Mol Breeding (2016) 36:64
DOI 10.1007/s11032-016-0487-6

Molecular mapping of stripe rust resistance gene *YrHu* derived from *Psathyrostachys huashanica*

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Received: 1 January 2016 / Accepted: 3 May 2016 / Published online: 18 May 2016

Abstract Wheat stripe rust is a destructive disease that affects most wheat-growing areas worldwide. Resistance genes from related species and genera add to the genetic diversity available to wheat breeding programs. The stripe rust-resistant introgression line H9020-17-25-6-4 was developed from a cross of resistant *Psathyrostachys huashanica* with the susceptible wheat cultivar 7182. H9020-17-25-6-4 is resistant to all existing Chinese stripe rust races, including the three most widely virulent races, CYR32, CYR33, and V26. We attempted to characterize this new line by genomic in situ hybridization (GISH) and genetic analysis. GISH using *P. huashanica* genomic DNA as a probe indicated that the translocated segment was too small to be detected. Genetic analysis involving F_1, F_2, and $F_{2:3}$ materials derived from a cross of Mingxian 169 and H9020-17-25-6-4 indicated that a single dominant gene from H9020-17-25-6-4, temporarily designated *YrHu*, conferred resistance to CYR29 and CYR33. A genetic map consisting of four simple sequence repeat, two sequence-tagged site (STS), and two sequence-related amplified polymorphism markers was constructed. *YrHu* was located on the short arm of chromosome 3A and was about 0.7 and 1.5 cM proximal to EST-STS markers *BG604577* and *BE489244*, respectively. Both the gene and the closely linked markers could be used in marker-assisted selection.

Keywords *Puccinia striiformis* f. sp. *tritici* · *Psathyrostachys huashanica* · Stripe rust resistance · Molecular mapping

Electronic supplementary material The online version of this article (doi:10.1007/s11032-016-0487-6) contains supplementary material, which is available to authorized users.

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Introduction

Stripe rust is a destructive disease caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*) and occurs in most wheat-growing areas worldwide (Chen 2005). In China, stripe rust is one of the most

damaging diseases of wheat and has been considered the most important disease of wheat since 1950 (Wan et al. 2007). The disease is more prevalent in the in the winter wheat-growing areas, especially in the areas of the Yellow River-Huai River. The northwestern regions of Gansu and Qinghai provinces in China are major over-summering areas for this disease. The major sources of strip rust inoculum in spring are from Sichuan and Hubei provinces, which are the over-wintering areas in China (Li and Zeng 2002). In the past 60 years, major epidemics in 1950, 1964, 1990, and 2002 resulted in yield losses of 6.0, 3.2, 1.8, and 1.3 million tons, respectively (Chen et al. 2009). Although chemical control can be very effective, resistant cultivars are the most effective, economic and environmentally friendly approach to reduce yield losses caused by stripe rust (Chen 2007).

To date, over 60 permanently named and many temporarily designated *Yr* genes conferring resistance to stripe rust have been described in wheat, and some have been widely used in different areas of the world (Lu et al. 2014; Zhou et al. 2014a, b). Most of the resistance genes are race-specific and are either currently ineffective or lose effectiveness within a few years of being introduced into agriculture. In recent years, the races CYR32 and CYR33 have caused severe losses in many high-yielding wheat cultivars. The critical issue in stripe rust control is the limited number of efficient resistance genes being used by breeders. In order to increase genetic diversity, both the resistance resources available in wheat and related species should be applied for agriculture.

The related species of *Psathyrostachys huashanica* exists only in the Huashan Mountain area of Shannxi province in China. Successful hybridization of *P. huashanica* with common wheat (*Triticum aestivum* L) was achieved 20 years ago. A number of translocation lines, substitution lines, and addition lines were used as intermediate breeding materials to improve various traits in common wheat. It has been demonstrated that wheat derivatives of *P. huashanica* can be used as a genetic resource to study stripe rust resistance (Ma et al. 2013a), take-all resistance (Fu et al. 2003; Wang and Shang 2000), barley yellow dwarf virus-GAV resistance, and novel storage protein genes (Zhao et al. 2010). In order to use *P. huashanica* for improving common wheat cultivars, the F_1 hybrid H881 ($2n = 28$, ABDNs) was obtained from common wheat cv. 7182 and *P. huashanica* by embryo rescue. Spontaneous chromosome doubling was induced by backcrossing with the maternal parent 7182, and the germplasm H8911 was generated. (Chen et al. 1991). "H8911" was crossed with "7182" again, and the line "H9020" was selected. To produce wheat-like derivative lines, 7182 was used as a recurrent parent for backcrossing, and selfing of four generations was conducted for selection. The alleged wheat translocation H9020-17-25-6-4 was isolated from a large number of progenies BC_2F_4, which showed resistance to all tested Chinese *Pst* races in the field and greenhouse. The objectives of the present study were to characterize and map the gene(s) for resistance to stripe rust in H9020-17-25-6-4 and to identify closely linked markers for resistance breeding.

Materials and methods

Plant materials

H9020-17-25-6-4, an awned hexaploid winter wheat genotype, was provided by Fu Jie, Institute of Botany, Northwest A&F University. This line is resistant in seedling and adult plant tests with races CYR29, CYR30, CYR31, CYR32, CYR33, Su11-4, Su11-7, Su11-11, and V26. F_1, F_2, and $F_{2:3}$ materials for genetic and molecular studies were produced from a cross between H9020-17-25-6-4 and Mingxian 169, a susceptible variety. Thirty-five leading wheat cultivars from the areas of the Yellow River-Huai River of China and four wheat-*P. huashanica* translocation lines (H9020-17-5, H9020-1-6-8-3, H9020-14-4-6-1, and H9014-121-5-5-9) were used to evaluate the polymorphisms of molecular markers close to the resistance gene in H9020-17-25-6-4. The four translocation lines were also used to determine whether the resistance gene in H9020-17-25-6-4 was identical to those in the four translocation lines.

H9020-17-25-6-4 and *P. huashanica* were also used in cytogenetic analyses to determine the size of the alien introgression.

Genomic in situ hybridization (GISH)

Root tips from germinating seeds were pretreated in ice water for 24–28 h, fixed in ethanol:acetic acid (3:1 v/v) for 24 h at room temperature, and stored in a refrigerator. Root tips were squashed in a drop of 45 %

acetic acid and frozen in a refrigerator at −80 °C. Total genomic DNA was extracted from fresh leaves of *P. huashanica* using the cetyl trimethyl ammonium bromide (CTAB) method. GISH analysis was performed according to a published method (Reader et al. 1994). *Psathyrostachys huashanica* DNA was labeled with a digoxigenin-11-dUTP probe by nick translation according to the manufacturer's protocol (Roche, Mannheim, Germany). Denatured hybridization solution (40 µl) containing 2× sodium chloride-sodium (SSC), 0.25 % sodium dodecyl sulfate, 10 % dextran sulfate, 50 % deionized formamide, 0.125 µg/µl salmon sperm DNA (5 µg/µL), and 2.5 ng/µl labeled probe DNA was overlaid on each slide. Denaturation was conducted for 10 min at 95 °C. After hybridization in a humid petri dish, post-hybridization washes were performed with 2× SSC buffer. Anti-digoxigenin-fluorescein (Roche, Mannheim, Germany) was used to detect and visualize the labeled chromosomes. Fluorescence signals were captured using an Olympus BX60 microscope.

Evaluation of stripe rust reactions in greenhouse

Seedling tests were performed under controlled greenhouse conditions. To determine the resistance type of H9020-17-25-6-4, 15 plants each of H9020-17-25-6-4, *P. huashanica*, cv. 7182, and Mingxian 169 were tested with 9 *Pst* races, viz. CYR29, CYR30, CYR31, CYR32, CYR33, Su11-4, Su11-7, Su11-11, and V26. Inoculations were done at the two-leaf stage. Seedlings were inoculated with fresh urediniospores, kept in a dark dew chamber at 10 °C for 24 h without light, and then grown in a growth chamber operating with 16 h light:8 h darkness with diurnal temperatures gradually changing from 4 °C at 2:00 a.m. to 20 °C at 2:00 p.m. Infection type (IT) data were recorded 17 days after inoculation based on a 0–4 scale where the plants with IT 0–2^+ were considered to be resistant and those with IT 3^-–4 susceptible (Zhou et al. 2011).

Seedlings of parents and F_2 and $F_{2:3}$ populations were grown in the greenhouse. About 10 seeds from each parent, 150 F_2 seeds, and 10 seeds from each 148 $F_{2:3}$ line were planted in 10-cm-diameter pots. The F_2 population was inoculated with race CYR29, and the $F_{2:3}$lines were subsequently inoculated with CYR29 and CYR33 in separate tests to confirm that the same gene confers resistance to the two races. IT data were recorded as described above.

Evaluation of stripe rust reactions in the field

In the 2010–2011 cropping season, the parents and $F_{2:3}$ lines were sown in duplicate at Jingzhou, Hubei province. Inoculation of $F_{2:3}$ lines was performed with races CYR29and CYR33. The test plots were surrounded by Mingxian 169 as a spreader. IT data were recorded on a 0–4 scale when disease severity reached 80 % based on the susceptible control. If all plants in a row had uniform susceptible ITs, the line was scored as homozygous susceptible (IT 3^-–4), or if uniformly resistant (IT 0–2^+), it was scored as homozygous resistant. Lines with variable ITs were scored as segregating.

DNA extraction and bulk construction

Genomic DNA was extracted from individual F_2 plants at the third or fourth healthy leaf using the CTAB method. DNA concentration was adjusted to 30 ng/µl for use in PCR (Li et al. 2011). Based on phenotypic data, resistant and susceptible DNA bulks were constructed for screening with resistance gene analog polymorphism (RGAP), simple sequence repeat (SSR), and expressed sequence tag (EST)-STS markers. For constructing resistant and susceptible bulks, equivalent volumes of DNA from 12 resistant and 12 susceptible F_2 samples were prepared. The 12 resistant and 12 susceptible F_2 plants were confirmed as homozygous resistant or homozygous susceptible by progeny testing in the greenhouse.

Molecular marker screen and segregation analysis

A total of 128 sequence-related amplified polymorphism (SRAP) markers (Li and Quiros 2001), 778 SSR markers covering all wheat chromosomes, and 110 EST-STS markers specific to chromosome 3AS were used to screen the parents and bulks (Table 1). SSR primer sequences were obtained from the GrainGenes 2.0 website, and the EST-STS markers were obtained from the Wheat Haplotype Polymorphisms website (http://wheat.pw.usda.gov/SNP/new/pcr_primers.shtml). PCRs were run in either a PTC200 Peltier Thermo-cycler or a Gene Amp PCR 9700 (ABI) system. The 15-µl reaction mixtures comprised 30 ng of template DNA; 1.5 µl Mg-free 10× PCR buffer; 0.8 units of Taq DNA polymerase; 5 mM of $MgCl_2$; 0.2 mM each of dATP, dTTP dGTP, and dCTP; and

Table 1 SSR, EST-STS, and SRAP primers used to identify markers for the stripe rust resistance gene in H9020-17-25-6-4

Primer	Sequence (5′–3′)	Tm	References
SSR			
Xwmc11	F: TTGTGATCCTGGTTGTGTTGTGA	62	Somers et al. (2004)
	R: CACCCAGCCGTTATATATGTTGA		
Xwmc532	F: GATACATCAAGATCGTGCCAAA	62	Somers et al. (2004)
	R: GGGAGAAATCATTAACGAAGGG		
Xcfd79	F: TCTGGTTCTTGGGAGGAAGA	60	Somers et al. (2004)
	R: CATCCAACAATTTGCCCAT		
Xgwm2	F: CTGCAAGCCTGTGATCAACT	50	Somers et al. (2004)
	R: CATTCTCAAATGATCGAACA		
EST-STS			
BE490440	F: CACGCTCAGGGAGGAGATAC	60	
	R: CCCATTCCTGTCGAGTTTGT		
BE489244	F: AGAGCCGTGCTACATCTGGT	60	
	R: CACAGATAGCCTTCATGCGA		
BG604577	F: CGTGTTGTTCGTGAAATTGG	60	
	R: CAGACAGGAGTGGTTCAGCA		
SRAP			
Me1-em9	F: TGAGTCCAAACCGGATA	50	Li and Quiros (2001)
	R: GACTGCGTACGAATTCGA		
Me3-em13	F: TGAGTCCAAACCGGAAT	50	Li and Quiros (2001)

5 µl of each primer pair. The PCR program consisted of initial denaturation at 94 °C for 5 min, followed by 40 cycles of 30 s denaturation at 94 °C, 1 min annealing at 50–65 °C (depending on individual primers), 1 min extension at 72 °C, and a final extension at 72 °C for 10 min. After amplification, 6 µl formamide-loading buffer containing 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 products. A mixture (about 6 µl) of the PCR product and loading buffer for each sample was loaded for electrophoresis in 8 % polyacrylamide gels. Gels were stained and visualized as previously described (Bassam et al. 1991).

Statistical analysis and genetic mapping

Chi-squared (χ^2) tests were used to evaluate the goodness of fit of observed and expected segregation ratios for stripe rust reaction and molecular markers. The "chitest" procedure in Microsoft Office Excel 2007 software was used to calculate *P* values. Linkage analysis and construction of a genetic map containing the SSR and SRAP markers and resistance loci were performed using MapMaker 2.0 software (Lander et al. 1987). A LOD ratio of 3.0 was adopted for significance, and recombination values were converted to map distances using the Kosambi mapping function (Kosambi 1943).

Polymorphisms of adjacent markers in wheat genotypes

The usefulness of a molecular marker in breeding depends on polymorphism between the gene donor and recipient genotypes. To evaluate the usefulness of the proximal markers *BE490440* and *BE489244* in marker-assisted selection, the 35 most prominent varieties from the Yellow River-Huai area of China and four wheat-*P. huashanica* translocation lines (Table 2) were tested.

Results

GISH identification in H9020-17-25-6-4

Somatic cells of the resistant line H9020-17-25-6-4 probed with genomic DNA from *P. huashanica* showed no visible translocation in H9020-17-25-6-4.

Table 2 Simple sequence (SSR), EST-STS, and SRAP markers linkage to *YrHu* and marker pair size, genotype A/B, and presence (+) or absence (−) H9020-17-25-6-4, resistance bulk, Mingxian 169, and susceptible bulk

Markers	Primer pair	Size(bp)	A/B or (+)/(−)				Inheritance
			RP	RB	SP	SB	
Xwmc11	WMC11	670	+	+	−	−	Co-dominant
Xwmc532	WMC532	181/188	A	A	B	B	Dominant
Cfd79	CFD79	251/254	A	A	B	B	Dominant
Xgwm2	GWM2	105/110	A	A	B	B	Dominant
BE490440	BE490440	950	+	−	+	−	Co-dominant
BE489244	BE489244	650	+	−	+	−	Co-dominant
BG604577	BG604577	490	+	−	+	−	Co-dominant
Me1-em9	Me1/em9	975	−	+	−	+	Co-dominant
Me3-em13	Me3/em13	490	+	−	+	−	Co-dominant

RP resistance parent H9020-17-25-6-4, RB resistance bulk, SP susceptible parent Mingxian 169, SB susceptible bulk, A genotype A, B genotype B, + presence, − absence

This result suggested that if H9020-17-25-6-4 carried an alien translocation, it was too small to be detected by GISH (fig. S1).

Evaluation of morphological traits

The morphological features of H9020-17-25-6-4 and cv. 7182 were quite similar (Fig. 1). H9020-17-25-6-4 produced rounded, plump, white seeds, which were slightly different from the red seeds produced by the male parent *P. huashanica*. The spike morphology of H9020-17-25-6-4 was wheat-like, and it had an awn. The mean spike length of H9020-17-25-6-4 was 12 cM, that of 7182 was 8 cM, and that of *P. huashanica* was 6 cM. In addition, H9020-17-25-6-4 produced red seeds, which resembled those of *P. huashanica*.

Inheritance of stripe rust resistance

H9020-17-25-6-4 and *P. huashanica* were resistant to all tested *Pst* races at the seedling stage, whereas 7182 and Mingxian 169 were susceptible. A total of 150 F_2 seeds produced 148 F_2 plants, which were tested with CYR29 for stripe rust reaction; 113 were resistant (IT 1–2^+), and 35 were susceptible (IT 3^-–4), a 3:1 ratio

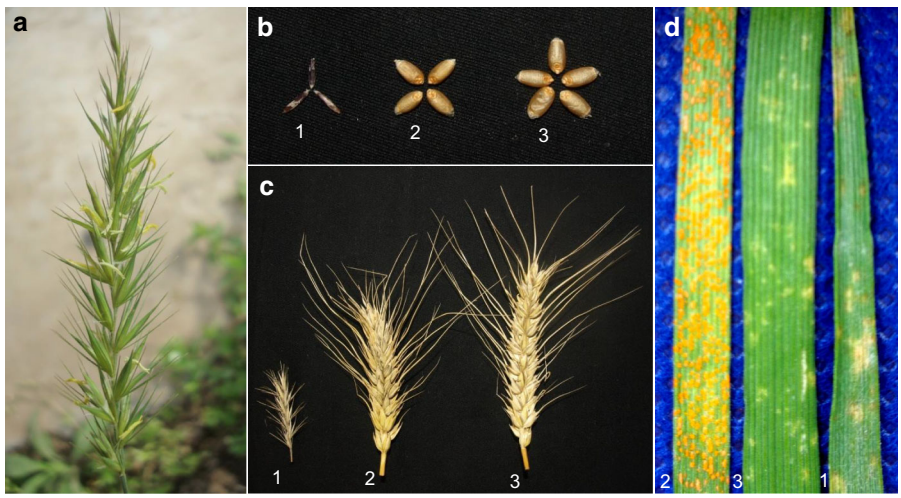

Fig. 1 The *Psathyrostachys huashanica*, spikes, spikelets, and seed features of *wheat-Psathyrostachys huashanica* disomic translocation line H9020-17-25-6-4 and its parents, common wheat cv. 7182 and *P. huashanica*. **a** *P. huashanica*, **b** spikes, spikelets, and seeds. **c** Infection types *1 P. huashanica*, *2* 7182, *3* H9020-17-25-6-4

($\chi^2 = 0.08$, $P = 0.70$). Among the $F_{2:3}$ 148 lines, 38 were homozygous resistant, 75 were segregating, and 35 were homozygous susceptible, thereby showing a 1:2:1 ratio ($\chi^2 = 0.15$, $P = 0.93$). The homozygous susceptible lines were all derived from susceptible F_2 plants, and the resistant and segregating lines were all derived from resistant F_2 plants. To confirm that the same gene confers resistance to various races, the $F_{2:3}$ lines were tested with race CYR33. All $F_{2:3}$ lines were scored identically to race CYR29. The 148 $F_{2:3}$ lines were also scored identically to race CYR29 and CYR33 in the 2010–2011 field test. The results indicated that a gene in H9020-17-25-6-4 conferred resistance to both CYR29 and CYR33. We tentatively designated this gene as *YrHu*.

Molecular mapping of stripe rust resistance gene in the translocation line

The F_2 genotypes of Mingxian 169/H9020-17-25-6-4 were used to map the resistance gene. Of 778 microsatellite primers chosen for initial screening, 265 (40.2 %) were polymorphic between the parental lines. The *Xwmc11*, *Xwmc532*, *Xcfd79*, and *Xgwm2* were polymorphic between the parents and bulks.

Given that the four SSR markers were previously mapped on the short arms of chromosome 3A, 110 additional EST-STS primer pairs for chromosome 3A were tested. Three EST-STS markers (*BE490440*, *BE489244*, and *BG604577*) that generated repeatable polymorphisms between the parents and contrasting bulks were used to genotype all 148 surviving plants from the F_2 population. The EST-STS markers behaved as dominant markers and amplified products for H9020-17-25-6-4 only (Fig. 2).

Saturation of a chromosome 3AS genetic map

Of 128 SRAP markers, two showed polymorphism between the parents and contrasting bulks. These markers were genotyped on the entire F_2 population. A linkage map consisting of nine markers (4 SSR, 3 EST-STS, and 2 SRAP markers) was constructed using phenotypic and genotypic data from the F_2 population (Table 2; Fig. 3). The three EST-STS markers, *BG604577*, *BE489244*, and BE490440, were assigned to chromosome bin 3AS-0.45-1.00. Linkage analyses confirmed the genetic association of the four SSR markers, three EST-STS markers, and two SRAP markers with stripe rust resistance. Analyses with MapMaker 3.0 software confirmed linkage between the markers and *YrHu*. *BG604577* and *BE489244* were proximal to the resistance gene, and *Xwmc11* and *Xgwm2* were distal (Fig. 3). The linkage map spanned a total genetic distance of 37.6 cM.

Validation of *YrHu* linked markers

To determine the origin of the resistance gene in H9020-17-25-6-4, the two closest flanking EST-STS markers (*BG604577*, *BE489244*) were tested with 7182 and *P. huashanica*. EST-STS analyses showed that *P. huashanica* possessed the same bands as those of H9020-17-25-6-4, whereas the target bands were absent in common wheat parent 7182 (fig. S2). The result indicated that the resistance gene in H9020-17-25-6-4 originated from *P. huashanica*. Markers *BG604577* and *BE489244* were also genotyped on a set of 39 wheat cultivars and lines to check for the presence of *YrHu*-linked alleles in these markers (table S3). Marker *BG604577* amplified a 490-bp product in resistant parent H9020-17-25-6-4 and was null in the susceptible parent Mingxian 169. The *YrHu*-linked 490-bp allele failed to amplify in all tested cultivars, thereby indicating the usefulness of this marker for marker-assisted selection of *YrHu*. Marker *BE489244* amplified a 650-bp product that was present in four cultivars susceptible to CYR33. Therefore, *BG604577* can be used for marker-assisted introgression of *YrHu* in wheat genotypes lacking the resistance-linked 490-bp allele.

Discussion

In recent years, an increasing number of researchers have focused on *P. huashanica* because of its excellent agronomic characteristics, especially resistance to stripe rust. Thus, transferring stripe rust resistance gene(s) from *P. huashanica* to wheat cultivars would be very beneficial to the wheat agriculture. Indeed, four stripe rust resistance genes, i.e., *YrHua* in chromosome 6AL of H9020-17-5 (Cao et al. 2008), *YrH9020* in chromosome 2DS of H9020-1-6-8-3 (Li et al. 2012), *YrH9014* in chromosome 2BS of H9014-14-4-6-1 (Ma et al. 2013a), and *YrHA* in chromosome 1AL of H9014-121-5-5-9 (Ma et al. 2013b), have been stably transferred from *P. huashanica* to hexaploid wheat germplasm. The two closest EST-STS markers

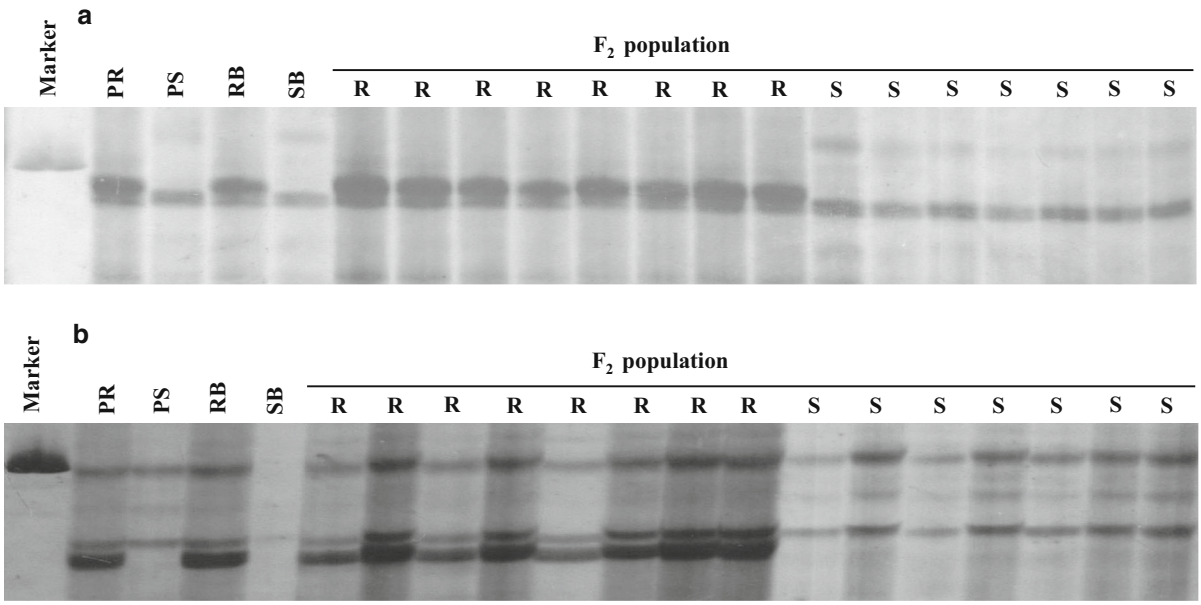

Fig. 2 Polyacrylamide gels showing EST-STS markers *BG604577* (**a**) and *BE489244* (**b**) that were polymorphic among resistance parent H9020-17-25-6-4 (PR), susceptible parent Mingxian169 (PS), resistant bulks (RBs), and susceptible bulks (SBs) and among F2 progeny of the Mingxian169/9020-17-25-6-4 cross

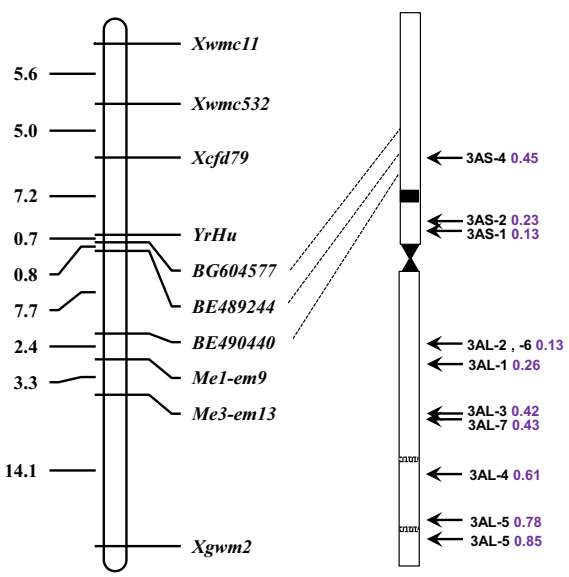

Fig. 3 Linkage maps of stripe rust resistance genes *YrHu*, flanking with four SSR, three EST-STS, and two SRAP markers markers on chromosome 3AS. The deletion bin location of *YrHp* in 3AS is based on the deletion map at the Wheat Genetic and Genomic Center of Kansas State University (http://www.k-state.edu/wgrc/Germplasm/Deletions/grp3.html)

(*BE490440* and *BE489244*) *YrHu* were tested on the four earlier translocation lines, and the specific bands were absent in all four lines. These results suggest that the resistance gene in H9020-17-25-6-4 was different from those in the other translocation lines. In this study, we identified a new resistance gene *YrHu* in translocation line H9020-17-25-6-4.

Based on rust response and molecular genotyping, *YrHu* was localized on the short arm of chromosome 3A. To date, none of the officially named *Yr* genes is located on chromosome 3A. Only one stripe rust resistance gene, temporarily named *YrTr2* from cultivar Tres, was previously located on chromosome 3A using monosomic analysis (Chen et al. 1995).

H9020-17-25-6-4 was selected for stripe rust resistance from a cross of susceptible common wheat cv. 7182 and *P. huashanica*. However, based on GISH, we found no cytological evidence for an alien translocation. The gene on chromosome 3AS named *YrHu* must be present in either a small alien segment derived from *P. huashanica* or a small wheat segment derived from gene recombination. More studies are needed to determine the source of *YrHu*. Currently, there is no resistance gene to stripe rust found on chromosome 3AS (McIntosh et al. 2013). Hence *YrHu* may be a new stripe rust resistance gene.

Interestingly, H9020-17-25-6-4 has an increased spike length, which may facilitate the production of more spikelets and kernels per spike, thereby improving the grain yield. Moreover, *YrHu* confers all-stage resistance. Therefore, H9020-17-25-6-4, which combined significant characteristics of high yield and stripe rust resistance and inherited stably, could be used directly by wheat breeders and therefore has a high application value. Usually, such genes provide excellent resistance when first identified and used, but the virulence race(s) inevitably overcome them. However, their wide effectiveness against currently predominant and newer races indicates that *YrHu* is potentially useful for wheat breeding programs in China. Moreover, the linked EST-STS marker alleles are absent or very rare in current Chinese varieties, indicating that they could be reliably used for marker-assisted introgression and selection of novel stripe rust resistance gene *YrHu* in wheat breeding programs.

Acknowledgments The research was supported by the National Basic Research Program of China (2013CB127700), National Science Foundation of China (31501620), Natural Science Funds of Hubei Province of China (2014CF367) and Hubei Key Laboratory of Food Crop Germplasm and Genetic Improvement (2014lzjj06), and the Open Project Program of State Key Laboratory of Crop Stress Biology for Arid Areas (CSBAA2015012). We thank Prof. R. A. McIntosh and Dr. Meinan Wang for critical review of the manuscript.

Compliance with ethical standards

Conflicts of interest None.

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