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ORIGINAL ARTICLE

Homoeologous recombination-based transfer and molecular cytogenetic mapping of powdery mildew-resistant gene *Pm57* from *Aegilops searsii* into wheat

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Abstract

Key message ***Pm57, a novel resistant gene against powdery mildew, was transferred into common wheat from Ae. searsi and further mapped to 2S^s#1L at an interval of FL0.75 to FL0.87.***

Abstract Powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *tritici*, is one of the most severe foliar diseases of wheat causing reduction in grain yield and quality. Host plant resistance is the most effective and environmentally safe approach to control this disease. Tests of a set of Chinese Spring–*Ae. searsii* (S^sS^s, $2n=2x=14$) Feldman & Kislev ex K. Hammer disomic addition lines with a mixed isolate of the powdery mildew fungus identified a novel resistance gene(s), designed as *Pm57*, which was located on chromosome 2S^s#1. Here, we report the development of ten wheat–*Ae. searsii* recombinants. The wheat chromosomes involved in five of these recombinants were identified by FISH and SSR marker analysis and three of them were resistant to powdery mildew. *Pm57* was further mapped to the long arm of chromosome 2S^s#1 at a fraction length interval of FL 0.75 to FL 0.87. The recombinant stocks T2BS.2BL-2S^s#1L 89-346 (TA5108) with distal 2S^s#1L segments of 28% and 89(5)69 (TA5109) with 33% may be useful in wheat improvement. The PCR marker *X2L4g9p4/HaeIII* was validated to specifically identify the *Ae. searsii* 2S^s#1L segment harboring *Pm57* in T2BS.2BL-2S^s#1L against 16 wheat varieties and advanced breeding lines, and the development of more user-friendly KASP markers is underway.

Keywords *Blumeria tritici* · FISH · GISH · *Pm57* · *Triticum aestivum* · *Aegilops searsii*

Introduction

Common or bread wheat, *Triticum aestivum* L. ($2n=6x=42$, AABBDD), is an allohexaploid species and grown worldwide. Yield stability and wheat quality are globally important. However, the wheat crop is frequently affected by diseases, pests, and abiotic stresses including lodging, heat, drought, salt and alkali, leading to yield instability and reduced grain quality.

Powdery mildew of wheat, caused by the fungus *Blumeria graminis* f. sp. *tritici*, is one of the most damaging foliar diseases of wheat (Bennett 1984). The disease often occurs in fields rich in nitrogen content and high in stand density in regions of high humidity and moderate temperature. Once infection occurs, the fungus can rapidly grow on the leaves, leaf sheaths, and spikes of the wheat plant, decreasing photosynthesis, diverting nutrients, and increasing respiration and transpiration in infected plants, leading to serious losses of both yield and grain quality (Fried et al. 1981; Everts et al. 2001; Conner et al. 2003; Wang et al. 2005).

Although powdery mildew can be controlled by fungicides, breeding resistant varieties is widely considered to be the most economical and environmentally safe

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approach to prevent or slow the disease spread. At present, 55 powdery mildew-resistance genes have been named (http://maswheat.ucdavis.edu/CGSW/2013-2014_Supplement.pdf) (McIntosh et al. 2014; Hao et al. 2015; Ma et al. 2015; Zhang et al. 2016). Eighteen of the resistance genes were derived from wheat relatives including *Aegilops tauschii* Coss. *(Pm19, Pm34*, and *Pm35), Ae. speltoides* Tausch (*Pm12, Pm32*, and *Pm53*), *Ae. geniculata* Roth. (*Pm29*), *Ae. longissima* (Schweinf. & Muschl. in Muschl.) Eig (*Pm13*), *Secale cereale* L. (*Pm7, Pm8, Pm17* and *Pm20*), *Dasypyrum villosum* (L.) Candargy (*Pm21* and *Pm55*), *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey (*Pm40* and *Pm43*), *Thinopyrum elongatum* (Host) Barkworth & D.R. Dewey (*Pm51*), and *Agropyron cristatum* (L.) Gaertn. (*Pm2b*) (http://maswheat.ucdavis.edu/CGSW/2013-2014_Supplement.pdf). However, new sources of powdery mildew resistance are constantly sought.

Aegilops searsii Feldman & Kislev ex Hammer ($2n=2x=14$, S^sS^s), native to sub-Mediterranean regions, is one of the S-genome diploid species belonging to the section *Sitopsis* (Jaub. & Spach) Zhuk. (Feldman and Kislev 1977), which also includes *Ae. speltoides* (SS, $2n=2x=14$), *Ae. bicornis* (Forsskål) Jaub. & Spach (S^bS^b, $2n=2x=14$), *Ae. longissima* (S^lS^l, $2n=2x=14$), and *Ae. sharonensis* Eig ($S^{sh}S^{sh}$, $2n=2x=14$) (van Slageren 1994).

Since *Ae. searsii* was first reported by Feldman and Kislev (1977), a Chinese Spring wheat–*Ae. searsii* amphiploid ($2n=8x=56$, $AABBDDS^sS^s$) (Feldman et al. 1979), 7 derived disomic chromosome addition lines, 14 ditelosomic addition lines, 21 disomic chromosome substitutions, and 31 ditelosomic substitution lines have been produced (Friebe et al. 1995).

Several *Ae. searsii* genes controlling high-molecular-weight glutenin subunits were identified (Sun et al. 2006; Garg et al. 2009) and Liu et al. (2011a) reported a novel gene, *Sr51*, conferring resistance to the stem rust race Ug99, located on the short arm of chromosome $3S^s$#1. Recent screening of the complete set of wheat–*Ae. searsii* chromosome addition lines in China showed that addition line with chromosome $2S^s$#1 confers resistance to powdery mildew. In this paper, we describe the development of compensating $2S^s$#1 wheat–*Ae. searsii* recombinants and the mapping of this powdery mildew-resistance gene, designated *Pm57*, to a distal segment of the long arm of $2S^s$#1.

Materials and methods

Plant materials

Lines TA3581 and TA3809 were used to develop the wheat–*Ae. searsii* recombinant population. TA3581 is a wheat–*Ae. searsii* disomic chromosome addition line where a pair of $2S^s$#1 chromosomes is added to the chromosome complement of Chinese Spring wheat (DA $2S^s$#1) (Friebe et al. 1995). The #1 designation is used to distinguish between the same *Ae. searsii* chromosome derived from different *Ae. searsii* accessions (Raupp et al. 1995). TA3809 is a Chinese Spring *ph1b* mutant stock lacking the *Ph1* gene and thereby permitting homoeologous recombination. TA3581, TA3809, and Chinese Spring wheat were used to develop $2S^s$#1-specific markers and evaluate response to powdery mildew following inoculation with an isolate mixture of *B. graminis* f. sp. *tritici* collected in Henan Province, China. All stocks used in this study were provided by the Wheat Genetics Resource Center at Kansas State University and are maintained at the experimental station of Henan Agricultural University.

Developing segregating populations for $2S^s$#1 recombinant selection

DA $2S^s$#1 (TA3581) was crossed as a male with the *ph1b* mutant stock (TA3809), and the F_1 plants were either self-pollinated or backcrossed with TA3809 to produce F_2 or BC_1F_1 populations. Individuals homozygous for *ph1b* and monosomic for $2S^s$#1 ($2n=43$) were selected using the *ph1b*-specific marker ABC302.3 (Wang et al. 2002) and $2S^s$#1-specific markers developed in this study. The homozygous (*ph1b*/*ph1b*) plants with monosomic addition of $2S^s$#1 ($2n=43$) were self-pollinated to produce the $2S^s$#1 recombinant population. Three mapped FlcDNA-based markers and STS-PCR markers located in the distal and proximal regions of each arm of chromosome $2S^s$#1 were used to identify putative recombinants. Plants missing at least one of the $2S^s$#1-specific markers were chosen for further genomic in situ hybridization (GISH) analysis to select $2S^s$#1 recombinants.

Screening of $2S^s$#1-specific markers

STS-PCR, SSR, and mapped full-length cDNA (FlcDNA)-based markers of wheat group-2 chromosomes were used to select $2S^s$#1-specific PCR markers. SSR primers used in the study were selected based on the SSR physical map of Sourdille et al. (2004) and the consensus SSR map of Somers et al. (2004). PCR was performed in 15 µL reaction mixtures containing 1X PCR buffer (Bioline USA Inc., Taunton, MA, USA), 2 mM $MgCl_2$, 0.25 mM dNTPs, a 10 pmol mixture of forward and reverse primers, 0.02 unit/µl of Taq DNA polymerase (Bioline USA Inc., Taunton, MA, USA), and 100–200 ng of genomic DNA. The procedure of PCR amplification procedure was according to Liu et al. (2011a). STS-PCR primers specific for wheat group-2 chromosomes were designed by Qi et al. (2007)

based on wheat expressed sequence tags (EST) mapped to wheat homoeologous group 2. STS-PCR amplification and PCR-amplified product digestion with six different four-base recognition restriction enzymes (*Alu*I, *Hae*III, *Mse*I, *Msp*I, *Rsa*I, and *Mbo*I) were according to Liu et al. (2011a). Mapped FlcDNA-based markers were selected based on the location of fluorescence in situ hybridization (FISH)-mapped FLcDNAs on the group-2 chromosome arms (Danilova et al. 2014). PCR primers were designed based on group-2-specific FlcDNA sequences flanking the introns by primer 3 (Liu et al. 2016). PCR amplification and PCR product digestion were performed following STS-PCR protocols. SSR-PCR products were resolved in 2.5% agarose gels; STS-PCR and mapped FlcDNA-based PCR products were resolved in 1.5% agarose gels following four-base recognition enzyme digestion, and visualized by ethidium bromide staining under UV light. Genomic DNA was isolated from 5- to 10-cm segments of young leaves using a BioSprint 96 workstation following the protocol described in the BioSprint DNA Plant Handbook (Cat. no. 941558, QIAGEN Inc., Valencia, CA, USA), or isolated following DNA mini-preparation protocols (Qi et al. 2007).

GISH and FISH analysis of recombinants

Chromosome identification was according to Gill et al. (1991). Genomic DNA isolation and GISH probe labeling were according to Liu et al. (2011a). Root tips were collected from putative recombinants missing at least one 2S^s#1-specific marker, treated in an N_2O gas chamber for 2.5 h followed by a treatment with 90% acetic acid on ice for 30 min (Danilova et al. 2012). The root tips were washed three times with 75% ethanol and fixed in an ethanol:glacial acetic acid (3:1 v/v) solution for 2–7 days. Squash preparations were made after staining with 1% acetocarmine. GISH was according to Liu et al. (2011a) with minor modifications. The ratio of genomic *Ae. searsii* DNA and CS blocking DNA was 1:100–120. For FISH, pAs1 and $(GAA)_9$ oligonucleotide probes were used to identify wheat and wheat–*Ae. searsii* recombinant chromosomes. Probe pAs1 preferentially paints tandem repeats on D-genome chromosomes, and $(GAA)_9$ paints all A- and B-genome chromosomes except 1 A and also labels 1D, 2D, and 7D (Danilova et al. 2012, 2014). After hybridization and slide washing, a drop (25–30 µl) of Vectashield mounting medium containing 1 µg/ml PI (Vector laboratories Inc, Burlingame, CA, USA) for GISH or DAPI (Vector laboratories Inc, Burlingame, CA, USA) for FISH was added to each slide and then covered with a 24×30 cm glass coverslip. Fluorescent images were captured with a SPOT2.1 charge-coupled device (CCD) camera (Diagnostic Instruments, Sterling Heights, MI, USA) using an epi-fluorescence Zeiss Axioplan 2 microscope. Images were further processed with Adobe Photoshop CS3 (Version 10.0.1) (Adobe Systems Inc., San Jose, CA, USA).

Powdery mildew assay

Powdery mildew assays were conducted at the Institute of Plant Protection, Henan Academy of Agricultural Sciences. A mixture of *Blumeria graminis* f. sp. *tritici* isolates were collected in Henan Province, and inoculated after the first leaf of each seedling had fully unfolded. Powdery mildew infection types (IT) were scored 7–10 days post-inoculation, when the susceptible controls were heavily infected. A 0–4 infection-type scale, as described by Wang et al. (2005) and Xiao et al. (2013), was used to evaluate the resistance. Plants with infection types of 0–2 were considered resistant, whereas an infection type of 3–4 was considered susceptible.

Results

Selecting *Ae. searsii* 2S^s#1-specific molecular markers

A total of 96 STS-PCR primers, 44 SSR primers, and 95 mapped FlcDNA-based markers were screened to select chromosome 2S^s#1-specific PCR markers. One STS-PCR marker (*Xbe586063*) and three mapped FlcDNA-based markers (*X2S3-2, X2S13*, and *X2L4g9p4*) were selected as 2S^s#1-specific markers (Fig. 1). None of the SSR markers screened in this study was specific for 2S^s#1. Markers *Xbe586063* and *X2L4g9p4* were located on the long arm of 2S^s#1 in deletion bins 2BL6-0.89-1.00 and 2AL1-0.85-1.00, respectively. Markers *X2S3-2* and *X2S13* were mapped to the short arm of group-2 chromosomes in deletion bins 2AS5-0.78-1.00 and 2DS1-0.33-0.47, respectively (Table 1). These markers were used to screen the recombinant population.

Developing wheat–*Ae. searsii* recombinants

A total of 398 plants derived from F_2 or BC_1F_1 plants homozygous for *ph1b* and monosomic for 2S^s#1were screened with four 2S^s#1-specific markers. Nineteen plants lacking the 2S^s#1 short-arm markers and 21 plants missing the long-arm markers were selected as putative recombinants. GISH analysis initially identified one Robertsonian translocation (RobT) (89(5)185) and 10 recombinants (89-152, 89-160, 89-346, 89-358, 89(2)362, 89(2)378, 89(5)12, 89(5)69, 89(5)102, and 89(6)88). Line 89-160, 89-358, 89(5)12, and 89(5)102 did not survive and the other six surviving recombinants (89-152, 89-346, 89(2)362, 89(2)378, 89(5)69 and 89(6)88) and the RobT 89(5)185 were further characterized (Fig. 2). Of the remaining plants

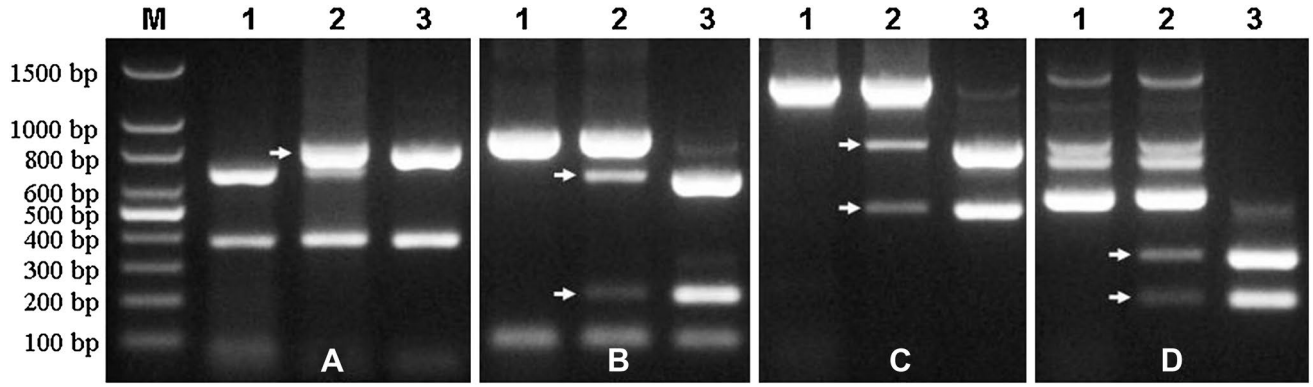

Fig. 1 PCR patterns of Chinese Spring (*lane 1*), Chinese Spring–*Ae. searsii* disomic addition line 2S^s#1 (TA3587) (*lane 2*) and *Ae. searsii* (TA1840) (*lane 3*). **a** *Xbe586063/RsaI* (2BL6-0.89-1.00); **b** *X2L4g9p4/HaeIII* (2AL1-0.85-1.00); **c** *X2S13/MspI* (2DS1-0.33-0.43); **d** *X2S3-2/MseI* (2AS5-0.78-1.00). Polymorphic fragments of 2S^s#1 are marked by *arrows*

Table 1 EST-STS markers specific to chromosome 2S^s#1 of *Ae. searsii*

Marker	Forward primer sequence	Reverse primer sequence	Enzyme	Deletion bin
X2S3-2/MseI	AGCCAGAGGCTGACACTGAT	CTTGGGCTGTCTTGGACATT	*Mse*I	2AS5-0.78-1.00
X2S13/MspI	TGAGCAGGAGGAGGAAGTGT	GCATGTTGTGCTTCTGGATG	*Msp*I	2DS1-0.33-0.47
X2L4g9p4/HaeIII	TTGGGCCAGGCTATGCAAAT	GGGAAGAAACCCTCCTTGAGC	*Hae*III	2AL1-0.85-1.00
Xbe586063/RsaI	CGTCGTGTTTGGGTAGGAGT	CCTGGGTGCTTGTTCTTCTC	*Rsa*I	2BL6-0.89-1.00

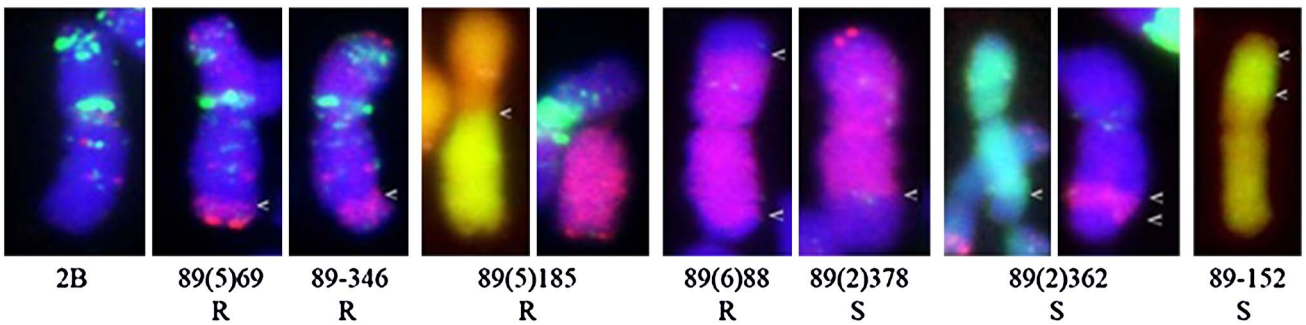

Fig. 2 GISH/GAA-FISH patterns of wheat–*Ae. searsii* recombinants. *Ae. searsii* chromatin is visualized in red in 89(5)69, 89-346, 89(5)185 telosome on the *right*, 89(6)88, 89(2)378, and 89(2)362 chromosomes on the *right*, in *yellow-green* in 89(5)185 RobT on the *left* and 89-152, and in *green* in 89(2)362 chromosome on the *left*; and GAA FISH sites are visualized in *green*. Powdery mildew reactions are indicated by *R* (resistant) and *S* (susceptible); *arrowheads* point to the translocation breakpoints. (Color figure online)

with dissociated 2S^s#1 specific markers, 5 had telosomes, 19 had complete 2S#1 chromosomes, and 5 plants were not identified.

Based on the analyses of 2S^s#1-specific markers and GISH patterns, line 89(5)69 and 89-346 had translocated chromosomes with the terminal segments of 2S^s#1 long arm replacing the distal regions of wheat chromosome long arms. Further measurements of ten recombinant chromosomes indicated that the fragment length (FL) of the translocation breakpoints (FL equals distance from the centromere to the translocation breakpoint divided by the complete lengths of this chromosome arm) averaged 0.67 in line 89(5)69 and 0.72 in line 89-346, with distal 2S^s#1 segments of 33 and 28% of the long arms, respectively. Line 89(2)378 and 89(2)362 had recombinant chromosomes of *Ae. searsii* 2S^s#1 where the distal 36 and 25% of the arms were replaced by wheat segments. Recombinant line 89(6)88 had translocated chromosomes where both distal segments of 2S^s#1 were replaced by wheat segments, with one breakpoint at FL 0.87 at long arm and another at FL 0.70 at the short arm. Line 89-152 had interstial translocations where proximal portions of the short arm were

replaced by 2S^s#1 derived segments between FL 0.82 and FL 0.35 (Fig. 2).

Powdery mildew responses and mapping of resistance gene *Pm57*

In powdery mildew tests of self-pollinated offspring of recombinant lines 89(5)69, 89-346 and 89(6)88 and the RobT 89(5)185 were resistant with infection types 0–1, and recombinants 89(2)362, 89(2)378, and 89-152 and Chinese Spring were susceptible with infection types of 3–4 (Fig. 3). Heterozygous recombinant 89(5)69, 89-346, and 89(6)88 plants were also resistant, indicating that the gene conferring powdery mildew resistance is dominant and was designated as *Pm57*. The three resistant recombinants 89(5)69, 89-346, and 89(6)88, and the RobT 89(5)185, all share a distal segment of the 2S^s#1L, limiting the *Pm57* gene to this segment.

Identifying wheat chromosome segments in the recombinant chromosomes

FISH, using *Ae. searsii* genomic DNA together with pAs1 and $(GAA)_9$, was used to identify six homozygous 2S^s#1L recombinants and to the wheat chromosomes involved in these recombinants. In recombinants 89(5)69 and 89-346, a distal segment of 2S^s#1L was translocated to the long arm of wheat chromosome 2B resulting in a T2BS.2BL-2S^s#1L recombinant chromosome (Fig. 2). The recombinant chromosome in 89(2)378 consisted of 2S^s#1S, part of 2S^s#1L, and a distal segment derived from the long arm of wheat chromosome 2A (T2S^s#1S.2S^s#1L-2AL) (Fig. 2). The recombinant

Fig. 3 Powdery mildew responses of Chinese Spring, Chinese Spring–*Ae. searsii* disomic addition line 2S^s#1, and derived wheat–*Ae. searsii* recombinant stocks. Photographs were taken 10 days post-inoculation with a mixed culture of *Blumeria graminis* f. sp. *tritici*

89(6)88 had a pair of chromosomes where the distal parts of the short and long arms of 2S^s#1 were replaced by homoeologous segments derived from wheat chromosome 2A resulting in Ti2AS-2S^s#1S.2S^s#1L-2AL (Fig. 2). GISH analysis of the self-pollinated offspring of line 89(2)362 identified a new recombinant with a shortened *Ae. searsii* segment. The original recombinant present in line 89(2)362 consisted of 2S^s#1S, part of 2S^s#1L, and a distal segment derived from an unidentified wheat chromosome, whereas in the progeny of this plant a new Ti2DS.2DL-2S^s#1L-2DL recombinant was identified; (Fig. 2). The recovery of a new recombinant in the progeny of 89(2)362 suggests that this plant originally may have had a dicentric wheat–*Ae. searsii* chromosome that underwent undetected breakage fusion-bridge cycles. Similarly, modified recombinants derived from cycling dicentric chromosomes were reported previously during the production of wheat–*Th. intermedium* recombinants (Liu et al. 2011b, 2013).

In the progeny of line 89(5)185, which originally had a RobT where the short arm of an unidentified wheat chromosome was translocated to 2S^s#1L, we recovered plants with a 2S^s#1L telosome (Fig. 2) that was most likely produced by centric misdivision of the univalent RobT. Homozygous recombinant stocks of the remaining lines were obtained by combining GISH and SSR marker analysis of self-pollinated progenies of the recombinant plants.

In addition to FISH identification, wheat group 2-specific SSR markers were also used for identifying the wheat segments involved in the recombinants, and SSR marker analysis supported the FISH data. Figure 4 shows recombinant 89(6)88 (Ti2AS-2S^{s}S.2S^s#1L-2AL), retaining both distal wheat 2A SSR markers *Xbarc124* and *Xgwm265*, but losing the 2A-specific proximal markers *Xgwm425* and *Xbarc15*. The markers *Xbarc124* and *Xgwm265* are located in the deletion bins 2AS5-0.78-1.00 and 2AL1-0.85-1.00, respectively, and the markers *Xgwm425* and *Xbarc15* were mapped in the deletion bins C-2AS5-0.78 and C-2AL1-0.85. Thus, the recombinant stock 89(6)88 was confirmed to have two independent recombination events in both arms of 2S^s#1, as suggested by FISH analysis (Fig. 2).

Further comparison of the 2S^s#1 segments showed that all resistant recombinants share an *Ae. searsii* segment ranging between FL 0.72 and FL 0.87, whereas in the susceptible recombinant 89(2)362, the 2S^s#1L segment is located between FL 0.51 and FL 0.75 and between FL 0.00 and FL 0.64 in the susceptible recombinant 89(2)378. These data map *Pm57* onto chromosome arm 2S^s#1L and between FL 0.75 and FL 0.87, corresponding to 12% the long arm length of the recombinant chromosomes (Fig. 5).

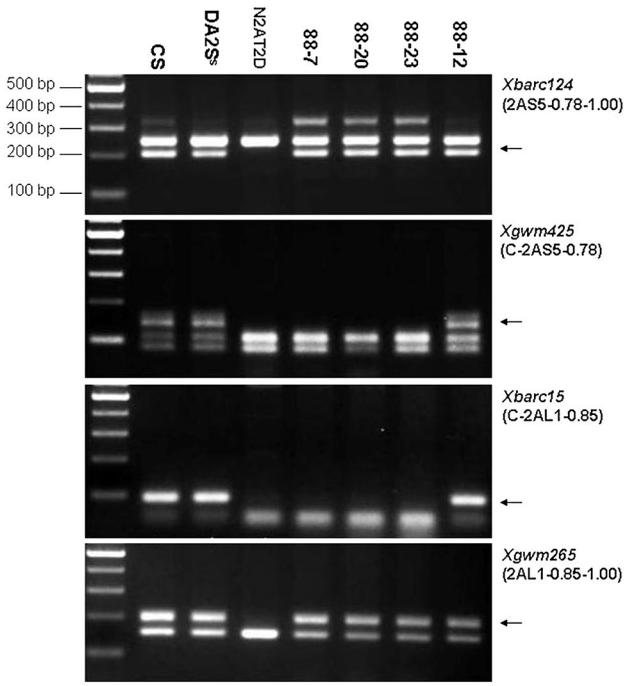

Fig. 4 SSR marker analysis of wheat–*Ae. searsii* recombinant line 89(6)88. From *left* to *right, Lane 1* 100 bp DNA marker; *lane 2* Chinese Spring; *lane 3* Chinese Spring–*Ae. searsii* disoimc addition line of 2S^s#1 (DA2S^s); *lane 4* N2AT2D; *lanes 5–7* homozygous plant 88-7, 88-20, 88-23 derived from heterozygous recombinant 89(6)88; *lane 8* plant 88-12 without translocated chromosomes derived from heterozygous recombinant 89(6)88 (the negative control line). All the homozygous recombinants have retained the wheat 2A distal markers of the short (*Xbarc124*) and long arms (*Xgwm265*) but have lost the wheat 2A proximal markers *Xgwm425* (short arm specific) and *Xbarc15* (long arm specific). SSR marker analysis showed recombinant 89(6)88 contained translocated chromosomes where both distal segments of 2S^s#1 were replaced by homoeologous segments of wheat chromosome 2A, confirming that line 89(6)88 can be designed as Ti2AS-2S^{s}S.2S^s#1L-2AL

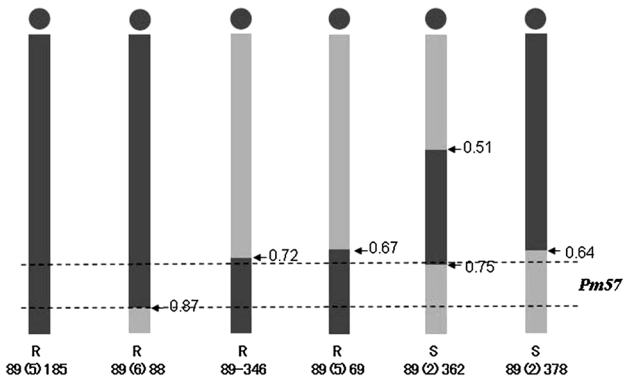

Fig. 5 Mapping of powdery mildew-resistance gene *Pm57*. Chromosome 2S^s#1L chromatin is shown in *black* and wheat chromatin in *grey, arrows* point to the translocation breakpoints, and *numbers* on the *right* indicate the fragment lengths (FL) of the breakpoints. All resistant recombinants share a 2S^s#1L segment between FL 0.75 and FL 0.87, limiting *Pm57* to this interval

Validation of *Pm57* linked markers

X2L4g9p4/HaeIII, an FLcDNA-based PCR marker is specific for the 2S^s#1 long arm and was used to identify putative 2S^s#1 recombinants tagging the *Ae. searsii* segments in 89(5)69 and 89-346 (T2BS.2BL-2S^s#1L) (Fig. 1). The corresponding full-length cDNA (AK331687) of *X2L4g9p4* was mapped at FL0.86, 0.91 and 0.89 2A, 2B and 2D long arms by single gene FISH (Danilova et al. 2014). The usefulness of marker *X2L4g9p4/HaeIII* in tagging the 2S^s#1L segments carrying *Pm57* was validated with 12 wheat varieties and advanced breeding lines. The result showed that *X2L4g9p4* PCR primers generated polymorphic DNA bands of 660 bp in the CS-*Ae. searsii* 2S^s#1 addition line, 89(5)185 (2S^s#1L telosome), recombinants 89(5)69 and 89-346 (T2BS.2BL-2S^s#1L), compared to both CS and 11 different wheat cultivars and advanced breeding lines from USA and China (Fig. 6). Thus marker *X2L4g9p4/HaeIII* can be used to track the transfer of *Pm57* in breeding programs and the development of more user friendly KASP markers is underway.

Discussion

Developing wheat–alien recombinants is a very efficient approach for utilizing the gene pool of distantly related relatives of wheat in crop improvement. Although wheat–alien translocations occur spontaneously, their frequency is very low. Several strategies have been used to transfer alien genes to wheat. The transfer of alien chromosome arms can be achieved using the centric breakage–fusion mechanism of univalents at meiotic metaphase I (Sears 1952). Radiation treatment (Sears 1956) and induced homoeologous recombination (Riley et al. 1968a, b) can be used for transferring alien chromosome segments smaller than complete chromosome arms. In this study, we identified ten wheat–*Ae. searsii* recombinants from 398 individuals derived from F_2 or BC_1F_1 plants homozygous for *ph1b* and monosomic for chromosome 2S^s#1. The frequency of recovered recombinants in this study was 2.76%, which is slightly higher than that previously reported for *ph1b*-induced wheat–*Ae. searsii* 3S^s#1 recombinants (1.94%) (Liu et al. 2011a). Of the five 2S^s#1 recombinants identified by FISH, all involved homoeologous group-2 chromosomes, indicating that they are genetically compensating and agronomically useful.

A single *Ae. searsii*-derived gene *Sr51*, conferring stem rust resistance was identified and transferred to wheat in the form of T3AL.3S^s#1S, T3BL.3S^s#1S, and T3DL.3S^s#1S RobTs (Liu et al. 2011a). In this study, we report the transfer and mapping of a novel powdery mildew-resistance gene, *Pm57*, to the wheat–*Ae. searsii* recombinant

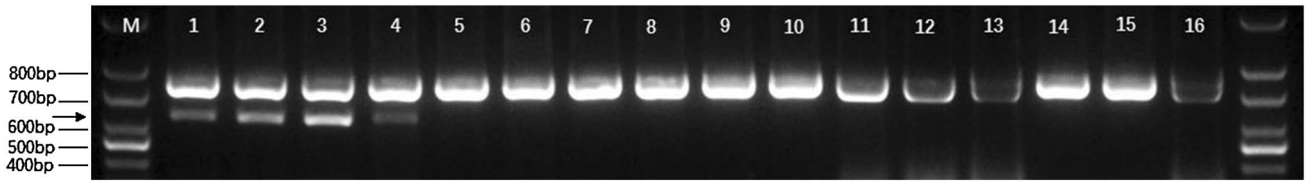

Fig. 6 PCR amplification of marker *X2L4g9p4/HaeIII. Lane M* 100 bp DNA ladder, *lanes 1–4* TA3581 (CS-*Ae. searsii* 2S^s#1 disomic addition line); TA5108 (89(5)69; T2BS.2BL-2S^s#1L); TA5109 (89-346 T2BS.2BL-2S^s#1L); and 89(5)185 (2S^s#1L telosome); *lanes 5–16* wheat varieties Chinese Spring, Lakin, Postrock, Pingan 8, Jingmai 66, Zhengzhou 366, Xinong 979, Pingan 0518, Pingan 602, Yanzhan 4110, Zhoumai 18, and advanced breeding line 1113-411-5-243. The *arrow* points to the polymorphic *X2L4g9p4/HaeIII* band of 660 bp amplified from the distal *Ae. searsii* 2S^s#1L segments carrying *Pm57*

chromosome T2BS.2BL-2S^s#1L. Ten of the catalogued powdery mildew-resistance genes are located on group-2 chromosomes, however, none are derived from *Ae. searsii*, indicating that *Pm57* is most probably new source of resistance.

Powdery mildew of wheat can cause severe yield loss, especially under high nitrogen conditions and stand densities. In a recent study of 908 wheat varieties and advanced lines from 2009 to 2013 in Henan, 63.9% of entries had the T1BL.1RS translocation with *Pm8*, and only 18 entries (1.98%) had *Pm2, Pm4a, Pm21*, or more than one resistance gene (Cao et al. 2015). To broaden the genetic variability, employing additional powdery mildew-resistance genes in cultivar improvement is highly recommended. The resistant powdery mildew recombinant lines 89(5)69 and 89-346 identified in this study as T2BS.2BL-2S^s#1L and *Pm57* linked maker *X2L4g9pe/HaeIII* will be useful in breeding powdery mildew-resistant wheat cultivars. The distal 2S^s#1L segments in those lines differ in size, being 28% in 89-346 (designated as TA5108) and 33% in 89(5)69 (designated as TA5109). We are currently transferring the T2BS.2BL-2S^s#1L recombinant chromosomes to locally adapted Chinese cultivars, which will then be evaluated for various agronomic traits under field conditions. Small quantities of TA5108 and TA5109 are available upon request.

Author contribution statement WL, D-HK, QY, and FB performed the molecular marker and GISH analyses; YS and CL evaluated the powdery mildew resistance; and WL, D-HK, BSG, and BF participated in writing the manuscript.

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Compliance with ethical standards

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

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