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ORIGINAL PAPER

Characterization and molecular mapping of stripe rust resistance gene *Yr61* in winter wheat cultivar Pindong 34

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Abstract
Key message **We report a new stripe rust resistance gene on chromosome 7AS in wheat and molecular markers useful for transferring it to other wheat genotypes.**
Abstract Several new races of the stripe rust pathogen have established throughout the wheat growing regions of China in recent years. These new races are virulent to most of the designated seedling resistance genes limiting the resistance sources. It is necessary to identify new genes for diversification and for pyramiding different resistance genes in order to achieve more durable resistance. We report here the identification of a new resistance gene, designated as *Yr61*, in Chinese wheat cultivar Pindong 34. A mapping population of 208 F_2 plants and 128 derived $F_{2:3}$ lines in a cross between Mingxian 169 and Pindong 34 was evaluated for seedling stripe rust response. A genetic map consisting of eight resistance gene analog polymorphism (RGAP), two sequence-tagged site (STS) and four simple sequence repeat (SSR) markers was constructed. *Yr61* was located on the short arm of chromosome 7A and flanked by RGAP markers *Xwgp5467* and *Xwgp5765* about 1.9 and 3.9 cM in distance, which were successfully converted into STS markers *STS5467* and *STS5765b*, respectively. The flanking STS markers could be used for marker-assisted selection of *Yr61* in breeding programs.

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Introduction

Stripe rust (or yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), is one of the most destructive diseases of wheat worldwide (Stubbs 1985). Growing resistant cultivars have proven to be the best way to control the disease and pyramiding of effective resistance genes has provided durable control of the disease (Chen 2005, 2007; Wellings 2007). In China, especially the northwestern and southwestern regions, stripe rust has caused significant economic losses during the last 60 years (Li and Zeng 2000; Wan et al. 2007). During the last 20 years, races CYR31, CYR32 and CYR33 have become predominant across the wheat growing regions because they are virulent on widely grown wheat cultivars (Chen et al. 2009). The appearance and spread of new races are related to the widespread use of a single gene in one or more widely grown cultivars (Chen et al. 2002).

Two major types of resistance to rust have been described and used in breeding programs, all-stage resistance (also called seedling resistance; ASR) and adult-plant resistance (APR). All-stage resistance can be detected at the seedling stage, and is effective throughout the entire growth cycle. This type of resistance is highly effective, but is prone to being overcome by virulent races. APR and high-temperature adult-plant (HTAP) resistance are usually more durable but are often expressed at adult-plant stage (Line 2002; Chen 2005) and/or as temperatures increase. HTAP resistance can be controlled by single or multiple genes with partial effects on disease response. The levels of protection therefore may not be adequate, especially when conditions remain cold (Qayoum and Line 1985; Chen 2005, 2013). In many situations early inoculum build-up can occur on cultivars with only APR, and the inoculum contributes to the overall spore load that may affect all cultivars across large regions, which is typical in China. For this reason many researchers believe that all-stage resistance has a role in crop protection despite its reputed lack of durability. The best strategy then is either to combine two or more ASR genes or combine ASR with APR. In this case, there is still a need for continued discovery and documentation of all types of stripe rust resistance genes (Chen 2007, 2013; Ren et al. 2012a).

So far, more than 60 permanently named and many temporarily named stripe rust resistance genes and QTL have been reported (Basnet et al. 2013; Chen 2013; McIntosh et al. 2013; Rosewarne et al. 2013; Xu et al. 2013; Zhou et al. 2014). Most of these genes are ASR genes, and only very few are effective against all current Chinese and world populations of *Pst* such as *Yr5* and *Yr15*. (Sharma-Poudyal et al. 2013).

The hexaploid winter wheat (*Triticum aestivum* L.) cultivar Pindong 34 [(Yan7578/81/128)//176 (15) 9-26/Dongda 2] was obtained from the Institute of Crop Germplasm Resources, CAAS, Beijing. It has been resistant in seedling tests with Chinese *Pst* races CYR29, CYR31, CYR32 and CYR33 and in field nurseries in Shaanxi and Gansu provinces since 2008 (Kang et al. unpublished data). The objectives of this study were to characterize and map the stripe rust resistance gene in wheat cultivar Pindong 34, and to identify closely linked markers for resistance breeding.

Materials and methods

Plant materials

Pindong 34 was crossed to Mingxian 169 (M169), a Chinese landrace winter wheat genotype susceptible to all *Pst* races in China. An F_2 population consisting of 208 plants derived from a single F_1 plant was grown in the greenhouse and tested with race CYR32. After scoring, the F_2 and the parental plants were transferred outdoors for vernalization during the winter, and subsequently transplanted in the field in the spring and tested with artificially inoculated CYR32 at adult-plant stage. A total of 128 F_2 plants survived and produced sufficient $F_{2:3}$ seeds. The 128 $F_{2:3}$ lines were tested with CYR32 at the seedling stage in the greenhouse.

Chinese Spring (CS) and 21 CS nulli-tetrasomic or mono-tetrasomic lines (Sears 1966) were used to determine the chromosomal location of resistance gene analog polymorphism (RGAP) markers linked to the resistance gene in Pindong 34. To determine the usefulness of the molecular markers flanking the resistance gene in Pindong 34 in marker-assisted selection, 59 leading wheat cultivars and breeding lines from ten wheat producing provinces were selected and tested in a greenhouse with race CYR32 and in field trails in Gansu province in 2011 (Zeng et al. 2014). Those wheat cultivars and lines were also used to determine polymorphism of the marker locus among various wheat germplasm.

Evaluation of parents and progeny of M169/Pindong 34 for reaction to stripe rust

Races CYR29, CYR31, CYR32, CYR33, PST-Ch42, PST-Su4 and PST-Su11 of *Pst* representing predominant races from the 1980s to 2010 were used to evaluate the stripe rust responses of Pindong 34 and M169. Race CYR32 was used to test the F_2 population, $F_{2:3}$ lines and 59 wheat cultivars and breeding lines. Homozygous $F_{2:3}$ lines selected based on their phenotypic data from the test with CYR32 were subsequently tested with four races (CYR29, CYR31, CYR33 and PST-Su4) to confirm that the same gene confers resistance to all races. Seedling tests were conducted under controlled greenhouse conditions. About 20 seeds for each of the $F_{2:3}$ lines and parents were planted in 8 × 8 cm pots filled with soil mixture. Seedlings at the two-leaf stage were inoculated with selected *Pst* races. The plants were dust inoculated with a mixture of fresh urediniospores and talc at a 1:50 ratio and kept in a dew chamber at 10 °C without light. After 24 h the inoculated plants were transferred to an environmentally controlled greenhouse with a daily cycle of 16 h of light at 16 °C and 8 h of darkness at 12 °C for seedling tests. Infection type (IT) data were collected 16–20 days after inoculation based on the 0–9 scale of Line and Qayoum (1992). Infection type was recorded for each F_2 plant, and IT 0–3, 4–6 and 7–9 were considered resistant, intermediate and susceptible, respectively. For genetic analysis and mapping, the $F_{2:3}$ lines were classified as resistant, segregating and susceptible based on IT data.

DNA extraction

Genomic DNA was extracted from fresh leaves of the parents and each F_2 plant of M169/Pindong 34. The genomic

Table 1 Resistance gene analog polymorphism (RGAP), simple sequence repeat (SSR) and primers used to identify markers for the stripe rust resistance gene in wheat cultivar Pindong 34

Primer[a]	Sequence (5′–3′)	Tm (°C)[b]	Reference
RGA			
rga53 (Lr10_P-looP_F)	GGGAAGACGACTCTTGCT	45	This study
rga54 (Lr35_P-looP_F)	GCAGGAAAAACAACG	45	This study
rga55 (Lr21_P-loop_F)	GGAAGACCACGTTTGCA	45	This study
rga57 (Mla1_P-looP_F)	TTGGGCAAGACCACT	45	This study
rga60 (Pm3b_Kin2)	GTCCCAAACATCATC	45	This study
rga62 (Lr35_Kin2)	GAGGACAACCAAGTA	45	This study
rga65 (Yr10_NBS-B)	TGCGATTACTCTACTACC	45	This study
rga66 (Lr10_NBS-B)	TGTGGTCATTATTAC	45	This study
rga67 (Pm3b_NBS-B)	ACGAGTTGTTGTCAA	45	This study
rga70 (Mla1_NBS-B)	TGTGGTGATTAGCCG	45	This study
rga72 (Lr10_GLPL)	AAGGTCAAAATAACA	45	This study
rga75 (Lr35_GLPL)	TGGTAGGTCCTTGTA	45	Ren et al. (2012a)
SSR			
Xcfa2174F	ACGGCATCACAGGTTAAAGG	60	Paillard et al. (2003)
Xcfa2174R	GGTCTTTGCACTGCTAGCCT	60	Paillard et al. (2003)
Xbarc127F	TGCATGCACTGTCCTTTGTATT	52	Somers et al. (2004)
Xbarc127R	AAGATGCGGGCTGTTTTCTA	52	Somers et al. (2004)
Xwmc283F	CGTTGGCTGGGTTATATCATCT	62	Somers et al. (2004)
Xwmc283R	GACCCGCGTGTAAGTGATAGGA	65	Somers et al. (2004)
Xwmc168F	AACACAAAAGATCCAACGACAC	63	Somers et al. (2004)
Xwmc168R	CAGTATAGAAGGATTTTGAGAG	52	Somers et al. (2004)

[a] *rga53*, *rga54*, *rga55*, *rga57*, *rga60*, *rga62*, *rga65*, *rga66*, *rga67*, *rga70*, *rga72* and *rga75* are RGA primers designed by Dr. Meinan Wang (Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430, USA)

[b] Annealing temperature

DNA was extracted following the method of Song et al. (1994) with some modifications. Five gram of fresh leaves was vacuum dried and ground to powder with two small steel balls in 2 mL tubes using TissueLyser II (QIAGEN, Germany) and then mixed with 800 μL extraction buffer (2 % sodium lauroylsarcosine, 0.1 M Tris–HCl, 10 mM EDTA, pH 8.0). An equal volume of phenol/chloroform/isoamylalcohol (25:24:1) was added; the mixture was shaken for 20 to 30 s and kept for 30 min on ice (<4 °C). After separation of the phases by 12,000 rpm centrifugation for 12 min, 600 μL of the upper phase solution was transferred to another 1.5 mL microcentrifuge tube. The solution was mixed with 30 μL of sodium acetate (3 M, pH 4.8) and 800 μL of cold (−20 °C) 95 % ethanol for DNA precipitation. After centrifugation at 12,000 rpm for 5 min, the DNA was rinsed twice with 75 % ethanol and air dried. The DNA was dissolved in 300 μL TE (10 mM Tris–HCl and 1 mM EDTA, pH 8.0) and stored at −20 °C until used. DNA quality and concentrations were determined using a NanoDrop ND-1000 (Thermo scientific, Wilmington, DE, USA). Stock DNA solutions were diluted to 50 ng/μL as templates for use in PCR amplification.

Bulk segregant analysis

Resistant and susceptible bulks were made with equal amounts of DNA from 10 resistant and 10 susceptible F_2 plants, which were selected based on the homozygous reactions of their derived $F_{2:3}$ lines. Twenty-seven RGAP primers based on wheat resistance gene sequences were designed by Dr. Meinan Wang (Ren et al. 2012a; Zhou et al. 2014). To confirm the chromosomal location of the resistance gene indicated by RGAP markers tested with the 21 Chinese Spring nulli-tetrasomic or mono-tetrasomic lines, an additional 126 SSR markers on chromosomes 7A, 7B and 7D were screened for polymorphic markers linked to the resistance gene with genomic DNA from the parents and two bulks. The SSR primers were obtained from the GrainGenes website (http://wheat.pw.usda.gov/GG2/index.shtml). The RGA and SSR primers (Table 1) were synthesized by the Shanghai Generay Biotech Co. Ltd, Shanghai. DNA samples from Pindong 34, M169 and bulks were used to identify molecular markers linked to the resistance locus.

PCR amplification, electrophoresis and gel visualization

The RGA primers were randomly paired and screened on the parents and bulks. Polymorphic bands specific to the resistant parent and resistant bulk, or susceptible parent and susceptible bulk, were used to genotype the F_2 plants that generated the 128 $F_{2:3}$ lines. RGAP markers were used in a modified previously described amplification program (Chen et al. 1998). PCR was performed in 15 μL reaction mixtures containing 1.2 μL (50 ng/μL) template DNA, 1.5 μL Mg-free 10 × PCR

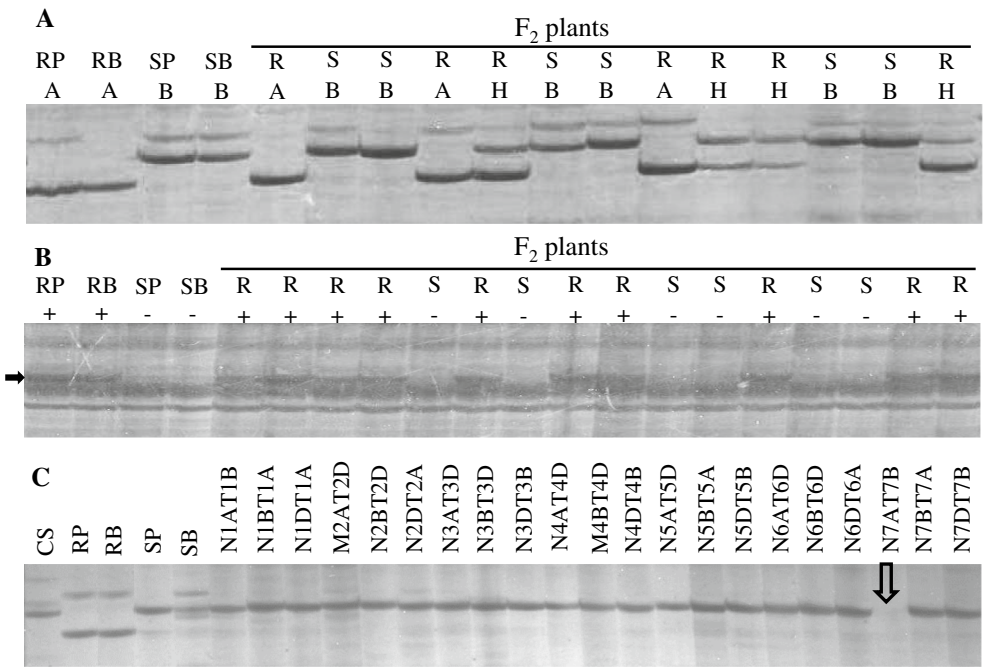

Fig. 1 Polyacrylamide gels showing RGAP marker *Xwgp5765b* (**a**) and *Xwgp5467* (**b**) amplifications in the resistant parent (RP, Pindong 34), resistant bulk (RB), susceptible parent (SP, M169), susceptible bulk (SB) and some F_2 plants of cross M169/Pindong 34. RGAP marker *Xwgp5765b* (**c**) amplification in Chinese Spring (CS), resistance parent (RP, Pindong 34), resistant bulk (RB), susceptible parent (SP, M169), susceptible bulk (SB) and 21 nullisomic (N) or monosomic (M)-tetrasomic (T) CS lines. The *arrow* shows the absence of the marker in line N7AT7B, indicating its location on chromosome 7A

buffer (MBI Fermentas Inc., Ontario, Canada), 1.5 μL 25 mM $MgCl_2$, 1.2 μL 2.5 mM of each dNTP, 1.2 μL (5 μM) of each primer solution, 0.12 μL Taq DNA polymerase solution (5 unit/μL) and 7.08 μL sterilized dd H_2O. Polymerase chain reactions were performed in a PCR S1000 thermo-cycler. After 5 min of denaturation at 94 °C, amplifications were programmed for 40 consecutive cycles, each consisting of 1 min at 94 °C, 1 min at 45 °C, 2 min at 72 °C and followed by an 8 min extension at 72 °C. A modified previously described touchdown amplification program was performed with SSR primers (Korbie and Mattick 2008). After 4 min of denaturation at 94 °C, amplifications were programmed for 15 consecutive cycles, each consisting of 30 s at 94 °C, 30 s at 65 °C and followed by 14 further cycles with each cycle at 1 °C less than the previous one, and 1 min at 72 °C; then 20 consecutive cycles of 30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C, followed by an 8 min extension at 72 °C. After amplification, 6 μL of formamide loading buffer [98 % formamide, 10 mM EDTA (pH 8.0), 0.5 % (W/V) xylene cyanol and 0.5 % (W/V) bromophenol blue] were added to the PCR product. After 4 min of denaturation at 94 °C, 4.5 μL of the PCR product and loading buffer mixture for each sample were loaded for electrophoresis in 6 % polyacrylamide gels. A 100 bp ladder (Cat: CW0636, www.cwbiotech) was used for determining the size of target bands. After electrophoresis, the gels were silver stained as previously described (Chen et al. 1998).

Development of STS markers

Sequence-tagged-site (STS) markers were developed from the RGAP markers flanking the resistance gene using the software Primer Premier 5 (Premier Biosoft, Palo Alto, CA, USA). PCR fragment cloning, sequencing and PCR product detection were performed using the previously described procedures (Xu et al. 2013). PCR mixture, amplification conditions and product detection for STS markers were the same as for the original RGAP markers, except the primers, initial denaturation at 94 °C for 4 min and 35 cycles of 94 °C for 35 s, 55 °C for 35 s and 72 °C for 35 s.

Data analysis and linkage map construction

RGAP and SSR markers linked to the resistance locus were used to construct a linkage map. Using the publicly available software QTL IciMapping V3.3 (Wang 2009; http://www.isbreeding.net/download_software_ICIM.aspx), a genetic map was developed using the phenotypic and genotypic data of the 128 F_2 plants with phenotypic data confirmed or clarified in their derived $F_{2:3}$ lines. The Kosambi map function was applied to calculate genetic distances in centiMorgans (cM). A LOD threshold of 3.0 was used for grouping, and the algorithm "nnTwo Opt" was used for ordering. The

Table 2 Resistance gene analog polymorphism (RGAP), simple sequence repeat (SSR) and sequence-tagged-site (STS) markers linked to the Pindong 34 (Pd34) stripe rust resistance locus and their primer pairs, size, presence (+) and absence (−) in Pd34, Mingxian169 (M169) and Chinese Spring (CS)

Marker	Primer pair	Size (bp)[a]	Presence (+)/absence (−)			Inheritance
			Pd34	M169	CS	
RGAP						
Xwgp5370	rga53/rga70	425	425	–	–	Dominant
Xwgp5467	rga54/rga67	1,302	1,302	–	–	Dominant
Xwgp5560	rga55/rga60	>1,500	>1,500	–	–	Dominant
Xwgp5765a	rga57/rga65	175	175	–	–	Dominant
Xwgp5765b	rga57/rga65	>1,500	>1,500	>1,500	>1,500	Co-dominant
Xwgp5766	rga57/rga66	>1,500	>1,500	–	–	Dominant
Xwgp6067	rga60/rga67	252	252	–	–	Dominant
Xwgp6270	rga62/rga70	>1,500	>1,500	–	–	Dominant
STS5467	*STS5467*	175	175	–	ND[b]	Dominant
STS5765b	*STS5765b*	300	300	285	ND	Co-dominant
SSR						
Xcfa2174	CFA2174	263	263	–	–	Dominant
Xbarc127	BARC127	500	500	–	500	Dominant
Xwmc283	WMC283	262/260	262	260	260	Co-dominant
Xwmc168	WMC168	1,250/1,245	1,245	1,250	1,250	Co-dominant

[a] Fragment sizes were visually estimated based on the 100 bp DNA ladder

[b] ND no data

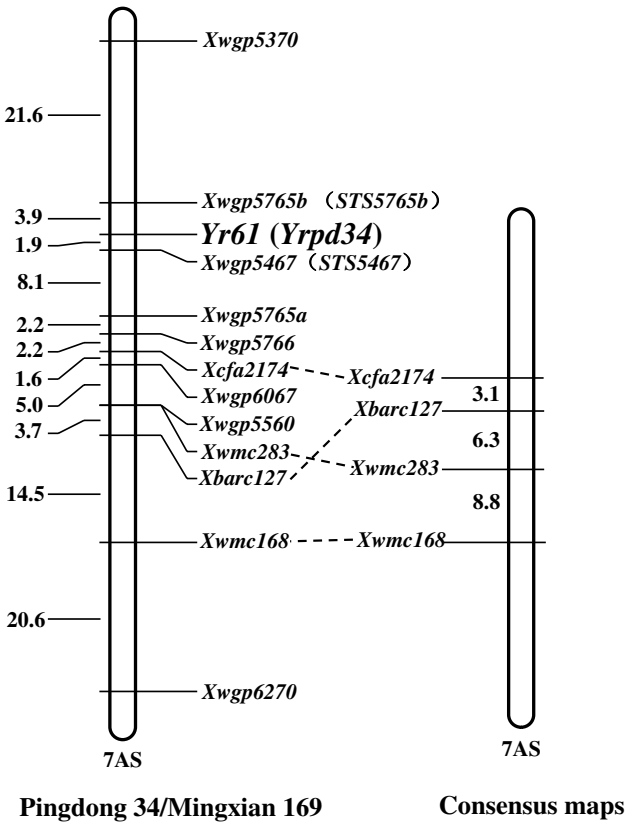

Fig. 2 Genetic map showing the location of the *Yr61* locus on wheat chromosome 7AS based on the M169/Pindong 34 mapping population of 128 $F_{2:3}$ lines. Linked SSR markers were assigned to chromosomes 7AS based on the consensus map (Somers et al. 2004; http://wheat.pw.usda.gov). Locations of markers amplified by the four pairs of SSR primers are also shown in the figure

linkage group was assigned to a chromosome by testing RGAP markers with the set of nulli-tetrasomic Chinese Spring lines and a chromosomal arm with SSR markers referring to the wheat SSR consensus map (Paillard et al. 2003; Somers et al. 2004; http://wheat.pw.usda.gov).

Results

Pindong 34 was highly resistant (IT 0–1) to all 7 races whereas the susceptible parent, M169, was highly susceptible (IT 9) at the seedling stage in the greenhouse tests. Pindong 34 was also highly resistant (IT 0–1) with disease severities of 0–5 % in field nurseries at Yangling, Shaanxi province and Tianshui, Gansu province (Supplementary Fig. 1). In contrast, M169 was consistently susceptible (IT 8–9; DS 70–100 %). The results showed that Pindong 34 has a high level of ASR to stripe rust.

Inheritance of stripe rust resistance in Pindong 34

Of the 208 F_2 plants from cross M169/Pindong 34 tested with CYR32 at the seedling stage, 159 were resistant (IT 0–2), 49 were susceptible (IT 8–9) ($\chi^2_{3:1} = 0.16$, $P_{1df} = 0.63$) and no plants had an intermediate IT, indicating a single dominant gene for resistance. Among the 128 $F_{2:3}$ lines, 29 homozygous susceptible lines were derived from susceptible F_2 plants, 23 homozygous resistant lines were from resistant F_2 plants, and of 76 segregating lines 73 were from resistant F_2 plants and three were from plants that had been mistakenly scored susceptible (IT

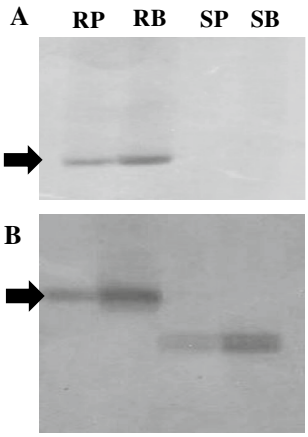

Fig. 3 STS markers *STS5467* (**a**) and *STS5765b*, (**b**) (*arrows*) amplified in cultivar Pindong 34 (RP), resistant bulk (RB), susceptible parent (SP, M169) and susceptible bulk (SB). The PCR products were run in 6 % polyacrylamide gels

8). The distribution of $F_{2:3}$ lines was marginally acceptable for a 1:2:1 ratio ($\chi^2 = 5.06$, $P_{2df} = 0.08$). To confirm that the same gene conferred resistance to various races, six homozygous resistant ($F_{2:3}$-26, $F_{2:3}$-32, $F_{2:3}$-42, $F_{2:3}$-47, $F_{2:3}$-61 and $F_{2:3}$-84) and six homozygous susceptible ($F_{2:3}$-57, $F_{2:3}$-72, $F_{2:3}$-87, $F_{2:3}$-89, $F_{2:3}$-114 and $F_{2:3}$-182) lines selected based on the test with CYR32 were tested with races CYR29, CYR31, CYR33 and PST-Su4. All twelve $F_{2:3}$ lines were scored identically to race CYR32. The 128 $F_{2:3}$ lines were also scored identically to race CYR32 in the field test. Thus, Pindong 34 carries a single dominant gene, temporarily designated *Yrpd34*, for widely effective ASR, and the phenotypic data of the 128 $F_{2:3}$ lines were reliable for mapping the resistance gene.

Mapping of *Yrpd34* using RGAP markers

The 128 F_2 plants with phenotypes confirmed or clarified with data of their derived $F_{2:3}$ lines DNA were used to identify molecular markers. Eight RGAP markers generating repeatable differences in band size between resistant and susceptible bulks were selected to test the mapping popualtion. As examples, Fig. 1a, b shows the banding patterns of RGAP markers *Xwgp5765b* (*Mla1_P-looP_F/Yr10_NBS-B*) and *Xwgp5467* (*Lr35_P-looP_F/Pm3b_NBS-B*), among the parents, bulks and some F_2 plants. *Xwgp5765b* was a co-dominant marker and the other seven RGAP markers were dominant, with bands amplified in the resistant parent and resistant bulk but not in the susceptible parent and susceptible bulk. A list of markers, with amplicon sizes and amplicon presence versus absence in Pindong 34, M169, and Chinese Spring is given in Table 2.

Chromosomal location of *Yrpd34*

Eight RGAP markers linked with *Yrpd34* were used to amplify Chinese Spring (CS), and RGAP marker *Xwgp5765b* present in CS was used to test the 21 nulli-tetrasomic or mono-tetrasomic lines. The unique bands were detected in all lines except N7AT7B, as shown by marker *Xwgp5765b* in Fig. 1c, indicating that the RGAP marker and the linked resistance gene were located on wheat chromosome 7A. SSR markers were therefore screened in bulk segregant analysis to identify additional markers. To confirm the chromosome location, 126 SSR markers covering the 7A, 7B and 7D chromosomes were screened across the parents and bulks. Four markers, *Xbarc127*, *Xcfa2174*, *Xwmc168* and *Xwmc283* on the short arm of chromosome 7A (7AS) (Paillard et al. 2003; Somers et al. 2004), showed polymorphisms between the parents and resistant and susceptible DNA bulks. *Xwmc168* and *Xwmc283* were co-dominant, and *Xbarc127* and *Xcfa2174* were dominant producing bands of 263 and 500 bp, respectively, in Pindong 34 and the resistant bulk, but not in M169 and the susceptible bulk (Table 2). The putatively linked markers were genotyped with DNA of all 128 F_2 plants used to generate the 128 $F_{2:3}$ lines. These markers were confirmed to be linked to the resistance gene locus. Three SSR markers *Xbarc127*, *Xwmc168* and *Xwmc283* present in CS were used to test the 21 nulli-tetrasomic or mono-tetrasomic lines. The expected bands were detected in all lines except N7AT7B, confirming that the SSR markers and the linked resistance gene were located on wheat chromosome 7A. According to published data, all four SSR markers are located on chromosome 7AS thus placing *Yrpd34* on the same chromosome arm.

Linkage map for *Yrpd34*

A linkage map consisting of eight RGAP and four SSR markers was constructed for *Yrpd34* using the phenotypic and genotypic data of the 128 $F_{2:3}$ lines. The map spanned 85.3 cM (Fig. 2). SSR markers *Xbarc127*, *Xcfa2174*, *Xwmc168* and *Xwmc283* were 24.7, 14.4, 39.2 and 21.0 cM proximal to the resistance locus. RGAP markers *Xwgp5765b* (*Mla1_P-looP_F/Yr10_NBS-B*) at 3.9 cM distal and *Xwgp5467* (*Lr35_P-looP_F/Pm3b_NBS-B*) at 1.9 cM proximal to *Yrpd34* were the closest flanking markers.

STS markers

Because the two markers most closely linked with *Yrpd34* were RGAP markers which are not very easy to use. The sequences of the markers were used to design STS primers. Two STS markers, *STS5467* (forward primer:

Table 3 Infection types produced by race CYR32 of *Puccinia striiformis* f. sp. *tritici* on seedlings in the greenhouse and on adult plants in the field at Tianshui (Gansu province) on various wheat genotypes and polymorphisms of STS markers flanking the *Yr61* locus in these genotypes

Wheat genotype	Province	Infection type		Presence (+)/absence (−) of marker	
		Seedling	Field	*STS5467*	*STS5765b*
Pindong 34 (Control)	Beijing	0–1	1	+	+
Fuyang 936	Anhui	9	9	−	−
Wan 9949	Anhui	5	3	−	−
Wanmai 369	Anhui	6	3	−	−
Wanmai 50	Anhui	8	9	−	−
Wanmai 53	Anhui	9	9	−	−
Jing 5642	Beijing	8	3	−	−
Jinghe 2	Beijing	8	0	−	−
Zhongmai 12	Beijing	7	2	−	+
Aikang 58	Henan	8	3	−	−
Fanmai 5	Henan	9	9	−	−
Huapei 5	Henan	8	2	−	−
Lankao 10	Henan	8	9	−	−
Luo 4518	Henan	9	9	−	−
Pumai 9	Henan	9	9	−	−
Wenmai 19	Henan	9	9	−	−
Xinmai 22	Henan	9	8	−	−
Xinmai 9408	Henan	9	9	−	−
Yumai 66	Henan	8	3	−	−
Yumai 70	Henan	9	3	−	−
Zhengmai 005	Henan	8	9	−	−
Zhengmai 9023	Henan	8	8	−	−
Zhongyu 6	Henan	7	8	−	+
Zhoumai 13	Henan	9	2	−	−
Zhoumai 18	Henan	8	2	−	−
Zhoumai 22	Henan	8	2	−	−
Emai 25	Hubei	9	8	−	−
Huamai 8	Hubei	9	8	−	−
Jing Mai 66	Hubei	8	3	−	−
Xiangmai 81	Hubei	9	8	−	−
Ningmai 13	Jiangsu	9	9	−	−
Shuangkang 7438	Jiangsu	8	3	−	−
Xumai 954	Jiangsu	5	9	−	−
Yangmai 17	Jiangsu	9	9	−	−
Ningchun 27	Ningxia	9	8	−	+
Ningchun 47	Ningxia	9	9	−	−
Ningdong 10	Ningxia	8	9	−	+
Ningdong 11	Ningxia	8	9	−	−
Changwu 357-9	Shaanxi	9	2	−	−
Qinmai 1148	Shaanxi	9	8	−	−
Qinnong 142	Shaanxi	8	3	−	−
Shaanmai 139	Shaanxi	2	2	−	−
Shaanmai 175	Shaanxi	2	2	−	−
Xiaoyan 216	Shaanxi	9	3	−	−
Xiaoyan 22	Shaanxi	9	8	−	−
Xinong 389	Shaanxi	3	2	−	−
Hemai 13	Shandong	8	9	−	+

Table 3 continued

Wheat genotype	Province	Infection type		Presence (+)/absence (−) of marker	
		Seedling	Field	*STS5467*	*STS5765b*
Jimai 19	Shandong	9	9	−	−
Jimai 22	Shandong	8	8	−	−
Lumai 21	Shandong	9	8	−	−
Shandong 664	Shandong	9	9	−	−
Shannong 16	Shandong	8	8	−	−
Taishan 23	Shandong	8	9	−	−
Taishan 9818	Shandong	8	8	−	−
Yannong 19	Shandong	8	9	−	−
Jinmai 47	Shanxi	8	9	−	−
Linmai 4	Shanxi	9	9	−	−
Linyu 867	Shanxi	9	2	−	−
Mianmai 42	Sichuan	1	1	−	−
Mianyang 19	Sichuan	9	9	−	+

3′-CACACACCTCAGCTCTTGGATG-5′ and reverse primers: 3′-CAAGAAGGCGACGAGGATGTTG-5′) and *STS5765b* (forward primer: 3′-GATTTGCGATTACTC-TACTA-5′ and reverse primer: 3′-GGCTTTCGTGTC-CGTCTC-5′), were successfully developed from RGAP markers $Xwgp5467_{1302}$ and $Xwgp5765b_{>1500}$, respectively. The STS markers *STS5467* (Fig. 3a) and *STS5765b* (Fig. 3b) showed the same patterns as the original RGAP markers when tested on the parents, bulks and the mapping population.

Polymorphism of the flanking STS markers among wheat cultivars and breeding lines

When tested on the 59 wheat cultivars and breeding lines, *STS5467* was absent in all and *STS5765b* was present in only 6 cultivars (Zhongmai 12, Zhongyu 6, Ningchun 27, Ningdong 10, Hemai 13 and Mianyang 19), showing 100 and 89.8 % polymorphism, respectively (Table 3). Interestingly, the six cultivars scored positive for the resistant marker allele were all susceptible to CYR32 (IT 7–9). These results indicated that none of the cultivars likely have *Yrpd34* and that the STS can be used for marker-assistant incorporation of the genes into various wheat cultivars.

Discussion

Pindong 34 has shown a high level of resistance to stripe rust in greenhouse and field tests since 2008. In the present study, we identified and mapped a gene on chromosome 7AS for resistance to predominant *Pst* races in China.

Currently, there are more than 60 permanently designated *Yr* genes and many others with temporary designations (Chen 2013; McIntosh et al. 2013; Rosewarne et al. 2013; Xu et al. 2013; Basnet et al. 2013; Zhou et al. 2014). Prior to the current study, six temporarily designated genes/QTL for stripe rust resistance were reported on wheat chromosome 7AS. *Yrxy1* is a temperature-sensitive resistance gene linked with SSR marker *Xwmc422* that is near the centromere (Somers et al. 2004; Zhou et al. 2011), while SSR markers *Xwmc283* and *Xbarc127* placed *Yrpd34* distal in 7AS. *QYr.inra-7A* in cultivar Recital (Dedryver et al. 2009), *QYr.sun-7A* in CPI133972 (Zwart et al. 2010), *QYr.caas-7AS* in Jingshuang 16 (Ren et al. 2012b), *QYrst.orr-7AS* in Stephens (Vazquez et al. 2012), and *QRYr7A.1* in Avocet (Rosewarne et al. 2012, 2013) were reported to have small and inconsistent effects on APR (Chen 2013; Rosewarne et al. 2013). In contrast, *Yrpd34* provides a consistently high level of resistance at all growth stages. Therefore, *Yrpd34* is different from those genes on chromosome 7A, and we permanently named it *Yr61*.

The markers linked to *Yr61* identified in this study can be used for marker-assisted selection in wheat breeding programs. As the most closely linked markers, *Xwgp5467* and *Xwgp5765b*, are RGAP markers, they need to be detected with a vertical gel electrophoresis system or a Li-Cor system. We successfully converted the flanking RGAP markers to STS markers, *STS5467* and *STS5765b*. Compared to the original RGAP markers, the STS markers are relatively robust and easy to score. The STS marker loci were highly polymorphic among 59 cultivars and breeding lines, suggesting that they can be used in marker-assisted selection for incorporating *Yr61* into elite wheat lines to develop wheat cultivars with effective resistance to stripe rust. As a small percent of wheat cultivars may be positive when marker *STS5765b* is used, cautions should be taken to interpret the data, and this marker should be used together with *STS5467*.

Because of its wide effectiveness against the predominant and emerging races of *Pst*, *Yr61* is a useful gene for wheat breeding programs in China. However, based on past experience the use of such ASR genes alone may result in the appearance of a virulent race(s); the gene must be combined with other effective all-stage resistance or adult-plant resistance genes, in order to prolong its effectiveness, and to provide farmers with at least some protection in case of lost effectiveness. Further characterization of *Yr61* by developing near-isogenic lines, fine mapping and eventually cloning is underway.

Author contributions XLZ conducted the phenotyping and genotyping experiments, analyzed the data, and drafted the manuscript. DJH identified the resistant parental line, made the cross and participated in the field experiments. ZSK conceived and directed the project and revised the manuscript. LLH codirected the project and revised the manuscript. XMC provided unpublished RGA primer sequences and revised the manuscript. HLG, SJG, LR and QLW participated in field and greenhouse experiments and data collection. All authors have read and approved the submitted version of the manuscript.

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Conflict of interest This manuscript does not have any conflicts of interest.

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