Theoretical and Applied Genetics (2020) 133:1149–1159
https://doi.org/10.1007/s00122-020-03538-8

ORIGINAL ARTICLE

A spontaneous wheat-*Aegilops longissima* translocation carrying *Pm66* confers resistance to powdery mildew

Huanhuan Li[1] · Zhenjie Dong[1] · Chao Ma[1] · Qing Xia[1] · Xiubin Tian[1] · Sunish Sehgal[3] · Dal-Hoe Koo[4] · Bernd Friebe[4] · Pengtao Ma[2] · Wenxuan Liu[1]

Received: 22 June 2019 / Accepted: 3 January 2020 / Published online: 13 January 2020

Abstract

Key message **A spontaneous Robertsonian T4S^{l}S·4BL translocation chromosome carrying *Pm66* for powdery mildew resistance was discovered and confirmed by RNA-seq, molecular marker, and in situ hybridization analyses.**

Abstract Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is a severe disease of bread wheat worldwide. Discovery and utilization of resistance genes to powdery mildew from wild relatives of wheat have played important roles in wheat improvement. *Aegilops longissima*, one of the S-genome diploid wild relatives of wheat, is a valuable source of disease and pest resistance for wheat. Chromosome 4S^{l} from *Ae. longissima* confers moderate resistance to powdery mildew. In this study, we conducted RNA-seq on a putative Chinese Spring (CS)-*Ae. longissima* 4S^{l}(4B) disomic substitution line (TA3465) to develop 4S^{l}-specific markers to assist the transfer of a *Bgt* resistance gene from 4S^{l} by induced homoeologous recombination. A pairwise comparison of genes between CS and TA3465 demonstrated that a number of genes on chromosome 4BS in CS were not expressed in TA3465. Analysis of 4B- and 4S^{l}-specific molecular markers showed that 4BS and 4S^{l}L were both missing in TA3465, whereas 4BL and 4S^{l}S were present. Further characterization by genomic and fluorescent in situ hybridization confirmed that TA3465 carried a spontaneous Robertsonian T4S^{l}S·4BL translocation. Powdery mildew tests showed that TA3465 was resistant to 10 of 16 *Bgt* isolates collected from different regions of China, whereas CS was susceptible to all those *Bgt* isolates. The powdery mildew resistance gene(s) in TA3465 was further mapped to the short arm of 4S^{l} and designated as *Pm66*.

Communicated by Albrecht E. Melchinger.

Huanhuan Li and Zhenjie Dong have contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00122-020-03538-8) contains supplementary material, which is available to authorized users.

✉ Pengtao Ma
ptma@ytu.edu.cn

✉ Wenxuan Liu
wxliu2003@hotmail.com

[1] College of Life Sciences, Henan Agricultural University, Zhengzhou 450002, People's Republic of China

[2] College of Life Sciences, Yantai University, Yantai 264005, People's Republic of China

[3] Department of Agronomy, Horticulture and Plant Science, South Dakota State University, Brookings, SD 57006, USA

[4] Department of Plant Pathology, Wheat Genetics Resource Center, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA

Introduction

Powdery mildew, caused by the biotrophic parasitic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*), is an important disease of bread wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD) worldwide and can cause severe yield losses in a relatively short period of time (Fried et al. 1981; Conner et al. 2003; Morgounov et al. 2012). Development and cultivation of wheat varieties with resistance to powdery mildew are considered the most effective, economical, and environmentally friendly way to control losses caused by this disease (Bennett 1984; Wang et al. 2005). Currently, a total of 89 cataloged resistance genes/alleles (*Pm1–Pm65*) are cataloged. They are located on nearly all wheat chromosomes. Forty-four of these genes originated from progenitors and wild relatives of wheat, including *Triticum urartu* Tumanian ex Gandilyan, *T. turgidum* L., *Aegilops tauschii* Coss, *Ae. speltoides* Tausch, *Ae. geniculata* Roth, *Ae. longissima* Schweinf. & Muschl., *Secale cereale* L, *Dasypyrum villosum* (L.) P. Candargy, *Thinopyrum*

intermedium (Host) Barkworth & D. R. Dewey, *Th. elongatum* (Host) D. R. Dewey, and *Agropryron cristatum* (L.) Gaertn. (McIntosh et al. 2017; Liu et al. 2017; Zou et al. 2018; Tan et al. 2018; Zhang et al. 2019). Some of these wheat relative-derived resistance genes, such as *Pm8* derived from *S. cereale*, have been used in wheat cultivars worldwide (Zeller 1973; Zeller and Fuchs 1983; Luo et al. 2009). *Pm21*, on the short arm of chromosome 6V from *D. villosum* conferring a high level of powdery mildew resistance, has been used in many wheat cultivars in China (Chen et al. 1995; Xing et al. 2018). However, up to now, only a few powdery mildew resistance (*Pm*) genes from wild relatives have been successfully used in wheat cultivars because of associated deleterious traits. *Pm* genes often break down once they are widely deployed in wheat cultivars. Thus, further discovery, identification, and transfer of new *Pm* genes for wheat improvement are still needed.

Aegilops longissima Schweinf. & Muschl. ($2n = 2x = 14$, S^lS^l), one of the S-genome diploid species belonging to section *Sitopsis* (Jaub. & Spach) Zhuk. (Feldman et al. 1995; Liu et al. 2002), is an important source of disease and pest resistance for wheat. For example, *Pm13* located on chromosome $3S^lS$ (Ceoloni et al. 1992) is one of the resistance genes utilized widely in wheat breeding programs in China (He et al. 2011) and chromosomes $4S^l$ and $6S^l$ also carry genes that confer resistance to powdery mildew (Xia et al. 2018). QTL on $1S^l$, $3S^l$, $5S^l$, and $7S^l$ confers resistance to eyespot in wheat (Sheng et al. 2012), and chromosome $2S^l$ carries genes that confer resistance to leaf rust.

Next-generation sequencing (NGS) technologies developed in the past decade provide new avenues for gene discovery and isolation with massive reductions in time, labor, and cost (Wang et al. 2009; van Dijk et al. 2014). Transcriptome sequencing, or RNA-seq based on NGS, provides extensive data for the analysis of the continuously changing transcriptome. RNA-seq has been used widely to discover resistance genes and reveals transcriptional expression patterns of targeted genes involved in pathogen-defense response pathways in crops, including hexaploid wheat with its closely related diploidized A, B, and D genomes constituting a huge genome exceeding 17 Gbp (Xin et al. 2012; Zhang et al. 2016; Boni et al. 2017). In recent years, RNA-seq has been used to develop molecular markers for diagnosing chromosomes, chromosome segments, or genes in wild relatives. This was previously difficult because of limited sequence data (Dai et al. 2012; Li et al. 2016, 2017; Wang et al. 2018).

We previously showed that chromosome $4S^l$ of *Ae. longissima* carried moderate resistance to powdery mildew (Xia et al. 2018). Here, we report the discovery and identification of a spontaneous CS-*Ae. longissima* Robertsonian $T4S^lS{\cdot}4BL$ translocation conferring resistance to powdery mildew by integrating RNA-seq, molecular markers, and in situ hybridization analyses.

Materials and methods

Plant materials

Three accessions were used in the present study: TA3465, a putative wheat-*Ae. longissima* $4S^l(4B)$ disomic substitution line, produced and kindly provided by Drs. R.S. Kota and J. Dvorak, University of California, Davis, USA (Kota and Dvorak, unpublished). Because previous *Ae. longissima* introgression lines were made from six different *Ae. longissima* parental accession designated from #1 to #6, the full designation of the group-4 *Ae. longissima* chromosome in TA3465 is designated $4S^l$#7; TA7546, a wheat-*Ae. longissima* $4S^l$#3 disomic addition line (DA$4S^l$#3, Friebe et al. 1993), and CS, a common wheat landrace and genetic background of TA3465 and TA7546. All materials are maintained by the Wheat Genetics Resource Center (WGRC) at Kansas State University, USA, and the Experimental Station of Henan Agricultural University.

Plant growth and tissue collection

Seeds of TA3465 and CS were sterilized with 5% sodium hypochlorite solution for 10 min at room temperature, and then planted in 8 cm diameter pots filled with autoclaved vermiculite. The seedlings were grown in an illuminated incubator at 18–20 °C, 18 h light and 6 h darkness, and 75% relative humidity. Three cm segments from the first leaves of 10 individual plants were pooled for each line. The samples were immediately frozen in liquid nitrogen for subsequent RNA isolation. Two biological replicates were collected for each accession.

Construction of cDNA libraries for illumina sequencing

Total RNA was extracted from leaf samples using a mirVana miRNA Isolation Kit (Cat. No. AM1561, Ambion, Thermo Fisher Scientific Inc., Waltham, MA, USA) following the manufacturer's protocol. RNA integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA Integrity Number (RIN) ≥ 7 were subjected to subsequent analyses. Libraries constructed using a TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions were sequenced on the Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeq X Ten) by OE Biotech (Shanghai, China), and 125-bp/150-bp paired-end reads (raw reads) were generated.

RNA-seq data analysis

Paired-end reads were pre-treated using an NGS QC Toolkit v2.3.3 (Patel and Jain 2012) to remove sequences containing adapters or poly-N above 5%, and low-quality reads to produce valid data for downstream sequence assembly. The validity ratio, Q30, and GC content of each sample were calculated after pre-treatment.

De novo assembly of valid data was applied using Trinity (vesion: trinityrnaseq_r20131110) (Grabherr et al. 2011), followed by removal of abundant sequences using TGICL (Pertea et al. 2003). The assembled sequences were designated as unigenes. Read counts of unigenes were normalized as fragments per kilobase per million mapped reads (FPKM). Genes with an FPKM ≥ 0.5 were considered expressed, whereas genes with FPKM = 0 were not expressed. Unigenes of CS and TA3465 were pairwise compared to identify genes present in CS and absent in TA3465. All the unigenes were assigned to chromosomes or chromosome arms based on blastn alignment against wheat reference genomic sequences (Wheat_IWGSC_RefSeq_v1_chromosomes) with cutoff of expect ≤ 1e−10 and qcov ≥ 75%) at UGRI BLAST (https://urgi.versailles.inra.fr/blast/blast.php).

Molecular marker analysis

Genomic DNA (gDNA) was isolated from 5 to 10 cm segments of young leaves with a DNeasy Plant Mini Kit (Qiagen, Cat No. 69104) following the instruction guide. Total RNA was isolated using an RNA prep Pure Plant Kit (Tiangen Biotech Co. Ltd., Code No. DP432), and cDNA was synthesized using a PrimeScript™ Double Strand cDNA Synthesis Kit (Takara, Code No. 6111A).

A total of 23 specific PCR primer pairs for 4B and $4S^{l}$ were used in the study (Table 1). Twelve primer pairs specific for 4B and seven specific for $4S^{l}S$ were designed based on RNA-seq sequences of CS and TA3465; the remaining four $4S^{l}L$-specific markers came from Wang et al. (2018). Specificity of chromosome $4S^{l}$ markers was validated using gDNA templates of TA3465 and TA7546. PCR amplification by "Touch-down 63" followed Liu et al. (2017). PCR products were resolved in 1.5% agarose gels and visualized by ethidium bromide staining under UV light.

Chromosome preparation and in situ hybridization

Root tips were collected from TA3465 and TA7546 and treated in a gas chamber with nitrous oxide for 2 h followed by a treatment with 90% acetic acid on ice for 10 min, and then kept in 70% ethanol at − 20 °C. Squash preparations were made in a 45% acetic acid solution. Genomic in situ hybridization (GISH) was according to Liu et al. (2017) with *Ae. longissima* genomic DNA probes labeled with fluorescein-12-dUTP. The ratio of genomic *Ae. longissima* DNA and CS blocking DNA was 1:120. For fluorescent in situ hybridization (FISH), eight oligonucleotide probes including six TAMRA (6-carboxytetramethylrhodamine)-modifed oligonucleotides (pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, and AFA-4) and two FAM (6-carboxyfuorescein)-modifed oligonucleotides [pSc119.2-1 and $(GAA)_{10}$] were used to identify wheat and *Ae. longissima* chromosomes (Du et al. 2017; Huang et al. 2018). After hybridization and slide washing, a drop (25–30 µl) of Vectashield mounting medium containing 1 µg/ml DAPI (Vector Laboratories Inc, Burlingame, CA, USA) was added to each slide and then covered with a 24 × 30 cm glass cover slip. Fluorescent images were observed with a Zeiss Axio Scope A1 fluorescence microscope (Germany) and captured with an AxioCam MRc5 CCD camera. Images were further processed with Adobe Photoshop CS3 (Version 10.0.1) (Adobe Systems Inc., San Jose, CA, USA).

Response to *Bgt* isolates

A mixture of *Bgt* isolates collected from different regions in the Henan province is maintained by Henan Agricultural University and used for preliminary identification of powdery mildew resistance of TA3465. To accurately confirm the resistant spectrum of TA3465, sixteen single-pustule-derived *Bgt* isolates collected from different regions in China and maintained at Yantai University, Shandong province, China, were used to inoculate TA3465. Each set of material inoculated with a single *Bgt* isolate was placed in an isolated space. For each isolate, five seedlings of each of CS and TA3465 were planted in rectangular trays with 32 cells (3 × 3 cm) in a spore-free space. Three replications were conducted for each test. When seedlings reached the one- to two-leaf stage, fresh conidiospores collected from Mingxian 169 seedlings were dusted onto the materials. The trays were then placed in a controlled greenhouse with a daily cycle of 14 h light at 22 ± 2 °C and 10 h of darkness at 18 ± 2 °C. Inoculations were repeated once a day for three consecutive days. When the pustules were fully developed on the first leaf of CS, the infection types (IT) of each plant were assessed on a 0–4 scale, where 0 = no visible symptoms; 0 = hypersensitive necrotic flecks; 1 = small and sparse conidial development; 2 = colonies with moderately developed hyphae, but few conidia; 3 = colonies with well-developed hyphae and abundant conidia, but colonies not joined; and 4 = colonies with well-developed hyphae and abundant conidia with mostly overlapping colonies. Plants with IT 0–2 were considered resistant, whereas those with IT 3–4 were susceptible (Shi et al. 1987).

Table 1 List of PCR primers used in the study

Name	Forward	Reverse	Tm (°C)	Specificity	Position (Mb)	References
CL67293Contig1	ACACCTGACTGACTG AGACT	CCAGGCCAAGAGTCC AGTTA	57	4BS	2	–
CL116905Contig1	GCCGGCCTATCTAAC TGAGG	TGGTCAATCAGTTTC CCACGT	57	4BS	9	–
CL47978Contig1	TTGCTTCGGGGAATT GGGTT	ACACCAAGAAGAAGC CCTCG	57	4BS	18	–
CL26655Contig1	GGTTCCCCATCGACA CATGT	TATTTGGACCTCAGC AGCGG	57	4BS	109	–
CL38954Contig1	GGGCTCTGAATGAAG ATATGGGT	TCATGTAGATGCACC TCTCAC	57	4BS	207	–
CL109675Contig1	GCTGCATTCTCTCCC GACTC	GGAAAGAATGCGGAG GGGG	57	4BS	287	–
CL34Contig1	ACTCGCTCGCAAAGA TCACA	TGCATCGCGGTTCAC AAAAG	57	4BS	315	–
CL40713Contig1	TCCTCCTCCCAGAAC CACAT	CCATGCGTCTTAGTG GGGAG	57	4BL	358	–
CL51285Contig1	GGGTGTTTTGGATGA GGGCT	TGCATTTCCAAGGAC CTGGA	57	4BL	426	–
CL3273Contig2	TCCTGTGTAGTTTGC CCGTC	CATCCGCTGAACTCC GAGAA	57	4BL	489	–
CL33860Contig1	AGACTATAGAGCGGC AATGTCA	CAAAATAAGTCTCTG GGATGCCA	57	4BL	595	–
CL47322Contig1	CCAGGTCTTCTCCCA GCTCA	TGGTTTCATTTCATT CGTCGCC	57	4BL	639	–
CL113827Contig1	CAGGGTCTCCAGGCT CTA	CACTCGGGACCAGAT TTT	58	$4S^lS$	3	–
CL115356Contig1	CCATGCTCAAGCAGG AAGAG	GTACCGAAACGCAAA GGGAT	60	$4S^lS$	34	–
CL103255Contig1	TCACCCTACGCATCG CCT	CCCTCCGGTTTTCAG CTC	58	$4S^lS$	68	–
CL107718Contig1	GATTCCGAGTCATTT CAAGCC	GAGACTAAACGCCGC ATCAG	61	$4S^lS$	95	–
CL112181Contig1	GCTTCAGGAGCATAC AACAA	TATCCACCCAACCAT ACTTT	58	$4S^lS$	95	–
CL116906Contig1/*Hae*III	ACAAGCCGATTGATA ACC	TGACATAACGCTACC AGAAT	56	$4S^lS$	658	–
CL107914Contig1	TAGGTCGGTTATCTA ATCTTGC	AAGGCATTCGGATGT ATTG	58	$4S^lS$	692	–
$4S^lL$-03	TTAACCAATGAGACC GACAA	CAAGTAGGACGTATT AGGCATA	55	$4S^lL$	464	Wang et al. (2018)
$4S^lL$-05	TTAACCAATGAGACC GACAA	CCTCCATTCCACAAT ATAGTAC	55	$4S^lL$	464	Wang et al. (2018)
$4S^lL$-06	ACCAATGAGACCGAC AAC	CCTCCATTCCACAAT ATAGTAC	55	$4S^lL$	464	Wang et al. (2018)
$4S^lL$-07	TTAACCAATGAGACC GACAA	AGGTACAAGTAGGAC GTATTAG	55	$4S^lL$	464	Wang et al. (2018)

Infected leaves 10 days post-*Bgt* inoculation were cut into 1–2 cm segments and stained with Coomassie Brilliant Blue-R-250 following Li et al. (2016) for microscopic examination.

Results

Powdery mildew response of TA3465

Primitively, isolate mixture of *Bgt* collected from different regions in Henan province was used to inoculate seedlings with the first leaf fully extended to assay the resistance of

TA3465, TA7546, and susceptible control CS. Seedling reactions of 10 days post-inoculation *Bgt* isolate mixture indicated that TA3465 and TA7546 were moderately resistant with IT 2, whereas CS was highly susceptible with IT 4 (Fig. 1). Thus, chromosomes $4S^{l}$#3 and $4S^{l}$#7 have a gene(s) conferring moderate resistance to powdery mildew.

RNA-seq quantity analysis, sequence assembly

Totals of 77,962,967 and 75,045,904 clean reads > 100 bp were obtained for CS and TA3465, respectively. An average of 91.29% of clean reads were mapped to genomic sites. The Q30 ratio inferred a base call accuracy of 99.90%, was 93.06% for CS, and 90.27% for TA3465, both higher than the threshold value of 85.00%, thus indicating that the RNA-seq quality was satisfactory for sequence assembly.

Clean reads of both accessions were assembled further into a total of 158,953 unigenes with a median length of 1247.95 bp using the short-read assembly software Trinity. The N50 value of the assembled sequences was 1725 bp.

Comparison of expressed genes between CS and TA3465

Expression levels of the 158,953 genes were normalized based on FPKM values of the sequences for further analysis of differential genes. This analysis revealed a total of 69,808 transcribed unigenes in CS at an FPKM ≥ 0.5 in both biological replicates, of which 37,058 (53.09%) could be assigned to wheat chromosomes by blastn to wheat reference genomic sequences (Wheat_IWGSC_RefSeq_v1_chromosomes) with a cutoff of expectation ≤ 1e−10 and qcov ≥ 75.00%. An analysis of the chromosomal distribution of 37,058 mapped genes in CS revealed that these genes were unevenly distributed on different chromosomes, of which chromosome 2B had the maximum number (2066, 5.58%), followed by 5B (2035, 5.49%), 2D (1937, 5.23%) and 6B (1931, 5.21%), whereas chromosome 6A had the least number of mapped genes (1385, 3.74%) (Fig. 2; Supplementary Tables 1, 2).

The 37,058 mapped genes were then pairwise compared between CS and TA3465, and yielded 913 non-expressed genes (NEGs) (2.46%) with FPKM values of 0.00 in both replicates of TA3465. Chromosome distribution analysis of these NEGs showed that each chromosome in TA3465 had an average of 42.3 NEGs (2.44%), whereas 4B had a significantly higher number; 282 of 1646 genes (17.13%) mapping to chromosome 4B of CS were not expressed in TA3465 (Fig. 3). Further analysis of these 282 NEGs revealed that 259 of 710 genes mapped to the short arm of 4B (4BS) in CS were missing, with a NEG frequency of 36.48%, significantly higher than the average ratio of 2.46% in TA3465. On the contrary, only 23 of 936 genes located on 4BL were

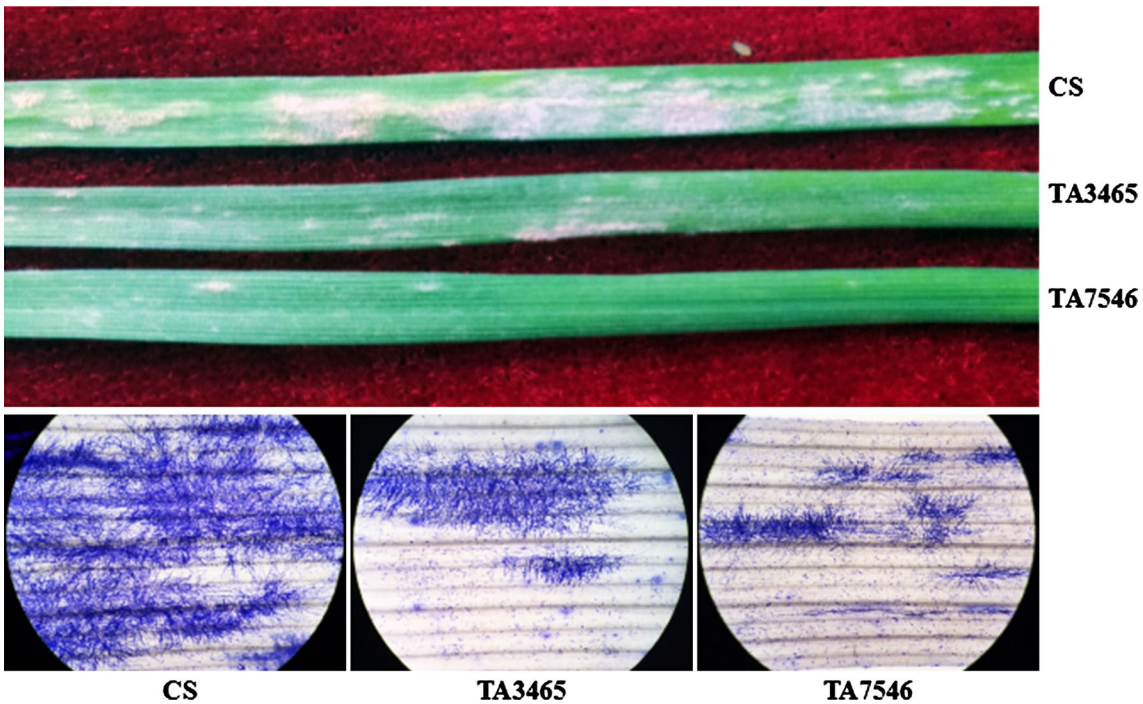

Fig. 1 IT of wheat-*Ae. longissima* putative $4S^{l}$#7(4B) disomic substitution line (TA3465), $4S^{l}$#3 disomic addition line (TA7546), and susceptible CS. ITs were scored 10 days post-inoculation using a mixture of *Bgt* races collected from Henan province. Upper images show the reactions of the first leaves, and lower images show *Bgt* colonies and hyphal developed on leaf segments stained with Coomassie Brilliant Blue-R-250 and observed at 400 × magnification

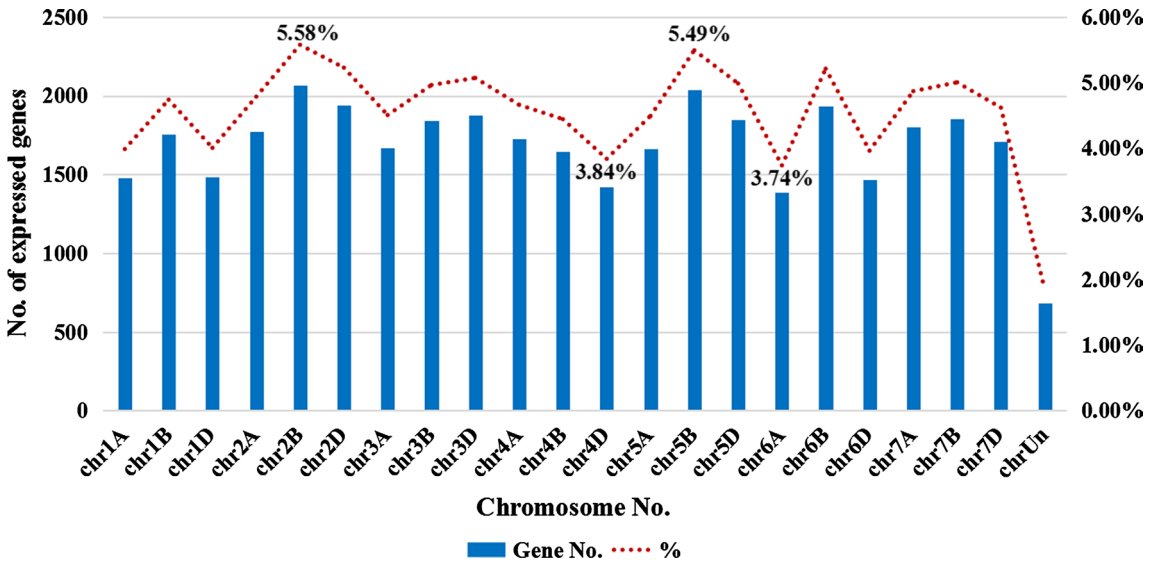

Fig. 2 Chromosomal distribution of mapped unigenes in CS

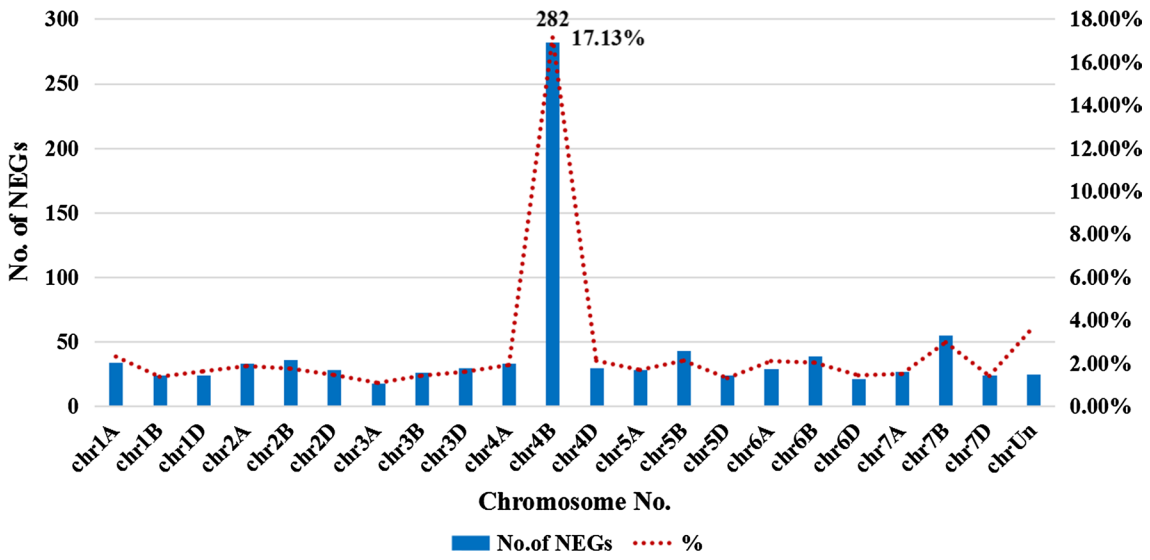

Fig. 3 Chromosomal distribution of CS-mapped genes in putative disomic 4S^{l}#7(4B) substitution TA3465, showing the number and ratio of CS-mapped genes not expressed in TA3465. NEGs: non-expressed genes

absent, with a NEG ratio of 2.46%, similar to the overall average NEG ratio for TA3465 (Supplementary Tables 1, 3). The abnormally high NEG frequency for 4BS in TA3465 led to the subsequent study of whether those NEGs were caused by non-expression of 4BS-located genes or by the absence of 4BS in TA3465.

Molecular analysis of 4S^{l}- and 4B-specific markers in TA3465

To verify that the 282 NEGs for chromosome 4B were either not expressed or missing in TA3465, 12 pairs of 4B-specific primers, including seven for 4BS-specific and five 4BL-specific, were designed based on RNA-seq (Table 1; Supplementary Table 4). PCR amplification using genomic DNA and cDNA as templates showed that all markers produced specific bands in CS, confirming that the unigenes were present and expressed in that genotype. All five 4BL-specific markers were present and the other seven 4BS-specific markers were absent in TA3465 (Fig. 4a), indicating that the short arms of 4B were missing in TA3465.

Analysis of 11 4S^{l}-specific markers, including seven for the short arm and four for the long arm of 4S^{l}, indicated the presence of all seven markers on 4S^{l}#7S and the absence of

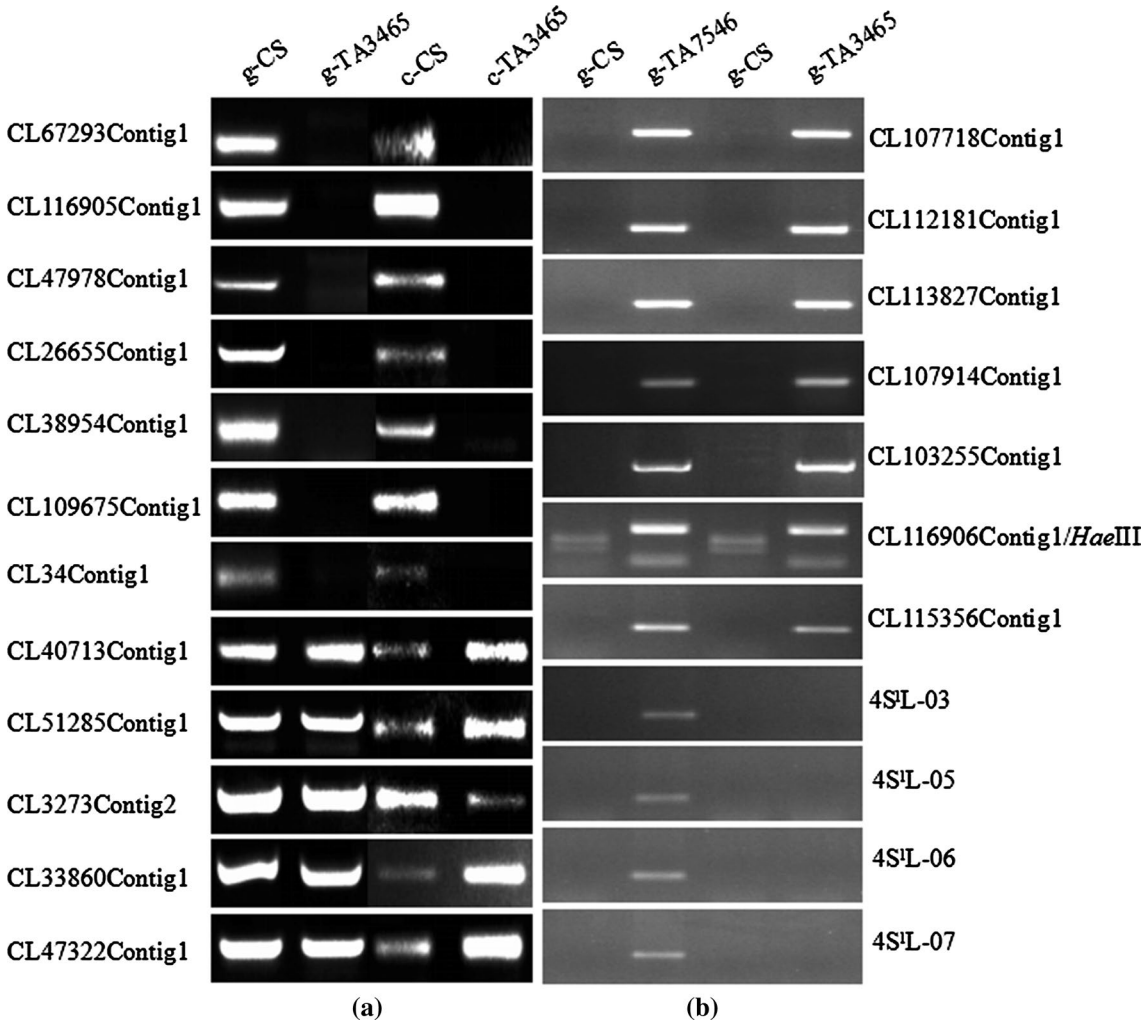

Fig. 4 PCR patterns of CS, putative 4S^{l}#7(4B) disomic substitution line TA3465, and 4S^{l}#3 disomic addition line TA7546. **a** PCR amplifications of CS and TA3465 by 4B-specific primer pairs using gDNA and cDNA as templates; and **b** specific PCR products of CS, TA3465, and TA7546 amplified using 4S^{l}-specific primer pairs. g: gDNA and c: cDNA. All specific molecular markers for 4BL and 4S^{l}#7S specific are present, whereas all markers for 4S^{l}#7L and 4BS are missing in line TA3465

the 4S^{l}#7L markers in TA3465 (Fig. 4b). Thus, the 4B- and 4S^{l}#7-specific molecular marker analyses demonstrated that line TA3465 was either a Robertsonian translocation consisting of 4S^{l}#7S derived from *Ae. longissima* and 4BL derived from CS, or a double ditelosomic line containing telosomes of 4S^{l}#7S and 4BL in a CS background.

Chromosome identification of TA3465 by in situ hybridization

GISH and FISH were subsequently applied to root-tip cells of both the disomic 4S^{l} addition line TA7546 and the putative translocation line TA3465 using gDNA probes of *Ae. longissima* and a set of eight repetitive wheat sequences as probes, respectively. GISH of TA7546 confirmed the presence of a pair of 4S^{l}#3 chromosomes (Fig. 5a). FISH was able to distinguish the short and long arm of 4S^{l}#3 (Fig. 5b). GISH and FISH analysis of TA3465 revealed the presence of a pair of Robertsonian translocation chromosomes consisting of 4S^{l}#7S and 4BL, that is, T4S^{l}#7S·4BL (Fig. 5c, d), confirming the results of the molecular marker analysis. Thus, TA3465 was redesignated as a Robertsonian T4S^{l}#7S·4BL translocation line. GISH and FISH analysis of original seeds obtained by Drs. Kota and Dvorak revealed that the line was heterogeneous for a complete 4S^{l}#7 and T4S^{l}#7S·4BL Robertsonian translocation chromosomes (Fig. 6).

Multi-isolate powdery mildew tests of TA3465

To accurately evaluate the response spectrum of TA3465, 16 *Bgt* isolates collected from different regions in China were used to inoculate TA3465. As a result, TA3465 was

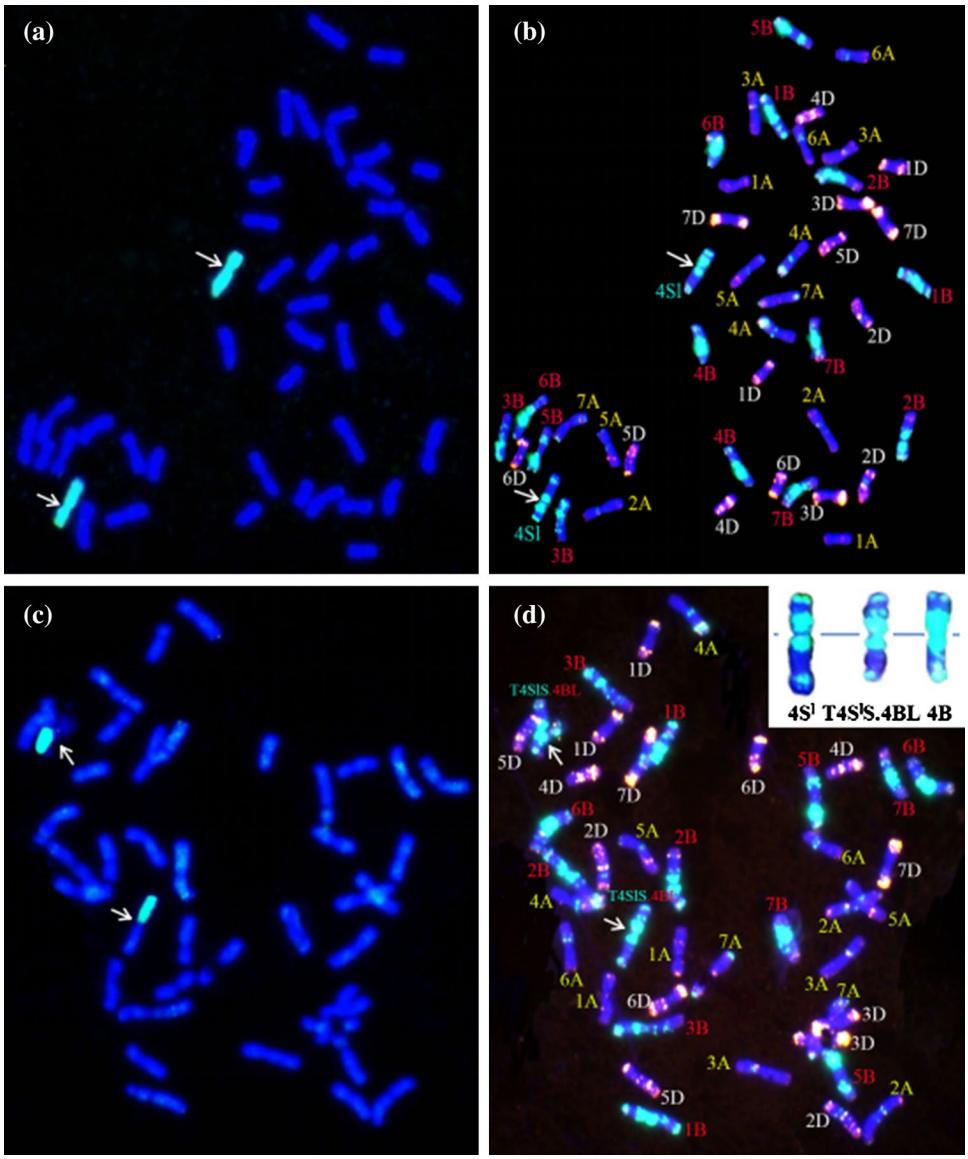

Fig. 5 GISH and FISH patterns of CS-*Ae*. *longissima* derivative TA3465, 4S^{l}#3 disomic addition line (TA7546), and CS. GISH (**a**) and FISH (**b**) patterns for disomic 4S^{l}#3 addition line TA7546; GISH (**c**) and FISH (**d**) patterns for T4S^{l}#7S·4BL translocation stock TA3465. Arrows indicate chromosome 4S^{l}#3 from *Ae*. *longissima* (**a**, **b**) and T4S^{l}#7S·4BL translocated chromosomes (**c**, **d**). *Ae*. *longissima* chromatin was visualized by green FITC florescence, and wheat chromosomes were counterstained with DAPI and fluoresce blue. FAM-modifed oligonucleoties pSc119.2-1 and $(GAA)_{10}$ are in green color; TAMRA-modifed oligonucleoties (pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, and AFA-4) are red (color figure online)

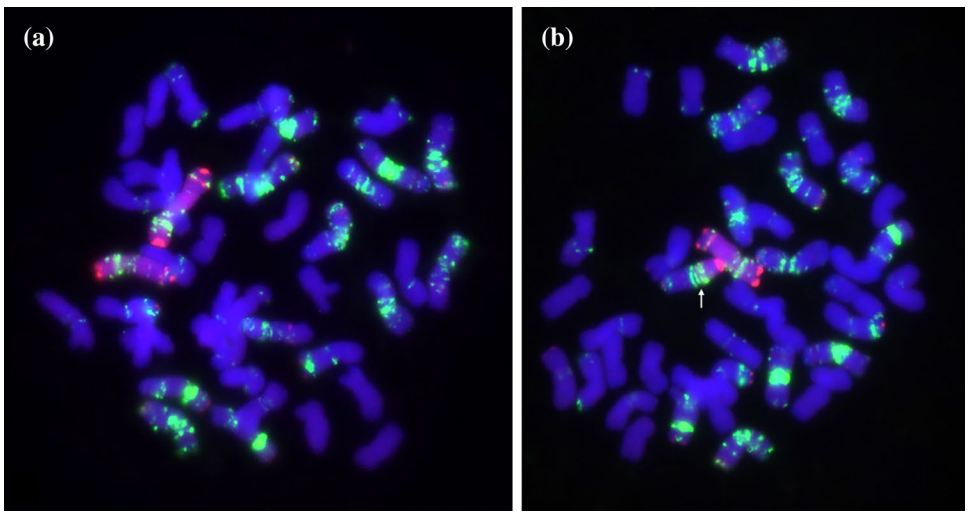

Fig. 6 GISH and $(GAA)_{10}$ FISH pattern in a seedling from the original seeds of TA3465 showing that this line was heterogeneous for a complete 4S^{l}#7 chromosome (visualized in red) (**a**) and a T4S^{l}#7S·4BL Robertsonian translocation indicated by an arrow (**b**) (color figure online)

Table 2 Reaction patterns of TA3465 and CS to *Bgt* isolates

Bgt isolates	Y02	Y03	Y04	Y15	Y06	Y07	Y08	Y10	Y11	Y14	Y05	Y16	Y17	Y18	Y21	B18
TA3465	3	0	2	1	0	0	1	2	1	3	4	3	0	0	4	3
CS	4	3	4	4	4	4	4	4	4	4	4	4	4	3	4	4

resistant to 10 of the 16 *Bgt* isolates with IT ranging from 0 to 2, whereas CS was highly susceptible to all those isolates with ITs of 3–4 (Table 2). Six *Bgt* races were virulent on TA3465. The phenotypes of three repetitions were consistent. Therefore, the *Pm* gene that confers broad-spectrum resistance to *Bgt* isolates was further mapped to the short arm of chromosome 4S^{l}#7 in TA3465.

Discussion

Powdery mildew is a significant disease of wheat worldwide. Exploring the gene pools of wild relatives for *Bgt* resistance is desirable for developing resistant wheat cultivars. Over time, the genetic basis for disease resistance in wheat cultivars has been narrowed by wide use of a few resistance genes and/or loss of effectiveness due to virulent isolates. This situation leads to unprecedented challenges in resistance breeding (Johnson 1992; Gupta et al. 2010; Karsai et al. 2012). The T4S^{l}#7S·4BL translocation stock TA3465 identified in the present study confers resistance to many *Bgt* isolates. The gene for resistance in TA3465 was designated as *Pm66* and is potentially useful for resistance breeding in China.

With decreasing genomic sequencing costs, RNA-seq is commonly used to develop molecular markers specific for alien chromatin in order to assist transfer to wheat and analyze chromosome structure in existing germplasm (Li et al. 2017; Rey et al. 2018; Wang et al. 2018). For example, Rey et al. (2018) identified a 36 Mb deletion at the distal end of chromosome 7AL in a wheat–barley 7HL addition line based on the RNA-seq pattern. Here, we showed that a putative progeny of a wheat-*Ae. longissima* 4S^{l}#7 substitution line was, in fact, a T4S^{l}#7S·4BL Robertsonian translocation line by combined analysis of RNA-seq, molecular markers, and in situ hybridization.

Linkage drag caused by undesirable or deleterious genes located in introgressed alien chromosome segments is considered a major constraint to exploitation of genes derived from wheat relatives in wheat cultivars. However, the molecular mechanisms underlying the impact of foreign chromatin on plant phenotype are kept unexploited by far. Recently, Rey et al. (2018) reported that a ditelosomic addition 7HL from *H. vulgare* into CS led to a 3% differential expression of genes in the 7HL stock. In this study, the translocated 4S^{l}#7 also leaded to a genome-wide suppression of gene expression in CS wheat by an average of 2.46% (Fig. 3). In addition, expression levels of a large number of genes were either up- or down-regulated in TA3465 compared to that in CS. For example, of five genes used to develop 4BL-specific markers, four genes were significantly up-regulated in TA3465, with expression levels of 3.83–6.51 times higher than that in CS, whereas expression level of gene CL3273Contig2 in TA3465 was only 1/5 that in CS (Fig. 4; Supplementary Table S4). The possible effects of those significantly affected genes of CS on the agronomic characteristics of TA3465 are currently under investigation.

It is likely that during development line TA3465 when the 4B and 4S^{l}#7 were both monosomic simultaneous misdivision at the centromeres and rejoining led to the centric fusion of the 4S^{l}#7S and 4BL chromosome arms (Sears 1952; Friebe et al. 2005). This centric fusion chromosome then remained undetected in the derived line TA3456. The nature of the translocation was resolved in this study by using more recently developed cytogenetic tools.

Acknowledgements We thank Dr. Yuli Song from Institute of Plant Protection, Henan Academy of Agricultural Sciences, for providing *Bgt* isolates collected in Henan Province, Drs. Rama S. Kota and Jan Dvorak for providing seeds of TA3465, and W. John Raupp and Dr. Robert McIntosh for critical reading of the manuscript. This project was financially supported by National Natural Science Foundation of China (Grant No. 31571658), the State Key Laboratory of Crop Genetics and Germplasm Enhancement at Nanjing Agricultural University, China (ZW2011002), Henan Province Special Major Science and Technology Project (161100110400), and the State Key Laboratory of Wheat and Maize Crop Science at Henan Agricultural University, China (39990022).

Author contribution statement This study was conceived by WL and PM; HL performed in situ hybridization. ZD and SS conducted RNA-seq data analysis and molecular marker design; CM conducted molecular marker analysis; QX and PM evaluated responses to *Bgt* isolates; XT prepared RNA samples; WL and HL wrote the manuscript; and D-HK and BF verified the chromosomal constitution of TA3465 maintained in KSU and amended the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

References

Bennett F (1984) Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programs. Plant Pathol 33:279–300

Boni R, Chauhan H, Hensel G, Roulin A et al (2017) Pathogen-inducible *Ta-Lr34res* expression in heterologous barley confers disease resistance without negative pleiotropic effects. Plant Biotechnol J 16:245–253

Ceoloni C, Signore G, Ercoli L, Donini P (1992) Locating the alien chromatin segment in common wheat-*Aegilops longissima* mildew resistant transfers. Hereditas 16:239–245

Chen P, Qi L, Zhou B, Zhang S et al (1995) Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91:1125–1128

Conner R, Kuzyk A, Su H (2003) Impact of powdery mildew on the yield of soft white spring wheat cultivars. Can J Plant Sci 83:725–728

Dai C, Zhang J, Wu X, Yang X et al (2012) Development of EST markers specifc to *Agropyron cristatum* chromosome 6P in common wheat background. Acta Agron Sin 38:1791–1801

Du P, Zhuang L, Wang Y, Yuan L et al (2017) Development of oligonucleotides and multiplex probes for quick and accurate identification of wheat and *Thinopyrum bessarabicum* chromosomes. Genome 60:93–103

Feldman M, Lupton F, Miller T (1995) Wheats. In: Smartt J, Simmonds N (eds) Evolution of crops. Longman Group, London, pp 184–192

Friebe B, Tuleen NB, Jiang J, Gill BS (1993) Standard karyotype of *Triticum longissimum* and its cytogenetic relationship with *T. aestivum*. Genome 36:731–742

Friebe B, Zhang P, Linc G, Gill BS (2005) Robertsonian translocations in wheat arise by centric misdivision of univalents at anaphase I and rejoining of broken centromeres during interkinesis of meiosis II. Cytogenet Genome Res 109:293–297

Fried P, Mackenzie D, Nelson R (1981) Yield loss caused by *Erysiphe graminis* f. sp. *tritici* on single culms of "Chancellor" wheat and four multilines. Z Pflanzenschutz 88:256–264

Grabherr M, Haas B, Yassour M, Levin J et al (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 29:644–652

Gupta P, Langridge P, Mir R (2010) Marker-assisted wheat breeding: present status and future possibilities. Mol Breed 26(1):145–161

He Z, Xia X, Chen X, Zhang Q (2011) Progress of wheat breeding in China and the future perspective. Acta Agron Sin 37:202–215

Huang X, Zhu M, Zhuang L, Zhang S et al (2018) Structural chromosome rearrangements and polymorphisms identified in Chinese wheat cultivars by high-resolution multiplex oligonucleotide FISH. Theor Appl Genet 131:1967–1986

Johnson R (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3–22

Karsai I, Vida G, Petrovics S, Petcu E et al (2012) Assessment of the spatial genotypic and phenotypic diversity present in the various winter wheat breeding programs in Southeast Europe. Euphytica 186:139–151

Li Q, Niu Z, Bao Y, Tian Q et al (2016) Transcriptome analysis of genes related to resistance against powdery mildew in wheat-*Thinopyrum* alien addition disomic line germplasm SN6306. Gene 1:5–17

Li S, Lin Z, Liu C, Wang K et al (2017) Development and comparative genomic mapping of *Dasypyrum villosum* 6V#4S-specific PCR markers using transcriptome data. Theor Appl Genet 130:2057–2068

Liu X, Richard R, Jia J, Dong Y (2002) Assessing genetic diversity of *Aegilops longissima* Schweinf and Muschl. by RAPD markers. J Plant Genet Resour 3:1–6

Liu W, Koo D, Xia Q, Li C et al (2017) Homoeologous recombination-based transfer and molecular cytogenetic mapping of powdery mildew-resistant gene *Pm57* from *Aegilops searsii* into wheat. Theor Appl Genet 130:841–848

Luo P, Zhang H, Shu K, Wu X et al (2009) The physiological genetic effects of 1BL/1RS translocated chromosome in "stay green" wheat cultivar CN17. Can J Plant Sci 89:1–10

McIntosh R, Dubcovsky J, Rogers W, Morris C et al (2017) Catalogue of gene symbols for wheat. 2017. Supplement. Annu Wheat Newsl 53:107–128

Morgounov A, Tufan H, Sharma R, Akin B et al (2012) Global incidence of wheat rusts and powdery mildew during 1969–2010 and durability of resistance of winter wheat variety Bezostaya 1. Eur J Plant Pathol 132:323–340

Patel R, Jain M (2012) NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS ONE 7:e30619

Pertea G, Huang X, Liang F, Antonescu V et al (2003) TIGR Gene Indices Clustering Tools (TGICL): a software system for fast clustering of large EST datasets. Bioinformatics 19:651–652

Rey E, Abrouk M, Keeble-Gagnere G, Karafiifiatova M et al (2018) Transcriptome reprogramming due to the introduction of a barley telosome into bread wheat affects more barley genes than wheat. Plant Biotechnol J 16:1767–1777

Sears ER (1952) Misdivision of univalents in common wheat. Chromosoma 4:535–550

Sheng H, See D, Murray T (2012) Mapping QTL for resistance to eyespot of wheat in *Aegilops longissima*. Theor Appl Genet 125:355–366

Shi Q, Zhang X, Duan X (1987) Identification of isolates of *Blumeria graminis* f. sp. *tritici*. Sci Agric Sin 20:64–70

Tan C, Li G, Cowger C, Carver B et al (2018) Characterization of *Pm63*, a powdery mildew resistance gene in Iranian landrace PI 628024. Theor Appl Genet 132:1137–1144

van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C (2014) Ten years of next-generation sequencing technology. Trends Genet 30:418–426

Wang Z, Li L, He Z, Duan X et al (2005) Seedling and adult plant resistance to powdery mildew in Chinese bread wheat cultivars and lines. Plant Dis 89:457–463

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57–63

Wang K, Lin Z, Wang L, Wang K et al (2018) Development of a set of PCR markers specific to *Aegilops longissima* chromosome arms and application in breeding a translocation line. Theor Appl Genet 131:13–25

Xia Q, Mai Y, Dong Z, Liu W (2018) Identification of powdery mildew resistance resources from wheat-wild relative disomic addition lines and development of molecular markers of alien chromosome-specificity. J Henan Agric Sci 47:64–69

Xin M, Wang X, Peng H, Yao Y et al (2012) Transcriptome comparison of susceptible and resistant wheat in response to powdery mildew infection. Genom Proteom Bioinform 10:94–106

Xing L, Hu P, Liu J, Witek K et al (2018) *Pm21* from *Haynaldia villosa* encodes a CC-NBS-LRR protein conferring powdery mildew resistance in wheat. Mol Plant 11:874–878

Zeller FJ (1973) 1B/1R wheat-rye chromosome substitutions and translocations. In: Sears ER, Sears LMS (eds) Proceedings of the 4th international wheat genetics symposium. University of Missouri Agricultural Experimental Station, Columbia, pp 209–221

Zeller F, Fuchs E (1983) Cytology and disease resistance of 1A/1R and some 1B/1R wheat-rye translocation cultivars. Z. Pflanzenzuecht 90:285–296

Zhang J, Zheng H, Li Y, Li H et al (2016) Coexpression network analysis of the genes regulated by two types of resistance responses to powdery mildew in wheat. Sci Rep 6:23805

Zhang D, Zhu K, Dong L, Liang Y et al (2019) Wheat powdery mildew resistance gene *Pm64* derived from wild emmer (*Triticum turgidum* var. *dicoccoides*) is tightly linked in repulsion with stripe rust resistance gene *Yr5*. Crop J. https://doi.org/10.1016/j.cj.2019.03.003

Zou S, Wang H, Li Y, Kong Z et al (2018) The NB-LRR gene *Pm60* confers powdery mildew resistance in wheat. New Phytol 218:298–309