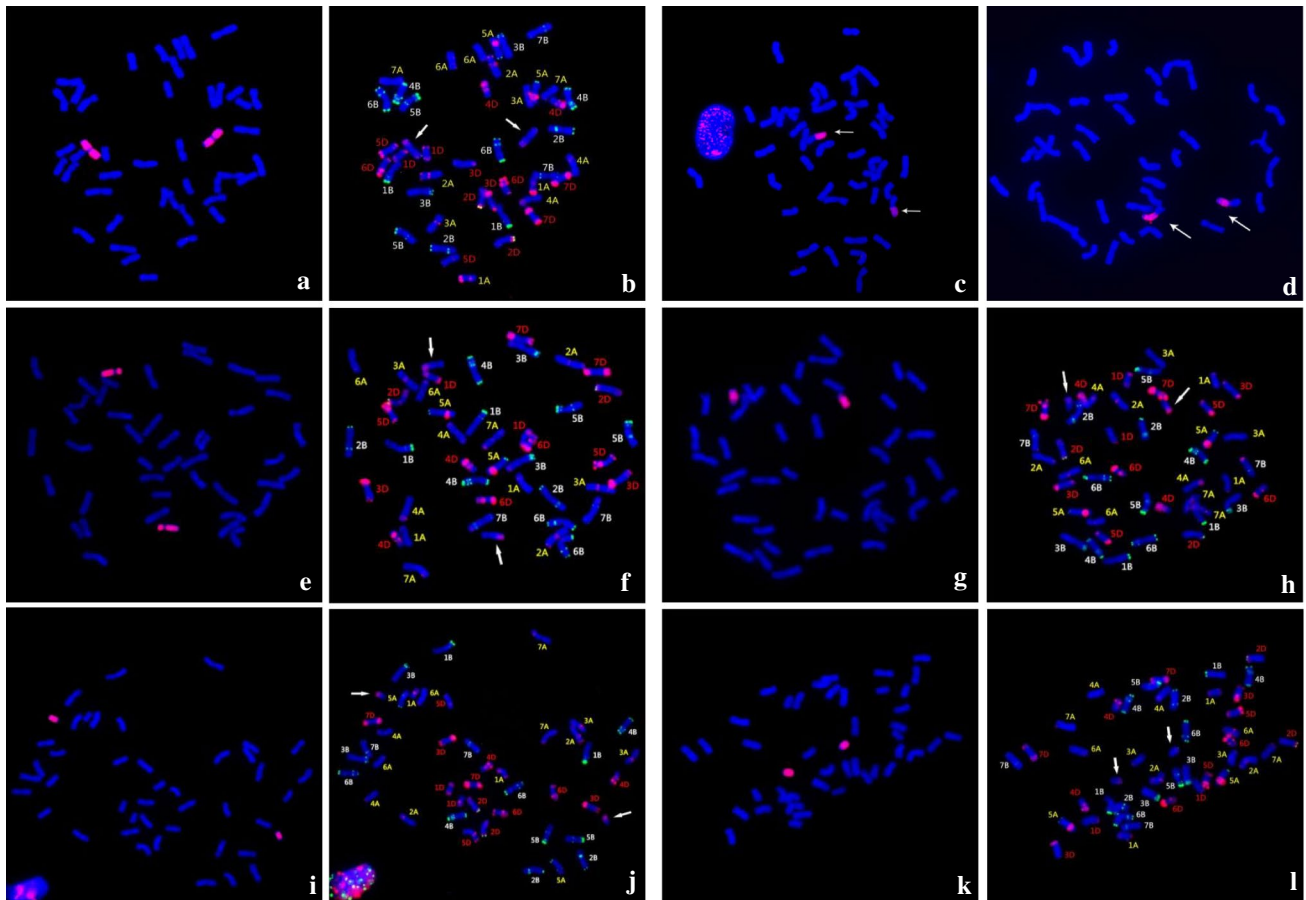
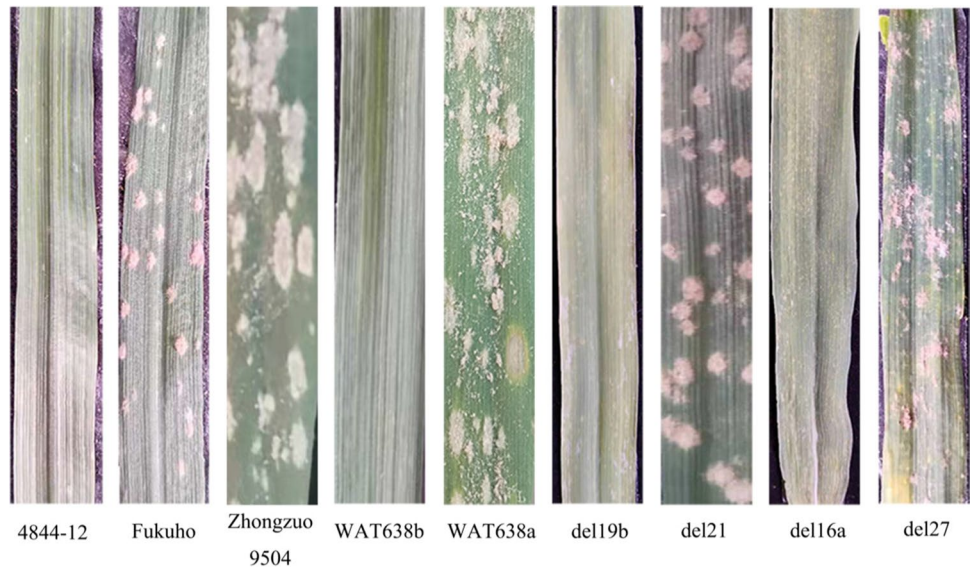
350-bp paired-end (PE) libraries were prepared using the NEBNext Ultra DNA Library Prep Kit. Sequencing was performed through HiSeq 2500 (Illumina, San Diego, CA, USA) with a paired-end read length of 150 bp at Huazhi Biotech Co., Ltd (Changsha, China). About 5 $\times$  Illumina short reads were generated. Raw sequencing data were processed using 'fastp' for removing adapters, detecting poor quality reads and over-represented sequences (Van der Auwera et al. 2013; Winfield et al. 2012). The clean reads were mapped to the integrated reference sequences of *A. cristatum* Z1842 (unpublished) and Chinese Spring RefSeqv2.0 ([https://urgi.versailles.inra.fr/download/iwgsc/IWGSC\\_RefSeq\\_Assemblies/v2.0/](https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v2.0/)) using BWA-MEM (Oliva et al. 2021). PCR duplicates were detected and removed using Picard's MarkDuplicates (<https://broadinstitute.github.io/picard/>). Finally, bedtools were used to calculate the sequencing coverage of reads to reference genome (Quinlan and Hall 2010). Introgression regions were identified by the distribution of coverage. The visualization of coverage was performed by ggplot2 package of R (Maag 2018). The disease resistance genes were obtained using a disease resistance gene analogue (RGA) prediction pipeline and homologous gene alignment with *Arabidopsis thaliana* and *Oryza sativa*. The gene markers were developed based on the sequence differences between orthologous genes of Chinese spring and *A. cristatum*.

**Table 1** Powdery mildew resistance assessment of wheat-*A. cristatum* 6P chromosome derived lines

Materials	Powdery mildew response	IT	Number of plants
4844–12	HR	2	20
Fukuho	S	7	20
Zhongzuo9504	S	9	20
WAT638a	S	7	20
WAT638b	HR	2	20
del16a	HR	2	20
del19b	HR	2	20
del21	S	7	20
del27	S	7	20

Zhongzuo9504 and the recurrent parent Fukuho served as negative control. IT represents powdery mildew resistance scale (Sheng and Duan 1991)

**Fig. 1** Powdery mildew resistance assessment of 4844–12, WAT638a, WAT638b, del16a, del19b, del21 and del27. Fukuho and Zhongzuo9504 as the susceptible control



**Fig. 2** Cytogenetic analysis by in situ hybridization. **a** GISH analysis of 4844–12; **b** FISH analysis of 4844–12; **c** GISH analysis of WAT638b; **d** GISH analysis of WAT638a; **e** GISH analysis of del16a; **f** FISH analysis of del16a; **g** GISH analysis of del27; **h** FISH analysis of del27; **i** GISH analysis of del21; **j** FISH analysis of del21; **k**

GISH analysis of del19b; **l** FISH analysis of del19b; FISH probes Oligo-pTa535-1 (red), Oligo-pSc119.2-1 (green) in (**b, f, h, j, l**), GISH probes (red) in (**a, c, d, e, g, i, k**). Chromosomes were counterstained with DAPI and visualized with blue fluorescence

## Results

### Powdery mildew resistance response of the *A. cristatum* 6P chromosome

At the adult stage, the following lines were inoculated with the *Bgt* races E20: the common wheat line Fukuho, Zhongzuo9504, and the wheat-*A. cristatum* 6P chromosome derived lines 4844–12, del16a, del19b, del21, del27, WAT638a and WAT638b with a genetic background of the common wheat cultivar Fukuho. Afterward, disease resistance was assessed once the susceptible control Zhongzuo9504 was thoroughly infected. The results showed that Zhongzuo9504 was extremely susceptible to powdery mildew, with an IT score of 9. The wheat-*A. cristatum* derived lines del21, del27, WAT638a and the recurrent parent

Fukuho were highly susceptible to powdery mildew, each with an IT score of 7. In contrast, 4844–12, del19b, del16a and WAT638b were highly resistant to powdery mildew, each with an IT score of 2 (Fig. 1, Table 1). These results suggested that a resistance locus was present on the *A. cristatum* 6P chromosome.

### Cytological characterization

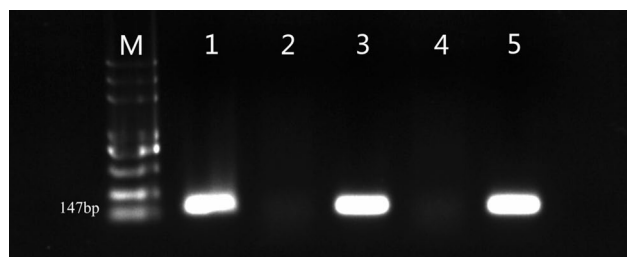
To determine the chromosomal composition, 4844–12 and the derived lines del16a, del19b, del21, del27, WAT638a and WAT638b were further characterized by GISH and FISH.

The results showed that the addition line 4844–12 has two chromosomes of Z559 and 42 chromosomes of Fukuho, with a clear green signal at the end of 6PL. It is a 6P chromosome additional line as previously reported (Wu et al. 2006)

**Table 2** Analysis of 6P specific STS molecular markers

Marker	4844–12	Fukuho	del19b	del21	del16a	del27	WAT638a	WAT638b	Bin
<i>AgC37790</i>	+	–	–	+	+	+	+	–	6PS14
<i>AgC4282</i>	+	–	–	+	+	+	+	–	6PS13
<i>AgC5604</i>	+	–	–	+	+	+	+	–	6PS12
<i>AgC2861</i>	+	–	–	+	+	+	+	–	6PS11
<i>AgC7094</i>	+	–	–	+	+	+	+	–	6PS10
<i>AgC2637</i>	+	–	–	+	+	+	+	–	6PS9
<i>AgC8950</i>	+	–	–	+	+	+	+	–	6PS8
<i>AgC1368</i>	+	–	–	+	+	+	+	–	6PS7
<i>AgC3896</i>	+	–	–	+	+	+	+	–	6PS6
<i>AgC10775</i>	+	–	–	+	+	+	+	–	6PS5
<i>AgC9835</i>	+	–	–	+	+	+	+	–	6PS4
<i>AgC4527</i>	+	–	–	+	+	+	+	–	6PS3
<i>AgC9581</i>	+	–	–	+	+	+	+	–	6PS2
<i>AgC7155</i>	+	–	–	+	+	+	+	–	6PS1
<i>AgC4399</i>	+	–	+	–	+	+	–	+	6PL1
<i>AgC27067</i>	+	–	+	–	+	+	–	+	6PL2
<i>AgC6900</i>	+	–	+	–	+	–	–	+	6PL3
<i>AgC3466</i>	+	–	+	–	+	–	–	+	6PL4
<i>AgC11930</i>	+	–	+	–	+	–	–	+	6PL5
<i>AgC2970</i>	+	–	+	–	+	–	–	+	6PL6
<i>AgC5103</i>	+	–	+	–	+	–	–	+	6PL7
<i>AgC15870</i>	+	–	+	–	–	–	–	+	6PL8
<i>AgC5532</i>	+	–	+	–	–	–	–	+	6PL9
<i>AgC2453</i>	+	–	+	–	–	–	–	+	6PL10
<i>AgC32015</i>	+	–	+	–	–	–	–	+	6PL11
<i>AgC9467</i>	+	–	+	–	–	–	–	+	6PL12
<i>AgC3960</i>	+	–	+	–	–	–	–	+	6PL13
<i>AgC10192</i>	+	–	+	–	–	–	–	+	6PL14
<i>AgC11295</i>	+	–	+	–	–	–	–	+	6PL15
<i>AgC12840</i>	+	–	+	–	–	–	–	+	6PL16
<i>AgC4348</i>	+	–	+	–	–	–	–	+	6PL17

‘+’ indicates that the material can be detected by this marker; ‘–’ indicates that the material cannot be detected by this marker



**Fig. 3** Analysis of STS molecular marker *Agc2970*. M: Marker D2000 puls; 1: Z559; 2: Fukuho; 3: del16a; 4: del27; 5: PB74

(Fig. 2a, 2b). WAT638a and WAT638b both have 42 chromosomes, and a full *A. cristatum* 6PS and 6PL chromosome segments were translocated onto wheat chromosomes as previously reported (Song et al. 2016) (Fig. 2c, 2d). Del16a, del19b, del21 and del27 all have 44 chromosomes with two obvious incomplete chromosomes from Z559, and the other 42 chromosomes are from wheat. No obvious abnormality of wheat chromosomes was observed among them (Fig. 2e, 2f). In summary, cytological identification revealed that del16a, del19b, del21 and del27 are wheat-*A. cristatum* 6P chromosomal deletion lines.

### Molecular marker analysis

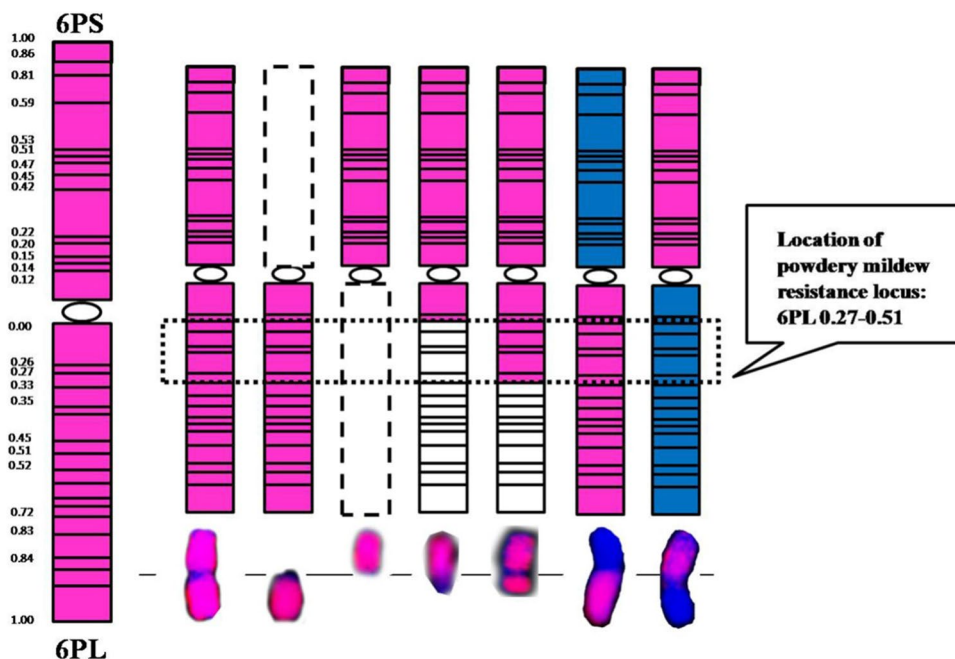
In previous work, the 6P chromosome was divided into 31 segments: 14 segments of 6PS and 17 segments of 6PL (Song et al. 2016). To determine the segments of the *A. cristatum* 6P chromosome introduced to wheat-*A. cristatum* derived lines, a total of 31 representative 6P-specific

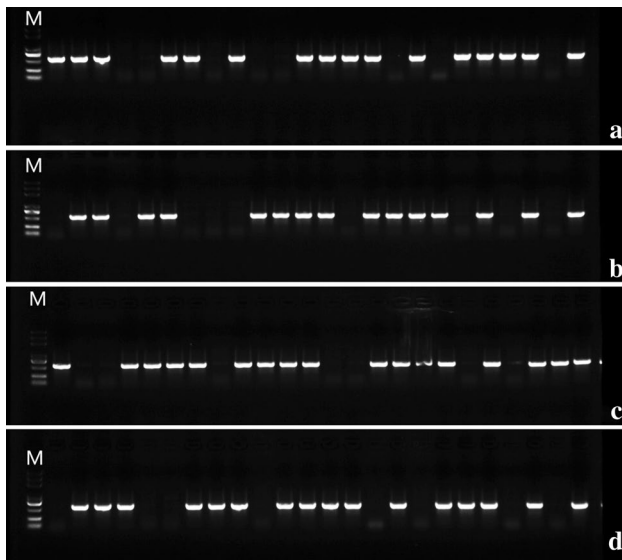
molecular markers in each segment were screened within 4844–12, del16a, del19b, del21, del27, WAT638a and WAT638b. All 31 markers of 6P showed amplification with prominent bands specific to *A. cristatum* 4844–12. However, these bands were absent in the recurrent parent Fukuho, indicating that 4844–12 is a wheat-*A. cristatum* addition line with a pair of complete 6P chromosomes. Fourteen markers belonging to 6PS were not amplified in del19b and WAT638b, indicating that the 6P chromosome in the del19b and WAT638b genome are 6PL. Del21 and WAT638a, with a complete 6PS; del27, with complete 6PS and 6PL bin (0–0.27), and del16a, with 6PS and 6PL bin (0–0.51) were identified in the same way (Table 2). In addition, a specific marker of 6PL bin (0–0.51), *Agc2970*, on the 6P chromosome was detected in powdery mildew-resistant wheat-*A. cristatum* introgressive line Pubing74 (Fig. 3). The powdery mildew resistance gene in Pubing74 may be the same as the powdery mildew resistance genes in 6PL(0.27–0.51). Based on the above analysis of the cytology results and molecular markers and the assessment of powdery mildew, the powdery mildew resistance gene was located on 6PL (0.27–0.51) (Fig. 4).

### Genetic analysis of the powdery mildew resistance locus

To further determine the genetic effect of the powdery mildew resistance locus of 6PL (0.27–0.51), the del16a and del27 BC<sub>3</sub>F<sub>2</sub> populations were inoculated with *Bgt* races E20 at the adult stage and then genotyped using the repeat sequence marker *AcPR3a* (Fig. 5). Both positive and

**Fig. 4** GISH patterns and chromosome diagrams of the 4844–12 and the 6P deletion lines. On the left is 31 chromosome bins of 6P. Pink represents *A. cristatum* chromatin, blue represents wheat chromatin

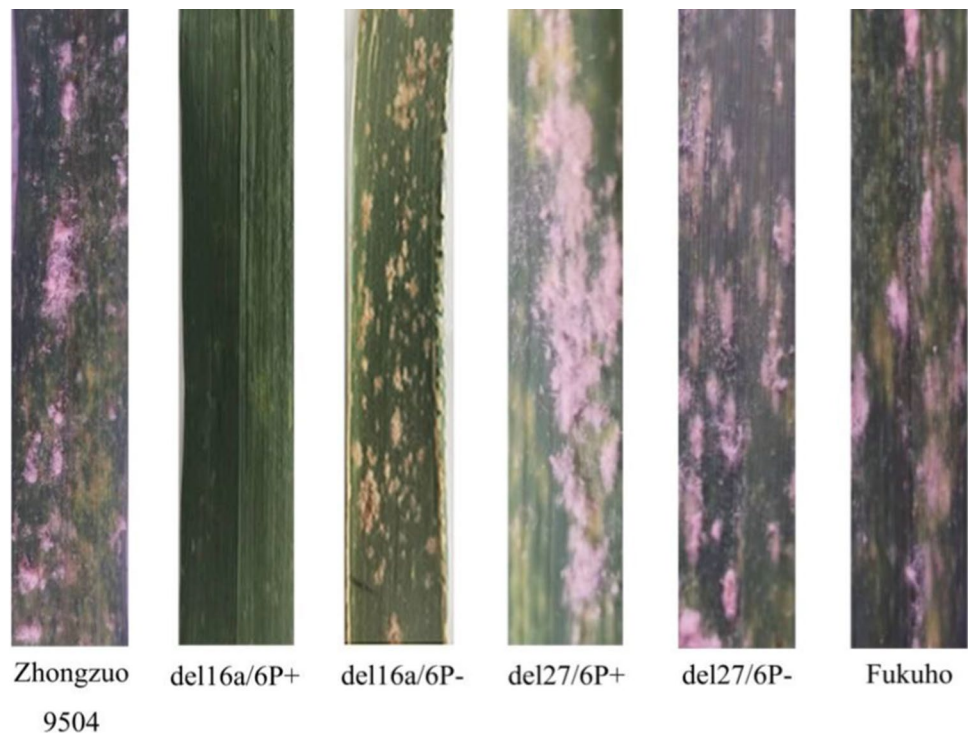




**Fig. 5** Genotype detection of part of the  $BC_3F_2$  population of del16a and del27 by repeat sequence markers *AcPR3a*, **a** and **b** are part of the  $BC_3F_2$  population of del16a, **c** and **d** are part of the  $BC_3F_2$  population of del27. M is marker D2000 plus

negative del27 plants were highly susceptible to powdery mildew with IT score was 7. All 110 positive del16a plants were highly resistant to powdery mildew, with an IT score of 2, and all 36 negative plants were highly susceptible to powdery mildew, with an IT score of 7 (Fig. 6, Table 3).

**Fig. 6** Powdery mildew resistance assessment of  $BC_3F_2$  population of del16a and del27, negative control Fukuho and Zhongzuo9504



$\chi^2$  tests further confirmed that powdery mildew resistance was significantly affected by the 6PL (0.27–0.51) segment.

### Evaluation of agronomic traits and breeding potential of the resistance locus

Exogenous resistance genes can be transferred into the common wheat through distant hybridization in the form of addition lines, substitution lines and translocation lines, but the large fragments of exogenous chromosomes introduced into wheat will produce negative agronomic traits, especially affect the development of wheat yield, resulting in resistance genes that are difficult to directly use for wheat improvement (Dai et al. 2020). To evaluate the breeding potential of the *A.cristatum* segments carrying this powdery mildew resistance locus, agronomic traits of all the wheat-*A.cristatum* derived lines (4844–12, del16a, del19b, del21, del27) and the 6PL translocation line WAT638b were evaluated. Compared to Fukuho, all lines, including 4844–12, del16a and WAT638b, had resistance to powdery mildew (spontaneously inoculated) (Fig. 7). Del19b, del16a and WAT638b carried 6PL (0.27–0.51) could increase spikelets number per spike (SNS) by 3.2, 5.0, 0.7, average kernel number per spikelet (KNS) could be increased by 1.5, 1.4, 1.0, and average grain number per spike (GNS) could be increased by 22.0, 19.0, 16.6 compared to Fukuho by Duncan's multiple-range test, respectively, None of these materials had obvious negative response to other agronomic traits, such as thousand-grain

**Table 3** Powdery mildew resistance assessment of BC<sub>3</sub>F<sub>2</sub> population of del16a and del27

Materials	BC <sub>3</sub> F <sub>2</sub>		Total
	6P+	6P–	
Zhongzuo9504		S(20)	20
Fukuho		S(20)	20
Del16a*	R(110)	S(36)	146
Del27*	S(111)	S(39)	150

‘+’ indicated the BC<sub>3</sub>F<sub>2</sub> population of the deletion lines contained *A. cristatum* 6P chromatin;

‘–’ indicated the BC<sub>3</sub>F<sub>2</sub> population of the deletion lines contained no *A. cristatum* 6P chromatin; R: resistant to powdery mildew; S: susceptible to powdery mildew

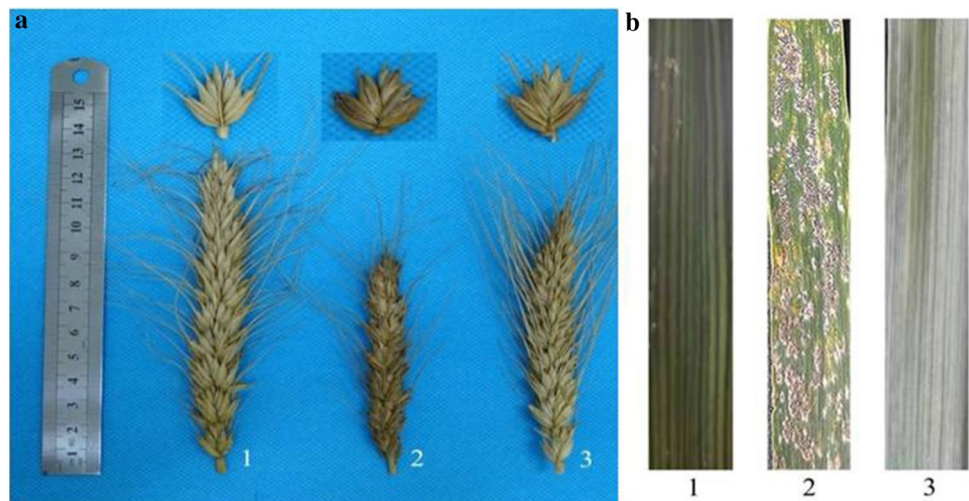
Numbers in brackets indicated the number of the progenies of deletion lines corresponding to resistance or susceptibility to powdery mildew. \*Chi-square statistics: *p* value < 0.01

weight. (Table 4). This shows that the 6PL resistance segment (0.27–0.51) also has the genetic effect of increasing the grain number per spike, which is consistent with the results of previous studies (Zhang et al. 2019). In summary, powdery mildew resistance locus 6PL (0.27–0.51) carried high grain number and disease resistance genes, no negative effect on thousand-grain weight, had potential application value for wheat breeding by creating small translocation lines in the future.

### Identification of candidate genes and development of markers

To determine the accuracy of the 6PL powdery mildew resistance segment (0.27–0.51) genomic region, the del16a and del27 were re-sequenced and mapped on *A. cristatum* Z1842 genome (Unpublished). By calculating the chromosome coverage, we determined that del27 carried ~ 387.02Mb of 6P, while del16a carried ~ 500.13Mb

**Fig. 7** Investigation of agronomic traits in the field. **a** Spike morphology diagram. **b** Assessment of powdery mildew in the field. 1: 4844–12; 2: Fukuho 3: WAT638b

**Table 4** Agronomic traits for four deletion lines and their parents in 2020–2021

Materials	Type	Spike length (cm)	Spikelet number per Spike	Kernel number per Spikelet	Grain number per Spike	Grain length(mm)	Thousand-Grain Weight(g)
4844–12		11.80 ± 0.82*	24.60 ± 1.07**	6.20 ± 0.63**	102.63 ± 10.21**	5.88 ± 0.16	37.35 ± 1.18
Fukuho		10.30 ± 0.86	20.80 ± 1.32	4.10 ± 0.32	64.00 ± 3.87	5.90 ± 0.07	35.84 ± 1.79
del21	6PS	11.40 ± 0.81*	21.20 ± 1.14	3.80 ± 0.42	58.22 ± 8.64	6.32 ± 0.26**	43.21 ± 4.91**
del19b	6PL	10.50 ± 0.71	24.00 ± 1.56**	5.60 ± 0.70**	86.00 ± 8.12**	5.54 ± 0.15	31.19 ± 2.85**
del27	6PS-6PL(0–0.27)	9.70 ± 0.67	23.80 ± 1.14**	4.10 ± 0.32	60.25 ± 10.08	5.90 ± 0.18	35.01 ± 3.73
del16a	6PS-6PL(0–0.51)	11.05 ± 0.64*	25.80 ± 1.03**	5.50 ± 0.71**	83.00 ± 6.59**	5.95 ± 0.19	37.23 ± 3.80
WAT638b	6AS-6PL	10.40 ± 0.52	21.50 ± 1.43*	5.10 ± 0.57**	80.56 ± 4.36**	5.61 ± 0.20	35.28 ± 2.82

\* and \*\* indicate significant differences from Fukuho by Duncan’s multiple-range test at the probability levels of *p* = 0.05 and *p* = 0.01, respectively



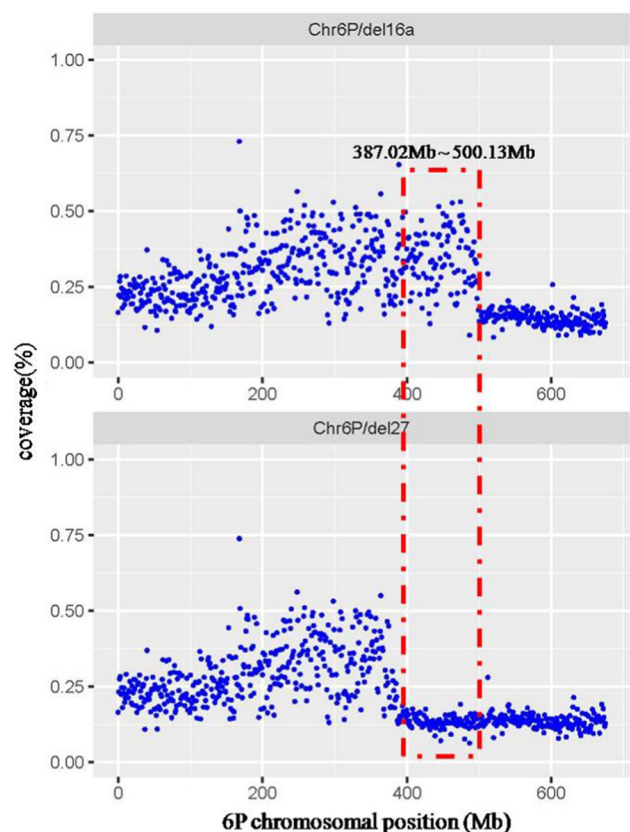
of 6P (Fig. 8). Therefore, the powdery mildew resistance gene(s) was located in the 387.02Mb~500.13Mb of 6P chromosome. According to the gene annotation, a total of 813 genes (*Acristatum06G039150*~*Acristatum06G047420*) were deposited in this interval. Among these genes, 17 resistance genes were predicted to be disease resistance genes by the RGAugury pipeline and 5 resistant genes were obtained homologous gene alignment with *Arabidopsis thaliana* and *Oryza sativa* (Table 5). Through sequence alignment with orthologous genes in Chinese Spring, we developed 3 *A. cristatum* specific gene markers (*Ac41770-2*, *Ac41790-2* and *Ac44250-2* correspond to gene *Acristatum06G041770*, *Acristatum06G041790* and *Acristatum06G044250*, respectively) (Fig. 9, Table 6). It indicated that these markers could detect chromatin of resistant regions in wheat-*A.cristatum* derived lines and beneficial to use molecular marker-assisted selection breeding in wheat.

## Discussion

Many powdery mildew resistance genes in wheat have lost their original resistance to new races. In this case, transferring genes from wild relatives to common wheat is an effective approach to expand genetic resources. At present, approximately 40 powdery mildew resistance genes have been discovered from wheat relatives. As an excellent gene donor parent for wheat breeding, *A. cristatum* has many resistance genes. A broad-spectrum powdery mildew resistance-associated segment located in 2PL bin 0.66–0.86 and the *Pmpb74* gene from the wheat-*A.cristatum* introgressive line Pubing74 have been reported (Li et al. 2017; Lu et al. 2016). In the present study, a new 6P powdery mildew resistance gene whose effects are apparent at the adult stage was identified. Hence, our study could broaden the pool of resistance genes for wheat breeding.

It is feasible method to use deletion lines and translocation lines carried different segments to divide chromosomal regions to ultimately locate foreign genes. In recent years, genes with providing broad-spectrum resistance to powdery mildew and leaf rust were located on 2PL (0.66–0.86), and stripe rust resistance genes and leaf rust resistance genes were located on 6PS (0.81–1.00) by mapping deletion lines and translocation lines (Jiang et al. 2018; Li et al. 2017; Song et al. 2016; Zhang et al. 2017b). In addition, the blue-grained gene from *Thinopyrum ponticum* chromosome 4Ag was successfully located by the use of a similar method (Liu et al. 2018). In our study, four deletion lines (del116a, del119b, del121, del127) and two translocation lines (WAT638a, WAT638b) were used to locate powdery mildew resistance genes, and their location was narrowed to 6PL (0.27–0.51). On the other hand, developing a large number of specific molecular markers of wheat relatives

is a convenient way to determine the segments of derived lines. Thirty-one expressed sequence tag-sequence-tagged site (EST-STS) markers were designed according to transcriptome sequencing data, and the loci of the deletion lines del116a, del119b, del121 and del127 were accurately determined. Based on the high similarity of the transcriptome sequences between *A. cristatum* and wheat (Chinese Spring), Ma et al. (2019) developed specific single-nucleotide polymorphism (SNP) markers for the P genome of *A. cristatum* under the background of multiple wheat varieties on a large scale. Moreover, using a wheat 660 K SNP array, Zhou et al. (2018) constructed high-density genetic linkage maps of *Agropyron* Gaertn. In this study, we developed 3 molecular markers, which could effectively detect chromatin of resistant locus in wheat-*A. cristatum* derived lines. Through the process of distant hybridization, a growing number of addition lines and translocation lines have been generated. Although many derived lines carry excellent exogenous resistance genes, most of these genes cannot be directly used in wheat breeding due to genetic linkage drag, and only a few excellent gene clusters are used in wheat breeding, for example, *Lr26/Sr31/Yr9/Pm8* resistance gene



**Fig. 8** Reads coverage analysis of del27 and del116a. The horizontal coordinate represents the position of 6P chromosome. The ordinate represents reads coverage at the corresponding location

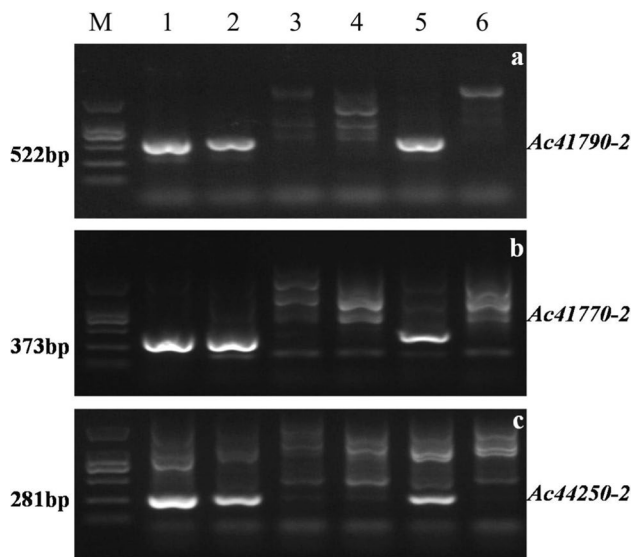
**Table 5** Resistant genes prediction of 6PL (0.27–0.51) by RGAugury pipeline and homologous annotation

Prediction method	Gene ID	Structural domain annotation/Homologous annotation
RGAugury pipeline	<i>Acristatum06G040060</i>	TM-CC
	<i>Acristatum06G040340</i>	TM-CC
	<i>Acristatum06G041180</i>	TM-CC
	<i>Acristatum06G041330</i>	TM-CC
	<i>Acristatum06G042660</i>	TM-CC
	<i>Acristatum06G043190</i>	TM-CC
	<i>Acristatum06G043890</i>	TM-CC
	<i>Acristatum06G039990</i>	RLP
	<i>Acristatum06G039170</i>	RLK
	<i>Acristatum06G039330</i>	RLK
	<i>Acristatum06G041460</i>	RLK
	<i>Acristatum06G041500</i>	RLK
	<i>Acristatum06G041910</i>	RLK
	<i>Acristatum06G042480</i>	RLK
	<i>Acristatum06G043460</i>	RLK
	<i>Acristatum06G044990</i>	RLK
	<i>Acristatum06G046540</i>	RLK
Homologous annotation	<i>Acristatum06G041770</i>	<i>OsPAL</i>
	<i>Acristatum06G041790</i>	<i>OsZB8</i>
	<i>Acristatum06G042750</i>	<i>OsTRITD</i>
	<i>Acristatum06G044250</i>	<i>OsDJA6</i>
	<i>Acristatum06G046630</i>	<i>AtMIZ1</i>

clusters of the 1B/1R translocation line from *Secale cereale* (Lazaridou et al. 2017; Mater et al. 2004); and *Lr24/Sr24* resistance gene clusters from *Thinopyrum elongatum*

(Rai et al. 2021). These gene clusters provide stable broad-spectrum resistance and have almost no negative effects on agronomic traits. Thus, some materials such as WAT638b and del16a, have 6PL (0.27–0.51) segments, maybe carries genes clusters for resistance to powdery mildew and increasing grain numbers and spikelet numbers per spike. Therefore, create the wheat-*A. cristatum* translocation line of small fragment with carrying 6PL (0.27–0.51) have great application potential for wheat resistance breeding. At the same time, we are working on transferring 6PL (0.27–0.51) to some main varieties, which maybe play an important role in disease resistance breeding in the future.

With the development of sequencing technology and the reduction of sequencing cost, more exogenous relatives of wheat have been sequenced, which speeds up the cloning foreign genes in the wheat, such as *Fhb7* has been cloned from *Thinopyrum elongatum* (Wang et al. 2020a). Genome resequencing is an effective technology to quickly determine the physical segments and discovering candidate genes of the exogenous fragment in translocation lines and deletion lines. In this study, del16a and del27 were re-sequenced to determine powdery mildew resistance locus about 113.11Mb from 387.02Mb to 500.13Mb on chromosome 6P. A total of 22 resistance genes have been annotated by RGAugury pipeline and homologous annotation with *Arabidopsis thaliana*



**Fig. 9** Development of gene markers. **a** *Ac41790-2* **b** *Ac41770-2* **c** *Ac44250-2*. 1: Z559; 2: WAT638b 3: Fukuho 4: Gaocheng8901; 5: del16a; 6: del27

**Table 6** Gene markers development based on the sequence differences between orthologous genes of Chinese spring and *A. cristatum*

Marker name	Gene ID	Left primer(5'-3')	Right primer(5'-3')	Annealing temperature
<i>Ac41770-2</i>	<i>Acristatum06G041770</i>	GACTTGTCCACC GTCGATCTAT	CCAGATCATG CAGCGGTT	56 °C
<i>Ac41790-2</i>	<i>Acristatum06G041790</i>	TGGTGAGGTGC AGGTTCATT	GCAGCGACTG GGTCATGAC	60 °C
<i>Ac44250-2</i>	<i>Acristatum06G044250</i>	GAAGACTTGTA CAATGGTGCC	CTGTTCTACAG AAGCACCCAG	56 °C

and *Oryza sativa*, and 3 gene markers have been developed, it is benefit to molecular marker-assisted selection breeding. In summary, this study provides a basic to clone powdery mildew resistance genes in the future.

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**Author's contribution statement** LHL conceived the research. YDL performed the research. YDL and SHZ wrote the paper. BH and XZL participated in part of cytology work. BJG, HMH, JPZ, YQL, ZZ, XMY, XQL and WHL participated in the preparation of the reagents and materials used in this study.

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## Declarations

**Competing interest** The authors declare no conflict of interest.

**Available of data and material** Data supporting the current study can be obtained by contacting the corresponding authors.

**Code availability** Not applicable.

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