Identification, Characterization, and Evaluation of Novel Stripe Rust-Resistant Wheat–*Thinopyrum intermedium* Chromosome Translocation Lines

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Abstract

Stripe rust is an important disease in wheat, and development of genetic resistance in cultivars is an effective approach to control the disease. Wild species of wheat, such as *Thinopyrum intermedium*, are an excellent gene source for wheat improvement. In this study, two stripe rust-resistant wheat–*Th. intermedium* chromosome translocation lines, CH4131 and CH4132, were characterized by cytogenetic and pathological methods. The introgressed chromosome fragment was tagged using amplified fragment-length polymorphism-derived sequence-characterized amplified region (SCAR) markers and intron targeting markers, indicating that CH4131 and CH4132 both possess a homologous group 3 chromatin of *Th. intermedium*. Genomic in situ hybridization results suggested that a very small *Th. intermedium* chromosome segment was translocated to the terminal region of wheat 1BS for both

lines, forming a configuration of T3Ai-1BS.1BL. The two translocation lines were resistant to stripe rust, and the resistance gene, temporarily designated *YrCH-1BS*, was likely derived from *Th. intermedium*. The translocated chromosome fragments have no genetic linkage drag to agronomic performance. The grain quality indexes of these two translocations were higher than local wheat varieties. Therefore, CH4131 and CH4132 could be used as potential gene sources in wheat improvement programs. The SCAR markers are useful to select stripe rust resistance from *Th. intermedium*.

Keywords: cereals and grains, cultivar/resistance, disease management, field crops, fungi, genomic in situ hybridization, stripe rust, *Thinopyrum intermedium*

Crop diseases threaten global food security. The genetic improvement of disease resistance greatly impacts the improvement of crop yield. Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* Erikss (*Pst*), is one of the most devastating diseases of wheat (*Triticum aestivum* L.). Statistical data showed that the area covered by the disease was 4.00 to 5.34 million ha per year in China, and the yield loss was estimated within the range of 10 to 20% and up to 30% in the years with serious occurrence (Chen 2005; Wellings 2011). More virulent and aggressive *Pst* races that began to appear around the year 2000 have spread rapidly over most of the wheat-growing regions of the world, causing significant yield losses (Hovmøller et al. 2008, 2016). In 2002, 2003, and 2009, wheat yield losses in China were up to 13.0, 9.8, and 8.1 million metric tons, respectively (Bai et al.

2014; Zhang et al. 2017a, b). The utilization of resistance genes is considered an economical and effective approach to control wheat diseases. Presently, >80 stripe rust resistance genes have been permanently named (McIntosh et al. 2016, 2017, 2018; Wu et al. 2018a). However, the rapid appearance of new virulent *Pst* races often leads to loss of effectiveness and necessitates searches for new sources of resistance. Therefore, there is a need to discover new stripe rust resistance genes to broaden the wheat gene pool for breeding stripe rust-resistant wheat cultivars.

Introducing disease resistance genes from wheat-alien species into wheat through chromosome engineering is an important method in achieving wheat resistance diversity (Brown 2015). Some important disease resistance genes have already been transferred from alien species to wheat, such as *Yr8*, *Yr17*, *Pm12*, and *Pm13* from the genus *Aegilops* (Cenci et al. 1999; Chen and Zhao 2007; Robert et al. 1999; Song et al. 2007); *Yr9*, *Pm8*, *Pm7*, *Pm17*, and *Pm20* from the genus *Secale* (Huang and Röder 2004; Hurmi et al. 2013; Mago et al. 2005); and *Pm21* from the genus *Dasyphyrum* (Liu et al. 1999). Wheat–*Thinopyrum* translocations T3DS.3DL-3Ae#1L (Agent), T6AS.6AL-6Ae#1L (K2046), and T7DS.7DL-7Ae#1L (Agatha); wheat–*Secale* translocations T1BL.1R#2S (Aurora and Kavkaz) and T1AL.1R#2S (Amigo); and wheat–*Dasyphyrum* translocation T6AL.6V#1S (KS04WGRC48) have made important contributions to both breeding disease-resistant wheat cultivars and increasing yield (Friebe et al. 1994; Wetzel and Rayburn 2000; Xie et al. 2012). However, most wheat-alien species chromosome translocations cannot be used directly in wheat breeding programs owing to genetic drag. Therefore, creating and identifying small chromosome segment translocations in wheat-alien species have become key research topics, because they could be used to overcome this disadvantage.

Thinopyrum intermedium ($2n = 6x = 42$, E^cE^cE^bE^bStSt) possesses many agronomically useful genes and is one of the most successfully used species in wheat breeding (Friebe et al. 1994; Li et al. 2016). Many examples have shown that *Th. intermedium* chromosomes could be easily exchanged and recombined with wheat chromosomes using

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chromosome engineering inducement (Lang et al. 2018; Liu et al. 2011a, b, 2013a, b; Tang et al. 2014; Xin et al. 2001; Zhan et al. 2015). Therefore, transfer of useful genes from *Th. intermedium* into wheat through creating wheat–*Th. intermedium* chromosome recombinants is feasible. In this study, two noncompensating wheat–*Th. intermedium* small chromosome segment translocations of T3Ai-1BS.1BL were identified from progenies of a complex cross involving four common wheat varieties and a *Th. intermedium* line using molecular cytogenetic methods. The stripe rust resistance, agronomical traits, and grain quality of both lines were investigated. Both lines were highly resistant to stripe rust and had no negative effect on agronomical traits and quality. Therefore, both translocation lines are useful materials for wheat breeding programs.

Materials and Methods

Plant material. Materials used in this study included four BC₃F₆ translocation lines, CH4131 to CH4134, that were derived from the complex cross Chinese Spring (CS)/Z1141//Taichung29 (TC29)//PH82-2-2//3*Shan 225. Z1141 is a genotype of *Th. intermedium* ($2n = 6x = 42$, E^eE^eE^bE^bStSt). PH82-2-2 and Shan 225 are a breeding line and a cultivar, respectively, of common wheat with good end use qualities. TC29 is a breeding line from Sichuan province. This study also included three *Th. intermedium*-related wild species, *Thinopyrum elongatum* PI98526 ($2n = 2x = 14$, E^eE^e), *Thinopyrum bessarabicum* PI531712 ($2n = 2x = 14$, E^bE^b), and *Pseudoroegneria strigosa* PI313960 ($2n = 2x = 14$, StSt), for comparison; cultivar Mingxian169 (MX169) is as a susceptible line to stripe rust, Zhongyou9507 is as a positive control with known

gluten gene subunit 5 + 10, and cultivar Liangxing 99 (LX99) is a middle-gluten wheat variety. These materials were provided by the Wheat Research Institute, Shanxi Agricultural University, Linfen, Shanxi, China. To confirm the origin of the stripe rust resistance, a doubled haploid (DH) population (85 lines) was developed from a cross between the resistant line CH4131 and the susceptible line CH4133 using the wheat by maize pollination method (Yang et al. 2017) by operation in Yunnan Academy of Agricultural Sciences, Kunming, China.

Stripe rust testing. Z1141, CS, TC29, Shan 225, PH82-2-2, and CH4131 to CH4134 were planted in a randomized complete block nursery with three replicates in a field at Linfen, Shanxi province in 2011 to 2012, 2012 to 2013, 2013 to 2014, and 2014 to 2015. Twenty seeds of each line were planted evenly in single rows of 1-m length with 33 cm between adjacent rows. MX169 was planted surrounding the nursery as an inoculum spreader to produce an epidemic environment. Eight races of *Pst* (CY29, CY31, CY32, CY33, CY34, Shuiyuan4, Shuiyuan5, and Shuiyuan7) were used in a mixture of equal quantity for inoculating plants at the wheat elongation stage. Stripe rust reaction were rated for infection type and recorded based on the 0 to 9 scale for signs and symptoms (Line and Qayoum 1992), with 1 being very resistant and 9 very susceptible. MX169 and Z1141 were used as the susceptible and resistant controls, respectively.

F₁ plants and DH lines originating from the cross CH4131/CH4133 were assessed at the seedling stage using races CY32 and CY34 in the greenhouse at the College of Plant Protection, Northwest A&F University, Yangling, Shaanxi province, in 2017 and 2018. Seedlings at the two-leaf stage were inoculated using urediniospores of each race mixed with talc (approximately 1:20). Plants were kept in the dark at 90% humidity and 10°C for 24 h, and then, they were transferred into a greenhouse at 17 ± 2°C with a 14-h photoperiod (22,000 lx) (Wu et al. 2018b). Stripe rust reactions were recorded 20 to 22 days after inoculation using the 0 to 9 scale as described above.

Cytogenetic analysis. Slide preparations were conducted according to Kato (1999) with some modifications (Huang et al. 2018). Total genomic DNA from *Th. intermedium* and *Secale cereale* was labeled with Texas red-5-dCTP by the nick translation method and used as probes. The detailed procedures of genomic in situ hybridization (GISH) were described by Liu et al. (2011a, b). Eight single-strand oligonucleotides were used to detect the wheat chromosomes, including TAMRA (6-carboxytetramethylrhodamine)-modified AFA-3, AFA-4, pAs1-1, pAs1-3, pAs1-4, and pAs1-6 and FAM (6-carboxyfluorescein)-modified oligonucleotides pSc119.2-1 and (GAA)₁₀. The general procedure of fluorescence in situ hybridization (FISH) was as described by Huang et al. (2018). Images were captured with an Olympus BX-60 fluorescence microscope (Olympus) equipped with a SPOT CCD (Olympus DP72) camera.

Amplified fragment-length polymorphism analysis. DNA was isolated from young leaves using an SDS (sodium dodecyl sulfate) protocol (Yang et al. 2006b). The amplified fragment-length polymorphism (AFLP) procedure was performed according to the protocol of Dhariwal et al. (2015). The restriction reaction contained 5 U of *Eco*RI and 5 U of *Mse*I in a final volume of 15 µl. The adaptors, preselective primers, and selective primers are listed in Table 1. The methods for cloning and sequencing relative polymorphic bands used were according to Sardesai et al. (2002).

PCR-based landmark unique gene PCR, intron targeting PCR, and ω-sec PCR. PCR-based landmark unique gene (PLUG) and other EST (expressed sequence tags)-based molecular markers proved to be powerful tools in identifying the chromosome homologous group of alien species in wheat background (Gong et al. 2017; Liu et al. 2015, 2019). Therefore, in this study, a total of 526/841 PLUG/intron targeting (IT) primer pairs were synthesized according to Ishikawa et al. (2007, 2009) and Zhang et al. (2017a, b), respectively, to identify wheat–*Th. intermedium* cross progenies. Among these PLUG/IT primer pairs, 57/135, 67/175, 85/120, 71/89, 78/140, 59/71, and 109/111 pairs belonged to chromosome homologous groups 1 to 7, respectively. PLUG and IT primer pairs were

Table 1. Sequences of amplified fragment-length polymorphism adaptors and primers used in this research

	Sequence (5'-3')
Adaptors ^a	
<i>Eco</i> R I-Forward	CTCGTAGACTGCGTACC
<i>Eco</i> R I-Reverse	AATTGGTACGCACTCTAC
<i>Mse</i> I-Forward	GACGATGAGTCTCTGAG
<i>Mse</i> I-Reverse	TACTCAGGACTCAT
Preselective primers	
<i>Eco</i> R I-A	GACTGCGTACCAATTCA
<i>Mse</i> I-C	GACGATGAGTCTCTGAGTAAC
Selective primers	
<i>Eco</i> R I-ACT+ <i>Mse</i> I-CAG	GAC TGC GTA CCA ATT CA
<i>Eco</i> R I-ACT+ <i>Mse</i> I-CAA	GAT GAG TCC TGA GTA AC

Fig. 1. Stripe rust responses of wheat lines A, CH4131, B, CH4132, C, CH4133, and D, CH4134 at adult plant stage.

synthesized by Chengdu Ruixin Biological Technology Co., Ltd. and Qingdao Qingke Biological Co., Ltd., and PCR protocol followed that according to Ishikawa et al. (2007, 2009) and Zhang et al. (2017 a, b). To obtain high levels of polymorphism, the PLUG PCR products were digested with the four-base cutter enzymes *Hae*III and *Taq*I according to Ishikawa et al. (2007, 2009). Moreover, a pair of primers ω -sec-P1 and ω -sec-P2 for detecting rye 1RS chromosome in a wheat background was synthesized according to Chai et al. (2006). All PCR products were separated on 2% agarose gels.

Table 2. Stripe rust infection types produced in the adult plant-stage material tested in 2015

Cultivar	Infection type									
	0	1	2	3	4	5	6	7	8	9
Z1141	16	4	—	—	—	—	—	—	—	—
Chinese Spring	—	—	—	—	—	—	—	—	—	20
Taichung29	—	—	—	—	—	—	—	—	—	20
Shan 225	—	—	—	—	—	—	—	—	—	20
CH4131	—	—	20	—	—	—	—	—	—	—
CH4132	—	—	20	—	—	—	—	—	—	—
CH4133	—	—	—	—	—	—	—	—	—	20
CH4134	—	—	—	—	—	—	—	—	—	20
PH82-2-2	—	—	—	—	—	—	20	—	—	—
Mingxian169	—	—	—	—	—	—	—	—	—	20

Agronomic traits assessment. The four translocation lines were assessed for agronomic performance in the field at Taiyuan and Linfen, Shanxi province, in 2015 and 2016. The experimental design consisted of three biological replicates arranged in a randomized block, with all plots and sites following the same standard cultivation practices and grown under irrigated conditions. The lines were sown in large-scale yield plots (1.2×6 m), and they were sown by grain number for comparable plant densities per plot ($345 \text{ seeds meter}^{-2}$). Ten randomly selected plants in each plot were used to measure winter hardness, heading date, maturity date, spike length, spikelet number per spike, plant height, grain number per spike, effective tiller number, and 1,000-kernel weight (TKW). The harvested grain was used in quality analysis.

Quality analysis. Quality analysis was performed in the four translocation lines CH4131 to CH4134 and control LX99. The grains were milled using wheat grinding machine 3100 (Perten). Protein content was measured using a DA7200 diode array near-infrared spectrometer (Perten). Wet gluten content was analyzed using a Glutomatic 2200 (Perten) according to the American Association of Cereal Chemists International (2000). A farinograph (Brabender) was used to examine the dough stability time and formation time, and an extensograph (Brabender) was used to examine the maximum resistance to drag and stretch area. The seed high-molecular weight glutenin subunits (HMW-GSs) were extracted and examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis according to the method described by Zhao et al. (2018).

Fig. 2. Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) analysis on metaphase chromosomes of **A** to **C**, CH4131, **D**, cultivar Shan 225, and **E**, PH82-2-2. **A**, GISH using genomic DNA of *Thinopyrum intermedium* as probe and genomic DNA of Chinese Spring as block. **B**, FISH using oligonucleotide multiplex probe. Green signals show pSc119.2-1 and (GAA)₁₀ modified with 6-carboxyfluorescein, and red signals show oligonucleotides pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, and AFA-4 modified with 6-carboxytetramethylrhodamine. **C**, GISH using genomic DNA of rye as probe. **D**, GISH of Shan 225 using genomic DNA of *Th. intermedium* as probe. **E**, GISH of PH82-2-2 using genomic DNA of *Th. intermedium* as probe. Scale bar: 10 μ m.

Results

Reaction to stripe rust inoculation in the four translocation lines. Z1141 showed an immune reaction (IT 0) on 16 plants and high resistance (IT 1) on 4 plants. CH4131 and CH4132 showed high resistance with IT 2, whereas CH4133 and CH4134 showed high susceptibility with IT 9 (Fig. 1). The four wheat lines used to develop CH4131 to CH4132 were either moderately susceptible (IT 6) for PH82-2-2 or highly susceptible (IT 9) for CS, TC29, and Shan 225 (Table 2). These results showed that CH4131 and CH4132 had stripe rust resistance and indicated that the resistance most likely was from the *Th. intermedium* line Z1141.

The chromosome composition of wheat-*Th. intermedium* introgressions. Chromosome counting indicated that the total number of chromosomes of CH4131 to CH4134 was 42. Using genomic DNA of *Th. intermedium* as the probe and genomic DNA of CS as the block, GISH was performed on the metaphase chromosomes of CH4131 to CH4134. As a result, a pair of small chromosome segment translocations was detected in both CH4131 (Fig. 2A) and CH4132 with the same signals, whereas no GISH signal was detected in susceptible lines CH4133 and CH4134.

To identify the translocated wheat chromosomes, after washing off the GISH signals, FISH was subsequently performed using the oligonucleotide multiplex probe on the metaphase chromosomes of the same cell as the one used for GISH. As a result, the translocated wheat chromosomes in CH4131 (Fig. 2B) and CH4132 (FISH signals were the same as in CH4131) were 1B. GISH using the DNA from *S. cereale* (Fig. 2C) showed no signals; meanwhile, rye 1RS-specific marker ω -sec-P1/ ω -sec-P2 did not amplify the 1,100-base pair band in CH4131 to CH4134 (Supplementary Fig. S1), indicating that they were not 1BL/1RS translocation lines.

Among the parents of CH4131 to CH4134, CS and TC29 are wheat cultivars, whereas PH82-2-2 and Shan 225 are wheat-*Thinopyrum ponticum* derivatives. To clearly identify the origin of translocated alien chromosome segments, GISH was also performed on the metaphase chromosomes of PH82-2-2 and Shan 225; however, no GISH signals were detected (Fig. 2D and E), indicating that the translocated alien chromosome segments are from Z1141.

Markers for precisely identifying wheat-*Th. intermedium* introgressions. In wheat-*Th. intermedium* introgressions lines CH4131 and CH4132, chromosomes 1B of wheat were involved in the chromosome translocation (Fig. 2A and B). However, the homologous group of *Th. intermedium* chromosome in the translocation remains unknown. To elucidate this, AFLP was conducted on the material tested to gain specific sequences of *Th. intermedium* in the introgression lines. As a result, six polymorphic bands were obtained (Supplementary Fig. S2). All of these six polymorphic bands were cloned and sequenced. The sequence lengths of 258, 201, 242, 224, and 187 base pairs were obtained from primer combinations A- to E (Table 3 and Supplementary Text S1). BlastN searches revealed that three sequences (A, D, and E) were highly homologous to sequence fragments of *T. aestivum* chromosome 3B, whereas sequence C was highly homologous to *Aegilops speltoides* mitochondrial DNA, and no sequence was found homologous to sequence B. Within these five cloned sequences, three sequences belonged to chromosome homologous group 3.

To further identify the homologous determination of the translocation segments of *Th. intermedium*, PLUG and IT primer pairs that belonged to the chromosome homologous groups 1 to 7 were used to amplify the genomic DNA of *Th. bessarabicum*, *Th. elongatum*, *P. strigosa*, *Th. intermedium*, CS, TC29, PH82-2-2, Shan 225, and CH4131 to CH4134. As a result, no polymorphic marker was generated by PLUG primer pairs. However, four IT primer pairs (CINAU1231, CINAU1233, CINAU1204, and CINAU1171) could generate additional polymorphism DNA bands in lengths of 480, 520, 100, and 550 base pairs in *Th. elongatum*, *Th. intermedium*, CH4131, and CH4132 (Fig. 3A to C), respectively. All of these four markers were derived from the chromosome homologous group 3 of *Dasypyrum villosum*. Therefore, the *Th. intermedium* chromosome segments in CH4131 and CH4132 belong to homologous group 3, which was consistent with the AFLP marker result.

Based on the cloned AFLP sequence of B, a pair of sequence-characterized amplified region (SCAR) primers D05-A4-1F (5'-CCGATGGAGGACTTTGTAG-3') and D05-A4-1R (5'-GCTTACTTGTGTGGACCTTG-3') was designed to develop SCAR markers. PCR using this pair of primers was performed on the material tested; the result showed that a polymorphic band (D05-A4-1), 163 base pairs in length, was generated in Z1141, CH4131, and CH4132, but no amplicon was detected in the other material tested (Fig. 3D), indicating that D05-A4-1 together with the four IT polymorphic bands could be used as molecular marker for tracing the *Th. intermedium* chromatin in wheat breeding programs.

PCR using the SCAR and IT primer pairs on *Th. bessarabicum*, *Th. elongatum*, *P. strigosa*, *Th. intermedium*, CS, TC29, PH82-2-2, Shan 225, and CH4131 to CH4134 showed that only *Th. elongatum*, *Th. intermedium*, CH4131, and CH4132 had the targeted segments (Fig. 3), indicating that the chromosome segments of *Th. intermedium* in CH4131 and CH4132 belonged to chromosome homologous group 3. Because the *Th. elongatum* used in this experiment was only from one population, the chromosome configurations of the two translocation lines were named as T3Ai-1BS.1BL instead of T3E^a-1BS.1BL.

Verification of the source of stripe rust resistance using the SCAR marker. Stripe rust responses were evaluated on seedlings of

Fig. 3. PCR patterns of infection-type markers **A**, CINAU1231; **B**, CINAU1171; and **C**, CINAU1233 and **D**, sequence-characterized amplified region marker D05-A4-1F/1R. Arrows show the polymorphism DNA bands. M, marker; 1, *Thinopyrum bessarabicum*; 2, *Thinopyrum elongatum*; 3, *Pseudoroegneria strigosa*; 4, *Thinopyrum intermedium*; 5, Chinese Spring; 6, Taichung29; 7, PH82-2-2; 8, cultivar Shan 225; 9, CH4131; 10, CH4132; 11, CH4133; 12, CH4134; 13, ddH₂O.

Table 3. Sequencing results of amplified fragment-length polymorphism special bands

No.	Primer combination name	Length (base pairs)	Homologous sequence	E value
A	<i>EcoRI</i> -ACT + <i>MseI</i> -CAG-258	258	<i>Triticum aestivum</i> chromosome 3B (FN564426.1)	5e-21
B	<i>EcoRI</i> -ACT + <i>MseI</i> -CAG-201	201	None	–
C	<i>EcoRI</i> -ACT + <i>MseI</i> -CAA-242	242	<i>Aegilops speltoides</i> mitochondrial DNA (AP013107.1)	4e-110
D	<i>EcoRI</i> -ACT + <i>MseI</i> -CAA-224	224	<i>Triticum aestivum</i> chromosome 3B (FN645450.1)	9e-61
E	<i>EcoRI</i> -ACT + <i>MseI</i> -CAA-187	187	<i>Triticum aestivum</i> chromosome 3B (FN564428.1)	2e-54
F	<i>EcoRI</i> -ACT + <i>MseI</i> -CAA-NONE	None	–	–

F₁ and the DH population originating from the cross CH4131/CH4133. CH4131 showed broad spectrum resistance to eight stripe rust races, and therefore, a mixture of prevalent races (CY32 and CY34) was utilized to inoculate the F₁ plants and the DH lines. All F₁ plants and 44 DH lines were highly resistant to CY32 and CY34 (IT 0), whereas 41 DH lines were highly susceptible (IT 9) (Fig. 4). The seedling assay of the DH population segregated in a 1:1 ratio (Supplementary Table S1), and F₁ plants were resistant, indicating that a single dominant gene was involved in the CH4131 resistance.

Fig. 4. Stripe rust responses of doubled haploid lines inoculated with races CY32 and CY34 of *Puccinia striiformis* f. sp. *tritici*. Doubled haploid lines that are highly resistant to **A**, CY32 and **D**, CY34. Doubled haploid lines that are highly susceptible to **B**, CY32 and **E**, CY34. Cultivar (Mingxian169) is highly susceptible to **C**, CY32 and **F**, CY34.

Genotyping the DH population with the SCAR marker showed that stripe rust resistance was closely linked with the SCAR marker, because all resistant lines had the SCAR amplification, whereas the susceptible lines had no amplification (Supplementary Fig. S3), confirming that the stripe rust resistance was from the translocated *Th. intermedium* 3Ai.

Agronomic performance of the translocation lines. Both CH4131 and CH4132 possess the stripe rust resistance gene, suggesting that they were an excellent gene source for wheat improvement. However, it remains unknown whether they have any genetic drag for undesirable agronomic traits. To evaluate these lines, agronomic traits investigation and quality analysis were performed. Based on the field data, CH4131 to CH4134 are facultative winter wheat. Leaves are dark green. There were no significant differences among CH4131 to CH4134 in spike length, spikelet number, and effective tiller number, but there were differences in the TKW value, plant height, and maturity (Fig. 5A and Supplementary Table S2). The translocation lines were later and taller, and they had smaller TKW than the non-translocation lines (Fig. 5B).

Quality analysis. Quality analysis suggested that there were no significant differences in protein content, sedimentation volume, wet gluten content, dough stability time, formation time, and maximum resistance to drag and stretch area among CH4131 to CH4134. The quality indexes of the four lines were higher than middle-gluten wheat variety LX99 (Table 4). HMW-GS analysis showed that the components of subunits *Glu-A1*, *Glu-B1*, and *Glu-D1* of these four lines were null: 14 + 15 and 2 + 12 (Fig. 6). Therefore, CH4131 to CH4134 are well suitable for making noodles and steamed bread.

Discussion

Thinopyrum species are important gene sources for wheat improvement. *Th. intermedium* can be crossed with wheat easily, and the derived wheat-*Th. intermedium* introgression lines have played an important role in both wheat chromosome engineering research and wheat breeding programs. So far, different research groups have developed disease-resistant wheat-*Thionopyrum* amphiploids or partial amphiploids, such as Zhong1 to Zhong5 (Han et al. 2004; Tian et al. 2000), Otrastsjuskaya (Fedak et al. 2000), TE-3 (Yang et al. 2006a), TAI8335 (Chang et al. 2010), TE253 (Bao et al. 2009), and

Fig. 5. Morphologies of **A**, plants and **B**, grain width of CH4131 to CH4134.

Table 4. Quality traits of the material tested

Cultivar	Protein content (%)	Sedimentation volume (ml)	Wet gluten content (%)	Dough stability time (min)	Formation time (min)	Maximum resistance to drag (EU)	Stretch area (cm ²)
CH4131	14.36 ± 1.42 a ^z	29.51 ± 1.34 ab	32.88 ± 2.76 a	6.7 ± 0.45 a	3.7 ± 0.12 a	212 ± 15.44 a	47 ± 3.93 a
CH4132	14.87 ± 1.58 a	32.84 ± 3.21 a	33.01 ± 3.23 a	7.9 ± 0.49 a	3.6 ± 0.33 a	220 ± 14.23 a	48 ± 2.65 a
CH4133	14.49 ± 1.16 a	31.67 ± 3.09 a	32.86 ± 1.98 a	7.7 ± 0.56 a	3.6 ± 0.24 a	214 ± 18.87 a	47 ± 2.15 a
CH4134	14.71 ± 1.25 a	32.05 ± 2.78 a	33.44 ± 3.02 a	7.3 ± 0.77 a	3.5 ± 0.28 a	209 ± 17.34 ab	46 ± 3.72 a
Liangxing 99	13.32 ± 0.84 b	26.81 ± 1.26 b	30.61 ± 1.82 b	2.1 ± 0.44 b	2.7 ± 0.23 b	193 ± 15.27 b	42 ± 3.72 b

^z Different letters are used to indicate means that differ significantly ($P < 0.05$, least significant difference test).

CH13-21 (Zhan et al. 2015). Using these amphiploids/partial amphiploids as a bridge, Wan et al. (1999) created seven wheat–*Th. intermedium* addition lines and concluded that at least two pairs of St chromosomes of *Th. intermedium* possess stripe rust resistance genes. Later, Li et al. (2006) transferred an unnamed stripe rust resistance gene from chromosome St of *Th. intermedium* ssp. *trichophorum* to common wheat. Subsequently, Liu et al. (2007) reported that they transferred stripe rust resistance gene *YrSt* from chromosome St of *Th. ponticum* to a cultivated wheat variety. Hao et al. (2012) identified a stripe rust-resistant wheat–*Th. ponticum* 4D/4St translocation line and speculated that the resistance was from 4St of *Th. ponticum*. Luo et al. (2009) transferred resistance gene *YrYU25* from *Th. intermedium* to hexaploid wheat. Hu et al. (2011) transferred the resistance from chromosome 6J⁸ of *Th. ponticum* to wheat and created a 6J⁸(6B) substitution line. Liu et al. (2013a, b) mapped a putative *Th. intermedium*-derived stripe rust resistance gene *Yr50* on wheat chromosome arm 4BL. Hou et al. (2016) mapped *Yr69* on 2AS of a stripe rust-resistant wheat–*Th. ponticum* cryptic introgression. However, none of the alien resistance genes mentioned above were from chromosome homologous 3. Ma et al. (1999) mapped a primary designated gene *YrE* on chromosome 3E⁶ of *Th. elongatum*. Nevertheless, *YrE* does not exist in the form of a wheat–*Th. elongatum* chromosome translocation; therefore, it is not available for breeding. In this study, we identified two stripe rust-resistant wheat–*Th. intermedium* T3Ai-1BS.1BL translocations CH4131 and CH4132. Both translocation lines are an excellent gene source for wheat breeding. The stripe rust resistance gene described in this study is different from *Yr50* (4BL) and *Yr69* (2AS) and might be the same gene or an allele of the temporarily named *YrE*. Therefore, this gene is clearly different from permanently named *Yr* genes and is designated as *YrCH-1BS*.

Germplasm innovation based on chromosome engineering has always played an important role in discovering exogenous excellent genes. It can be applied to rapid, accurate, and consistent improvement in breeding high-yielding and high-quality wheat cultivars that have resistance to multiple diseases. There are several hundred disease resistance genes that have been designated. Some of these genes that have come from wild relatives of wheat were highly valued by wheat breeders and pathologists (Liu et al. 2012). However, not all of the resistance genes from wild wheat species could be used widely in breeding programs because of the linkage of genes for undesirable traits. In this study, we comprehensively evaluated the agronomic traits and quality of the stripe rust-resistant wheat–*Th. intermedium* lines with the T3Ai-1BS.1BL translocation. The translocated

chromosome fragments have no obvious linkage drag to agronomic and grain quality performance. Therefore, they could be widely used in wheat improvement. The SCAR and IT markers, which were cosegregating with *YrCH-1BS*, may be useful in stripe rust improvement.

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Fig. 6. High-molecular weight glutenin subunit analysis of the material tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis. 1, Zhongyou 9507; 2, CH4131; 3, CH4132; 4, CH4133; 5, CH4134; 6, PH82-2-2; 7, Taichung29; 8, cultivar Shan 225.

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