Identification of an Elite Wheat-Rye T1RS·1BL Translocation Line Conferring High Resistance to Powdery Mildew and Stripe Rust

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Abstract

Wheat-rye T1RS·1BL translocations have been widely used worldwide in wheat production for multiple disease resistance and superior yield traits. However, many T1RS·1BL translocations have successively lost their resistance to pathogens due to the coevolution of pathogen virulence with host resistance. Because of the extensive variation in rye (*Secale cereale* L.) as a naturally cross-pollinating relative of wheat, it still has promise to widen the variation of 1RS and to fully realize its application value in wheat improvement. In the present study, the wheat-rye breeding line R2207 was characterized by comprehensive analyses using genomic in situ hybridization (GISH), multicolor fluorescence in situ hybridization with multiple probes, multicolor GISH, and molecular marker analysis, and then was proven to be a cytogenetically

stable wheat-rye T1RS·1BL translocation line. Based on the disease responses to different isolates of powdery mildew and genetic analysis, R2207 appears to possess a novel variation for resistance, which was confirmed to be located on the rye chromosome arm 1RS. Line R2207 also exhibited high levels of resistance to stripe rust at both seedling and adult stages, as well as enhanced agronomic performance, so it has been transferred into a large number of commercial cultivars using an efficient 1RS-specific kompetitive allele specific PCR marker for marker-assisted selection.

Keywords: powdery mildew, stripe rust, *Secale cereale*, *Triticum aestivum*, T1RS·1BL translocation, MAS, KASP

Powdery mildew and stripe rust, caused by *Blumeria graminis* (DC.) E.O. f. sp. *tritici* (Bgt) and *Puccinia striiformis* Westend. f. sp. *tritici* (Pst), respectively, are two devastating diseases of wheat (*Triticum aestivum* L.), significantly reducing grain yield and quality worldwide (Chen 2014; Dean et al. 2012). Host resistance is considered to be the most efficient, cost-effective, and environmentally friendly measure to control these diseases (Chen 2013; Ma et al. 2020). Up to now, more than 80 formally designated powdery mildew (*Pm*) resistance genes and alleles (*Pm1* to *Pm66*) and more than 80 formally designated stripe rust (*Yr*) resistance genes (*Yr1* to *Yr83*) have been identified, but few of them have actually been applied in large-scale commercial production (Li et al. 2020a, 2020b; McIntosh et al. 2019; Qie et al. 2019; Tan et al. 2018). Due to the coevolution of pathogen virulence with host resistance, many race-specific genes have been quickly overcome by the new virulent pathotypes, particularly in areas where resistance genes were widely used (An et al. 2019). For instance, *Pm3a*, *Pm8*, and *Pm17* have been reported to have lost their resistance in the southeastern soft red winter wheat region in the United States (Cowger et al. 2009, 2018; Parks et al. 2008); *Yr10* and *Yr26*, which were effective against all previously

identified *Pst* races, have been defeated by the new *Pst* race CYR34/V26 (Qi et al. 2016). Therefore, exploring new gene resources for powdery mildew and stripe rust resistance is urgently needed for improvement of wheat.

For a long time, conventional interbreeding between wheat cultivars and large-scale use of a few limited resistance genes has greatly narrowed the genetic basis of wheat resistance, resulting in a bottleneck in wheat improvement (Gupta et al. 2010). Wheat relatives have significant genetic diversity and abundant valuable genes; therefore, they play an important role in wheat improvement (Mujeeb-Kazi et al. 2013). A successful example of the introgression of elite alien genes into common wheat was the utilization of chromosome arm 1RS of rye (*Secale cereale* L., 2n = 2x = 14, RR) (Lukaszewski 1990; Tester and Langridge 2010). That translocation derived from the rye cultivar Petkus carried valuable resistance genes, including *Pm8* against powdery mildew, *Yr9* against stripe rust, *Lr26* against leaf rust, and *Sr31* against stem rust (Mago et al. 2005; McIntosh et al. 2011), as well as genes associated with superior agronomic traits and abiotic-stress tolerance (Howell et al. 2014, 2019; Kumlay et al. 2003; Shearman et al. 2005). For those reasons, the wheat-rye T1RS·1BL translocations have been used worldwide in breeding programs. Approximately 1,050 wheat cultivars that carry T1RS·1BL translocation inherit the chromosome arm 1RS of the rye cultivar Petkus (Schneider et al. 2016), which indicates that few resources in the rye species have served as the 1RS ancestors in current wheat cultivars (Kim et al. 2004). Owing to the narrow genetic basis and their long-term use in production, the resistance genes *Pm8*, *Yr9*, and *Lr26* have successively lost their resistance to new virulent pathotypes of diseases since the 1990s (Lutz et al. 1992), and later virulence for *Sr31* was reported (Pretorius et al. 2000, 2010). Despite the breakdown of resistance, the T1RS·1BL translocations have continued to be widely utilized in wheat breeding because of their yield potential and wide environmental adaptability (Ren et al. 2017; Wang et al. 2017). In addition, rye is a cross-pollinating crop, so it

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contains significant genetic heterogeneity within and among cultivars (Targońska et al. 2016). Abundance of genetic variations in IRS, such as *Sr50* from the rye cultivar Imperial and *SrIRS^{Amigo}* from the rye cultivar Insave, still provide high resistance to the highly virulent wheat stem rust race Ug99, whereas the highly resistant gene *Sr31* has been overcome by Ug99 (Mago et al. 2015; Rahmatov et al. 2016). Therefore, it is still important to unceasingly widen the variation of IRS to achieve the full potential of IRS in wheat improvement.

Once the alien chromatin was integrated into the wheat genome, genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) were widely used as effective detection methods to identify and analyze the chromosome composition due to their ease of use and accuracy (An et al. 2006, 2013). Meanwhile, molecular markers specific for alien chromosomes also have been powerful for detecting alien chromatin in a wheat background (Hechanova et al. 2018).

Combined with GISH/FISH, efficient alien-chromosome-specific markers have been extensively utilized to accelerate the process and improve the outcome of wheat-alien breeding programs (Edet et al. 2018). With the increased alien segments introduced into common wheat, it is critical to use tightly linked, high-resolution, and stable alien-specific markers to rapidly and efficiently detect the targeted progenies, especially the competitive allele specific PCR (KASP) markers, which have been widely used in gene mapping, and marker-assisted selection (MAS), due to their advantages of high throughput, high flexibility, desirable genetic stability, and locus specificity (Rasheed et al. 2016; Semagn et al. 2014; Wu et al. 2017).

A wheat-rye breeding line, R2207, exhibited high resistance to diseases and elite agronomic traits in wheat-growing regions for consecutive years. The objectives of this study were to (i) analyze the chromosome composition of R2207 using molecular cytogenetic methods; (ii) assess its resistance to powdery mildew and stripe rust isolates and analyze the inheritance of its resistance gene(s); (iii) survey the agronomic traits for breeding availability evaluation; and (iv) transfer R2207 into a large number of commercial cultivars using a IRS-specific KASP marker for MAS breeding.

Materials and Methods

Plant materials. Line R2207 is a wheat-rye progeny of unknown pedigree. For consecutive years, it showed high resistance to powdery mildew and stripe rust in the field. Wheat cultivar Mingxian169, which does not have any documented *Pm* or *Yr* genes, was used as the susceptible control in the disease resistance tests for powdery mildew and stripe rust. Thirty-eight wheat genotypes carrying documented *Pm* gene(s) were used as controls for comparison with the powdery mildew resistance of R2207. The susceptible wheat cultivar Shixin733 was crossed with R2207 to generate F₂ progenies to determine the inheritance of the resistance of powdery mildew.

Total genomic DNA of Chinese Spring (CS) was used as a blocking DNA in GISH detection. Total genomic DNA of the rye cultivar Imperial, *Triticum urartu* (2n = 2x = 14, AA), *Aegilops speltoides* (2n = 2x = 14, BB), and *A. tauschii* (2n = 2x = 14, DD) were used in multicolor GISH (mc-GISH) detection. For molecular marker analysis of R2207, CS, rye cultivar Imperial, the complete set of disomic addition lines of CS-Imperial, IRS and 1RL ditelosomic addition lines of CS-Imperial, two triticales lines (10R2-193-2 and 10R2-194-2 [AABBRR]), and two T1BL·IRS wheat-rye translocation lines (Lovrin10 and Lovrin13) (Rabinovich 1998) were used as controls. All the seedlings were used for extracting total genomic DNA by the CTAB method (Gill et al. 1991).

Three wheat cultivars with the T1RS·1BL translocation, including Shimai18, Ji5265, and Heng4444, and three wheat cultivars without the T1RS·1BL translocation, including Shixin633, Shixin733, and Shixin828, were compared with R2207 to evaluate its agronomic traits in the field from 2015 to 2017.

To transfer the valuable genes into commercial cultivars, R2207 was crossed with 12 cultivars including Shixin633, Shixin733, Shixin828, Gao8901, Shiyou17, Shixin5071, Lumai13, Liangxing99, Jimai20, Jimai22, Tainong19, and Shannong17. CS and the rye

cultivar Imperial were used as controls for genotyping of the IRS-specific KASP markers.

Sequential GISH and multicolor FISH (mc-FISH) analyses. GISH and mc-FISH analyses were conducted to determine the chromosomal composition of the wheat-rye line R2207. For GISH analysis, the root tip cells were prepared for mitotic chromosome observation and hybridization as previously described (Han et al. 2009). Total genomic DNA of the rye cultivar Imperial was labeled with fluorescein-12-dUTP (green) by the nick translation method and used as a probe, whereas genomic DNA of CS was used as block with a 1:50 ratio of probe to blocker.

After GISH analysis, two highly repetitive sequence clones, pAs1 (red) labeled with digoxigenin-11-dUTP and pSc119.2 (green) labeled with biotin-11-dUTP, respectively, were used as probes for mc-FISH (Zheng et al. 2014). Nondenaturing FISH (ND-FISH) analysis also was performed to determine the identity of the rye and wheat chromosomes after GISH analysis. The combination of the oligonucleotide probes Oligo-pSc119.2-2 and Oligo-pTa535-2 could distinguish all the 42 chromosomes of wheat (Tang et al. 2014). The probe Oligo-pSc119.2-2 (green) was 5' end-labeled with 6-carboxyfluorescein, and the probe Oligo-pTa535-2 (red) was 5' end-labeled with 6-carboxytetramethylrhodamine. These oligonucleotide probes were synthesized by Shanghai Invitrogen Biotechnology Co. (Shanghai, China). ND-FISH analysis was carried out according to the method of Tang et al. (2014).

All slides were counterstained with 4,6-diamidino-2-phenylindole after GISH or FISH. Then, detection and visualization of rye chromatin through an epifluorescence Olympus BX53 with a cooled charge-coupled device digital camera were performed. Program CellSens Standard 1.12 (Olympus Corporation, Tokyo, Japan) was used in image analysis.

mc-GISH analysis. Total genomic DNA of the rye cultivar Imperial and *T. urartu* labeled with fluorescein-12-dUTP, total genomic DNA of *A. tauschii* labeled with Texas-red-5-dUTP, and total genomic DNA of *A. speltoides* conducted as blocking (Fu et al. 2012) were used in combination for mc-GISH to determine the genomic identity of R2207. Image capture and analysis were performed as described above.

Molecular marker analysis. The IRS-specific marker O-SEC5'-A/O-SEC3'-R (F, CTATTAGTTCGAAAAGCTTATGA; R, GCA TATGACTCAAATTATTTTTT) was used to detect IRS chromatin (Shimizu et al. 1997). The marker O11B3/O11B5 (F, GGTACC AACAACAACAACCC; R, GTTGCTGCTGAGGTTGGTTC) was used as a diagnostic marker to detect the 1BS chromosome arm for the *Glu-B3* gene (Van Campenhout et al. 1995). In molecular marker analysis, three offspring plants of R2207 from three consecutive generations were involved to confirm its genetic stability. CS, rye cultivar Imperial, the complete set of disomic addition lines of CS-Imperial, and IRS and 1RL ditelosomic addition lines of CS-Imperial were used as controls to ensure the specificity of the IRS-specific marker O-SEC5'-A/O-SEC3'-R. Lovrin10 and Lovrin13 derived from rye cultivar Petkus (Rabinovich 1998) were used as controls because they are well-known T1RS·1BL translocation lines. Two triticales lines (10R2-193-2 and 10R2-194-2) of unknown origin of rye were also used as controls to indicate the universality of the marker O-SEC5'-A/O-SEC3'-R in different rye resources.

The PCR amplification system (10 µl total) contains 1 µl of template DNA (50 to 150 ng/µl), 4 µl of 2× Taq Master Mix (Vazyme Biological, Nanjing, China), 4 µl of ddH₂O, and 1 µl of primers (10 µmol). The PCR procedure was as follows: 94°C for 5 min, followed by 36 cycles of 94°C for 30 s, 51°C (O-SEC5'-A/O-SEC3'-R) or 60°C (O11B3/O11B5) for 30 s, and 72°C for 40 s, and a final extension at 72°C for 10 min. The PCR products were separated in 2% agarose gel in 1× TAE buffer.

Assessment of resistance to powdery mildew and stripe rust. Line R2207 was tested for seedling resistance to powdery mildew with 23 single-pustule-derived *Bgt* isolates by separate inoculations in a greenhouse, along with 38 wheat genotypes carrying known *Pm* gene(s) as controls (Table 1). Twenty seeds of each line were planted in rectangular trays (54 × 28 × 4 cm) with 128 wells

(3 × 3 × 4 cm), which were placed in the incubator, which was set at 20 ± 0.5°C with a daily photoperiod of 14 h. The susceptible control Mingxian169 also was randomly planted in each tray. At the one-leaf stage, all seedlings were separately inoculated with fresh spores of single *Bgt* isolates that were increased on Mingxian169 seedlings and were incubated in darkness at 18 ± 0.5°C with a relative humidity >80% for 24 h. The trays were subsequently transferred into a greenhouse with a diurnal cycle at 22 ± 2°C for 14 h of light and 18 ± 2°C for 10 h of darkness (Qie et al. 2019). When spore formation was observed on the first leaf of Mingxian169, the infection types (ITs) of powdery mildew at the seedling stage were scored using a 0 to 4 scale as described by Si et al. (1992), where 0 to 2 were considered resistant and 3 to 4 susceptible. To assess the resistance at the adult stage, R2207 and Mingxian169 were planted and inoculated with mixed *Bgt* isolates collected from northern China from 2014 to 2017 using three replicates at Luancheng Agro-Ecological Experimental Station, Chinese Academy of Sciences, Shijiazhuang, China. For each replicate, line R2207 and Mingxian169 were planted in two rows with 20 seeds per row. After the wheat reached full heading stage (Zadoks et al. 1974), the powdery mildew reactions for all the plants of R2207 and Mingxian169 were scored using a 0 to 9 scale, where 0 to 4 were considered resistant and 5 to 9 susceptible (Sheng and Duan 1991). In addition, a prevalent *Bgt* isolate, E09 (Li et al. 2011), was selected to test the reactions of seedlings of R2207, Shixin733, and their F₂ plants for the genetic analysis of the resistance. After evaluating the seedling reactions, these plants

were transplanted into the field to test their adult-plant reactions, respectively.

Four *Pst* races (CYR31, CYR32, CYR33, and CYR34), which are prevalent and considered as highly pathogenic in China (Chen et al. 2009, 2014; Gebreslasie et al. 2020; Han et al. 2015; Wan et al. 2004), were used to assess the response of R2207 to stripe rust at the seedling stage in another greenhouse. The environmental conditions and methods of disease inoculation were previously described in Xu et al. (2014). Wheat cultivar Mingxian169 was used as a susceptible control. The IT was recorded based on a 0 to 4 scale in which 0 to 2 were considered resistant and 3 to 4 susceptible (Volin and Sharp 1973). At the adult stage, a mixture of the *Pst* races CYR31, CYR32, and CYR33 was used for artificial inoculation from 2014 to 2017 for R2207 and susceptible control Mingxian169 (Wang et al. 2009) at Luancheng Agro-Ecological Experimental Station, Chinese Academy of Sciences, Shijiazhuang, China. The field trials were conducted the same as that for assessment of adult-plant resistance to powdery mildew. The IT of stripe rust at the adult stage was scored based on the 0 to 9 scale as described by Wan et al. (2004).

Evaluation of agronomic traits. The wheat-rye line R2207 and six winter wheat cultivars including Shimai18, Ji5265, Heng4444, Shixin633, Shixin733, and Shixin828 were planted at the Luancheng Agro-Ecological Experimental Station, Chinese Academy of Sciences, Shijiazhuang, China. Shimai18, Ji5265, and Heng4444 contain T1RS-1BL translocations, whereas Shixin633, Shixin733, and Shixin828 do not. An evaluation of agronomic traits was conducted

Table 1. Seedling disease responses of R2207, susceptible wheat cultivar Mingxian169, and 38 wheat genotypes possessing documented powdery mildew resistance (*Pm*) genes to 23 individual *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates^z

Cultivars/lines	<i>Pm</i> genes	R/S ratio	<i>Bgt</i> isolates																						
			E01	E02	E05	E06	E07	E09	E11	E13	E15	E16	E17	E18	E20	E21	E23- (1)	E23- (2)	E26	E30- (1)	E30- (2)	E31	E32	E49	E50
R2207	Unknown	16/7	R	S	R	S	R	R	R	S	R	R	S	R	R	R	R	R	R	R	S	S	S	R	R
Kavkaz	8	5/18	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	R	R
Amigo	17	1/22	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CI14189	7	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
TAM104/ Thatcher	20	13/10	S	R	R	R	S	S	R	R	R	S	R	S	R	S	S	R	R	R	R	S	S	S	R
Mingxian169	–	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Funo	–	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ulka/8cc	2	19/4	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	S	R	R
Maris Huntsman	2+6	19/4	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	S	R	R
Baimianmai3	4+8	18/5	R	R	R	R	R	R	R	R	R	S	R	R	S	S	R	R	R	R	R	S	R	R	R
Xiaobaiddongmai	XBD	13/10	R	S	R	S	S	R	S	S	S	R	S	R	R	S	S	R	R	R	R	R	S	R	R
Chancellor	–	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Axminster/8cc	R	2/21	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Asosan/8cc	3a	2/21	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S
Chul/8cc	3b	5/18	S	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Sonora/8cc	3c	1/22	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Kolibri	3d	8/15	R	S	S	R	S	S	R	R	S	S	S	S	S	S	R	S	S	R	R	S	S	R	S
Mich.Amber/8cc	3f	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
W150	3e	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Khapli/8cc	4a	18/5	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	R	R	R
Armada	4b	19/4	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	R	R	R
81-7241	4c	18/5	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	S	R	R
Hope/8cc	5a	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Aquila	5b	19/4	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	S	R	R
Coker 983	5+6	5/18	S	S	R	S	S	S	S	S	R	S	S	S	S	S	S	R	R	R	R	S	S	S	S
Timgalen	6	0/22	S	S	S	–	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Coker 747	6	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Wembley	12	23/0	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
R4A	13	15/7	R	R	S	R	R	R	R	S	S	R	R	–	R	R	S	R	R	S	R	R	S	S	S
Brigand	16	23/0	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
XX186	19	0/23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
DS6V(6A)	21	22/0	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	–
Chiyacao	24	10/13	R	R	S	S	R	R	R	S	S	R	R	S	S	S	S	R	R	S	R	S	S	S	S
Maris Dove	2+MLD	18/4	R	R	R	–	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	S	R	R
Mission	4b+5b	19/4	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	R	R	R
Normandie	1+2+9	1/22	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S
5P27	30	18/4	R	R	–	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	R	R	R
NCA5	25	7/16	S	S	S	S	S	S	R	S	R	S	S	S	S	S	S	S	R	R	R	S	S	R	R
NCD7	34	9/14	S	R	R	S	S	S	R	S	R	S	R	S	S	S	S	R	R	R	R	S	S	S	R
NCD3	35	16/7	R	R	R	S	S	S	S	S	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R

^z Infection types of powdery mildew at seedling stage were scored using a 0 to 4 scale as Si et al. (1992) described, in which 0 to 2 were considered resistant (R) and 3 to 4 susceptible (S). Dash (–) indicates no data.

from 2015 to 2017 with three replicates in a randomized complete block design. For each replicate, 20 seeds were evenly sown in 1.5-m-long rows spaced 0.25 m apart. At physiological maturity (Zadoks et al. 1974), plant height, spike number per plant, spike length, fertile spikelet number per spike, kernel number per spike, thousand-kernel weight, and grain yield of 10 randomly selected plants in the middle of the two internal rows were investigated. The ANOVA and least significant difference test were performed using SPSS 22.0 software (SPSS, Chicago, IL) to test the significance of differences between R2207 and the six wheat cultivars for each agronomic trait.

Use of R2207 in wheat breeding. To transfer the valuable resistance and yield-associated genes into commercial wheat, R2207 was crossed and back-crossed with 12 widely grown cultivars in Hebei and Shandong provinces including Shixin633, Shixin733, Shixin828, Gao8901, Shiyou17, Shixin5071, Lumai13, Liangxing99, Jimai20, Jimai22, Tainong19, and Shannong17. Combined with the phenotype evaluation in the field, the 1RS-specific KASP marker SWK5282 (forward primer 1 with rye allele, gaaggtgaccaagttcatgctGAGCTGATTTCCATGTA; forward primer 2 with wheat allele, gaaggtcggagt-caacggattGAGCTGATTTCCATGTC; and reverse primer, TACCAAGTCCTGAACCA) (Han et al. 2020) was used to efficiently trace 1RS chromosomes in the F_2 or BC_1F_1 populations for MAS. This KASP marker is developed based on a 1RS-specific single nucleotide polymorphism (SNP) allele between the rye and wheat genome, and it

has been validated that it can be applied to screen wheat-rye derived populations on large scale, unconstrained by different wheat and rye backgrounds (Han et al. 2020).

The KASP amplification system (8 μ l total) consisted of 4.5 μ l standard-carboxy-X-rhodamine KASP master mix, 0.13 μ l of assay mix, 0.97 μ l of ddH₂O, and 2.4 μ l of template DNA (50 ng/ μ l). The reaction was performed in a Bio-Rad CFX real-time PCR system (Bio-Rad Laboratories, Hercules, CA). The PCR procedure was as follows: 94°C for 15 min, 94°C for 20 s, followed by 10 touchdown cycles of 64 to 58°C (decreasing 0.6°C per cycle), 38 cycles of 94°C for 20 s, and 58°C for 60 s, and fluorescence was detected using Bio-Rad CFX Manage 3.1 software.

Results

Sequential GISH and FISH analyses of R2207. Using genomic DNA of the rye cultivar Imperial as a probe, GISH analysis showed one pair of intact rye chromosome arms, which displayed a strong green hybridization signal and contained an obvious secondary constriction, were translocated onto one pair of wheat chromosomes in R2207 with a total number of 42 chromosomes in a cell (Fig. 1A and B). After that, GISH analyses using the probes pAs1 combined with pSc119.2, and Oligo-pSc119.2-2 combined with Oligo-pTa535-2, determined the identity of the individual chromosomes of R2207. The probes pSc119.2 or Oligo-pSc119.2-2 produced strong green hybridization signals in the telomere regions and weak

Fig. 1. Genomic in situ hybridization (GISH) and multicolor fluorescence in situ hybridization (mc-FISH) analyses of chromosomes of R2207. For GISH, total genomic DNA of the rye cultivar Imperial (green) were used as a probe and Chinese Spring DNA as a blocker. Chromosomes were counterstained with 4,6-diamidino-2-phenylindole (blue). **A** and **B**, GISH analysis of R2207. **C**, mc-FISH analysis of the same metaphase cell after GISH analysis (**A**) with pSc119.2 (green) and pAs1 (red). **D**, Nondenaturing FISH analysis of the same metaphase cell after GISH analysis (**B**) with Oligo-pSc119.2-2 (green) and Oligo-pTa535-2 (red). The bar represents 10 μ m, and the stars indicate the translocation chromosomes T1RS-1BL.

hybridization signals in the subtelomere regions on both the long and short arms of the translocated chromosomes, indicating that the rye chromosome arm 1RS had translocated onto the wheat chromosome arm 1BL, thereby forming a wheat-rye T1RS·1BL translocation line (Fig. 1C and D). The seeds from selfed R2207 were used for the sequential GISH and mc-FISH analyses in three consecutive generations. The results revealed that R2207 was a cytogenetically stable T1RS·1BL translocation line, and its chromosome composition was $2n = 42 = 20''W + 1''T1RS \cdot 1BL$.

mc-GISH analysis of R2207. mc-GISH analysis was performed to further reveal the genomic identity of the chromosomes in R2207. Using genomic DNA of the rye cultivar Imperial and three diploid progenitors of common wheat as probes or blockers, the results of the cross-hybridization signal made the rye chromosomes display green fluorescent signals, whereas the wheat A-, B-, and D-genome chromosomes displayed yellow-green, brown or gray, and pink or red fluorescent signals, respectively. The results demonstrated that R2207 contained seven pairs of A-genome chromosomes (yellow-green), six pairs of B-genome chromosomes (brown or gray), and seven pairs of D-genome chromosomes (pink or red), along with a translocation of one pair of rye chromosome arms (green) to one pair of B-genome chromosomes (Fig. 2, arrows). Two chromosome genomic translocation events occurred in R2207. A standing chromosome arrangement event in hexaploid wheat that two small B-genome chromosome fragments translocated onto the two long arms of A-genome chromosomes (Dvorak et al. 2018) were detected (Fig. 2, diamonds). Simultaneously, two other small B-genome chromosome fragments were translocated onto the two long arms of D-genome chromosomes (Fig. 2, stars).

Molecular marker analysis of R2207. Molecular marker analysis was also conducted to confirm the chromosome composition of R2207. The 1RS-specific marker O-SEC5'-A/O-SEC3'-R amplified two specific fragment bands consisting of approximately 1,530 and 700 bp. The 1BS-diagnostic marker O11B3/O11B5 amplified an approximate 630-bp fragment band. As expected, O-SEC5'-A/O-SEC3'-R amplified the same size of specific bands in the rye cultivar Imperial and three offspring plants of R2207 from three consecutive generations, 1R disomic addition line and 1RS ditelosomic addition line of CS-Imperial, two T1BL·1RS translocation lines (Lovrin10 and Lovrin13), and two triticale lines (10R2-193-2 and 10R2-194-2),

Fig. 2. Multicolor genomic in situ hybridization analysis of R2207. Bright-green, yellow-green, brown or gray, and pink or red fluorescence signals indicate R-genome, A-genome, B-genome, and D-genome chromosomes, respectively. Two small B-genome chromosome fragments are translocated to A-genome chromosomes (diamonds), and other two small B-genome chromosome fragments are translocated to D-genome (stars). The bar represents 10 μ m, and the arrows indicate the translocation chromosomes T1RS·1BL.

but not in CS or the wheat-rye disomic addition lines except for the 1R addition line or the 1RL ditelosomic addition line of CS-Imperial (Fig. 3A). Meanwhile, the marker O11B3/O11B5 did not amplify fragments in Imperial, three offspring plants of R2207 from three consecutive generations, or two T1BL·1RS translocation lines (Lovrin10 and Lovrin13), but amplification occurred in CS, a complete set of disomic addition lines and 1RS and 1RL ditelosomic addition lines of CS-Imperial, and two triticale lines (10R2-193-2 and 10R2-194-2) (Fig. 3B). These results demonstrated that the chromosome arm 1BS of R2207 had been substituted by the chromosome arm 1RS of rye.

Analyses of R2207 for resistance to powdery mildew and stripe rust. At the seedling stage, line R2207 was highly resistant to 16 of 23 *Bgt* isolates, accounting for 69.6% of the isolates tested (Table 1). To confirm the source of resistance, the F₂ population of R2207 and susceptible cultivar Shixin733 were used to test for powdery mildew resistance against isolate E09. The results demonstrated that all the plants containing 1RS showed the same highly resistant reaction as R2207, but the plants without 1RS were susceptible (Fig. 4). Thus, the powdery mildew resistance in R2207 was confirmed to have been derived from 1RS. Meanwhile, R2207 showed a significantly different reaction pattern in comparison with all the wheat genotypes. Especially, for the genotypes with documented *Pm* gene(s) derived from rye, Kavkaz with *Pm8* derived from 1RS of Petkus rye was resistant to five of 23 *Bgt* isolates, Amigo with *Pm17* from 1RS of Insave rye was resistant to only one isolate, CI14189 with *Pm7* from 2RL of Rosen rye was susceptible to all 23 isolates, and TAM104/Thatcher with *Pm20* from 6RL of Prolific rye was resistant to 13 of 23 *Bgt* isolates (Table 1), indicating that R2207 most likely carries a new resistance variation. At the adult-plant stage, over three consecutive growing seasons, R2207 exhibited high resistance to a mixture of *Bgt* isolates in the field with an IT 0 to 2, whereas the control Mingxian169 showed a highly susceptible reaction with an IT 8 to 9. The corresponding F₂ plants of R2207 and Shixin733, which were transplanted to the field, showed a consistent disease response that agreed with that of the seedling resistance test.

Line R2207 showed high resistance to four stripe rust races (CYR31, CYR32, CYR33, and CYR34) at the seedling stage,

Fig. 3. PCR amplification: **A**, 1RS-specific marker O-SEC5'-A/O-SEC3'-R; and **B**, 1BS-diagnostic marker O11B3/O11B5. Lanes: M = D2000; 1 = Chinese Spring (CS); 2 = Imperial; 3 to 5 = R2207 from three consecutive generations; 6 to 12 = 1R to 7R disomic addition lines of CS-Imperial; 13 to 14 = 1RS and 1RL ditelosomic addition lines of CS-Imperial; 15 to 16 = two T1BL·1RS translocation lines (Lovrin10 and Lovrin13); and 17 to 18 = two triticale lines (10R2-193-2 and 10R2-194-2). The arrows indicate the diagnosed amplified fragments of the markers.

Fig. 4. Evaluation of powdery mildew resistance at seedling stage and molecular detection of the 1RS chromosome from R2207 using 1RS-specific markers O-SEC5'-A/O-SEC3'-R in F₂ plants crossed by R2207 and Shixin733. M = D2000; 1 = Chinese Spring; 2 = Imperial; 3 = Shixin733; 4 = R2207; and S and R = susceptible and resistant F₂ plants, respectively. The arrows indicate the diagnosed amplified fragment.

whereas Mingxian169 was highly susceptible (Fig. 5, Table 2). Among the documented *Yr* genes, *Yr9* that derived from IRS of the rye cultivar Petkus was susceptible to the four races. Also, the widely virulent race CYR34 has overcome most of the *Yr* genes currently used in wheat production, such as *Yr26* (Table 2). At the adult-plant stage, R2207 also showed a high level of resistance to a mixture of *Pst* races CYR31, CYR32, and CYR33.

Agronomic performance of R2207. Line R2207 showed neither morphological nor cytological segregation after strict bagging for self-pollinating for three consecutive generations. By comparison with six representative commercial cultivars in the production, R2207 had significantly higher spike number per plant, spike length, and fertile spikelet number per spike, as well as comparable or superior kernel number per spike, thousand-kernel weight, and grain yield per plant, suggesting that R2207 could be a valuable resource for wheat improvement (Fig. 6, Table 3).

Use of R2207 in wheat breeding. To efficiently transfer the resistance gene(s) into different wheat backgrounds, R2207 was crossed with 12 commercial cultivars including Shixin633, Shixin733, Shixin828, Gao8901, Shiyu17, Shixin5071, Lumai13, Liangxing99, and so on. The F_2 or BC_1F_1 progenies of those crosses were selected using the IRS-specific KASP marker SWK5282 (Fig. 7). The powdery mildew resistance of the offspring plants cosegregated with the detection results of the IRS alleles and was not restricted to the different wheat backgrounds. Based on the genotyping results, combined with the phenotype evaluation, the highly resistant derivative plants against powdery mildew and stripe rust with the IRS alleles and desirable agronomic performance have been retained for further MAS breeding.

Discussion

Rye, a naturally cross-pollinating relative of common wheat, is an important gene donor for wheat improvement. Through distant

hybridization and chromosome engineering, many wheat-rye addition, substitution, and translocation lines have been developed for further use in wheat breeding. Compared with addition lines and substitution lines, translocation lines are preferred by breeders because of less linkage drag, smaller alien genetic segments, and regular meiotic behavior (Falke et al. 2009). In the present study, a novel wheat-rye T1RS·1BL translocation line (R2207) was systematically characterized by GISH, mc-FISH with multiple probes, mc-GISH, molecular marker analysis, multiple pathogen tests, and agronomic traits assessment. Line R2207 possessed broad resistance to powdery mildew that was most probably conferred by a new variation on chromosome IRS. Also, it has superior resistance to stripe rust at both seedling and adult-plant stages and enhanced agronomic traits; thus, it should be a promising disease resistance donor for wheat improvement. Among all of the rye translocation lines, IRS translocation line was regarded as a particularly notable success in wheat breeding (Tester and Langridge 2010). Since the 1950s, when the IRS chromosome arm from the German rye cultivar Petkus was first transferred into common wheat (Schlegel and Korzun 1997), the wheat-rye T1RS·1BL translocations have made great contributions to wheat improvement due to their multiple disease resistance and superior yield traits. Unfortunately, owing to continual evolution of virulent isolates in the pathogen populations, the resistance genes on IRS have successively lost their resistance. After large numbers of released cultivars carrying T1RS·1BL translocation lost their disease resistance, the problem of a single source of origin causing a lack of genetic diversity was extensively revealed. Researchers have made great efforts to create new T1RS·1BL translocation lines from different resources (Li et al. 2016; Lukaszewski 2017; Molnár-Láng

Fig. 5. Stripe rust response of Mingxian169 and R2207 to races CYR31, CYR32, CYR33, and CYR34 of *Puccinia striiformis* f. sp. *tritici* at the seedling stage, respectively.

Table 2. Seedling disease responses of R2207 and susceptible wheat cultivar Mingxian169 to races CYR31, CYR32, CYR33, and CYR34 of *Puccinia striiformis* f. sp. *tritici*

Races	Virulence spectra on <i>Yr</i> genes ^y	Infection types ^z	
		R2207	Mingxian169
CYR31	<i>Yr1</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i> , <i>Yr9</i> , <i>Yr17</i> , <i>Yr18</i> , <i>Yr28</i> , <i>Yr29</i> , <i>Yr43</i> , <i>Yr44</i> , <i>YrExp2</i> , <i>YrSP</i>	0;	4
CYR32	<i>Yr1</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i> , <i>Yr9</i> , <i>Yr17</i> , <i>Yr18</i> , <i>Yr27</i> , <i>Yr28</i> , <i>Yr29</i> , <i>Yr31</i> , <i>Yr43</i> , <i>Yr44</i> , <i>YrExp2</i> , <i>YrSP</i>	0	4
CYR33	<i>Yr1</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr9</i> , <i>Yr17</i> , <i>Yr18</i> , <i>Yr28</i> , <i>Yr29</i> , <i>Yr31</i> , <i>Yr43</i> , <i>Yr44</i> , <i>YrExp2</i> , <i>YrSP</i>	0;	4
CYR34	<i>Yr1</i> , <i>Yr6</i> , <i>Yr7</i> , <i>Yr8</i> , <i>Yr9</i> , <i>Yr10</i> , <i>Yr17</i> , <i>Yr18</i> , <i>Yr24</i> , <i>Yr26</i> , <i>Yr27</i> , <i>Yr28</i> , <i>Yr29</i> , <i>Yr31</i> , <i>Yr32</i> , <i>Yr43</i> , <i>Yr44</i> , <i>YrExp2</i> , <i>YrSP</i>	0;	4

^y Refers to Wu et al. (2020).

^z Infection types of stripe rust at seedling stage were scored using a 0 to 4 scale as Volin and Sharp (1973) described, in which 0 to 2 were considered resistant and 3 to 4 susceptible.

Fig. 6. Morphological traits of R2207 plant.

et al. 2010; Qi et al. 2016; Ren et al. 2009, 2017, 2018; Yang et al. 2014). Those new T1RS·1BL translocation lines had either valuable resistance traits, or elite agronomic performance, or other elite genes/alleles, and hence served as new valuable donors for wheat improvement. Therefore, there is still potential to identify more new elite variations to broaden the genetic base, to enhance the disease resistance, and simultaneously to improve the yield traits. For this reason, R2207 is undoubtedly a promising resource for disease resistance breeding.

Rye is a naturally cross-pollinating crop, resulting in heterogeneity between and within different cultivars. This characteristic creates huge variation in rye for wheat improvement, which, to some extent, explained why new IRS variations have been continuously identified. Many instances have demonstrated this phenomenon. For example, Ren et al. (2017, 2018) developed T1RS·1BL translocation lines derived from the rye cultivars Weining and Baili, respectively. Both of them were resistant to powdery mildew and stripe rust. Li et al. (2016) developed three new T1RS·1BL translocation lines and a new 1R (1B) substitution line. Surprisingly although the four lines were all derived from the rye cultivar Aigan and the susceptible wheat cultivar Mianyang11, their responses to inoculated *Pst* pathotypes turned out to be significantly different from each other. This may due to the high genetic diversity in rye. There exists a high probability of genetic diversity in rye cultivars (Ren et al. 2011). In the present study, R2207 showed significantly different reaction patterns to 23 *Bgt* isolates from those of *Pm7*, *Pm8*, *Pm17*, and *Pm20* (Table 1). Combined with different reaction patterns of R2207 to pathogen isolates, the cosegregation of resistance with 1RS in F_2 individuals indicated that it should possess a new resistance variation for powdery mildew on 1RS. Meanwhile, R2207 exhibited superior resistance to four *Pst* races, especially against the widely virulent race CYR34 (Fig. 5, Table 2), which further revealed its high breeding value in wheat production. Of course, more genetic and molecular evidence between R2207 and other reported resistance genes on 1RS is still required to further determine their differences. Fine genomic sequencing of different wheat cultivars and the cloning of the key genes and their functional validation should explain the evolutionary relationship between the documented resistance stocks.

Although many IRS translocation lines have been developed, not every line is useful for breeding (Carver and Rayburn 1995; Kim et al. 2004; McKendry et al. 1996; Singh et al. 1998; Villareal et al. 1998; Zhou et al. 2007). Several reports indicated that T1RS·1BL translocations increased yield in only specific combinations of 1RS and wheat genetic background (Jung and Leleay 1985; Ren et al. 2017). Fortunately, R2207 showed elite agronomic performance accompanied with resistance to two diseases. Compared with three commercial cultivars carrying T1RS·1BL translocations (Shimai18, Ji5265, and Heng4444) and three commercial

cultivars without T1RS·1BL translocations (Shixin633, Shixin733, and Shixin828), R2207 showed significant enhancement of spike number per plant, spike length, and fertile spikelet number per spike, and comparable or superior levels of kernel number per spike, thousand-kernel weight, and grain yield per plant. Meanwhile, R2207 exhibited high resistance at adult stage to the mixed *Bgt* isolates in the field and to 16 of 23 *Bgt* isolates tested at the seedling stage, and it also showed high resistance to the mixture of *Pst* races at the adult stage and *Pst* races CYR31, CYR32, CYR33, and CYR34 at the seedling stage. Therefore, R2207 combined multiple valuable traits and could be a promising donor for wheat breeding.

To transfer the T1RS·1BL translocation into different wheat genetic backgrounds, 1RS-specific KASP marker SWK5282 was used to efficiently select the progenies of R2207 crossing with cultivars such as Shixin633, Shixin733, Shixin828, Gao8901, and so on. In this process, SWK5282 was shown to be more time-saving and convenient than traditional PCR markers and greatly accelerated the transfer of resistance genes and achieved high-throughput detection (Han et al. 2020). Then, combined with the phenotype investigation in the field, F_2 or BC_1F_1 individuals with the IRS allele and elite agronomic traits were selected, and the seeds were sown in the field. In the near future, more stable high-generation lines derived from R2207 should be obtained and serve for wheat improvement.

Fig. 7. The genotyping results of the 1RS-specific kompetitive allele specific PCR marker performed for marker-assisted selection in F_2 progenies of R2207 and commercial cultivar Shixin633. Orange circles represent the homozygous 1RS special single nucleotide polymorphism (SNP) (Allele 1/Allele 1), indicating control rye Imperial; blue squares represent the homozygous wheat special SNP (Allele 2/Allele 2), indicating wheat parent Shixin633, the F_2 plants without chromosome arms 1RS, and control Chinese Spring. Green triangles represent the presence of both Allele 1 and Allele 2, indicating R2207 and the F_2 plants containing chromosome arms 1RS. Black diamonds indicate no template control.

Table 3. Agronomic performance of R2207 compared with six representative commercial cultivars^z

Year	Lines	Plant height (cm)	Spike number per plant	Spike length (cm)	Fertile spikelet number per spike	Kernel number per spike	Thousand-kernel weight (g)	Grain yield per plant (g)
2015–2016	R2207	76.0 ± 1.4 a	12.7 ± 1.3 a	11.8 ± 0.2 a	22.3 ± 1.3 a	72.0 ± 2.9 a	48.3 ± 1.1 ab	33.4 ± 2.7 a
	Shimai18	68.3 ± 2.7 c	10.5 ± 0.9 c	10.0 ± 0.3 c	18.7 ± 1.2 c	61.1 ± 1.6 d	48.0 ± 1.7 ab	26.3 ± 2.4 c
	Ji5265	66.2 ± 1.5 d	11.5 ± 1.2 b	8.9 ± 0.5 d	19.8 ± 0.6 b	67.5 ± 3.1 b	45.8 ± 1.4 c	30.5 ± 3.2 ab
	Heng4444	71.2 ± 1.8 b	9.5 ± 1.1 d	10.6 ± 0.4 b	17.4 ± 1.2 d	64.1 ± 3.2 c	49.2 ± 1.5 ab	27.0 ± 3.4 bc
	Shixin633	65.7 ± 2.2 d	9.2 ± 1.0 d	9.1 ± 0.4 d	19.0 ± 1.1 bc	64.3 ± 3.3 c	47.4 ± 1.4 bc	26.1 ± 4.0 c
	Shixin733	70.5 ± 1.5 b	7.3 ± 1.1 e	8.3 ± 0.2 e	18.5 ± 0.5 c	70.6 ± 2.8 a	49.8 ± 1.6 a	24.1 ± 2.2 c
	Shixin828	68.8 ± 1.2 c	8.0 ± 1.0 e	8.9 ± 0.3 d	17.5 ± 0.5 d	70.9 ± 2.5 a	47.9 ± 1.4 ab	24.0 ± 4.2 c
2016–2017	R2207	74.2 ± 2.1 a	13.9 ± 1.2 a	11.6 ± 0.6 a	20.5 ± 0.7 a	62.5 ± 2.2 a	43.7 ± 1.4 a	31.1 ± 3.8 a
	Shimai18	63.7 ± 1.6 e	12.0 ± 1.3 bc	9.1 ± 0.2 b	17.0 ± 0.6 bc	54.1 ± 3.2 c	43.1 ± 1.6 ab	25.0 ± 2.7 bc
	Ji5265	64.3 ± 1.3 de	12.6 ± 1.3 b	8.3 ± 0.3 c	17.5 ± 0.8 b	61.8 ± 2.3 a	40.7 ± 1.6 c	27.2 ± 1.9 b
	Heng4444	69.4 ± 1.9 b	11.5 ± 1.2 c	8.9 ± 0.2 b	16.1 ± 0.7 d	57.8 ± 2.4 b	44.0 ± 1.6 a	26.3 ± 2.9 b
	Shixin633	65.2 ± 1.3 de	11.2 ± 1.2 cd	8.4 ± 0.3 c	17.2 ± 0.6 b	58.1 ± 3.2 b	41.0 ± 2.0 bc	24.4 ± 3.2 bc
	Shixin733	67.5 ± 2.2 c	10.0 ± 1.3 e	8.2 ± 0.2 c	17.0 ± 0.9 bc	60.9 ± 1.7 a	44.7 ± 1.9 a	23.9 ± 2.3 bc
	Shixin828	65.4 ± 1.8 d	10.4 ± 1.3 de	8.3 ± 0.3 c	16.4 ± 1.0 cd	61.5 ± 1.8 a	42.9 ± 1.5 ab	22.6 ± 2.6 c

^z Values followed by the same letters in the same column were not significantly different at the 0.05 probability level according to the LSD test.

In conclusion, this study provides a thorough methodology for identifying a novel wheat-rye T1RS·1BL translocation line with superior agronomic traits and high resistance to two diseases as well as an effective method for transferring the chromosome 1RS for MAS. In the future, the development of more T1RS·1BL translocation lines should be encouraged to increase genetic diversity and fully release the potential of 1RS for wheat breeding. Meanwhile, small segment translocation lines of R2207 will be produced for accurate utilization of the valuable traits in 1RS in the near future.

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