Genetic and Molecular Mapping of Stripe Rust Resistance Gene in Wheat–*Psathyrostachys huashanica* Translocation Line H9020-1-6-8-3

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Abstract

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat worldwide. The best strategy to control stripe rust is to grow resistant cultivars, but only a few effective genes are available. The wheat accession H9020-1-6-8-3 is a translocation line previously developed from interspecific hybridization between wheat genotype 7182 and *Psathyrostachys huashanica*, and is resistant to most Chinese *Puccinia striiformis* f. sp. *tritici* races. To identify the resistance genes in the translocation line, H9020-1-6-8-3 was crossed with susceptible genotype Mingxian 169, and seedlings of parents and F₁, F₂, and F₃ progenies were tested with prevalent Chinese *P. striiformis* f. sp. *tritici* races CYR32 and CYR33 under controlled greenhouse conditions. The genetic results indicated that two single dominant genes in H9020-1-6-8-3 confer resistance to CYR32 and

CYR33, respectively. The gene for resistance to CYR33 was temporarily designated as *YrH9020*. Six simple-sequence repeat markers were used to map the resistance gene to the short arm of wheat chromosome 2D, using 329 F₂ plants tested with CYR33 in the greenhouse. The genetic distances of the two closest flanking markers, *Xgwm261* and *Xgwm455*, were 4.4 and 5.8 centimorgans, respectively. Disease assessments and polymorphic tests of the flanking markers among the *Psathyrostachys huashanica* line and wheat lines 7182, H9020-1-6-8-3, and Mingxian169 suggested that the resistance gene *YrH9020* in H9020-1-6-8-3 was originally from *P. huashanica*. The exotic stripe rust resistance gene and linked molecular markers should be useful for pyramiding with other genes to develop wheat cultivars with high-level and durable resistance to stripe rust.

Wheat stripe rust, also known as yellow rust and caused by *Puccinia striiformis* f. sp. *tritici* Erikss., is one of the most devastating diseases that plagues wheat (*Triticum aestivum*) production globally, especially in China (5,6,20,31). China has the largest stripe rust epidemiologic region in the world in terms of wheat area affected by the disease, which is particularly destructive to autumn-sown wheat in the northwest and southwest of the country (20,32,34). Fifteen nationwide stripe rust epidemics have been recorded since 1950, and estimated losses of 6.0, 3.2, 1.8, and 1.3 million tons of wheat occurred in 1950, 1964, 1990, and 2002, respectively (20,34).

The most effective and economical approach to controlling the disease is growing resistant wheat cultivars (5,20,21). To date, more than 40 stripe rust resistance genes with official *Yr* designations have been reported in wheat, and many genes and quantitative trait loci (QTLs) have been temporarily named (5,9,12, 17,18,26,27). However, the majority of these genes are race-specific, and new *P. striiformis* f. sp. *tritici* races usually overcome the resistances used in cultivars within a few years of commercial production (8,20). Gene pyramiding, gene deployment, and multiline cultivar strategies are considered useful for protecting and prolonging the resistance of cultivars. The effective use of such strategies depends greatly on the availability of a range of resistance genes. However, only a few genes are available conferring effective all-stage resistance against the current populations of the pathogen worldwide. Therefore, it is urgent to identify new stripe rust resistance genes from various origins for diversifying resistance and pyramiding genes for different types of resistance in order to achieve high-level and durable resistance for sustainable control of stripe rust.

In addition to wheat landraces, wild wheat relatives are also important resources for stripe rust resistance in wheat breeding. Thus far, among the officially designated *Yr* genes, *Yr5*, *Yr8*, *Yr9*, *Yr15*, *Yr17*, *Yr19*, *Yr28*, *Yr35*, *Yr36*, *Yr37*, *Yr38*, *Yr40*, and *Yr42* were identified from wild wheat relatives. Among these, *Yr5* and *Yr15* are still resistant to most prevalent Chinese *P. striiformis* f. sp. *tritici* races (38).

Psathyrostachys huashanica Keng. (2n = 2x = 14, NsNs), a perennial cross-pollinating plant and a wild relative of common wheat, has been found only on Huashan Mountain in Shaanxi province, China. Therefore, it has been listed as an endangered and protected wild species in China. *P. huashanica* is characterized by early maturity and possesses many valuable traits such as winter hardiness, resistance to sterility drought tolerance, and broad-spectrum disease resistance. Through interspecific hybridization between wheat line '7182' and *P. huashanica*, embryo rescue technology, and subsequent extensive backcrossing with this wheat parent line, Chen et al. (4) have developed several translocation wheat lines, which greatly facilitates the utilization of *P. huashanica* in wheat breeding programs.

A preliminary study on inheritance of the resistance to stripe rust in *P. huashanica* accession 0503383 and some translocation lines indicated that *P. huashanica* has valuable disease resistance genes, which can be highly expressed in a wheat background (13). From translocation line, H9020-17-5, Cao et al. (3) identified a single dominant gene, *YrHua*, which confers resistance to *Puccinia striiformis* f. sp. *tritici* race CYR 31, and located the gene on wheat chromosome 6AL.

The current study focused on identifying the gene or genes conferring all-stage stripe rust resistance in another wheat–*Psathyrostachys huashanica* translocation line, H9020-1-6-8-3, which is resistant to most Chinese *Puccinia striiformis* f. sp. *tritici* races in both the seedling and adult-plant stage. Its resistance spectrum was different from H9020-17-5 in our preliminary tests using genetic analysis and molecular mapping.

Materials and Methods

Plant materials. H9020-1-6-8-3 is a wheat–*Psathyrostachys huashanica* translocation line which derived from the hybridization

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between donor parent *P. huashanica* accession 0503383 and receptor parent 7182 wheat. 'Mingxian 169' is a winter wheat cultivar that is susceptible to all Chinese *Puccinia striiformis* f. sp. *tritici* races identified thus far. To study the genetics of and develop molecular markers for its stripe rust resistance, H9020-1-6-8-3 was crossed with Mingxian 169, using Mingxian 169 as the female parent. F₁, F₂, and F₃ generations and parents were used in seedling tests in the greenhouse for identifying the genes for all-stage resistance in H9020-1-6-8-3.

The *Psathyrostachys huashanica* accession 0503383 and wheat 7182 were also tested in the greenhouse with 14 *Puccinia striiformis* f. sp. *tritici* races to identify the resistance spectrum of *Psathyrostachys huashanica* and deduce the origin of the stripe rust resistance gene in H9020-1-6-8-3. Also, wheat-*P. huashanica* translocation line H9020-17-5 was tested with these *Puccinia striiformis* f. sp. *tritici* races to distinguish the stripe rust resistance gene in H9020-17-5 from that in H9020-1-6-8-3.

Pathogen isolates and disease assessment. Seedling tests were conducted under controlled greenhouse conditions as described by Li and Zeng (20). In total, 14 *P. striiformis* f. sp. *tritici* races (CYR10, CYR21, CYR22, CYR23, CYR25, CYR27, CYR29, CYR29-mut3, CYR30, CYR31, CYR32, CYR33, Su11-4, and Su11-11), with various virulence gene combinations, were chosen to test seedlings of *Psathyrostachys huashanica* accession 0503383, wheat 7182 and Mingxian 169, and wheat-*P. huashanica* translocation lines H9020-1-6-8-3 and H9020-17-5. CYR32, which overcomes the resistance of most Chinese wheat cultivars and breeding lines, was the predominant race in China from 2001 to 2007 (34). CYR33 was the second most prevalent race after CYR32 from 2002 to 2007, and it has been the most prevalent race since 2008 (National Wheat Rust Research Group, unpublished observation). Thus, CYR32 and CYR33 were selected to test F₁, F₂, and F₃ populations derived from Mingxian169 × H9020-1-6-8-3, and the resistance gene to CYR33 in H9020-1-6-8-3 was selected to construct a genetic linkage map.

Seed of the two parents and F₁, F₂, and F₃ progenies were grown in pots 10 cm in diameter. About 15 seeds for each parent, 3 seeds for the F₁ lines, 300 seeds for the F₂ lines, and 15 seeds for each of the F₃ lines were planted, with about 15 seeds in each pot of the F₂ and F₃ progeny.

When the first leaves were fully expanded (about 10 days after planting), all seedlings were inoculated with fresh urediniospores of selected *Puccinia striiformis* f. sp. *tritici* isolates that were increased on susceptible wheat Mingxian 169, using the brushing method (20). After inoculation, the seedlings were placed in a dew chamber at 9°C and 100% relative humidity for 24 h, then trans-

ferred to a greenhouse maintained with 14 h of light and 10 h of darkness at 12 to 17°C. Infection types (ITs) were scored 15 to 16 days after inoculation, when rust was fully developed on the susceptible check, Mingxian 169, based on the scale of 0, 0+, 0-, 1, 1+, 2, 2+, 3-, 3, 3+, and 4 (2,19). Plants with ITs of 0 to 2+ were considered resistant and those with ITs of 3- to 4 susceptible (19,22).

DNA extraction and bulk segregant analysis. Genomic DNA was isolated from leaves using the cetyltrimethyl ammonium bromide method (30,37), dissolved in 1× Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and stored at -20°C. DNA was quantified using the mini-gel method (25) and a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific Inc., Wilmington, DE). The DNA stock solution was adjusted to 30 ng/μl with sterilized, deionized, and distilled water for use as the working solution for polymerase chain reaction (PCR).

The F₂ plants inoculated with CYR33 were used to construct the mapping population. Aliquots of DNA from the 10 most resistant and the 10 most susceptible F₂ plants were combined into resistant and susceptible bulks, respectively (29). The simple-sequence repeat (SSR) procedure was used to screen for potential markers linked to the resistance locus (14). Wheat SSR primers were synthesized by Shanghai Generay Biotech Co., Ltd. (Shanghai, China) according to the sequences published on the GrainGenes website (<http://wheat.pw.usda.gov/GG2/index.shtml>). In total, 355 SSR primer pairs covering all wheat chromosomes were screened on the parents and the bulks, among which were 36 SSR primer pairs located on chromosome 2DS and 34 SSR primer pairs located on chromosome 2DL, to further characterize the translocated line H9020-1-6-8-3 in detail. Primer pairs generating specific bands in both H9020-1-6-8-3 and the resistant bulks or Mingxian 169 and the susceptible bulks were used to genotype the 329 F₂ individual plants.

PCR amplification, electrophoresis, and gel visualization. The PCR reactions were performed in a PTC-200 Thermo Cycler (Bio-Rad Laboratories, Inc.). A 15-μl reaction mixture consisted of 45 ng of template DNA; 1.5 μl of Mg-free 10× PCR buffer; 0.6 unit of Taq DNA polymerase (Mbi Fermentas Inc., Ontario, Canada); 5 mM MgCl₂; 0.25 mM each of dCTP, dGTP, dTTP, and dATP; and 7.5 μM each primer. The PCR amplification conditions were as follows: after 4 min of denaturation at 94°C, amplifications were programmed for 35 consecutive cycles, each consisting of 1 min at 94°C, 1 min at 50, 55, or 60°C (depending on the individual primers), and 1 min at 72°C; and then a 10-min extension step at 72°C.

After amplification, 6 μl of formamide loading buffer (98% formamide, 10 mM EDTA [pH 8.0], 0.5% [wt/vol] xylene cyanol,

Table 1. Infection types on accessions of *Psathyrostachys huashanica*, wheat, and wheat-*P. huashanica* translocation lines tested with races of *Puccinia striiformis* f. sp. *tritici* as seedlings

Races	Virulence formula ^b	Infection types ^a				
		<i>P. huas</i>	7182	H9020-1-6-8-3	H9020-17-5	Mingxian 169
CYR10	2	0;	3	0	0-3	3
CYR21	1,2,3,4,6,7,8,9,10,11	0;	4	3	0	3
CYR22	1,2,3,4,6,7,8,9,10,11,13	0;	3-4	0-3	0	4
CYR23	1,2,3,4,6,7,8,9,11	0;	4	3	0	3-4
CYR25	1,2,3,4,5,6,7,8,9,11	0;	3	0;	0	3
CYR27	1,2,3,4,5,6,7,8,9,10,11,13	0;	3	3	0	4
CYR29	1,2,3,4,5,6,7,8,9,11,12,16	0;	3	0-0;	0;	3+-4
CYR29-mut3	1,2,3,4,8,11	0;	3	0-0;	0	3-4
CYR30	1,2,3,4,5,6,7,8,9,11,12,16,17	0;	4	0;-1	0;	4
CYR31	1,2,3,4,5,6,7,8,9,11,12,14,16,17	0;	4	0+;	0;-1	4
CYR32	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16,17	0;	4	0;-2	0-0;	4
CYR33	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16	0;	4	0;-2	0-0;	3+-4
Su11-4	1,2,3,4,6,7,8,9,10,11,13,14,16	0;	3+-4	0;	0;	4
Su11-11	1,2,3,4,6,7,8,9,10,11,13,14	0;	4	0+;-1	0;	4

^a *P. huas* indicates *Psathyrostachys huashanica* accession 0503383, 7182 and Mingxian 169 are common wheat cultivars, and H9020-1-6-8-3 and H9020-17-5 are translocation lines derived from the hybridization between *P. huashanica* accession 0503383 and 7182.

^b Chinese differential genotypes: 1, Trigo Eureka; 2, Fulhard; 3, Lutescens 128; 4, Mentana; 5, Virgilio; 6, Abbondanza; 7, Early premium; 8, Funo; 9, Danish 1; 10, Jubilejina 2; 11, Fengchan 3; 12, Lovirn13; 13, Kangyin 655; 14, Shuiyuan 11; 15, Zhong 4; 16, Lovirn 10; 17, Hybrid 46; 18, *Triticum spelta ablum*; 19, Guinong 22.

and 0.5% [wt/vol] bromophenol blue] was added to the PCR products. About 4 to 5 µl of the PCR product and loading buffer mixture for each sample was loaded for electrophoresis in a 6% polyacrylamide gel as previously described (7). After electrophoresis, the gel was silver-stained for visualization (7).

Data analysis. To evaluate the goodness of fit of observed and expected segregation ratios in F₂ and F₃ progenies to establish the number of stripe rust resistance genes and mode of inheritance, χ^2 tests were used. The "Chitest" procedure in the data analysis of Microsoft Office 2003 was used to calculate χ^2 and *P* values of χ^2 tests. Linkage analysis was conducted with Mapmaker 3.0b (16). Map distance in centimorgans was calculated according to the Kosambi mapping function (15), and a log of the likelihood ratio score of 3.0 was used as the threshold for a declaration of linkage. The Mapdraw 2.1 program was used to draw the linkage map.

Results

Origin of stripe-rust resistance in H9020-1-6-8-3. Seedling IT data from tests with the 14 *P. striiformis* f. sp. *tritici* races on H9020-1-6-8-3, Mingxian169 (as a control), and the direct parent lines of H9020-1-6-8-3 (*Psathyrostachys huashanica* accession 0503383 and wheat line 7182) are shown in Table 1. H9020-1-6-8-3 was resistant (IT 0; to 2) to 10 of the tested races (CYR10, CYR25, CYR29, CYR29-mut3, CYR30, CYR31, CYR32, CYR33, Su11-4, and Su11-11); susceptible (IT 3) to *P. striiformis* f. sp. *tritici* races CYR21, CYR23, and CYR27; and showed a variable IT to CYR22 (IT 0 to 3). By contrast, Mingxian169 was susceptible (IT 3 to 4) to all tested races. As for the parents of H9020-1-6-8-3, only *P. huashanica* accession 0503383 was highly resistant (IT 0;) to all tested races, similar to the reaction of H9020-1-6-8-3 to 10 of the races. In contrast, the other parent, wheat line 7182, was susceptible (IT 3 to 4) to all tested races. This result suggested that the stripe rust resistance of H9020-1-6-8-3 to these 10 races is controlled by an alien resistance gene originating from *P. huashanica* accession 0503383.

Inheritance of stripe rust resistance in H9020-1-6-8-3. The reactions of the F₁, F₂, and F₃ generations from the cross Mingxian 169 × H9020-1-6-8-3 to races CYR32 and CYR33 are shown in Table 2.

When tested with CYR32, F₁ plants responded with IT 0;, indicating dominance of resistance. In all, 227 F₂ plants segregated into 168 resistant and 59 susceptible, fitting a 3:1 resistant/susceptible ratio ($\chi^2 = 0.12$, *P* = 0.73), suggesting that a single dominant gene confers resistance to CYR32. The F₃ generation had 55 homozygous resistant, 109 segregating, and 41 homozygous susceptible lines, which fit a 1:2:1 (resistant/segregating/susceptible) ratio ($\chi^2 = 2.74$, *P* = 0.25), confirming that a single dominant gene is involved in stripe rust resistance to CYR32.

All three F₁ plants were resistant to CYR33. Of the 329 F₂ plants, 254 plants were resistant and 75 susceptible, fitting a 3:1 resistant/susceptible ratio ($\chi^2 = 0.85$, *P* = 0.36), indicating that a

single dominant gene is involved in resistance to CYR33. Among 235 F₃ families, 59 were homozygous resistant, 127 segregating, and 49 homozygous susceptible lines, showing a 1:2:1 (resistant/segregating/susceptible) ratio ($\chi^2 = 2.38$, *P* = 0.30). This further confirmed that a single dominant gene is associated with stripe rust resistance to CYR33, and the gene was temporarily designated as *YrH9020*.

Molecular mapping of the stripe rust resistance gene. Of the 355 SSR markers employed in this study, 116 markers (32.4%) showed clear polymorphisms between the two parents. Among these 116 markers, 16 were located on wheat chromosome 2DS and 10 were located on chromosome 2DL. All 116 markers were used for further investigation of resistant and susceptible DNA bulks. Of the 116 markers, only six SSR loci, (*Xgwm102*, *Xgwm455*, *Xgwm261*, *Xwmc503*, *Xcfd53*, and *Xgpm332*), all located on wheat chromosome 2DS, produced strong and repeatable bands that were polymorphic between the resistant and susceptible bulks as well as their parents. This indicated that *YrH9020* was located on chromosome 2DS. Subsequent linkage analysis, based on the phenotypic and genotypic data on the six polymorphic markers in the F₂ population, indicated that the resistance gene *YrH9020* was linked to the six SSR loci, with genetic distances of 4.4 to 14.6 centimorgans (cM) (Figs. 1 and 2). The two closest flanking SSR loci were *Xgwm261* and *Xgwm455*, with genetic distances of 4.4 and 5.8 cM, respectively. Among the six polymorphic markers, *Xgwm102*, *Xgwm455*, and *Xcfd53* were co-dominant, and *Xgwm261*, *Xwmc503*, and *Xgpm332* were dominant.

The linkage analysis based on the data from F₃ families genotyped with the most closely linked markers *Xgwm455* and *Xgwm261* confirmed the F₂ results (Table 3). For the 235 F₃ families, 55 of the 59 homozygous resistant genotypes carried the same allele as that of resistant parent H9020-1-6-8-3 at the SSR locus *Xgwm455*, and four were heterozygous. For the 127 segregating F₃ families, 112 were detected to have heterozygous genotypes; 4 had the same allele as H9020-1-6-8-3, and 11 the same allele as that of susceptible parent Mingxian 169. For the 49 homozygous susceptible genotypes, 39 carried the same allele as Mingxian 169, whereas 3 had the same allele as H9020-1-6-8-3 and 7 were heterozygous.

To further confirm the origin of the stripe rust resistance gene in H9020-1-6-8-3, four polymorphic SSR markers (*Xgwm261*, *Xgwm455*, *Xgwm102*, and *Xcfd53*) were used to test H9020-1-6-8-3, Mingxian 169, *P. huashanica* accession 0503383, and 7182. H9020-1-6-8-3 and *P. huashanica* produced the same polymorphic bands, whereas Mingxian 169 and 7182 had the same sized band, which further confirmed that the stripe rust resistance gene *YrH9020* in H9020-1-6-8-3 originated from *P. huashanica* accession 0503383.

Discussion

Because of the high variability of *Puccinia striiformis* f. sp. *tritici* races and a limited number of race-specific resistance genes

Table 2. Observed numbers, expected ratios, and χ^2 tests on plants or lines of parents and F₁, F₂, and F₃ generations from the cross Mingxian169 × H9020-1-6-8-3 tested with most prevalent Chinese *Puccinia striiformis* f. sp. *tritici* races CYR32 and CYR33

Test race	Parents or population ^b	Observed number of plants or lines ^a			Expected ratio	χ^2	<i>P</i> value
		Res.	Seg.	Susc.			
CYR32	H9020-1-6-8-3	14	—	0	...	...	...
	Mingxian169	0	—	13	...	...	...
	F ₁	3	—	0	...	...	...
	F ₂	168	—	59	3:1	0.12	0.73
	F ₃	55	109	41	1:2:1	2.74	0.25
CYR33	H9020-1-6-8-3	15	—	0	...	...	...
	Mingxian169	0	—	12	...	...	...
	F ₁	3	—	0	...	...	...
	F ₂	254	—	75	3:1	0.85	0.36
	F ₃	59	127	49	1:2:1	2.38	0.30

^a Res. = resistant, Seg. = segregating, and Susc. = susceptible.

^b H9020-1-6-8-3 = wheat-*Psathyrostachys huashanica* translocation line; Mingxian169 = winter wheat cultivar susceptible to all Chinese *Puccinia striiformis* f. sp. *tritici* races.

used in breeding programs, resistances in wheat cultivars have been continually overcome by emerging races, which has resulted in seven to eight large-scale replacements of cultivars since the 1950s in China (20,34). Furthermore, with the appearance and development of new virulent races CYR32 and CYR33, most commercial and candidate varieties lost resistance in recent years. Thus, extensive screening of potentially new sources of resistance, including those from wild wheat relatives, is urgently needed for wheat resistance breeding.

Indeed, utilization of exotic genes has made substantial contributions to wheat resistance improvement in China. Since the early 1970s, wheat cultivars with the 1B/1R translocation carrying *Yr9*, primarily derived from *Secale cereale* and carrier cultivars 'Lovrin 13', 'Predgorina 2', 'Kavkaz', 'Neuzucht', and their derivatives, have been widely used in wheat breeding programs in China (11). About 80% of Chinese wheat varieties contained *Yr9* and succumbed to destructive stripe rust epidemics in 1990 and 2002 (11,19,34,36). In addition, 'Zhong 4' and 'Guinong 22', the Chinese differential hosts of *P. striiformis* f. sp. *tritici*, show high-level resistance to all the known *P. striiformis* f. sp. *tritici* races and pathotypes and have also been utilized in wheat resistance breeding (10,35). The stripe rust resistance gene in Zhong 4 originated from *Thinopyrum intermedium* (33), and that in Guinong 22 was introgressed from a derivative of *Haynaldia villosa* and *Triticum durum* (39).

The wheat-*Psathyrostachys huashanica* translocation line H9020-1-6-8-3 used in this study was resistant to most prevalent Chinese *Puccinia striiformis* f. sp. *tritici* races in the seedling stage. The results of the genetic analysis indicated that the resistance to CYR32 and CYR33 in H9020-1-6-8-3 was controlled by two single dominant genes, one for CYR32 and one for CYR33, and the gene conferring resistance to CYR33 was located on chromosome 2DS by six SSR polymorphic markers. According to the genotypic data from the six markers in the F_2 and F_3 populations, some recombinant plants occurred in the F_2 and F_3 populations, and the result was similar with H9020-17-5 (3). When testing polymorphic amplified fragment length polymorphism (AFLP) markers on the 132 F_2 plants derived from the cross of wheat-*Leymus mollis* (Trin.) Hara translocation line 93784 and the susceptible wheat 'WL1', Zhou et al. (40) also found some recombinant plants. Research on the phenomenon of recombination within a translocated piece of an alien chromosome is underway.

To date, stripe rust resistance genes (QTLs) *Yr16*, *YrKat*, *QYr.inra-2DS*, and *QYr.caas-2DS* have been located on the short arm of chromosome 2D (1,23,24,28). *Yr16* originally derived from *T. aestivum* and is a non-race-specific, adult-plant resistance gene

(28). *YrKat* derived from 'Katepwa' and is a temperature-sensitive stripe rust resistance gene (1). Both *QYr.inra-2DS* and *QYr.caas-2DS* are QTLs for reduced stripe rust severity in the adult-plant stage that derived from French bread wheat 'Camp Rémy' and Italian common wheat 'Libellula', respectively (23,24). However, *YrH9020* in H9020-1-6-8-3 derived from *Psathyrostachys huashanica* and is a gene for race-specific, all-stage resistance. Therefore, *YrH9020* is unlikely to be identical to *Yr16*, *YrKat*, *QYr.inra-2DS*, or *QYr.caas-2DS*. *YrHua* derived from another wheat-*P. huashanica* translocation line, H9020-17-5, and was located on wheat chromosome 6AL by SSR marker *Xgwm169* (3). Polymorphic tests indicated that *Xgwm169* was not present in H9020-1-6-8-3, and the flanking markers for *YrH9020*, *Xgwm261*, and *Xgwm455* were also absent in H9020-17-5. Cao et al. (3) converted AFLP marker *PM14* (301) into an STS marker linked to

Fig. 2. Linkage map of stripe rust resistance gene *YrH9020* in wheat-*Psathyrostachys huashanica* translocation line H9020-1-6-8-3 and six polymorphic simple-sequence repeat markers on wheat chromosome 2DS based on 329 F_2 plants from cross of H9020-1-6-8-3 with susceptible wheat 'Mingxian 169', tested with most prevalent Chinese *Puccinia striiformis* f. sp. *tritici* race CYR33. Locus names are indicated on the right and genetic distances between loci in centimorgans on the left.

Fig. 1. Electrophoresis of polymerase chain reaction products amplified with simple-sequence repeat markers A, *Xgwm102* and B, *Xgpw332* in polyacrylamide gel. RP, resistant parent H9020-1-6-8-3 (wheat-*Psathyrostachys huashanica* translocation line); SP, susceptible parent, wheat 'Mingxian 169'; R, resistant F_2 individual plant; and S, susceptible F_2 individual plant from Mingxian 169 $\times$ H9020-1-6-8-3 cross tested with most prevalent Chinese *Puccinia striiformis* f. sp. *tritici* race CYR33. Arrow indicates the polymorphic band.

Table 3. F₂ genotypes inferred from seedling reactions of F₃ families from cross Mingxian 169 × H9020-1-6-8-3 tested with most prevalent Chinese *Puccinia striiformis* f. sp. *tritici* race CYR33 and the alleles of the H9020-1-6-8-3 resistance gene *YrH9020* at the two closest flanking simple-sequence repeat loci *Xgwm455* and *Xgwm261*

Markers, genotypes ^b	Allele ^a			Total
	A	H	B	
<i>Xgwm455</i>				
RR	55	4	0	59
Rr	4	112	11	127
rr	3	7	39	49
Total	62	123	50	235
<i>Xgwm261</i>				
RR	58	—	1	59
Rr	116	—	11	127
rr	9	—	40	49
Total	183	—	52	235

^a A, homozygous for H9020-1-6-8-3 (wheat–*Psathyrostachys huashanica* translocation line); H, heterozygous; and B, homozygous for Mingxian 169 (winter wheat cultivar).

^b RR, homozygous resistant; Rr, segregating; and rr, homozygous susceptible.

YrHua, and this STS marker was also absent in H9020-1-6-8-3. Furthermore, among 14 tested *Puccinia striiformis* f. sp. *tritici* races, H9020-1-6-8-3 was susceptible (IT 3) to CYR21, CYR23, and CYR27, and resistant-to-susceptible to CYR22 (IT 0 to 3). However, H9020-17-5 was resistant (IT 0 to 2) to all tested races, except for its resistant-to-susceptible reaction (IT 0 to 3) to CYR10 (Table 1). These results suggest that *YrH9020* should be different from *YrHua* and might be a new gene.

Psathyrostachys huashanica is a wild grass species related to wheat but different from other alien species from which stripe rust resistance genes have been reported and used in wheat breeding programs. Its resistance genes have been translated into a series of translocation, substitution, and addition lines through interspecific crossing between *P. huashanica* and wheat genotype 7182, which greatly facilitates utilization of resistance genes from *P. huashanica* in wheat resistance breeding programs. Unfortunately, *P. huashanica* resistance has not been used in wheat breeding programs thus far because of the poor understanding of its genetic basis and lack of linked molecular markers. In the present study, six SSR markers were identified to be linked to the resistance gene *YrH9020*, with genetic distance of 4.4 to 14.6 cM. Such linked SSR markers should be useful for marker-assisted selection. In wheat breeding programs, use of molecular markers may greatly facilitate resistance gene pyramiding and deployment, and improve breeding efforts in achieving durable stripe rust resistance.

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