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ORIGINAL ARTICLE

Fine mapping of *Pm58* from *Aegilops tauschii* conferring powdery mildew resistance

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Abstract

Key message **The powdery mildew resistance gene *Pm58* was traced to a 141.3-kb interval with the co-segregating marker *Xkasp68500* in wheat breeding.**

Abstract *Pm58* is a powdery mildew resistance gene identified in *Aegilops tauschii* accession TA1662 and effective in a common wheat background. To finely map *Pm58*, an F_2 population of 676 plants derived from the cross T093 × TA1662 was used for recombinant screening. We obtained 13 recombinants that occurred between the flanking markers *Xhnu670* and *Xhnu186*. Genotyping and phenotyping these recombinant $F_{2:3}$ families delimited *Pm58* to a 0.22-cM interval (*Xsts20220*–*Xkasp61553*) on chromosome arm 2DS. The region carrying the *Pm58* locus was approximately 141.3-kb, which contained eight annotated genes according to the reference genome sequence of *Ae. tauschii* AL8/78. Haplotype analysis of 178 *Ae. tauschii* accessions using the candidate gene-specific markers identified a disease resistance gene *AET2Gv20068500* as a candidate for *Pm58*. Comparative mapping of the *Pm58*-containing interval revealed two presence/absence variations (PAVs) between AL8/78 and common wheat Chinese Spring. PAV-1 resides in the 3′-end of *AET2Gv20068500*. The majority of 158 common wheat cultivars (84.8%) displayed the absence of a 14.1-kb fragment in the PAV-1 region, which was confirmed by aligning the targeted genome sequences of the other sequenced *Ae. tauschii* accessions and common wheat cultivars. A co-segregating marker *Xkasp68500* developed from *AET2Gv20068500* can distinguish TA1662 from all randomly selected common wheat cultivars and will be instrumental for tracking *Pm58* in breeding programs.

Introduction

Wheat (*Triticum aestivum* L.) powdery mildew, caused by the fungus *Blumeria graminis* (DC.) Speer f. sp. *tritici* emend. É. J. Marchal (*Bgt*), is a globally devastating disease particularly damaging in regions with high humidity and cool to moderate temperatures (Te Beest et al. 2008). This disease can cause reductions in the yield components of tiller number, grain number, and kernel weight (Bowen et al. 1991), and the resulting losses in yield are 30 to 40% in heavily epidemic years (Conner et al. 2003; Singh et al. 2016). Deployment of host resistance is the preferred choice for managing this disease and reducing pesticide dependency (Kang et al. 2020). Race-specific resistance is the basis of wheat powdery mildew resistance. Many cloned powdery mildew resistance genes encode nucleotide-binding leucine-rich-repeat-containing (NLR) immune receptors that can recognize pathogen effectors (Bourras et al. 2015, 2019; Praz et al. 2017; Hewitt et al. 2021). To prolong the usefulness of race-specific resistance, it is necessary to continuously broaden resistance gene pools, stack genes with different resistance modes, and allocate reasonable growing regions (Mundt 2002; Kang et al. 2020).

Wild relatives of common wheat (AABBDD, $2n = 6\times = 42$) are frequently used as sources of disease resistance

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despite the presences of crossing barriers and incompatibility (Kishii 2019). When a new race of the fungus *Puccinia graminis* f. sp. *tritici* (Ug99 or TTKSK) and wheat blast (*Magnaporthe oryzae Triticum*) appeared and threatened the global wheat production, breeders sought and deployed resistance genes in *Aegilops* species (Olson et al. 2013; Cruz et al. 2016). *Aegilops tauschii* Coss. (DD, $2n = 2x = 14$), the D-genome progenitor of common wheat (Huang et al. 2002), carries several genes for resistance to powdery mildew (Lutz et al. 1995; Miranda et al. 2006, 2007; Sun et al. 2006; Li et al. 2011; Wiersma et al. 2017). Some of them, such as *Pm2a*, *Pm19*, *Pm34*, *Pm35* and *Pm58*, have been transferred into common wheat through indirect or direct crossing approaches (Kishii 2019). Among these genes, only *Pm2a* has been cloned by mutant chromosome sequencing (Sánchez-Martín et al. 2016).

The powdery mildew resistance genes from the wild species or wheat relatives, such as *Pm12* (Jia et al. 1996), *Pm16* (Chen et al. 2005), *Pm20* (Friebe et al. 1994), and *Pm62* (Zhang et al. 2018), can usually provide durable resistance to *Bgt* pathogens. However, these genes are more difficult to directly use in breeding due to higher probabilities of having inferior agronomic characteristics dragged along with the target loci (Summers and Brown 2013). To eliminate the undesirable linkages associated with resistance genes, it is necessary to isolate them and develop their diagnostic markers. The releases of reference genome sequences of *T. urartu* (AA; Ling et al. 2018), *T. turgidum* ssp. *dicoccoides* (AABB; Avni et al. 2017), *T. turgidum* ssp. *durum* (AABB; Maccaferri et al. 2019), *Ae. tauschii* (DD; Luo et al. 2017), and *Secale cereale* (RR; Li et al. 2021) have greatly facilitated the development of new markers and map-based cloning of resistance genes from the wild relatives of wheat. Furthermore, re-sequencing of mapping parents has been used to identify single nucleotide polymorphisms (SNPs) and insertion/deletion variations (InDels) that have contributed to fine map the targeted genes (Qiu et al. 2021).

Powdery mildew resistance gene *Pm58* was identified in *Ae. tauschii* accession TA1662. It was previously mapped to an 8.6-Mb interval flanked by Kompetitive allele-specific PCR (KASP) markers *K-TP127986* and *K-TP61544* using 96 BC_2F_4 introgression lines and was confirmed to be effective at both seedling and adult stages (Wiersma et al. 2017, 2018). The objectives of this study were to (1) further narrow down the genomic region harboring the *Pm58* locus and identify its candidate genes, and (2) develop the co-segregating markers of *Pm58* allowing efficiently tracking the resistance allele in the hexaploid backgrounds.

Materials and methods

Plant materials

TA1662 (carrying *Pm58*) was crossed with T093, which was collected from Henan Province of China and was highly susceptible to powdery mildew. T093 and TA1662 were phylogenetically clustered into two distinct lineages of *Ae. tauschii*, i.e., lineage 1 (L1) and lineage 2 (L2), respectively (Zhou et al. 2021). A total of 676 F_2 plants and derived $F_{2:3}$ families were used for fine mapping of *Pm58*. One hundred and seventy-eight *Ae. tauschii* accessions used for haplotype analysis were collected from diverse regions of China, the Middle East and Central Asia (Supplementary Table 1). Furthermore, 158 worldwide common wheat cultivars were randomly selected and used to examine the situation of a presence/absence variation (PAV) resided in the *Pm58* region and detect the polymorphisms of the *Pm58* candidate gene-specific markers in the hexaploid backgrounds (Supplementary Table 2).

Powdery mildew evaluations

The prevalent Chinese *Bgt* isolate E09 (Lu et al. 2020) was used to inoculate the mapping populations and two parents TA1662 and T093. Each F_1 and F_2 seed was planted in a single cell of a 72-cell tray (6×12), and 20 to 25 seeds from each $F_{2:3}$ family were sown in five cells of a 50-cell tray (5×10). For fine mapping of *Pm58*, at least 25 F_3 plants were tested to confirm the phenotypes and genotypes of the corresponding F_2 recombinants. TA1662 and T093 were used as resistant and susceptible controls, respectively. All seedlings at the two-leaf stage were inoculated with fresh conidiospores and incubated under controlled chamber conditions with a daily cycle of 16 h of light at 22 °C and 8 h of darkness at 18 °C. Infection types (ITs) were recorded 5 to 7 d post-inoculation when T093 displayed severe symptoms using a scale from 0 to 4, in which 0, 0;, 1, and 2 were classified as resistant, and 3 and 4 were considered as susceptible (Lu et al. 2020).

Re-sequencing and SNP/InDel calling

The genomic DNA of TA1662 and T093 was extracted using a standard CTAB method (Huang et al. 2000), and 1 µg DNA per sample was fragmented by sonication to an average size of 300–400 bp. The libraries containing selected fragments were sequenced using a BGISEQ-500 platform with a paired-end read length of 150 bp. We filtered raw data using SOAPnuke (Chen et al. 2018) and

obtained clean reads with sequencing depths of more than 20 × for each accession.

The remaining high-quality reads were mapped to the *Ae. tauschii* AL8/78 reference genome (Luo et al. 2017) using the Burrows-Wheeler Alignment tool (BWA, Li and Durbin 2009). The duplicated reads were marked and removed using the Genome Analysis Toolkit (GATK, McKenna et al. 2010). The genomic variants (SNPs and InDels) were identified with the GATK HaplotypeCaller module. Variants' genic positions (e.g., intragenic, upstream, downstream, and intergenic) and associated functions were further annotated with ANNOVAR software (Wang et al. 2010) based on AL8/78 genome annotation information.

Marker development and linkage mapping

Pm58 has been previously mapped to the 13.5–22.1 Mb region on chromosome 2DS (Wiersma et al. 2017). The AL8/78 reference sequence corresponding to the target interval was used to screen for simple sequence repeat (SSR) markers with the Perl script MISA program (https://webblast.ipk-gatersleben.de/misa/). Linkage analysis with 12 newly-developed SSR markers and 179 T093/TA1662 F_2 plants delimited *Pm58* to the *Xhnu670–Xhnu186* interval on chromosome 2DS. Based on the re-sequencing results of the two mapping parents, the SNPs and InDels resided in the *Pm58*-containing interval were searched to develop KASP or sequence-tagged site (STS) markers for saturation mapping. All primers were designed with MacVector 11.0 (Accelrys, San Diego, CA, USA). Primer sequences of the markers detecting polymorphisms are listed in Supplementary Tables 3 and 4.

The SSR and STS markers were amplified in a T100™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) according to the following procedures: 95 °C for 3 min; 32 cycles of 95 °C for 15 s, 52–60 °C for 20 s, and 72 °C for 50 s; and a final extension at 72 °C for 10 min. The polymerase chain reaction (PCR) profile contained 7.5 µl of 2 × Taq Plus master Mix II (Vazyme, Nanjing, China), 1.2 µl of primer mix (3 µM for each primer), 1.5 µl (150 ng) of genomic DNA, and 4.8 µl of ddH_2O. The PCR products were analyzed by electrophoresis with 8% non-denaturing polyacrylamide gels or 1% agarose gels.

KASP genotyping assays

Twelve SNPs located within the *Pm58* region were converted to KASP markers (Supplementary Table 4). Two different tail sequences, FAM (5′-GAAGGTGACCAAGTTCATGCT-3′) and HEX (5′-GAAGGTCGGAGTCAACGGATT-3′), were added to the 5′-end of TA1662 allele (A1)- and T093 allele (A2)-specific primers, respectively. KASP assays were performed in 96-well formats with 10 µl reactions containing 5 µl of 2 × KASP Master mix (LGC Genomics, Middlesex, UK), 0.14 µl of primer mix (12 µM for each allele-specific primer and 30 µM for a common primer), 1.5 µl (150 ng) of genomic DNA, and 3.36 µl of ddH_2O. A LightCycler 480 II system (Roche, Indianapolis, IN, USA) was used for PCR thermocycling and fluorescence detection. The cycling programs were referred from Lu et al. (2020).

Physical and comparative mapping

The sequences of the polymorphic markers closely linked to *Pm58* were used as queries to search against the AL8/78 reference sequence (Luo et al. 2017). Based on the physical positions of two flanking markers *Xsts20220* and *Xkasp61553*, the *Pm58* interval was anchored onto the AL8/78 reference genome. According to the gene annotations of AL8/78 (http://aegilops.wheat.ucdavis.edu/jbrowse/index.html?data=Aet%2Fdata%2F&loc), eight annotated genes residing in the *Xsts20220–Xkasp61553* interval were extracted and used to search for their homologs in the D genome of common wheat cv. Chinese Spring (CS) (IWGSC 2018), using cutoff parameters of E-value ≤ $1E^{-10}$, identity ≥ 80%, and a minimum of 100 bp match length.

In the *Pm58*-containing interval, two PAVs were identified by aligning the targeted genome sequences of AL8/78 and CS, which were confirmed by comparative analysis of the other sequenced *Ae. tauschii* accessions (Zhou et al. 2021) and common wheat cultivars (Walkowiak et al. 2020; Sato et al. 2021). We also designed two pairs of primers (Supplementary Table 5) to analyze the situation of PAV-1 in 158 common wheat cultivars and 178 *Ae. tauschii* accessions.

Haplotype analysis

Eight candidate gene-specific markers (Supplementary Table 4) were designed and employed for genotyping 178 *Ae. tauschii* accessions. For association analysis, 5–10 plants (two-leaf stage) of each *Ae. tauschii* accession were evaluated for responses to *Bgt* isolate E09 in a chamber with three replicates.

Statistical analysis

Genetic analysis was conducted using an F_2 population derived from T093 × TA1662. The Chi-squared test was performed to test the hypothesis that *Pm58* was a dominant gene conferring powdery mildew resistance. The genetic linkage map was constructed with JoinMap 4.0 (Van Ooijen 2006) using the maximum likelihood algorithm and the Kosambi function.

Results

Inheritance of the powdery mildew resistance in TA1662

Aegilops tauschii accession TA1662 was immune to *Bgt* isolate E09 with IT 0 and T093 was highly susceptible with IT 4 (Fig. 1). To analyze the inheritance of *Pm58*, we crossed TA1662 with T093 and infected their F_1 plants with *Bgt* E09. The F_1 hybrids were highly resistant (IT 0–1), indicating the dominant nature of *Pm58*. Among the 179 F_2 plants, 142 were resistant with IT 0 to 1 and 37 were susceptible with IT 3 to 4, fitting a 3:1 ratio ($\chi^2 = 1.79$, $P = 0.18$). The $F_{2:3}$ populations segregated as 44 homozygous resistant, 98 segregating, and 37 homozygous susceptible fitting a 1:2:1 segregation ratio ($\chi^2 = 2.16$, $P = 0.34$). These results suggest that *Pm58* in TA1662 is a dominant powdery mildew resistance gene.

Rough mapping of *Pm58* with SSR markers

Using a population of 96 BC_2F_4 introgression lines, *Pm58* has been delimited to an 8.6-Mb interval flanked by *K-TP127986* and *K-TP61544* on chromosome 2DS (Wiersma et al. 2017). Based on the AL8/78 reference sequence in the targeted region, 12 SSR markers showed identical polymorphisms between the two parents and two contrasting bulks were developed (Supplementary Fig. 1). Using these newly developed SSR markers, we genotyped 179 F_2 plants previously used for inheritance analysis and initially localized *Pm58* into a 602.8-kb interval flanked by *Xhnu670* and *Xhnu186*, and co-segregated with *Xhnu415* (Fig. 2a).

Re-sequencing of TA1662 and T093

To identify more SNPs and InDels resided in the targeted interval, we re-sequenced TA1662 and T093 using a BGISEQ-500 platform with 23.3- and 24.8-fold coverage, respectively. The high-quality reads were mapped against the AL8/78 reference genome to identify genomic variants. In total, 28,835,554 homozygous SNPs and 3,327,243 homozygous InDels were detected between T093 and TA1662, which was similar to the number of variants between T093 and AL8/78 but 3.0-fold higher than that between two L2 accessions TA1662 and AL8/78 (Supplementary Fig. 2a). The SNPs and InDels between T093 and TA1662 were distributed unevenly among the *Ae. tauschii* chromosomes (Supplementary Fig. 2b). The number of variants per chromosome ranged from 3,933,976 for chromosome 1D to 5,099,799 for chromosome 7D. The SNP/InDel density also varied considerably among the chromosomes and ranged from 7500 variants per Mb for chromosome 2D to 8565 variants per Mb for chromosome 4D. A total of 5295 homozygous variants (including 4577 SNPs and 718 InDels) between the two parents were identified in the targeted 602.8-kb region containing *Pm58*. Among them, 1694 were located within genes, 341 were within the 1-kb upstream regions of transcription start sites, 259 were within the 1-kb downstream regions of transcription stop sites, and 3001 were in the intergenic regions. These variants facilitate the development of new molecular markers linked to *Pm58*.

Fine mapping of *Pm58* with KASP/STS markers

For saturation mapping of the *Pm58* region, 12 polymorphic KASP/STS markers were developed based on the variants resided in the *Xhnu670–Xhnu186* interval (Figs. 3 and 4).

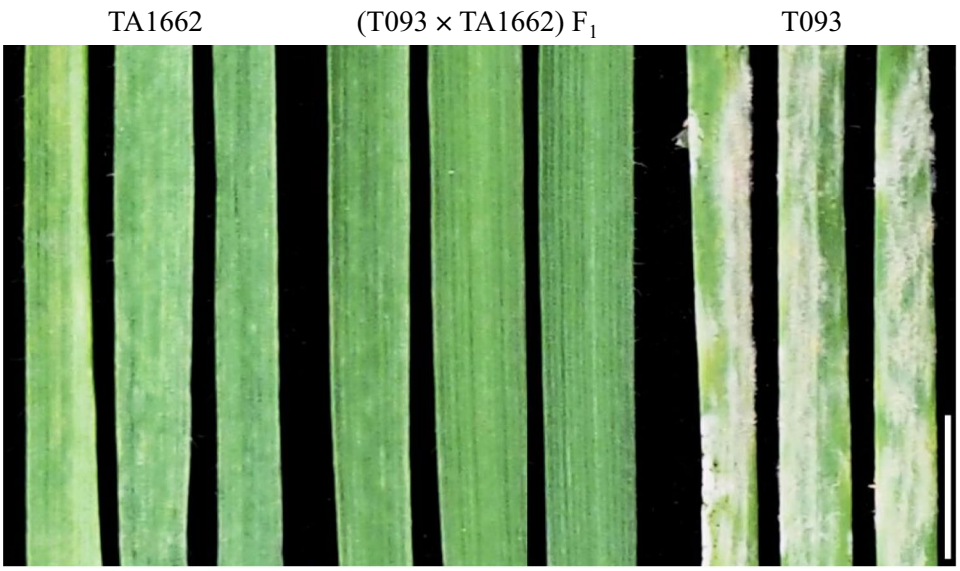

Fig. 1 Powdery mildew reactions of resistant parent TA1662, susceptible parent T093, and their F_1 hybrids to *Bgt* isolate E09. Representative leaves were photographed at 7 d post-inoculation. Scale bar, 5 mm

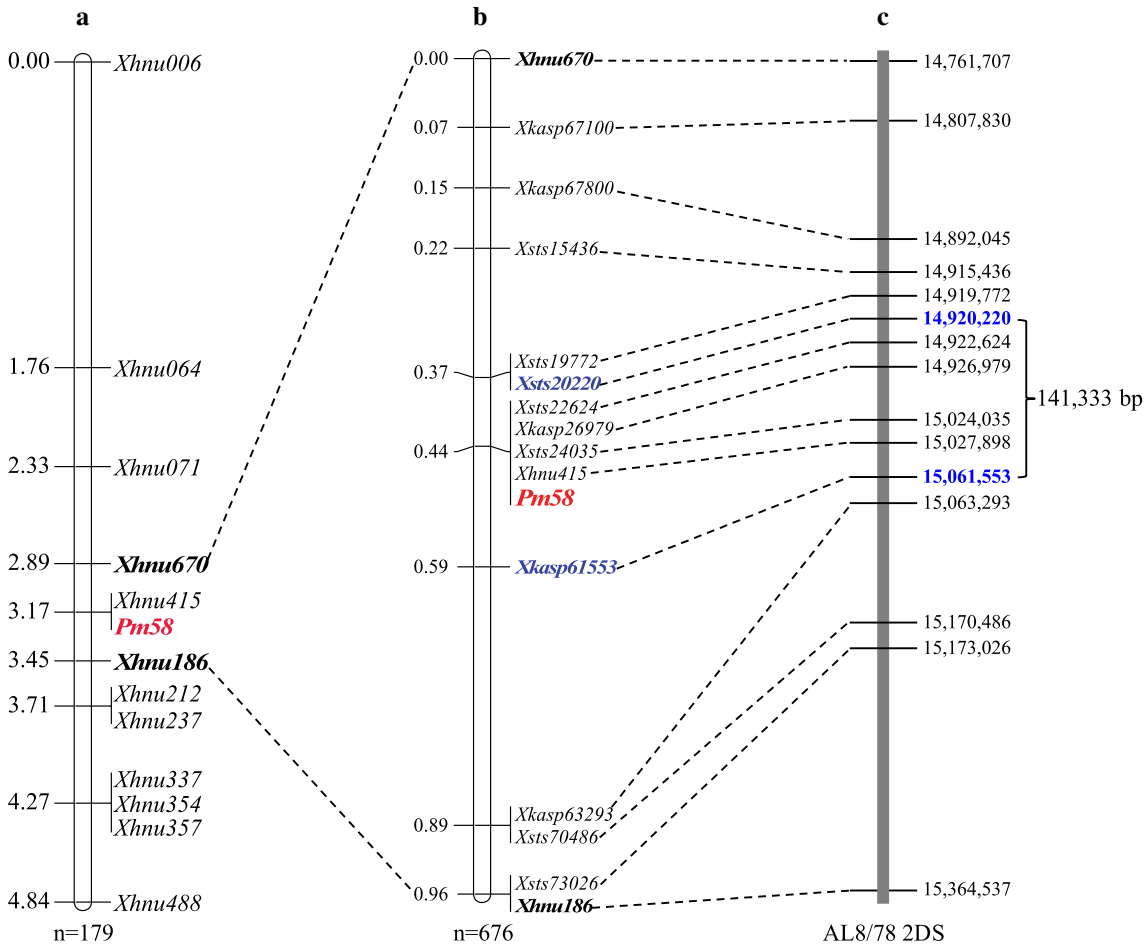

Fig. 2 Genetic and physical maps of the *Pm58* region. **a** Primary mapping of *Pm58*. **b** High-density genetic map saturated with the KASP and STS markers. Maps ***a*** and ***b*** were constructed based on 179 and 676 F_2 plants, respectively, derived from T093×TA1662. Genetic distance is shown on the left side of the maps in cM. **c** Partial physical map of AL8/78 chromosome 2DS. The physical positions (bp) of molecular markers are indicated on the right side of the physical map

These polymorphic markers were used to genotype 676 F_2 plants derived from T093×TA1662, and a high-density genetic map of the 2DS region containing *Pm58* was constructed (Fig. 2b). This map was comprised of 15 marker loci and spanned 0.96 cM. The average genetic distance between adjacent markers was about 0.06 cM.

A total of 13 recombinant F_2 individuals representing 12 types of genotypes were identified between the flanking markers *Xhnu670* and *Xhnu186* (Table 1). The $F_{2:3}$ families from these recombinants were evaluated for resistance to *Bgt* isolate E09, and five families were homozygous resistant, seven were segregating, and one was homozygous susceptible. With the help of recombinant types 1, 3, and 11, which were identified with *Xsts20220* and *Xkasp61553*, *Pm58* was fine mapped to a 0.22-cM *Xsts20220–Xkasp61553* interval (Table 1; Fig. 2b).

Noting that only one recombinant individual (P1-390) was identified between *Xsts20220* and *Pm58*, and only two (P1-008 and P2-106) were identified between *Pm58* and *Xkasp61553* (Table 1), we further checked these three critical crossovers by genotyping and phenotyping at least 40 $F_{2:3}$ progenies of them. The F_3 plants from three critical recombinants showed segregating responses to *Bgt* isolate E09, which recombined to the genotypes detected by *Xsts20220* or *Xkasp61553* and co-segregated with *Xsts22624*, *Xkasp26979*, *Xsts24035*, and *Xhnu415* (Supplementary Tables 6, 7, and 8). The genotypes of the remaining ten non-critical recombinants were also checked by genotyping their corresponding $F_{2:3}$ families (data not shown). Thus, we eventually delimited *Pm58* to a 141.3-kb region covering 14,920,220–15,061,553 bp on chromosome 2DS (Fig. 2c).

Candidate genes for *Pm58* and association analysis

According to the gene annotations of AL8/78 reference genome, eight annotated genes resided in the targeted

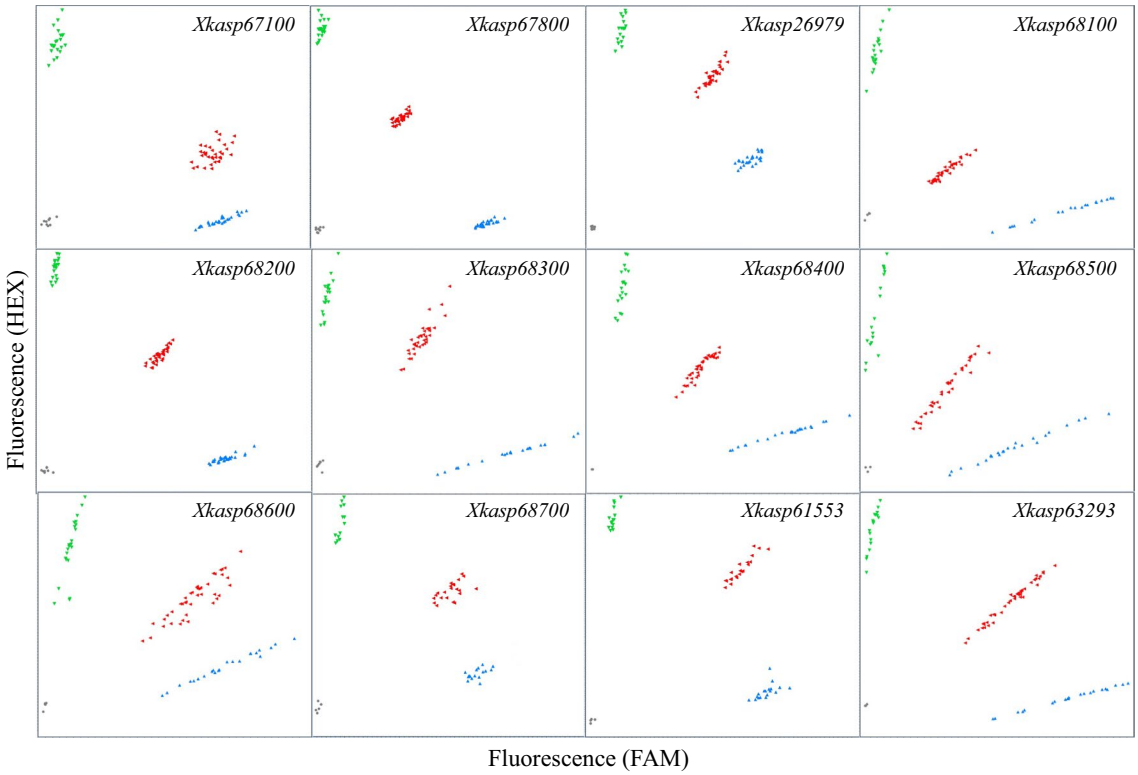

Fig. 3 Scatter plots for 12 SNP-derived KASP markers. The blue and green triangles represent TA1662 and T093 alleles, respectively, and the red triangles represent heterozygous alleles in the F_2 population derived from T093 × TA1662. The gray dots represent non-template control. *Xkasp68100*, *Xkasp68200*, *Xkasp68300*, *Xkasp68400*, *Xkasp68500*, *Xkasp68600*, and *Xkasp68700* were developed according to the SNPs resided in the *Pm58* candidate genes (color figure online)

Xsts20220–Xkasp61553 interval (Fig. 5a, b). Five of these genes encoded hypothetical or uncharacterized proteins, and another three (*AET2Gv20068500*, *AET2Gv20068600* and *AET2Gv20068700*) were annotated to encode disease resistance proteins (Supplementary Table 9). Comparisons of the re-sequencing reads of TA1662 and T093 identified some SNP/InDel variants located within the candidate genes. Based on these variations, one KASP/STS marker for each gene was developed (Figs. 3 and 4) and used for genotyping all the F_2 plants. As expected, these gene-specific markers displayed the consistencies between their genetic map positions and the sequence-based physical-map positions and were co-segregated with *Pm58* (Fig. 5a, b).

Pm58 haplotypes in 178 *Ae. tauschii* accessions were examined using eight candidate gene-specific markers (Fig. 5c). A total of nine haplotypes were detected, with three major haplotypes (Hap5, Hap8, and Hap9) representing 89.3% of the accessions used in this study. Hap9, as represented by susceptible parent T093, had the highest frequency (61.8%), followed by Hap5 (19.1%) and Hap8 (8.4%). TA1662 and another four accessions (AY001, AY007, AY034 and AY049) from Azerbaijan, Tajikistan, or Iran were assigned to Hap1 and were found to be highly resistant to *Bgt* isolate E09 (Supplementary Table 1). Compared to Hap1 with eight TA1662 alleles, two accessions carrying Hap3 with five TA1662 alleles were susceptible, indicating the importance of *Xkasp68500*, *Xkasp68600*, and *Xkasp68700* loci in powdery mildew resistance (Fig. 5c). However, multiple powdery mildew testes confirmed that *Ae. tauschii* AY072 (Hap4) carrying TA1662 alleles at both *Xkasp68600* and *Xkasp68700* loci was highly susceptible to *Bgt* E09 (Fig. 5c; Supplementary Table 1). Therefore, we speculate that the nonsynonymous SNP (A/G) at *Xkasp68500* locus is critical for powdery mildew resistance, and *AET2Gv20068500* is the most likely candidate gene for *Pm58*.

Comparative analysis of allelic genomic regions carrying *Pm58*

Alignments of the candidate genes to their homologs in the D genome of common wheat CS showed that the *Pm58*-containing interval was collinear to a 139.3-kb region (from 14,804,472 to 14,943,785 bp on CS chromosome 2DS) with eight annotated genes (IWGSC 2018; Supplementary Fig. 3). Except for *AET2Gv20068300*, which was

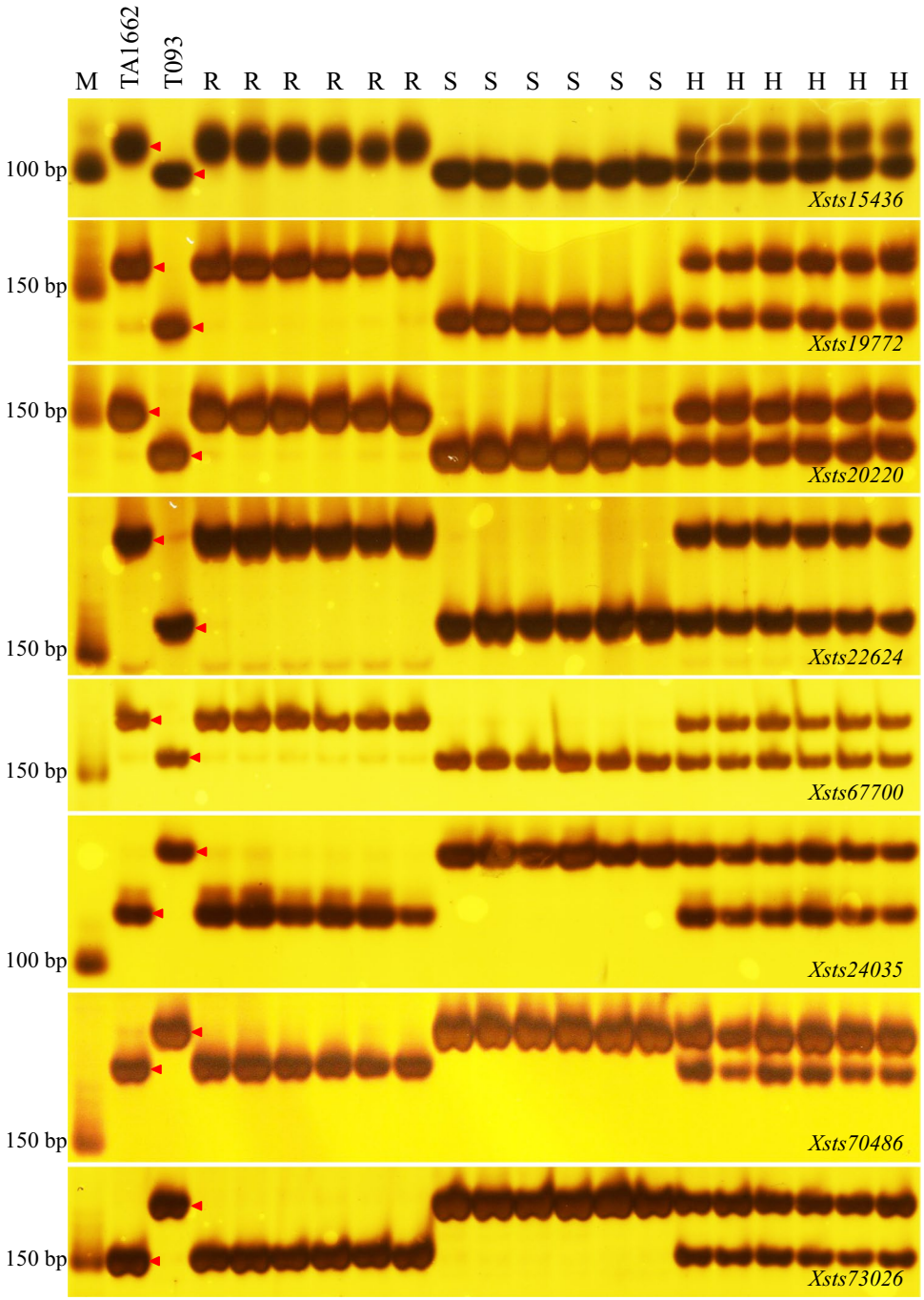

Fig. 4 PCR amplification patterns of eight InDel-derived STS markers. *R*, *S*, and *H* indicate homozygous resistant, homozygous susceptible and heterozygous resistant F_2 plants, respectively. *M* is the DNA size standard. *Xsts67700* was developed according to an 8-bp InDel in the candidate gene *AET2Gv20067700.32*

considered as a low-confidence gene with a small size (114-bp), homologs for the *Ae. tauschii* genes were found in the D genome of CS. The gene synteny was considerably well in the *Pm58* region, even though two PAVs were identified between *Ae. tauschii* and common wheat (Supplementary Fig. 3). PAV-1 was located between *AET2Gv20068400/TraesCS2D02G033600LC* and *AET2Gv20068500/TraesCS2D02G041300*. The 14.1-kb deletion in the CS genome caused truncated forms deficient in 3′-ends of both *TraesCS2D02G033600LC* and *TraesCS2D02G041300* (Fig. 6a; Supplementary Fig. 4). PAV-2 was located within the predicted second intron of *AET2Gv20068700/TraesCS2D02G041600*. The 12.0-kb insertion in the CS genome involved two tandem repeat sequences with 6016 bp each (Supplementary Fig. 4).

To gain more insights into these two structural variations on 2DS, we examined the situations of PAV-1 and PAV-2 in another four sequenced *Ae. tauschii* accessions (Zhou et al. 2021) and ten common wheat cultivars (Walkowiak et al. 2020; Sato et al. 2021) in addition to AL8/78 and CS

Table 1 Genotypes of 13 recombinants identified between markers *Xhnu670* and *Xhnu186* and their $F_{2:3}$ phenotypes

Recombinant F_2 plant	Genotype[a]										Recombination type	$F_{2:3}$ phenotype[c]
	Xhnu670	*Xkasp67100*	*Xkasp67800*	*Xsts15436*	*Xsts19772* *Xsts20220*	*Xsts22624* *Xkasp26979* *Xsts24035* *Xhnu415*	***Pm58***[b]	*Xkasp61553*	*Xkasp63293* *Xsts70486*	*Xsts73026* *Xhnu186*		
P1-390	B	B	B	B	B	H	H	H	H	H	1	0 (22), 1 (6), 3 (6), 4 (6)
P3-148	B	B	B	B	B	B	B	B	H	H	2	3 (8), 4 (26)
P1-008	H	H	H	H	H	H	H	B	B	B	3	0 (28), 1 (6), 3 (7), 4 (3)
P2-050	H	H	H	H	H	H	H	H	H	B	4	0 (12), 1 (6), 3 (5), 4 (2)
P1-130	A	A	H	H	H	H	H	H	H	H	5	0 (11), 1 (13), 4 (8)
P3-063	A	A	A	A	H	H	H	H	H	H	6	0 (23), 1 (7), 3 (4), 4 (2)
P1-215	A	A	A	A	A	A	A	A	H	H	7	0 (34), 1 (3)
P1-250	A	A	A	A	A	A	A	A	H	H		0 (26), 1 (3)
P2-108	H	A	A	A	A	A	A	A	A	A	8	0 (35), 1 (2)
P3-029	H	H	H	A	A	A	A	A	A	A	9	0 (29), 1 (2)
P3-074	H	H	H	H	A	A	A	A	A	A	10	0 (27), 1 (4)
P2-106	H	H	H	H	H	H	H	A	A	A	11	0 (27), 1 (10), 3 (6), 4 (4)
P3-018	H	H	H	H	H	H	H	H	A	A	12	0 (9), 1 (13), 4 (5)

[a]*A* TA1662 genotype, *B* T093 genotype, *H* heterozygous genotype

[b]Genotypes of 13 recombinants at the *Pm58* locus were inferred from the powdery mildew responses of their corresponding $F_{2:3}$ families

[c]Infection types (ITs) 0 and 1 represent resistant phenotypes; 3 and 4 represent susceptible phenotypes. The number of F_3 plants identified for each IT is shown in parenthesis

(Supplementary Fig. 4). All of the five *Ae. tauschii* accessions carried the 14.1-kb nucleotide sequences in the PAV-1 region, whether they belonged to lineage L1 or L2. However, the majority of the 11 genome-sequenced common wheat cultivars (90.9%) displayed extensive nucleotide sequence deletions in this region. For PAV-2, four *Ae. tauschii* accessions contained none of the duplicates, while XJ02 and nine common wheat cultivars contained one of the two repeat sequences. Wheat cultivar Julius had the 14.1-kb insertion and 12.0-kb deletion in the PAV-1 and PAV-2 regions, respectively, making it most similar to *Ae. tauschii* accessions in the *Pm58* region (Supplementary Fig. 4).

Allelic analysis of the critical *Xkasp68500* locus in common wheat cultivars

Due to the *Xkasp68500* locus was located within the PAV-1 region, we first analyzed the frequency of the 14.1-kb deletion in 158 common wheat cultivars with two pairs of primers (Fig. 6a, b). As expected, the PCR products were successively amplified in 24 cultivars with PAV-1-F1/R1 and in the others with PAV-1-F2/R2 (Supplementary Table 2), indicating that 84.8% of the wheat cultivars did not contain this 14.1-kb fragment in the PAV-1 region. By contrast, the targeted bands were amplified in all of the 178 *Ae. tauschii* accessions using PAV-1-F1/R1 (Fig. 6b). This result, plus the findings depicted in Supplementary Fig. 4, demonstrates that nucleotide sequence and gene losses in the PAV-1 region indeed occurred in a large number of wheat genotypes during hexaploidization.

We further analyzed the allelic forms of *Xkasp68500* in 24 wheat cultivars without the 14.1-kb deletion. Intriguingly, all of these cultivars, including ten from China and 14 from other countries, carried the susceptible alleles consistent with T093 (Fig. 6c). Therefore, *Xkasp68500* can clearly distinguish TA1662 from all of the 158 common wheat cultivars and has a polymorphism rate of 100%, which is much higher than that of other candidate gene-specific markers (Supplementary Fig. 5).

Discussion

Aegilops tauschii, the progenitor of common wheat D genome, constitutes a reservoir of genetic diversity for improvements in resistance to rust (Olson et al. 2013; Yu et al. 2015), powdery mildew (Miranda et al. 2006, 2007; Wiersma et al. 2017), Hessian fly (Raupp et al. 1993; Zhao et al. 2006), and pre-harvest sprouting (PHS) (Zhang et al. 2017). TA1662, an important *Ae. tauschii* accession from Azerbaijan, confers resistance to both Ug99 stem rust (Olson et al. 2013) and powdery mildew (Wiersma et al. 2017).

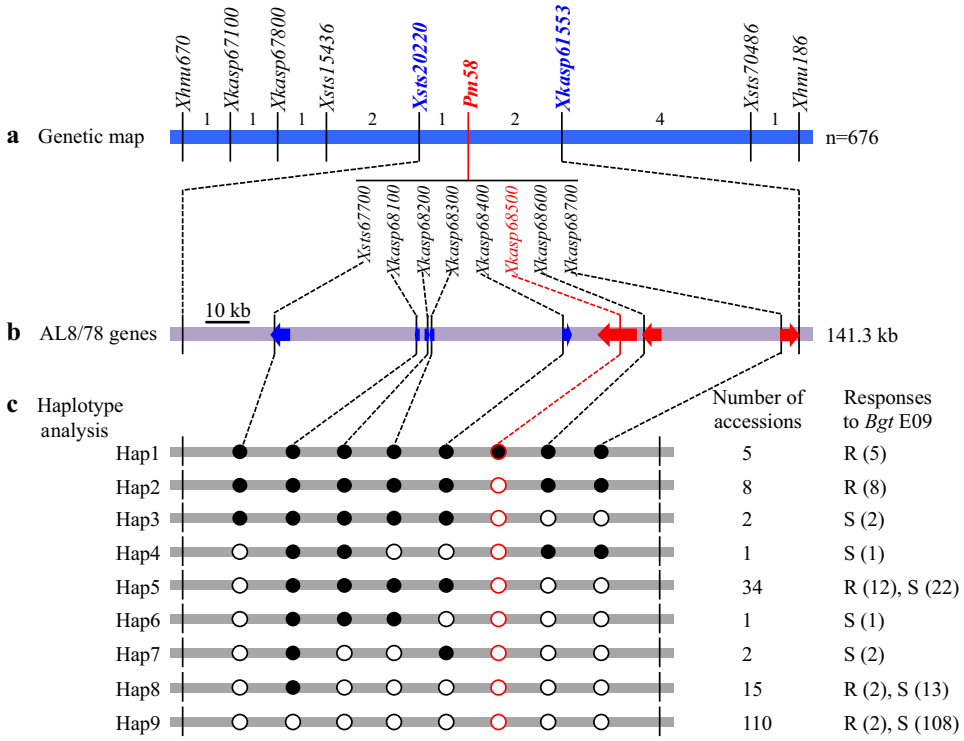

Fig. 5 Analysis of the *Pm58* candidate genes. **a** Genetic map of *Pm58*. The number of crossovers identified between the adjacent marker loci is shown above the rectangle. **b** Eight candidate genes annotated within the 141.3-kb targeted region according to the AL8/78 annotations. The candidate genes and their corresponding molecular markers are connected with dashed lines. The annotated genes associated with disease resistance are shown as red arrows. **c** Haplotype analysis of the *Pm58* region. A total of 178 *Ae. tauschii* accessions were analyzed using eight candidate gene-specific markers. The solid and blank circles indicate TA1662 and T093 genotypes, respectively. *R*, resistant, and *S*, susceptible. The number of accessions with resistant or susceptible phenotypes to *Bgt* E09 is shown in parenthesis

SrTA1662 has been cloned using association genetics with resistance gene enrichment sequencing (AgRenSeq, Arora et al. 2019), and *Pm58* was previously mapped within an 8.6-Mb interval on chromosome 2DS using a population of 96 introgression lines (Wiersma et al. 2017). In this study, we narrowed down the *Pm58*-containing interval to 141.3 kb by analyzing 13 recombinants from the cross *Ae. tauschii* T093 × TA1662 (Table 1; Fig. 5).

Common wheat evolved from hybridization between tetraploid *Triticum turgidum* and a single lineage of *Ae. tauschii* (Wang et al. 2013), which led to only 25% of the genetic diversity of *Ae. tauschii* contributing to the initial gene flow into wheat (Gaurav et al. 2021). Collections of *Ae. tauschii* germplasm exhibit wide diversity for various desirable traits and can be divided into two lineages (L1 and L2) or five sublineages (L1EY, L1EX, L1W, L2W, and L2E) according to phylogenetic analyses with genome-wide SNP data (Wang et al. 2013; Zhou et al. 2021). Recent population genomic analysis revealed a rare third lineage of *Ae. tauschii*, which was geographically restricted to Georgia (Gaurav et al. 2021). The susceptible parent T093 used in this study belongs to L1EY, while TA1662 and AL8/78 belong to L2W (Zhou et al. 2021). Genome re-sequencing revealed three times more SNP/InDel variants between T093 and TA1662 than between TA1662 and AL8/78, and with uneven distribution (Supplementary Fig. 2). Chromosome 4D had the highest SNP/InDel density, compared to the lowest marker density of 4D in other published intervarietal genetic maps of wheat (Cui et al. 2017; Liu et al. 2018). The abundant genetic variations between T093 and TA1662 facilitate the development of new molecular markers to finely map *Pm58*.

A search for annotated genes in the *Pm58*-containing region based on the AL8/78 genome annotations (Luo et al. 2017) identified eight candidate genes (Fig. 5a, b). Among these genes, three were disease-resistance genes (Supplementary Table 9). Eight functional markers derived from these candidate genes were developed and all of them were co-segregated with *Pm58* (Fig. 5a, b). A panel of 178 *Ae. tauschii* accessions were analyzed with these gene-specific markers, and a total of nine haplotypes were distinguished (Fig. 5c). At least one accession in each of the Hap3 to Hap9 exhibited susceptible phenotypes to *Bgt* E09, which was entirely consistent with the genotypes examined by *Xkasp68500*. *AET2Gv20068500*, comprised of the

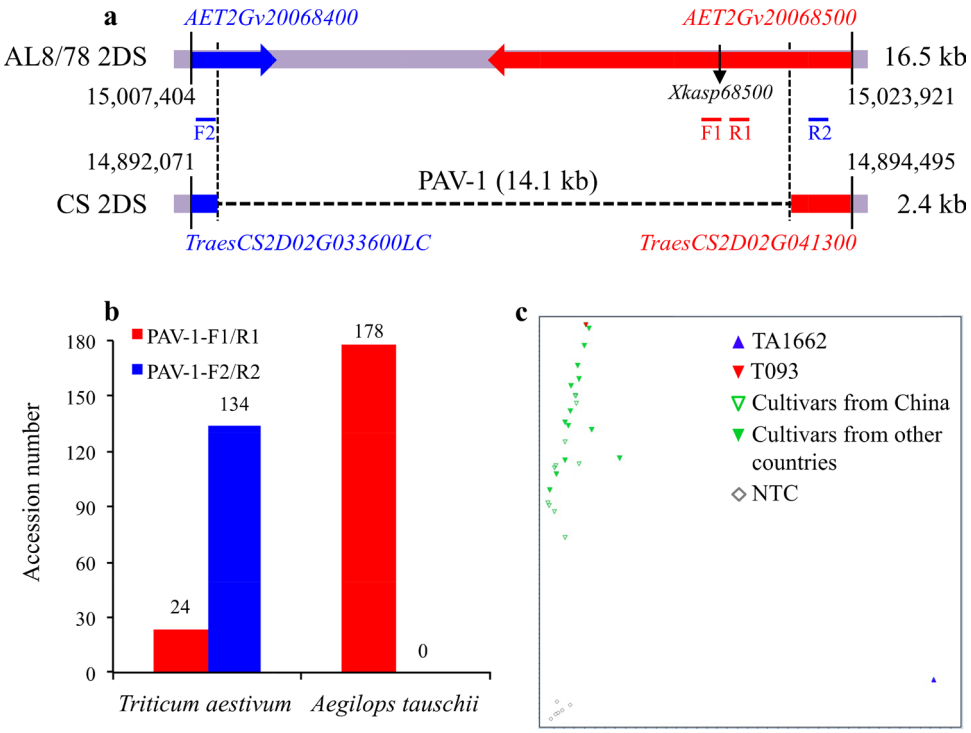

Fig. 6 Polymorphic patterns of *Xkasp68500* between TA1662 and common wheat cultivars. **a** *Xkasp68500* is located within the 14.1-kb PAV-1 region. Vertical dashed lines indicate the breakpoints flanking PAV-1; horizontal arrows represent the positions/directions of two predicted open reading frames; the vertical arrow indicates the position of *Xkasp68500*; and short horizontal lines represent the positions of PAV-1-F1/R1 and PAV-1-F2/R2. **b** Frequency analysis of the 14.1-kb deletion in 158 wheat cultivars and 178 *Ae. tauschii* accessions with two pairs of primers. **c** Allelic forms of *Xkasp68500* in 24 wheat cultivars without the 14.1-kb deletion. Scattered triangles with different colors show clustering of alleles of TA1662, T093, and wheat cultivars on the *x*-(FAM) and *y*-(HEX) axes. Gray diamonds represent non-template controls (NTC)

Xkasp68500 locus, encodes a typical NLR protein. In fact, most of the cloned powdery mildew resistance genes, such as *Pm1a* (Hewitt et al. 2021), *Pm2* (Sánchez-Martín et al. 2016), *Pm3* (Yahiaoui et al. 2004), *Pm5e* (Xie et al. 2020), *Pm8* (Hurni et al. 2013), *Pm17* (Singh et al. 2018), *Pm21* (He et al. 2018), *Pm41* (Li et al. 2020), and *Pm60* (Zou et al. 2018), are *NLR* genes and confer race-specific resistance. Although pathogens can overcome a single *NLR* gene through rapid evolution, stacking of multiple unlinked resistance genes has proven to provide durable and broad-spectrum resistance in wheat (Luo et al. 2021). Thus, identifying the most likely candidate for *Pm58* in this study may result in opportunities for broadening resistance gene pools and gene stacking. However, the association analysis presented here is preliminary because only partial sequence variations are taken into account. The next step will be to clone and sequence the candidate genes in 178 *Ae. tauschii* accessions and analyze the associations of all the SNPs/InDels with powdery mildew resistance.

Structural variations are typically defined as short InDels, long PAV, copy number variation (CNV), inversions and translocations (Saxena et al. 2014). Many important crops, such as oilseed rape and wheat, are polyploid species in which gene redundancies can buffer the deleterious effects of genomic variations (Walkowiak et al. 2020). Gene PAV has been implicated in the influence of many agriculturally important traits in crops, for example, stay-green and disease resistance in oilseed rape (Qian et al. 2016; Gabur et al. 2020), as well as heat tolerance, PHS and powdery mildew resistance in wheat (Lang et al. 2021; Xue et al. 2021; Zhai et al. 2021). Recently, more than 2000 PAV genes mainly related to pathogen resistance and stress adaptation have been identified, which were present in *Ae. tauschii* genome but absent from CS (Zhou et al. 2021). In this study, two PAVs between AL8/78 and CS were found in the *Pm58*-containing region, although the gene orders were very conserved (Supplementary Fig. 3). The 14.1-kb deletion in the PAV-1 region seemed widespread in common wheat cultivars (Supplementary Table 2; Supplementary Fig. 4), which greatly impacted the presences and functions of its surrounding genes *TraesCS2D02G033600LC* and *TraesCS2D02G041300* (Fig. 6a, b). PAV-2 can be considered as an extreme CNV, where two tandem duplicates in CS were completely missing in AL8/78 (Supplementary Fig. 4). Although PAV-2 was located within the intronic region of

AET2Gv20068700/TraesCS2D02G041600, further study is needed to determine whether it can change the splicing and/or expression of its residing genes.

There are two ways to utilize the useful genes of *Ae. tauschii* in wheat breeding, either through the synthetic hexaploid approach or the direct hybridization between *T. aestivum* and *Ae. tauschii* (Cox et al. 2017; Kishii 2019). *Pm58* has been transferred into a hard winter wheat line KS05HW14 through a direct cross (Wiersma et al. 2017). Two registered wheat lines fixed for *Pm58* exhibited resistant reactions to multiple *Bgt* isolates and good agronomic characteristics (Wiersma et al. 2018), confirming that *Pm58* has significant values for resistance improvement in modern wheat cultivars. However, both introgression lines had lower yield potentials than their recurrent parent KS05HW14 in most of the tested locations (Wiersma et al. 2018). To prevent the unfavorable linkage drags of resistance genes from the wild relatives, it is best to isolate the targeted genes and use them precisely in gene transferring and pyramiding. Here, we pick up a likely candidate gene for *Pm58* and provide a gene-specific KASP marker *Xkasp68500* that can distinguish TA1662 from all of the 158 randomly selected common wheat cultivars (Fig. 6; Supplementary Fig. 5). The fine mapping and candidate gene predictions conducted in this study will help to accelerate the verification of the causal genes for *Pm58*, and the co-segregating molecular marker *Xkasp68500* can be used for marker-assisted selection in wheat breeding programs.

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Author contribution statement SX, SH, XC, and YM conducted marker development, genotyping and data analysis; ML constructed the population; SB conducted bioinformatic analysis; XW, TS, YW, HW, and XA conducted phenotyping; SL supervised the project; SX and SL wrote the manuscript. All authors reviewed and approved the manuscript.

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Data availability All data generated during this study are included in this published article and its supplementary information files.

Declarations

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

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